LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

The Effects of Dietary Fat and Genotype on Insulin Sensitivity: The LIPGENE Dietary Intervention Study

A thesis submitted to the University of Dublin, Trinity College for the degree of Doctor of Philosophy (Ph.D.)

By

Audrey C Tierney

May 2008

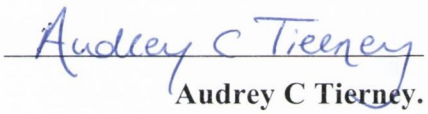
Department of Clinical Medicine
University Of Dublin
Trinity College

TRINITY COLLEGE
0 8 JAN 2009
LIBRARY DUBLIN
THOSIS
8678

Dedicated to my parents, Dermot and Mary

Declaration

I, the undersigned submit this thesis to the University of Dublin, Trinity College for the examination of the degree of Ph.D. The work herein is entirely my own except where acknowledged and has not been previously submitted for any other degree at this or any other University. The library may lend or copy this thesis upon request.



Audrey C Tierney.

Summary

The Metabolic Syndrome (MetS) is characterised by hyperinsulinaemia, low glucose tolerance, dyslipidaemia (increased triacylglycerol, (TAG) and non-esterified fatty acids (NEFA) and reduced high density lipoprotein (HDL)-cholesterol concentrations), hypertension and obesity. This cluster of factors has been recognised for many years and was formally labelled in 1988 by Gerald Reaven who suggested that insulin resistance is the key metabolic perturbation. Given recent evidence that dietary interventions have been more effective than pharmacological agents in preventing the development of the MetS and T2DM in high-risk individuals, exploration of the concept that altered dietary fat intake may attenuate the impact of the MetS warrants investigation. The objective of this thesis was to examine the relationship between dietary and plasma fatty acids and insulin sensitivity at baseline and after a 12-week dietary intervention in subjects with the MetS. It also served to determine if the IRS-1 genetic polymorphism Gly972R associated with increased risk of the MetS played a causal role in determining an individual's responsiveness to dietary fat modification.

In chapter three the design of the LIPGENE Dietary Intervention Study was outlined. In effect, this chapter introduced and provided the basis for the entire thesis. Over 15,500 subjects were screened to recruit 486 subjects with the MetS who initiated the study. At screening, waist circumference for males and females were 111cm and 103cm, respectively, with fasting plasma glucose 5.5mmol/L, TAG 1.8mmol/L, HDL cholesterol concentrations 1.30mmol/L and blood pressure 143/89mmHg.

The development of a food-exchange model to investigate the effects of four experimental diets, distinct in fat quantity and quality and suitable for use in free-living volunteers with the MetS, at eight centres across Europe was illustrated in chapter 4. The quantity of exchangeable fat in the diets were replaced by specifically designed study foods including spreads, cooking oils, mayonnaises and biscuits. Also critical to the food-exchange model were the use of carbohydrate exchanges to ensure the diets were isoenergetic. The results indicated that overall compositional targets were largely achieved with significant differences in fat quantity and quality between the four study diets.

Chapter 5 assessed, among MetS subjects, participating in the LIPGENE Dietary Intervention Study, the physiological, metabolic, biochemical and dietary characteristics between gender, age groups and BMI status and examined the association of these characteristics with the number of metabolic disorders that define the MetS. Females had greater insulin sensitivity at baseline ($p<0.05$) with no differences across the age categories. Body weight, BMI and waist circumference significantly decreased with increasing insulin sensitivity ($p<0.05$). Insulin sensitivity measured by QUICKI decreased with increasing number of MetS criteria and increasing body weight determined both S_I and HOMA-IR in linear regression analysis ($p<0.05$).

The LIPGENE Human Dietary Intervention Study was a multi-centre, trans–European, randomised, controlled trial with the aim of determining the relative efficacy of reducing dietary SFA consumption, by altering quality and reducing the quantity of dietary fat, on metabolic risk factors of the MetS. 417 free-living subjects with the MetS received one of four dietary treatments for 12 weeks: (1) High-fat SFA-rich diet (2) High-fat, MUFA-rich diet; (3) Low-fat, high-complex carbohydrate diet and (4) Low-fat, high-complex carbohydrate diet, with 1.24 g/d LC n-3 PUFA. An IVGTT, lipoprotein analysis, cytokines, adhesion molecules and coagulation factors were determined pre- and post-intervention. Dietary fat modification had no significant effect on insulin sensitivity (S_I) as illustrated in chapter 6. The effect of the four dietary treatments were determined in volunteers with a habitual high- or low-fat intake pre-intervention, defined as being above or below the median (36% of Total Energy). S_I was significantly lower following the high-fat, SFA-rich diet ($P=0.021$) in subjects with a habitual low-fat intake pre-intervention. The sensitivity to SFA was more applicable to females wherein S_I was reduced following the high-fat, SFA rich diet. HOMA-IR was also reduced in females following the high-fat MUFA diet ($P<0.05$).

The G972R variant is a potential contributor to the insulin resistance in T2DM. In chapter 7 the codon-972 aminoacid variant of IRS-1 in the heterozygous form was found in 11.7% of the WP1.2 MetS cohort. The GG homozygote group for rs1801278 in the IRS-1 gene had significantly higher QUICKI values than the AG/AA genotype ($p=0.019$). The effect of carrying an A allele in rs1801278 resulted in higher S_I values with plasma MUFA levels below the median of 28.2% ($p=0.032$).

Acknowledgements

I would like to sincerely thank all the following people for their help and support during this research:

Firstly I would like to thank Prof. Helen Roche for allowing me the opportunity to carry out this research and for her encouragement, patience, kindness and support shown to me throughout my Ph.D. I am grateful to Unilever for providing the study foods for the intervention study and to the EU Sixth Framework Programme for funding this research.

I would also like to thank Prof. Mike Gibney for his guidance and helpful advice and to Dr. Sinead McCarthy for her help in setting up the dietary intervention. To the PIs in the other European centres for their professional input, comments and constructive criticisms throughout this research. It was greatly appreciated.

To the other Ph.D students across Europe involved in the LIPGENE Dietary Intervention Study who without their dedicated time and effort, I would not have been able to complete this thesis, Thank you!

All of Mike's team in the Trinity Centre before the 'big move', Edel, Àine, Trìona, Sarah, Noelle, Aideen and Marianne for the much needed breaks and late evening banter during the first two years of the Ph.D. A special word of thanks to Jo, Catherine and Jennifer for your support and friendship throughout.

To everyone in the Lab: Catherine, Sinèad, Cathal, Vanessa, Mandy, Melissa, Elizabeth, Karen, Emma, Annalousie, Jane, Clare and Jolene. Thanks for your support and friendship and for proof reading the various chapters. Thanks especially to Catherine, Jane and Jolene for sharing the LIPGENE experience with me!!

I would like to express my sincere thanks to all the volunteers that took part in the LIPGENE Dietary Intervention Study and welcomed me into their homes for weekly weigh ins, 24-hour recalls and most importantly the cups of tea and friendly chats. Thanks especially to John D, Marie and the all lads in the buildings office for the continued support and lifelong friendships gained.

For those who assisted me in the human intervention study, Thank you! To those in the Diabetes Day Centre, Tallaght Hospital, especially Dr. James Gibney, Tommy, Neuman, Niamh and Corina who made those early Tuesday and Thursday mornings less laborious and somewhat fun and enjoyable. To Declan Gasparro and the staff in the

Endocrinology Lab for performing the insulin and C-peptide assays and to Dr. John Kearney (DIT) for all the statistical advice given for much of this thesis. I would also like to thank Dr. Lucy Norris and Jeanette Brosnan in the Department of Obstetrics and Gynaecology for help with the fibrinogen assay. Thanks to Dr. Fiona Lithander for encouraging chats in the last year of the Ph.D.

A special thanks to John, for proof reading the entire thesis and for the continued friendship, support and reality checks on a regular basis! Thanks also to Michael for the regular fine dining. Thanks to Deirdre and Sarah for the encouragement and support this past year.

To all my friends who have helped in different ways to keep me going and encouraged me to complete my thesis. A special thanks to Trish and Dan, Michelle, Laura, Hilda, Jennifer, Caroline, Edel and Joey, Mary, Charlene, Tess and Fin for your patience, understanding and constant encouragement. Thanks to the Spillane Family also.

To my family: Diarmaid for the acknowledgement that I am and always was your favourite sister! Marie, Brìd and Sheila, thanks for the support and for your own brand of sisterly encouragement for the duration of the project. Mam and Dad, thanks for the constant support and encouragement in everything I do. Your hard work and determination has been an inspiration and has been the driving force in the completion of this Ph.D.

Finally to Aidan ('my best friend'!), for your love and support throughout. Thanks for always reminding me what was important in life.

TABLE OF CONTENTS

Declaration		i
Summary		ii
Acknowledgements		iv
Table of Contents		vi
Abbreviations		xi
Publications, Abstracts and Presentations		xiii

Chapter One: **Introduction**

1.1	**Metabolic Syndrome**	1
1.1.1	Definitions used in the diagnosis of the MetS	2
1.1.2	Prevalence of the MetS	6
1.2	**Underlying factors of the MetS**	**8**
1.2.1	Insulin Resistance	8
1.2.2	Obesity	9
1.2.3	Dyslipidaemia	10
1.2.4	Inflammation	13
1.2.5	Coagulation Factors	14
1.2.6	Blood Pressure	14
1.2.7	Endothelial Dysfunction	15
1.2.8	Impaired Fasting Glucose and Impaired Glucose Tolerance	15
1.2.9	Lifestyle Determinants	16
1.2.10	Genetic Determinants	17
1.3	**Diseases associated with the MetS**	**18**
1.3.1	Type 2 Diabetes Mellitus	19
1.3.2	Cardiovascular Disease	20
1.4	Measuring Insulin Sensitivity and Insulin Resistance	**21**
1.4.1	Intravenous Techniques	21
	(i) Euglycaemic Hyperinsulinaemic Clamp	21
	(ii) Insulin Suppression Test	22
	(iii) Intravenous Glucose Tolerance Test	22
1.4.2	Fasting Indices - Non intravenous techniques	26
	(i) Fasting Insulin	26
	(ii) Oral Glucose Tolerance Test	27
	(iii) HOMA	27
	(iv) QUICKI	29
1.5	**Dysregulation of fatty acid metabolism in the aetiology of the MetS**	**32**
1.5.1	The effects of Non Esterified Fatty Acids on insulin resistance	32
1.5.2	Insulin Signalling - Disruption with fatty acids	33
1.5.3	Insulin Secretion - Disruption with fatty acids	38
1.5.4	Membrane phospholipid fatty acid composition and insulin action	39
1.5.5	Adipokines	39

1.6 Dietary Fat and Insulin Sensitivity **41**
1.6.1 Animal Data 43
1.6.2 Human Studies 45
1.6.3 n-3 PUFA 51
1.6.4 Plasma Fatty Acids 53
1.7 Effects of Dietary Fat on other features of the MetS **55**
1.8 Low-Fat, High-Carbohydrate Diets **58**
1.9 Impact of weight loss on insulin sensitivity **59**
1.10 Validity of Dietary Intake Data - Under-reporting **60**
1.11 Strategies to Prevent the MetS **61**
1.12 Gene-Nutrient Interactions: Implications for dietary fatty acids **61**
1.13 IRS-1 Gene **63**
1.14 Research Objectives **64**

Chapter Two: **Subjects, Materials and Methods**

2.1 Recrutiment **66**
2.1.2 Subjects 66
2.1.2 Inclusion/Exclusion Criteria 67
2.1.3 Recruitment Procedure 68
2.2 Screening Visit **68**
2.2.1 Body Weight, Height, Wasit and Hip Circumference and Blood Pressure 68
2.2.2 Bio-electrical Impedance Protocol 69
2.2.3 Collection of Screening Blood Sample 70
2.2.4 Determination of cholesterol concentration at screening 71
2.2.5 Determination of TAG concentration at screening 71
2.2.6 Determination of glucose concentration at screening 71
2.2.7 HDL isolation and analysis at screening 72
2.3 Randomisation and Study Design **72**
2.4 Dietary Intervention **73**
2.4.1 Dietary Assessments 73
2.4.2 Intervention Foods 74
2.5 Clinical Investigation Day **74**
2.5.1 Collection of Fasting Blood Samples 74
2.5.2 Sample Preparation Protocols 75
2.5.3 Intravenous Glucose Tolerance Test 78
2.6 Biochemical Analysis **78**
2.6.1 Triacylglycerol-rich lipoprotein (TRL) isolation protocol 78
2.6.2 LDL isolation protocol and analysis 79
2.6.3 Determination of cholesterol, TRL cholesterol, TAG, TRL TAG and HDL cholesterol concentration 79
2.6.4 Determination of NEFA concentrations 80
2.6.5 Determination of Plasma Glucose 80
2.6.6 Buffy coat isolation 80

2.6.7 Determination of Insulin and C-peptide 80
2.6.8 Plasma fatty acid compositional analysis 81
2.6.9 Plasma Apolipoprotein A1, B and E 83
2.6.10 Plasma apoCII and CIII 83
2.6.11 Plasma apoB48 83
2.6.12 Serum Cytokine Analysis 83
2.6.13 Determination of PAI-1 and tPA 84
2.6.14 Determination of Fibrinogen 84
2.7 IVGTT Modelling 86
2.8 Candidate Gene Identification, SNP selection and Genotyping 89
2.9 Statistical Power of Study 90

Chapter Three: **The LIPGENE Human Dietary Intervention Study: the interaction between dietary fatty acid modification and genetic risk of the MetS in Europeans - Cohort Details and Study Design**

3.1 Abstract 92
3.2 Introduction 93
3.3 Subjects and Methods 95
3.3.1 Study Design and Dietary Intervention Rationale 95
3.3.2 Recruitment and Screening Process 97
3.3.3 Clinical Investigations and Dietary Assessment, Pre- and Post-Intervention 100
3.3.4 Candidate gene identification, SNP selection and genotyping 103
3.3.5 Statistical power of study design 104
3.4 Results 106
3.5 Discussion 109

Chapter Four: **LIPGENE Food Exchange Model for alteration of dietary fat quantity and quality, in free-living participants with the MetS from eight European Countries**

4.1 Abstract 111
4.2 Introduction 112
4.3 Methods and Volunteers 114
4.3.1 Study Design 114
4.3.2 Food Exchange Model 115
4.3.3 Dietary Advice 117
4.3.4 Food Collection and compliance monitoring 118
4.3.5 Dietary Assessment 119
4.3.6 Statistical Analysis 119
4.4 Results 121
4.4.1 Achievement of dietary targets 121
4.4.2 Achievement of dietary targets by each centre 121

4.4.3 Compliance and Under-reporting 122
4.5 Discussion **131**

Chapter Five: **Determining age, gender and BMI differences in a MetS cohort and the associations between their plasma and dietary fatty acids and insulin sensitivity**

5.1 Abstract **136**
5.2 Introduction **137**
5.3 Subjects and Methods **142**
5.3.1 Dietary Assessment 143
5.3.2 Clinical Investigations 143
5.3.3 Collection of fasting blood samples 143
5.3.4 Intravenous Glucose Tolerance Test 144
5.3.5 Biochemical Analysis 145
5.3.6 Fatty Acid compositional analysis 146
5.3.7 Anthropometric measurements 146
5.3.8 Data preparation and statistical analysis 147
5.4 Results **150**
5.4.1 Characteristics of the cohort at baseline 150
5.4.2 Tertiles of Insulin Sensitivity 159
5.4.3 Ranking according to total fat, saturated fat and monounsaturated fat 161
5.4.4 Correlation Analysis 167
5.4.5 Linear Regression Analysis 174
5.4.6 Number of Criteria of the MetS 183
5.4.7 Binary Logistic Regression Analysis 183
5.5 Discussion **195**

Chapter Six: **The effects of different amounts and types of dietary fat on metabolic markers of the MetS - Findings from the LIPGENE Dietary Intervention Study**

6.1 Abstract **204**
6.2 Introduction **205**
6.3 Subjects and Methods **209**
6.3.1 Study Design 210
6.3.2 Dietary Assessment 212
6.3.3 Clinical Investigations 212
6.3.4 Collection of urine and blood samples 213
6.3.5 Intravenous Glucose Tolerance Test 214
6.3.6 Biochemical Analysis 214
6.3.7 Fatty acid compositional analysis 216
6.3.8 Anthropometric measurements 216
6.3.9 Data preparation and statistical analysis 217
6.4 Results **220**

6.4.1 Repeated Measures ANOVA 220
Anthropometry 220
IVGTT Parameters 231
Inflammatory Cytokines 240
Coagulation Markers 240
Lipids and apolipoproteins 247
Plasma Fatty Acids 250
6.4.2 Correlation Analysis 263
6.4.3 Linear Regression Analysis 285
6.4.4 The Effects of Four Dietary Treatments on Volunteers with a High and Low, Pre-Intervention Total Fat Intake 292
6.4.5 MetS at Post-Intervention 303
6.5 Discussion **304**

Chapter Seven: **The G972R variant of the insulin receptor substrate-1 (IRS-1) gene, insulin sensitivity and gene-nutrient interactions in a MetS cohort**

7.1 Abstract **316**
7.2 Introduction **317**
7.3 Subjects and Methods **319**
7.3.1 DNA extraction and genotyping 319
7.3.2 Statistical analysis 320
7.4 Results 321
7.4.1 Differences at Baseline 322
7.4.2 SNP*Fatty Acid Interactions 324
7.4.3 RM ANOVA 325
7.4.4 Linear Regression 327
7.5 Discussion **328**

Chapter Eight: **General Discussion** **331**

Chapter Nine: **Bibliography** **338**

Abbreviations

ACE	Angiotensin Converting Enzyme
ADA	American Diabetes Association
ADP	Adenosine diphosphate
AHA	American Heart Association
AIRg	Acute insulin response to glucose
ALA	Alpha Linoleic Acid
ALT	Alanine Transaminase
AMNCH	Adelaide & Meath Hospital, Dublin, incorporating the National Children's Hospital
ANOVA	Analysis of Variance
Apo	Apolipoprotein
ARIC	Atherosclerosis Risk in Communities Study
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
ATPIII	Adult Treatment Panel III
TAG	Triacylglycerol
AUC	Area Under the Curve
BHT	Butylated Hydroxy Toluene
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BP	Blood Pressure
BW	Body weight
C	Carbon
Ca^{2+}	Calcium
CETP	Cholesterol Ester Transfer Protein
CHD	Coronary Heart Disease
CHO	Carbohydrate
CI	Confidence Interval
cm	centimeters
CM	Chylomicrons
CO_2	Carbon dioxide
C-peptide	Connecting peptide
CRP	C-Reactive Protein
CV	Coefficient of variation
CVD	Cardiovascular Disease
DECODE	Diabetes Epidemiology Collaborative analysis of Diagnostic criteria in Europe
DHA	Docosahexaeoic acid
DI	Disposition Index
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
EGIR	European Group for the study of insulin resistance
ELISA	Enzyme linked immunosorbent assay
EM	expectation maximization
EPA	Eicosapentaenoic Acid
ER	Endoplasmic Reticulum
EU	European Union
FAME	Fatty Acid Methyl Esters
FFA's	Free fatty acids
FPIR	First Phase Insulin Response
FUQ	Food Use Questionnaire
g/d	grams per day
G0	Glucose at time 0 minutes
GC	Gas Chromatography
GLUT4	Glucose transporter 4
GP	General Practitioner
GSIS	Glucose Stimulated Insulin Secretion
H	Hydrogen
H(t)	Haplotype tagged
H_2O	Water
H_2O_2	Hydrogen Peroxide
HCL	Hydrochloric acid
HDL	High Density Lipoprotein
HFMUFA	High-Fat, Monounsaturated fat-rich diet
HFSFA	High-Fat, Saturated fat-rich diet
HGP	Hepatic glucose production
HLQ	Health and Lifestyle Questionnaire
HMW	High molecular weight
HOMA-IR	Homeostasis model assessment-insulin resistance
HOSO	High Oleic-acid Sunflower Oil
HPLC	High performance liquid chromatography
HRT	Hormone replacement therapy
hs	high sensitivity
HURS-UCO	Córdoba, Spain
Hx	history
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IKKb	I kappa B kinase beta
IL-6	Interleukin-6
IMT	Intima Medial Thickness
INSERM	U476, Marseille, France
IR	Insulin receptor
IRAS	Insulin Resistance Atherosclerosis Study
IRS-1	Insulin receptor-substrate-1
IV	Intravenous
IVGTT	Intravenous Glucose Tolerance Test
JNK1	C-jun N-terminal kinase 1
JUMC	Krakow, Poland
K^+	Potassium
kg	kilograms
LC A CoA	Long Chain acyl CoA
LC n-3 PUFA	Long-chain n-3 polyunsaturated fatty acids
LC	Long chain
LDL	Low Density Lipoprotein
LF n-3 PUFA	Low-Fat n-3 Polyunsaturated rich diet
LF	Low-Fat
LMW	Low molecular weight
LOG	Logarithmic

LPL	Lipoprotein Lipase
m	metres
m^2	Metre squared
MetS	Metabolic Syndrome
MI	Myocardial infarction
min	minute
MINMOD	Millennium softward package to compute insulin sensitivity
mmHG	millimetre of mercury
mmol/L	millimoles per litre
MMW	Medium molecular weight
MUFA	Monounsaturated Fatty Acids
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
NaOH	Sodium hydroxide
NCEP	National Cholesterol Education Panel
NDNS	National Diet and Nutrition Survey
NEFA	Non-esterified fatty acids
NF-kB	Nuclear factor-kappa B
ng/ml	nanograms/millilitre
NGT	Normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institute of Health
nm	nanometre
NO	Nitroc Oxide
O_2	Oxygen
OGTT	Oral glucose tolerance test
OR	Odds ratio
P2	Removal rate of insulin from the interstitial space
P3	Movement of circulating insulin to interstitial space
PAI-1	Plasminogen activated inhibitor-1
PEG	Polyethylene glycol
PGI2	Prostacyclin I_2
PGI3	Prostacyclin I_3
PI3K	Phosphatidlylinositol-3-kinase
PKB	Protein Kinase B
PKC	Protein Kinase C
PPAR	Peroxisome Proliferator Activated Receptor
PPARγ	Peroxisome proliferator Activated Receptor gamma
PUFA	Polyunsaturated Fatty Acid
QUICKI	Quantitative insulin sensitivity check index
RCF	Revolutions per minute
RM ANOVA	Repeated Measures Analysis of Variance
SAT	Subcutaneous adipose tissue
SD	Standard deviation
SEM	Standard error of the mean
SFA	Saturated Fatty Acids
S_G	Glucose Effectiveness
S_I	Insulin Sensitivity
sICAM	soluble Intracellular Adhesion Molecule
SPSS	Statistical Package for the Social Sciences
SQRT	Square root transformation
SSPG	Steady State Plasma Glucose
SSQ	Subject Suitability Questionnaire
SST	Serum separator tube
sVCAM	soluble Vascular Adhesion Molecule
T2DM	Type 2 Diabetes Mellitus
TCD	Trinity College Dublin
TCF7L2	Transcription Factor 7-Like 2
TGD	Total Glucose Disposal
TGF-β	Transforming Growth Factor Beta
TNFα	Tumour necrosis factor alpha
tPA	Tissue type plasminogen activator
TRL	Triacylglycerol rich lipoprotein
TZD	Thiazolidinedione
UM	University of Maastricht
UO	University of Oslo
UoR	University of Reading
UU	University of Uppsala
VAT	Visceral adipose tissue
VIF	Variance Inflation factor
VLDL	Very Low Density Lipoprotein
vWF	von Willebrand Factor
WGA	Whole genome association
WHO	World Health Organisation
WHR	Waist-Hip-Ratio
Δ	delta
%E	Percentage Energy
°C	degrees centigrade
-2LL	-2 Log Likelihood

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Papers

Tierney AC and Roche HM (2007). The potential role of olive oil-derived MUFA in insulin sensitivity. *Mol Nutr Food Res* **51**, 1235-1248.

Phillips C, **Tierney AC** and Roche HM (2008) Gene-Nutrient Interactions in the Metabolic Syndrome. *Journal of Nutrigenetics and Nutrigenomics* **1**, 136-151.

Shaw DI*, **Tierney AC***, McCarthy S, Upritchard J, Vermunt S, Lovedal-Gulseth H, Drevon CA, Blaak E, Saris WHM, Karlstrom B, Helal O, Defoort C, Gallego R, Siedlecka D, Malczewska-Malec M, Lopez-Miranda J, Roche HM, Lovegrove JA (2008) LIPGENE food exchange model for alteration of dietary fat quantity and quality, in free-living participants with the Metabolic Syndrome from eight European countries. *British Journal of Nutrition* (Accepted)
*Joint first author

Tierney AC, Mc Monagle J, Shaw DI, Lovedal-Gulseth H, Helal O, vanHees A, Marin C, Partkya L, Karslström B, Vessby B, Dembinska-Kiec A, López Miranda J, Blaak E, Defoort C, Drevon CA, Lovegrove JA, Roche HM (2008) LIPGENE Metabolic Paper, Dietary fat modification & metabolic markers of the metabolic syndrome. *In preparation*

Articles

Tierney AC, Roche HM (2008) The Genetics of Obesity. *Nutrition Wise*, January edition

Abstracts

Tierney AC, Lovegrove JA, Gulseth H, Defoort C, Blaak E, Marin C, Partyka L, Karlstrom B, Vessby B, Dembinska-Kiec A, Miranda JL, Saris WHM, Lairon D, Drevon CA, Williams CM, GIbney MJ, Roche HM (2007) LIPGENE dietary intervention: Cohort details and study design. *Proc Nutr Soc* **66-a**

Tierney AC, Mc Monagle J, Shaw DI, Lovedal-Gulseth H, Helal O, vanHees A, Marin C, Partkya L, Karslström B, Vessby B, Dembinska-Kiec A, López Miranda J, Blaak E, Defoort C, Drevon CA, Lovegrove JA, Roche HM (2008) LIPGENE Metabolic Paper, Dietary fat modification & metabolic markers of the metabolic syndrome. *Submitted to European Association for the Study of Diabetes,* Italy, September 2008.

Presentations

18th International Congress of Nutrition, Durban, South Africa, September 2008 **(Poster Presentation)**

2nd European Nutrigenomics Conference (NuGO), September 2005, Tuscany, Italy **(Poster Presentation)**

1st NuGO Introduction Course for Young Investigators, April 2005, Marseille, France **(Poster Presentation)**

Nutrition Society, Summer Meeting, Diet and Chronic Disease, July 2007, Coleraine, Ireland **(Poster Presentation)**

Workshop on Obesity and its link to type 2 Diabetes for young investigators, July 2007, Munich, Germany **(Poster Presentation)**

Chapter One

Introduction

1.1 METABOLIC SYNDROME

The Metabolic Syndrome (MetS) is characterised by hyperinsulinaemia, impaired glucose tolerance, dyslipidaemia (increased triacylglycerol, (TAG) and non-esterified fatty acids (NEFA) and reduced high density lipoprotein (HDL)-cholesterol concentrations), hypertension and obesity. This cluster of factors has been recognised for many years and was formally labelled in 1988 by Gerald Reaven who suggested that insulin resistance is the key metabolic perturbation (Reaven, 1988a). Other factors associated with the MetS or its consequences are hyperfibrinogenaemia, increased plasminogen activator inhibitor-1 (PAI-1), low tissue plasminogen activator (tPA), nephropathy, microalbuminuria and hyperuricaemia (Hansen, 1999). The aetiology of the MetS is unknown but predisposing factors include aging, obesity, sedentary lifestyle and genetics. The syndrome predicts cardiovascular disease (CVD) and Type 2 Diabetes Mellitus (T2DM) (Ford, 2005b). Among non-diabetic insulin resistant subjects, waist circumference, HDL-cholesterol, fasting glucose, proinsulin, insulin sensitivity, 2 hour insulin, 2 hour glucose and impaired glucose tolerance (IGT) were all significant predictors of the MetS in univariate models. Waist circumference was the optimum variable to predict those who were likely to develop the MetS and was a better predictor than body mass index (BMI) (Palaniappan *et al.*, 2004). There are several terms in existence to describe the clustering of components – Syndrome X, Deadly Quartet, Insulin Resistance Syndrome or Cardiometabolic Syndrome as well as numerous ways of defining the syndrome with seven definitions proposed in the past 8 years (*Table 1.1*). In a cross-sectional analysis of 1859 men and women aged ≥20 years from the National Health and Nutrition Examination Survey (NHANES) 2001-2002, a larger percentage of participants with the MetS had fair or poor health than participants without the MetS (Ford & Li, 2008). Individuals with the MetS are twice as likely to die from, and have a three-fold higher risk of developing heart attack or stroke compared with people without the syndrome, with a 5-fold greater risk of developing T2DM (Isomaa *et al.*, 2001).

Table 1.1: Timeline of most popular definitions of the Metabolic Syndrome

Year	Organization
1998	WHO, (World Health Organisation)
1999	EGIR (European Group for the Study of Insulin Resistance)
2001	NCEP ATP III (National Cholesterol Education Program/Adult Treatment Panel)
2002	ACE (American College of Endocrinology)
2004	AHA/NHLBI (National Heart, Lung, and Blood Institute/American Heart Ass.)
2005	IDF (International Diabetes Federation)
2005	AHA/NHLBI (National Heart, Lung, and Blood Institute/American Heart Ass.)

1.1.1 Definitions used in the Diagnosis of the Metabolic Syndrome

The clustering of metabolic risk factors pertaining to CVD within individuals has spurred several established learned bodies to publish criteria to diagnose the MetS (See *Table 1.1*). *Table 1.2* highlights the similarities and differences between the most widely used definitions. Differences between the definitions include the essential criteria, the emphasis on adiposity and the priority of assignment. Although the differences may seem subtle, they can have a significant impact when it comes to prevalence rates among certain communities. For example, among 4714 nondiabetic men, central obesity (in the diagnosis of the MetS) was present in 61.1% (WHO) or 19.5% (NCEP) or 50.4% (IDF) of men, depending on criteria used (Qiao, 2006). Only about 30% of people appear to be diagnosed by most definitions and about 35-40% of people diagnosed with the MetS are only eligible for such classification using one definition (Day, 2007).

In a recent report, Gerald Reaven stated that the approach of the WHO in attempting to create diagnostic criteria was the most rational effort and the recent version of the IDF was the most dangerous (Reaven, 2006). The WHO was the first organisation to outline the clinical criteria for diagnosing the MetS (Alberti & Zimmet, 1998). The WHO designated the MetS as a special classification for persons with the potential for diabetes presenting with impaired fasting glucose (IFG) or insulin resistance by hyperinsulinemic euglycaemic clamp. Other risk components included central obesity, dyslipidaemia, hypertension and microalbuminuria. Their reasoning for the diagnosis of the MetS was to identify persons at risk of CVD (Reaven, 2006). The WHO definition was formulated as a working definition, to be

improved upon but to use as a sound basis to develop research and for comparative purposes (Hu *et al.*, 2004). The European Group for the Study of Insulin Resistance (EGIR) proposed some modifications to the WHO working definition to enable the identification of MetS patients distinct from T2DM (Balkau & Charles, 1999). In addition, this group recommended the use of fasting insulin as opposed to the euglycaemic clamp due to its limited used in epidemiological studies and in a clinical setting. Fasting insulin values above the highest quartile for the non-diabetic general population were indicative of the presence of insulin resistance. They also suggested using fasting plasma glucose measurements (>6.1mmol/L) instead of an oral glucose tolerance test (OGTT), further simplifying the criteria. EGIR also recommended using waist circumference instead of BMI and waist-hip ratio (WHR) as it is a more straightforward measurement and correlates better with intraabdominal visceral adipose tissue mass (Pouliot *et al.*, 1994).

The National Cholesterol Education Program, Adult Treatment Panel III (ATPIII) proposed its definition of the MetS in 2001 (NCEP, 2001). Their aim was to "focus on primary prevention in persons with multiple risk factors" and considered the MetS to represent "multiple, interrelated factors that raise CVD risk". The specific factors considered were abdominal obesity, atherogenic dyslipidaemia, elevated blood pressure (BP), glucose intolerance and pro-thrombotic and pro-inflammatory states. The components are not distinguished qualitatively from each other, any 3 out of the 5 having an adverse effect as any other group of 3 abnormalities (Reaven, 2006). The ATPIII criteria does not require insulin resistance for the diagnosis. However, 3 or more components gives reasonable prevalence estimates of 12.9% for men and 11% for women (Hu *et al.*, 2004). Of the individuals fulfilling this definition, 59.5% of men and 60% of women fall within the highest quartile of plasma insulin levels (Hu *et al.*, 2004). While the EGIR and the ATPIII definitions both use waist circumference to define obesity, with relatively high cut-off points, obesity is not an essential component (Cameron *et al.*, 2007). In 2004 and 2005 the NCEP ATPIII definition was modified by the American Heart Association and National Heart, Lung and Blood Institute (AHA/NHLBI) (Grundy *et al.*, 2004; Grundy *et al.*, 2005). In the San Antonio Heart Study (SAHS), lowering the plasma glucose improved the predictive discrimination of the MetS in Mexican Americans (Lorenzo *et al.*, 2003). For large scale screening purposes, the NCEP ATPIII classification is more practical than the WHO criteria, as fasting glucose is more easily attained and determination of insulin

resistance is not required. The relationship between the presence of the MetS by the ATPIII criteria and insulin resistance is suggested not to be perfect and suffers mostly from lack of sensitivity (Sierra-Johnson *et al.*, 2006). To provide a more sensitive identification of those individuals at increased risk, Sierra-Johnson *et al.* (2006) suggest to recognise that insulin resistance may be the underlying metabolic disorder contributing to increased CVD risk or to reduce the number of required ATPIII components from three to two. The authors further demonstrate that simple measurement of just waist circumference may provide greater overall diagnostic accuracy than counting categorical components of the MetS (Sierra-Johnson *et al.*, 2006).

In 2005, a new version of the MetS was published after a consensus conference organised by the International Diabetes Federation (IDF). The new worldwide definition highlighted additional metabolic criteria which they stated should be included in all research conducted in the field (IDF, 2005). The IDF criteria takes into account a lower cut-off value for central obesity with an ethnicity specific adjustment. It has been questioned whether the IDF set their cut-offs for waist circumference too low in identifying high-risk individuals for CVD and whether it labels low risk individuals in already economically challenged health systems (Nilsson *et al.*, 2007).

The various definitions share the same goal: to identify high-risk individuals who would most benefit from early and more intensive disease prevention.

Table 1.2: Definitions of the Metabolic Syndrome

WHO 1999	EGIR 1999	ATPIII 2001	IDF
Glucose intolerance, IGT or diabetes and/or insulin resistance	Insulin resistance (defined as hyperinsulinaemia)		Central obesity—waist circumference ≥ 90 cm (M) or ≥ 80 cm (F)
Plus two or more of the following	Plus two or more of the following	Three or more of the following	Plus two or more of the following
1 Obesity: BMI > 30 kg/m^2 or WHR > 0.9 cm (M), > 0.85 cm (F)	1 Central obesity: waist circumference ≥ 94 cm (M), ≥ 80 cm (F)	1 Central obesity: waist circumference ≥ 102 cm (M), ≥ 88 cm (F)	1 Triglycerides ≥ 1.7 mmol/l or treatment for this abnormality
2 Dyslipidaemia: triglycerides ≥ 1.7 mmol/l or HDL cholesterol < 0.9 mmol/l (M), < 1.0 mmol/l (F)	2 Dyslipidaemia: triglycerides > 2.0 mmol/l or HDL cholesterol < 1.0 mmol/l or on treatment for this abnormality	2 Hypertriglyceridaemia: triglycerides ≥ 1.7 mmol/l	2 Low HDL cholesterol: < 1.03 mmol/l (M), < 1.29 mmol/l (F) or treatment for this abnormality
3 Hypertension: BP ≥ 140/90 mmHg and/or medication	3 Hypertension: BP ≥ 140/90 mmHg and/or medication	3 Low HDL cholesterol: < 1.04 mmol/l (M), < 1.29 mmol/l (F)	3 Hypertension: BP ≥ 130/85 mmHg or medication
4 Microalbuminuria: urinary albumin excretion rate ≥ 20 μg min^{-1} or albumin : creatinine ratio ≥ 30 mg/g	4 Fasting plasma glucose ≥ 6.1 mmol/l	4 Hypertension: BP ≥ 130/85 mmHg	4 Fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed Type 2 diabetes
		5 Fasting plasma glucose ≥ 6.1 mmol/l	
The 2005 update to the NCEP ATPIII MetS definition specifies that waist circumference of ≥ 80 cm (F) and ≥ 90 cm (M) are to be used for Asian Americans; the glucose cut-off was lowered to 5.6mmol/L and treatment of dylipidaemia and BP was included (Grundy *et al.*, 2005).			
ATPIII, Adult Treatment Panel III; BMI, body mass index; BP, blood pressure; EGIR, European Group for the study of Insulin Resistance; HDL, high-density lipoprotein; IDF, International Diabetes Federation; IGT, impaired glucose tolerance; MetS, metabolic syndrome; M/F, male/female; WHO, World Health Organization; WHR, waist–hip ratio.			

1.1.2 Prevalence of the Metabolic Syndrome

On the basis of the definition from the NCEP, the prevalence of the MetS is approximately 25% of the general population with variations in genetic background (Ford *et al.*, 2002). The prevalence of the MetS varies depending on definition used and also on the characteristics of the population being studied. In the Kupio Ischemic Heart Disease Study, the predictive value of both the WHO and NCEP definitions of the MetS were examined. The prevalence ranged from 8.8% to 14.3% depending on the definition. The study reported that all cause mortality associated with the MetS was increased 1.9-fold and CVD mortality 2.6-fold when using the WHO definition, whereas the NCEP definition less consistently predicted all cause and CVD mortality (Lakka *et al.*, 2002). However, in the Third National Health and Nutrition Examination Survey (NHANES III) approximately 24% of adult men and 23% of adult women have the MetS as assessed by the NCEP definition (Ford *et al.*, 2002). This study however, reported marked differences in prevalence among ethnic groups and noted that the prevalence was highest in Mexican Americans and lowest in blacks of both sexes (Park *et al.*, 2003). In the Atherosclerosis Risk in Communities (ARIC) study, the prevalence of the MetS was 28% in men and 34% in women using the same definition. In 1997, the Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study determined the presence of the MetS using a modification of the WHO definition for 11 European cohorts and showed that 15% of nondiabetic men and 14.2% of nondiabetic women had the MetS (DECODE Study Group, 1998).

The prevalence of the MetS increases with age, rising steeply after the third decade and reaching a peak in men aged 50-70 years and in women aged 60-80 years (Park *et al.*, 2003). Although the high prevalence of T2DM and glucose intolerance has been previously attributed to aging itself, data suggest that the age-related decline in insulin sensitivity is associated with abdominal obesity and inactivity, such that older persons who are physically active and do not have increased abdominal girth are much less likely to develop T2DM (Cefalu *et al.*, 1995).

The prevalence of the MetS increases with obesity as assessed by BMI or waist circumference (Park *et al.*, 2003; Janssen *et al.*, 2004). In NHANES (1988-1994) the MetS was present in 4.6% of normal weight, 22.4% of overweight and 59.6% of obese men (Park *et al.*, 2003). With the increase in obesity closely linked to the rising rates of T2DM and its preceding states such as IFG or IGT, it is alarming to

consider that in the USA, data from NHANES show that amongst adults (20-74yrs) the prevalence of obesity has more than doubled from 15% (1976-1980 survey) to 32.9% (2003-2004 survey) (National Centre for Health Statistics). Of concern, the prevalence of overweight in children has also risen from 5% to 13.9% in 2-5 year olds, 6.5% to 18.8% in 6-11 year olds and 5% to 17.4% in 12-19 year olds (National Centre for Health Statistics). This prompts for immediate intervention in this vulnerable group. Vanhala *et al.* (1998) showed that half of obese children (n=712) had become obese adults with an especially high risk of the MetS and that childhood obesity overall increases the risk for the MetS in adulthood. Furthermore, the risk of the syndrome was lower among obese adults who had not been obese as children. One possible mechanism proposed by the author is that continuous obesity from childhood to adulthood serves as a 'generator' for prolonged insulin resistance (Vanhala *et al.*, 1998).

The influence of gender on the prevalence of the MetS varies between populations, related to the population's characteristics and the definitions used. Hwang *et al.* (2007) found in their population-based survey that women had the first isolated component earlier but presented as cases of MetS later than men. Lipid components of the MetS occurred earliest in both genders, indicating that dietary fat intake may contribute to this positive association (Miller *et al.*, 1990). The presence of different sex steroid hormones and intrinsic sexual dimorphisms existing at the molecular and cellular level may account for the gender differences (Mittendorfer, 2005). Greater visceral fat accumulation and lower plasma adiponectin concentrations in men than in women may be important candidates for mediating gender differences in insulin sensitivity and CVD (Wajchenberg, 2000; Nishizawa *et al.*, 2002). The finding that the protection conferred on women is not lifelong and dissipates after the age of 50 years supports the protective effect of female sex hormones and the unfavourable effects of testosterone on substrate metabolism in view of the MetS (Hwang *et al.*, 2007).

1.2 UNDERLYING FACTORS OF THE METABOLIC SYNDROME

1.2.1 Insulin Resistance

Insulin resistance is defined as an impaired biological response to either exogenous or endogenous insulin (ADA, 1998). Kolterman *et al.*, (1980) stated that insulin resistance is a general term used to describe any defect in insulin action and divided insulin resistance into abnormalities of insulin sensitivity or insulin responsiveness. In a consensus development conference on insulin resistance, the panel suggested that insulin resistance does not have to be confined just to parameters of glucose metabolism but should apply to any of the biological actions of insulin and might include its effect on lipid and protein metabolism, vascular endothelial function and gene expression (ADA, 1998). Insulin resistance is now recognised as a characteristic trait of T2DM and contributes to abnormalities in liver, fat, muscle tissue and pancreatic β-cells (Saltiel, 2000). Insulin resistance is associated with aging and a sedentary lifestyle with a genetic predisposition also advocated (Saltiel, 2000). Obesity is also a major component, its cause or effect not wholly determined.

Results from the Bruneck Study, a population based study, indicated that insulin resistance is very common among subjects with metabolic diseases (Bonora *et al.*, 1998). Lower prevalence rates were found with hypercholesterolaemia, hyperuricaemia and hypertension. Higher rates of insulin resistance were found with T2DM and hypertriglyceridaemia and in the low HDL-cholesterol states, with the latter two almost never occurring as isolated disorders and nearly always associating with insulin resistance. This suggests that insulin resistance is almost ever-present when several metabolic disorders cluster together, more so than when metabolic disorders are isolated (Bonora *et al.*, 1998). As this finding was independent of age, gender and BMI, Reaven's hypothesis that insulin resistance is more likely to be the common denominator with a pathogenic effect (Reaven, 1988a) is further substantiated.

Values for insulin sensitivity vary widely in healthy populations and there is no agreed-upon cut-off for the definitions of insulin sensitivity or insulin resistant individuals.

1.2.2 Obesity

Obesity predisposes to insulin resistance and is the most important determinant of T2DM (Reaven, 1988a; Pi-Sunyer, 1993). The total direct (obesity related primary medical consultation, hospital treatment and medicines) and indirect (sick days as a result of obesity-related illnesses and from early deaths as a consequence of the same illnesses) cost of obesity for the fifteen member states of the EU in 2002 was estimated to be approximately €32,800 $X10^6$/year (Fry & Finley, 2005).

The relationship between obesity and insulin resistance is seen across all ethnic groups and across the full range of body weights (Kahn & Flier, 2000). Central obesity has become a defining factor in the development of the MetS more so than peripheral body fat distribution (Kissebah *et al.*, 1982) with visceral adipose tissue (VAT) accumulation having a more significant pathogenic impact on insulin resistance than subcutaneous adipose tissue (SAT) (Carr *et al.*, 2004). However, due to its mass size, SAT contributes to circulating free fatty acids (FFAs) (Ybarra *et al.*, 2008). In a recent study, liposuction removal of abdominal SAT improved the lipid profile without an impairment in insulin sensitivity or inflammatory markers (Ybarra *et al.*, 2008). Insulin resistance in obesity is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (Reaven, 1995). These fundamental defects may be as a result of impaired insulin signalling in muscle and liver tissue as well as in adipocytes. There may also be a downregulation of the major insulin-responsive glucose transporter, Glut4. In both muscle and adipocytes, insulin binding to its receptor, receptor phosphorylation, tyrosine kinase activity and phosphorylation of IRS are reduced (Kahn & Flier, 2000). In humans, the TAG content of muscle correlates directly with insulin resistance and the fatty acid composition of muscle phospholipids influence insulin sensitivity (Borkman *et al.*, 1993)

Adipose tissue is not purely a storage tissue but also acts as an endocrine organ with adipokines (adipose tissue-derived hormones and inflammatory cytokines) playing an essential role in overall insulin sensitivity and insulin resistance (Ruan & Lodish, 2004).

Both animal and human studies have repeatedly shown that the intake of dietary fat and total energy are strongly and positively associated with body weight gain (Riccardi *et al.*, 2004). Epidemiological data supports this finding (Astrup, 2001). The importance of being overweight in relation to risk of developing the MetS

and T2DM is corroborated by intervention studies demonstrating an improvement in all abnormalities clustering in the MetS in individuals who reduce their body weight (Riccardi *et al.*, 2004). Weight loss improves insulin sensitivity in peripheral tissues, increasing the capacity of non-oxidative glucose metabolism (Henry *et al.*, 1986; Albu *et al.*, 1995). In the Normative Aging Study, overall adiposity was found to be positively related to both fasting and postprandial serum insulin concentrations. When the relationship of diet and insulin were examined, both fasting and postprandial serum insulin increased with increasing saturated fatty acid, after adjustment for total energy, body adiposity and body-fat distribution (Parker *et al.*, 1993).

An important question to ask in unravelling the aetiology of insulin resistance is why a proportion of obese subjects are not insulin resistant. One possibility is that the high rate of conversion of insulin resistance into impaired glucose tolerance and T2DM may remove many obese individuals from a cohort selected on the basis of normal glucose tolerance (Ferrannini *et al.*, 1997). Another reasoning is that in some individuals, weight accumulation occurs in ways or at sites that do not interfere with insulin action or glucose uptake (Ferrannini *et al.*, 1997). Ferrannini *et al.*, (1997) in their quest to elucidate this hypothesis also suggest that the levels of insulin sensitivity in many obese subjects were even higher before they gained weight. It is known from longitudinal studies (Swinburn *et al.*, 1991b) that the risk of weight gain is higher in insulin sensitive individuals.

1.2.3 Dyslipidaemia

Dyslipidaemia is a principal feature of the MetS. It is characterised by a group of interrelated plasma lipid and lipoprotein abnormalities which includes hypertriglyceridaemia, low HDL-cholesterol and increased small dense LDL-cholesterol particles (Nieves *et al.*, 2003; Taskinen, 2003). Over production of very low density lipoproteins (VLDLs) is also attributed to this disarray and is dependent on fatty acid metabolism and insulin sensitivity (Sparks & Sparks, 1994; Nieves *et al.*, 2003; Taskinen, 2003). With lipid overaccumulation, ectopic deposition of lipids in non-adipose tissue (e.g. liver, skeletal muscle) occurs leading to insulin resistance (Kelley *et al.*, 2002). The increased delivery of lipolytic products to the liver results in a higher concentration of circulating VLDL and LDL particles (Lewis & Steiner, 1996; Sniderman *et al.*, 2001), with enhanced clearance of selected TAG-rich lipoproteins (TRL) decreasing HDL-cholesterol concentrations (Lamarche *et al.*,

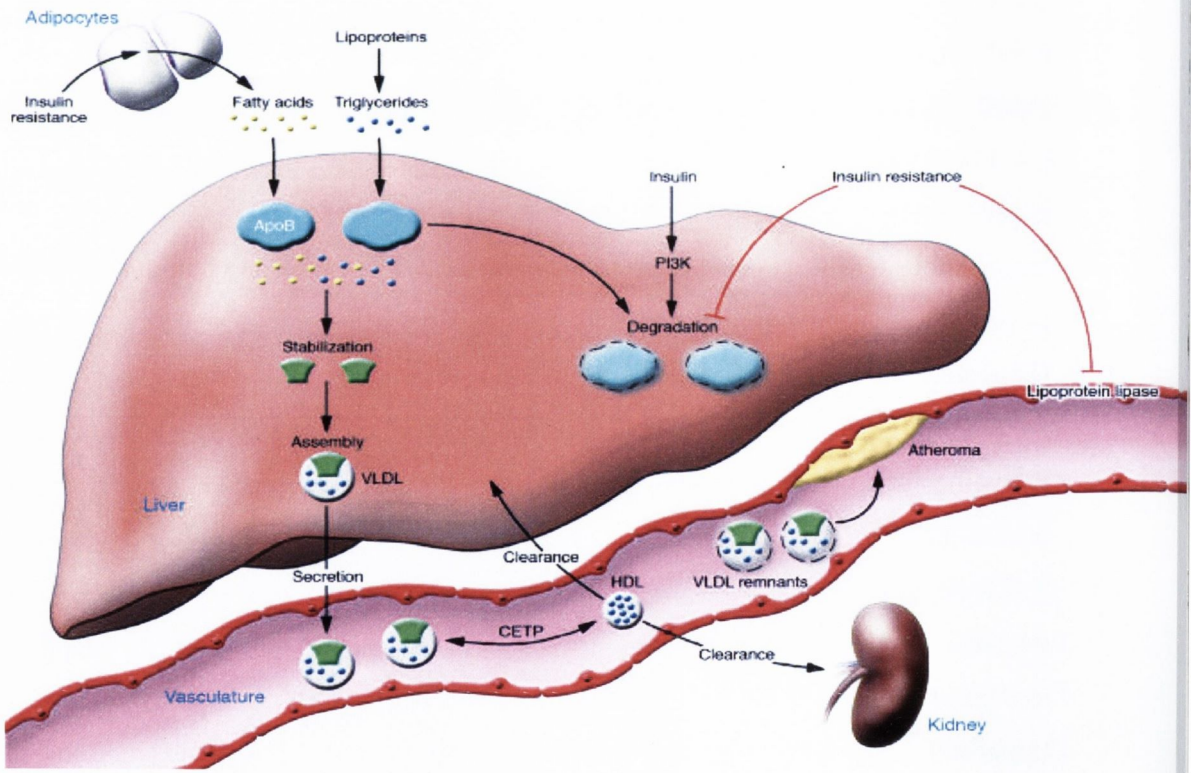
1999). Hepatic gluconeogenesis would also be promoted leading to hyperinsulinaemia as well as to the accumulation of fatty acid metabolites in the pancreas causing impaired insulin secretion (Unger & Zhou, 2001). Hornstra *et al.*, (1998), suggest that hyperinsulinaemia causes dyslipidaemia, as correction of the dyslipidaemic state leaves the insulin resistance unchanged, however, correction of the insulin resistance (by weight reduction, physical activity and a low-fat diet) results in the normalisation of dyslipidaemia.

Apolipoprotein (apo) B48 is essential for chylomicron formation in the intestine, the main pathway through which dietary fat enters the bloodstream. Intestinal TAG input varies on the basis of dietary fat intake. After chylomicrons enter the bloodstream, they rapidly undergo lipolysis via the action of lipoprotein lipase (LPL), lose much of their TAG and acquire cholesterol ester from other lipoproteins via the action of cholesterol ester transfer protein (CETP). During this process they also acquire apoE from HDL and become chylomicron remnants. These remnants are taken up by the liver via receptors that bind apoE (Schaefer, 2002). Apo E deficiency results in delayed apoB48 clearance and increases CHD risk (Schaefer *et al.*, 1985). ApoB100 is the major protein of VLDL and the sole protein of LDL. ApoB100 is combined with lipid by the liver by the actions of microsomal transfer protein. VLDL is also TAG rich. VLDL rapidly loses much of its TAG via lipolysis. LDL is the major cholesterol-carrying lipoprotein (Schaefer, 2002). LDL is cleared from plasma in part through the action of the LDL receptor by both the liver and peripheral cells (Schaefer, 2002). ApoA1 is essential for HDL formation. ApoA1 is present largely in an atheroprotective particle (with 2 to 4 apoA1 molecules per HDL particle). ApoCII is synthesised in the liver and intestine and activates LPL which hydrolyses TAG within the core of the lipoprotein particles and plays a key role in the regulation of TAG clearance (Jong *et al.*, 1999). ApoCIII is also synthesised in the liver and intestine and is thought to play several roles in the metabolism of TRLs with increased concentration of apoCIII inhibiting the binding and hydrolysis of lipoproteins by LPL and hepatic lipase (Jong *et al.*, 1999), and inhibiting the recognition and uptake of lipoproteins by the liver (Sehayek & Eisenberg, 1991; Mann *et al.*, 1997). Apo E which is synthesised in the liver, plays an important role in mediating the hepatic recognition and uptake of remnant particles by receptor mediated processes (Blum, 1982). The overexpression of Apo E on lipoproteins has

been shown to inhibit the LPL hydrolysis of TAG rich emulsions *in vivo* and *in vitro* (Rensen & van Berkel, 1996).

The disordered insulin signalling in peripheral tissues such as adipose cells results in abnormal lipid metabolism (*Figure 1.1*) (Semenkovich, 2006). With an increase in NEFAs, impaired insulin signalling leads to a loss or suppression of lipolysis. Insulin signalling through the PI3K dependent pathways, promotes the degradation of Apo B, which leads to the hypertriglyceridemic effect characteristic of insulin resistance. Insulin resistance also decreases LPL activity, which mediates VLDL clearance. VLDL is metabolised to remnant lipoproteins and VLDL which increases atherosclerotic risk (Semenkovich, 2006). The increased concentrations of TAG-rich VLDL particles contributes to reduced HDL metabolism in insulin resistance (Ginsberg, 2006).

Figure 1.1: Abnormal lipid metabolism in insulin resistance (Semenkovich, 2006).



1.2.4 Inflammation

Metabolic and immune pathways are closely linked and interdependent. Hormones, cytokines, signalling proteins, transcription factors and bioactive lipids can function in both metabolic and immune roles. In addition, metabolic and immune systems also regulate each other (Wellen & Hotamisligil, 2005). The normal inflammatory responses rely upon metabolic support and energy redistribution with the mobilisation of stored lipid playing an important role in fighting infection during the acute-phase response (Khovidhunkit *et al.*, 2004). Thus the basic inflammatory response favours a catabolic state and suppresses anabolic pathways including the insulin signalling pathway (Wellen & Hotamisligil, 2005).

Hotamisligil was one of the first to propose the concept of inflammation in relation to metabolic conditions, such as obesity and insulin resistance, with the demonstration that adipocytes constitutively express the proinflammatory cytokine tumor necrosis factor (TNF)- α and that expression of TNFα in adipocytes of obese animals is significantly increased (Hotamisligil *et al.*, 1993). These observations provided the first link between an increase in the expression of plasma concentration of a pro-inflammatory cytokine and insulin resistance. In humans, it was shown that expression of TNFα in adipose tissue fell after weight loss (Kern *et al.*, 1995). The inhibition of insulin signalling downstream of the insulin receptor (IR) is a primary mechanism through which inflammatory cytokines leads to insulin resistance (Wellen & Hotamisligil, 2005). Exposure of cells to TNFα or elevated NEFAs stimulates inhibitory phosphorylation of the serine residue of insulin receptor substrate (IRS)-1 (Hotamisligil *et al.*, 1996; Yin *et al.*, 1998; Aguirre *et al.*, 2000). This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the IR, thereby inhibiting downstream signalling and insulin action (Hotamisligil *et al.*, 1996; Aguirre *et al.*, 2002)

In a population based study, a statistically significant and positive correlation between CRP and total cholesterol, TAG, BMI, glucose and uric acid was observed, with a negative correlation between CRP and HDL cholesterol (Frohlich *et al.*, 2000). In a multicentre, population-based study, a strong association between CRP and body fat (BMI and waist circumference), insulin sensitivity and fasting insulin was reported (Festa *et al.*, 2000). In a multivariate linear regression model, BMI, systolic BP and insulin sensitivity were related to CRP levels (Festa *et al.*, 2000). This suggests that

low-grade systemic inflammation occurs in insulin resistance. However, whether it is a cause or effect of the disease is not clear.

1.2.5 Coagulation Factors

Although not included in the MetS criteria in most definitions, pro-thrombotic and pro-inflammatory states accompany the MetS the majority of the time.

Plasminogen Activator Inhibitor-1 (PAI-1) is the primary physiological inhibitor of plasminogen activation in blood and plays an important role in the regulation of the fibrinolysis cascade by binding and inactivating tissue-type plasminogen activator (tPA) (Kohler & Grant, 2000). Ultimately the action of PAI-1 inhibits the formation of plasmin and the degradation of fibrin clots and increases the risk for thrombus formation (Goralski & Sinal, 2007) and ultimately acute and chronic CVD and development of atherosclerosis. Visceral fat accumulation is considered an important regulator of PAI-1 and the elevated levels observed in insulin resistance could be as a result of overexpression by adipose tissue. It is suggested that PAI-1 may not just increase as a result of obesity and insulin resistance but may in fact have a direct causative role (Ma *et al.*, 2004).

Among people with the MetS, atherosclerosis is increased and fibrinolytic function is abnormal (Anand *et al.*, 2003). PAI-1 is increased among T2DM patients (Sobel *et al.*, 1998). Also, elevated endogenous tPA predicts mortality and myocardial infarction (MI) (Ridker *et al.*, 1993). Concentrations of PAI-1 in a group of apparently healthy persons was significantly correlated with degree of insulin resistance, fasting plasma insulin, TAG and HDL-cholesterol concentrations (Abbasi *et al.*, 1999).

Fibrinogen, considered an integral part of the acute phase response as well as the haemostatic system, is a strong and independent predictor of MI (Maresca *et al.*, 1999). It is suggested that adiposity is strongly associated with circulating levels of CRP and fibrinogen and that visceral as well as subcutaneous adipose tissue contribute (Festa *et al.*, 2001).

1.2.6 Blood Pressure (BP)

Serne *et al.*, (1999), demonstrated an inverse relationship between blood pressure and insulin sensitivity. On the basis of published reports, at least half of the patients with hypertension can be considered to have insulin resistance and hyperinsulinaemia (Reaven *et al.*, 1996). The possible mechanisms underlying a relationship between

insulin and BP are complex and can be divided into direct and indirect effects (Hornstra *et al.*, 1998). Hypertension may occur as a direct result of insulin resistance through the stimulatory effect which high insulin concentrations have on vascular smooth muscle proliferation (Banskota *et al.*, 1989). Insulin also enhances sodium retention directly via its effect on the proximal tubuli (DeFronzo, 1981) and indirectly through stimulation of the sympathetic nervous system and augmentation of angiotensin-II-induced aldosterone secretion (Rocchini *et al.*, 1990).

Reaven states that the relationship between insulin resistance, elevated BP and CVD is more complicated than that of any of the other components that make up the various definitions of the MetS (Reaven, 2006). To demonstrate the link, he presented the following three points: Firstly, patients with essential hypertension are insulin resistant and hyperinsulinaemic as a group; secondly, normotensive first degree relatives of patients with essential hypertension are more insulin resistant than matched controls without a family history of hypertension, and thirdly, hyperinsulinaemia has been shown in population based studies to predict the eventual development of essential hypertension (Reaven, 2006).

1.2.7 Endothelial Dysfunction

Damage of the endothelium leads to endothelial dysfunction which is characterised by enhanced expression of cytokines, cell adhesion molecules, von Willebrand factor (vWF), platelet activating factor and endothelin and decreased synthesis of prostacyclin (PGI_2) and transforming growth factor-β (TGF- β) (Hornstra *et al.*, 1998). Impaired endothelial release and/or action of nitric oxide (NO) has been proposed as a mechanism for reduced vasodilatation or "endothelial dysfunction" commonly associated with obesity and insulin resistance (Moller & Kaufman, 2005).

1.2.8 Impaired Fasting Glucose and Impaired Glucose Tolerance

Impaired glucose tolerance (IGT) is defined as hyperglycaemia following a glucose load (WHO., 1999). Subjects with IGT are at an increased risk of developing diabetes and CVD (Jarrett *et al.*, 1979; Fuller *et al.*, 1980; Burchfiel *et al.*, 1990). Due to the cumbersome nature of carrying out an OGTT, a new stage of impaired fasting glucose (IFG) (6.1-6.9mmol/L) was introduced by the ADA to replace IGT. One study (Tripathy *et al.*, 2000) clearly demonstrated that IFG and IGT represent 2 different populations with disturbed glucose metabolism. The major determinant of IFG was

defective insulin action rather than impaired β-cell function. This group also showed significantly elevated TAG and total cholesterol and lower HDL-cholesterol concentrations suggesting IFG subjects show several features of the MetS (Tripathy *et al.*, 2000). Subjects with IGT differed with respect to impaired insulin secretion relative to glucose and the degree of insulin resistance (Tripathy *et al.*, 2000). IGT remains a better predictor than IFG of risk of future diabetes and or mortality (Shaw *et al.*, 1999; Tuomilehto *et al.*, 2001).

1.2.9 Lifestyle Determinants

Adverse lifestyle factors independently associated with greater risk of having the MetS include physical activity, high carbohydrate (CHO) intake and smoking (Wannamethee *et al.*, 2006). Bjorntorp *et al.*, (1972), observed that insulin action is greater in physically active when compared to sedentary individuals. Insulin levels during an OGTT were lowest in men with the highest physical activity (independent of age, BMI, the ratio of subscapular to triceps skinfold thickness, cigarette smoking and energy intake) (Feskens *et al.*, 1994).

However, speculation exists as to whether exercise alone without a concomitant decrease in body weight can improve insulin sensitivity (Ross, 2003). The Diabetes Prevention Program has demonstrated that moderate intense physical activity for at least 150 minutes/week, combined with weight loss of 5% to 7% can reduce the development of the MetS in subjects with IGT (Orchard *et al.*, 2005). Physical activity could exert its protective effects on the MetS through improving the individual components (Zhu *et al.*, 2004). Zhu *et al.*, (2004), also observed a lower risk of having the MetS in light-to-moderate drinkers. With heavy drinkers, men had an increased risk of having the MetS consistent with a J-shaped effect of alcohol on CVD risk (Zhu *et al.*, 2004).

The term 'type A' was originally coined by Friedman and Rosenman (1959) in reference to what they believed to be a 'coronary-prone' behaviour pattern. This pattern is characterised by competetiveness, time urgency, impatience and hostility (Friedman & Rosenman, 1959). Individuals with a 'type A' behaviour pattern have been described as aggressive, dominant, quick-witted, self confident, autonomous and extraverted (Fry & Finley, 2005). Clinical features of the MetS are commonly observed among those individuals with a 'type A' coronary-prone behaviour pattern (Ravaja *et al.*, 1996), with gender differences observed (Fry & Finley, 2005).

Socio-economic deprivation is also associated with clinical features of the MetS (Bjorntorp & Rosmond, 2000), in individuals who have spent less time in education (Wamala *et al.*, 1999; Kerenyi *et al.*, 2000; Hidvegi *et al.*, 2001) and in those doing more menial jobs (Brunner *et al.*, 1997).

Recently, it was observed that whole-body insulin sensitivity was decreased in response to the combination of physical inactivity and a high-fat diet (Stettler *et al.*, 2005).

1.2.10 Genetic Determinants

The current global epidemic in the incidence of the MetS and T2DM is an important illustration of the shared contribution of both genetic and environmental factors to diet-related polygenic disorders. Evidence for a genetic basis for the MetS and T2DM has been derived from studies of families, twins and populations with genetic admixture. The familial nature, marked difference in the prevalence of the MetS among various racial groups and the difference in concordance rates between monozygotic twins is clearly consistent with the idea that there is a genetic component to susceptibility to these diseases. High heritability estimates have been reported for fasting glucose, insulin, TAG and HDL-cholesterol concentrations (Freeman *et al.*, 2002; Mills *et al.*, 2004). Poulsen *et al.*, (2001), have studied the relative impact of genetic versus environmental factors for the development of the components of the MetS amongst 303 elderly twin pairs. This study demonstrated that glucose intolerance, obesity and low HDL-cholesterol concentrations are significantly higher among monozygotic twins than among dizygotic twins, indicating a genetic influence on the development on these phenotypes (Poulsen *et al.*, 2001). In contrast, the heritability estimates for hyperinsulinaemia, hypertension and hypertriacylglycerolaemia are low, indicating a more important environmental influence on these components of the MetS. More recently, results from a comprehensive study examining young and elderly monozygotic and dizygotic twins provided further evidence for a role of genes in controlling insulin secretion, insulin action and nonoxidative glucose metabolism (Poulsen *et al.*, 2005). These data indicate a major role for genetic susceptibility, whilst also emphasising the important role of the environment. In particular, trends in diet and physical activity, coupled with genetic susceptibility, must account for the recent and dramatic rise in the

incidence of the MetS and T2DM, demonstrating the important impact of gene–environment interactions.

With the advent of high-throughput genetic analysis our understanding of the genetic architecture and biology of diet-related polygenic disorders is improving. 2006 heralded identification of the most important T2DM susceptibility gene known so far, transcription factor 7-like 2 (TCF7L2) (Grant *et al.*, 2006). The original study in Icelandic, Danish and US white cohorts reported that SNPs of the TCF7L2 gene, especially rs12255372 and rs7903146, were strongly associated with T2DM. This finding has been widely reproduced in several populations and also been shown to predict the conversion from impaired glucose tolerance (IGT) to overt diabetes (Florez *et al.*, 2006). More recently a Finnish study investigated the mechanisms of how these SNPs increase T2DM risk. They found that these variants altered TCF7L2 gene expression and are associated with decreased insulin secretion, but not insulin sensitivity, both in the general population as well as in the offspring of T2DM individuals (Wang *et al.*, 2007). As part of this research the Finnish Diabetes Prevention Study investigated the association of these SNPs with incident diabetes. Five hundred and twenty two overweight subjects with IGT were randomly assigned to either an intensive diet and lifestyle intervention group or to a control group. Subjects in the intervention programme were given individually tailored dietary advice aimed to reduce weight and intake of total and saturated fat and to increase intake of dietary fibre. In addition, subjects were guided to increase their level of physical activity. The control group received general information on the benefits of weight reduction, physical activity and healthy diet. The mean duration of follow-up was 3.9 years, at which point DNA for genotyping was available from 507 subjects. Interestingly the study found that variants of the TCF7L2 were associated with incident diabetes in the control group, but not the intervention group. This indicates that environmental factors such as lifestyle-intervention can reduce the risk conferred by genetic factors, even when risk genotypes are related to impaired insulin secretion (Wang *et al.*, 2007).

1.3 DISEASES ASSOCIATED WITH THE MetS

It is now established that the MetS predicts the development of T2DM and CVD (Wilson *et al.*, 2005). Coronary Heart Disease (CHD) remains the major cause of death in most affluent societies (Mann, 2002). Rates of this disease are declining in

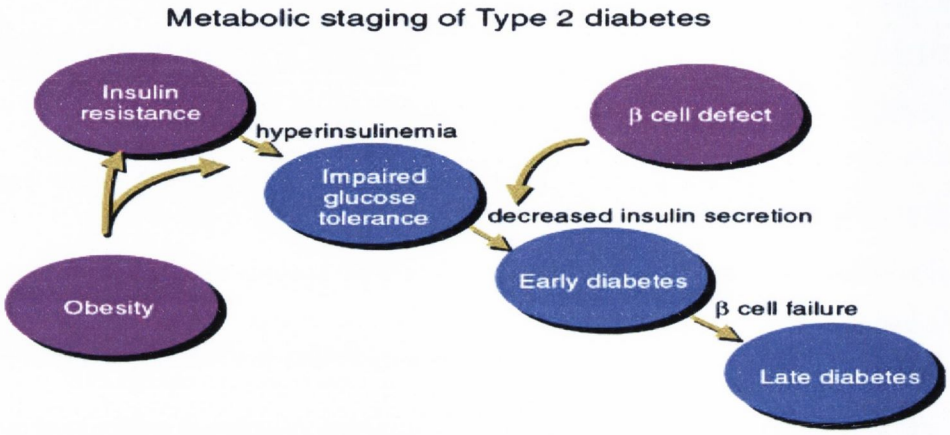
most developed countries, although they continue to rise in some parts of the world, especially in many countries in Eastern Europe and Asia. However, with respect to T2DM, unlike CHD, rates have not been declining in any country and statistics from the WHO suggest about 300 million adults worldwide will have developed T2DM by the year 2025 (King *et al.*, 1998). The rapid acculturation from a somewhat traditional lifestyle to one more in place with modern-day socieites, with an increase in the prevalence of a sedentary lifestyle and obesity (Zimmet, 1995) are attributuable to the growing numbers of individuals who will develop T2DM.

1.3.1 Type 2 Diabetes Mellitus (T2DM)

After the discovery of insulin in 1922, it was assumed that deficiency in this hormone, exclusively led to T2DM (Saltiel, 2000). However, further pioneering work by Himsworth (1936), and Yalow & Berson (1960), demonstrated that insulin insensitivity rather than insulin deficiency was the biochemical defect in this group of patients. *Figure 1.2* demonstrates that T2DM progresses from a worsening of insulin action, to peripheral insulin resistance to finally a loss of insulin secretion. β-cells of the pancreas normally compensate for the insulin resistant state by increasing basal and postprandial insulin secretion. With the eventual failure to compensate, the β-cells no longer respond appropriately to glucose leading to altered glucose homeostasis and the development of glucose intolerance. As insulin resistance increases, adipose tissue generates more fatty acids, the liver produces more glucose and the β-cells fail, resulting in progressive T2DM. Cross-sectional comparison of groups of various stages of impaired glucose tolerance (Knowler *et al.*, 1990) as well as prospective studies in high-risk populations (Bergstrom *et al.*, 1990; Haffner *et al.*, 1990) have suggested that insulin resistance is already evident in the early stage of disease. In an attempt to determine which is the earliest and perhaps primary abnormality in T2DM, Martin *et al.*, (1992) used a minimum model of glucose disposal and insulin secretion to estimate insulin sensitivity (S_I), glucose effectiveness (S_G-the propensity of high glucose concentrations (independent of insulin) to enhance glucose utilisation) and first-phase and second-phase β-cell responsiveness in normoglycaemic offspring of couples who both had T2DM (Martin *et al.*, 1992a). The study showed that the subject group had defects in S_I and S_G which both independently influence the risk of diabetes. The measures of insulin secretion, however, were non-predictive. The presence of these defects more than 10 years before the onset of clinical disease

suggests that they may be genetically determined traits that characterise a pre-diabetic state (Martin *et al.*, 1992a).

Figure 1.2: Metabolic staging of Type 2 diabetes (Saltiel, 2000).



1.3.2 Cardiovascular Disease (CVD)

The clinical features of the MetS are associated with an increased risk of CVD (Alexander *et al.*, 2003), including greater risk of CHD for a given level of LDL cholesterol and premature death (Trevisan *et al.*, 1998). The principle that the individual components can act synergistically or cluster together to amplify the risk is seen in the criteria used to establish the syndrome, however, the components can also act independently in the development of atherosclerotic CVD (Moller & Kaufman, 2005).

Trevisian *et al.*, (1998) examined the association of the MetS with the risk of CVD in a large population-based (22,256 men; 18,495 women) Italian study. They reported that the risk of death from all causes as well as CVD, increased with the number of metabolic abnormalities in both genders. Similarly, the Framingham Offspring Study (2406 men; 2569 women) examined the clustering of metabolic factors in relation to coronary heart disease (CHD) (Wilson *et al.*, 1999) and found that a cluster of 3 or more risk factors increased the risk of CHD 2.4 fold for men and 5.9 fold for women. In the Insulin Resistance Atherosclerosis Study (IRAS), higher S_I was associated with thinner intima-medial thickness (IMT) of the carotid artery wall in non-Hispanic whites and Hispanics (Howard *et al.*, 1996). This association remained significant even after adjustment for traditional CHD risk factors. It has been suggested that higher insulin levels are related to the development of

atherosclerosis both through a direct effect (Howard *et al.*, 1996) on the arterial wall and indirectly through the effects on lipids and BP (Stout, 1990).

1.4 MEASURING INSULIN SENSITIVITY AND INSULIN RESISTANCE

With the increasing number and types of methods to measure insulin sensitivity/resistance, the choice may seem confusing. However, depending on the given hypothesis, the goals and experimental limitations, one method may fit the criteria more than others.

1.4.1 Intravenous Techniques

(i) Euglycaemic Hyperinsulinaemic Clamp

The most widely accepted research method or "gold-standard" is the euglycaemic hyperinsulinaemic clamp technique, originally developed by Andres and co-workers (Andres *et al.*, 1966; DeFronzo *et al.*, 1979).

Briefly, exogenous insulin is infused, so as to maintain a constant plasma insulin level above fasting, while glucose is fixed at a basal level by infusing glucose at varying rates. The glucose infusion is delivered via an indwelling catheter at a rate based on plasma glucose which is measured every 5 minutes. When the plasma glucose level falls below basal, the glucose infusion rate is increased to return plasma glucose to basal levels and vice versa. The total amount of glucose infused over time is an index of insulin action on glucose metabolism. The more glucose that has to be infused per unit time, the more sensitive the patient is to insulin. In contrast, the insulin-resistant patient requires much less glucose to maintain basal plasma glucose levels. The advantage of this procedure is that the effect of insulin on fuel metabolism can be assessed without the confounding effects of hypoglycaemic counter-regulation, endogenous insulin secretion, or variable levels of hyperglycaemia (ADA, 1998). The use of isotopes allows for the assessment of insulin action on glucose uptake and production, inhibition of lipolysis and changes in protein metabolism (DeFronzo *et al.*, 1982). The insulin sensitivity index derived from clamp data can be defined as $SI_{CLAMP} = M/(G \times \Delta I)$, where M is normalised for G (steady-state blood glucose concentration) and ΔI (difference between fasting and steady state plasma insulin concentration) (Katz *et al.*, 2000).

During a clamp, insulin is administered as a constant infusion and, therefore does not reflect the variations inherent in endogenous secretion. Also, the peripheral

administration of insulin is unphysiological, which reverses the normal gradient between portal and peripheral insulin (Radziuk, 2000). The peripheral and hepatic responses to insulin are assumed to occur in parallel, which is unlikely to occur (Rizza *et al.*, 1981).

The main advantage of using the glucose clamp to estimate insulin sensitivity in humans is that it directly measures whole body glucose disposal at a given level of insulinaemia under steady-state conditions (Muniyappa *et al.*, 2008). The main disadvantage is that it is time-consuming, labour intensive, expensive and requires an experienced operator to manage the technical difficulties (Muniyappa *et al.*, 2008). Its use therefore in large clinical investigations or epidemiological studies is limited.

(ii) Insulin Suppression Test

The insulin suppression test was introduced by Shen *et al.* in 1970 and modified by Harano *et al.* (Shen *et al.*, 1970; Harano *et al.*, 1978). Briefly, after an overnight fast, somatostatin or the somatostatin analogue octreotide (Pei *et al.*, 1994) is intravenously infused to suppress endogenous secretion of insulin and glucagon. Simultaneously, insulin and glucose are infused into the same anticubital vein over 3 hours. From the contralateral arm, blood samples for the determination of glucose and insulin are taken every 30 minutes for 2.5 hours and then at 10 minute intervals for 150-180 minutes. The constant infusions of insulin and glucose will determine steady-state periods assumed to be from 150-180 minutes after initiation of the test (Muniyappa *et al.*, 2008). Steady state plasma glucose (SSPG) concentrations will be higher in insulin resistant subjects and lower in insulin sensitive subjects. This is a less labour-intensive and less technically demanding technique compared with the glucose clamp. Estimates of insulin sensitivity determined by SSPG correlate well with reference standard glucose clamp estimates in normal subjects (r=0.93) and in T2DM patients (r=0.91) (Greenfield *et al.*, 1981; Mimura *et al.*, 1994). Its limitations are similar to those cited with the clamp. In addition, SSPG under ideal conditions determines primarily skeletal muscle insulin sensitivity and is not designed to reflect hepatic insulin sensitivity (Muniyappa *et al.*, 2008).

(iii) Intravenous Glucose Tolerance Test (IVGTT)

Bergman *et al.*, (1986), developed an alternative approach for analysing the dynamic response to an injection of glucose, the 'minimal-model method', which used

computerised approaches to estimate β-cell function and insulin sensitivity from the frequently sampled IVGTT (Pacini & Bergman, 1986). To obtain an index of tissue sensitivity to insulin (S_I), a specific "minimal" mathematical model is used to account for the exact dynamics of glucose observed during the IVGTT (Bergman *et al.*, 1979; Bergman *et al.*, 1981). It is known that the ability to normalise blood glucose after administration of carbohydrate (CHO) depends on the combined abilities of secreted insulin and glucose itself to inhibit endogenous glucose production and accelerate glucose uptake (Lerner & Porte, 1971; Reaven & Olefsky, 1974b). The minimal model of glucose metabolism (Bergman *et al.*, 1979) had two major advantages over other methods at the time it was introduced (DeFronzo *et al.*, 1979). The IVGTT allowed individuals to be characterised by means of complex mathematical processing of the data obtained from a single experiment, and it avoided the difficulty of modeling feedback between glucose and insulin metabolism by treating plasma insulin as a measured input to the glucose system (Araujo-Vilar *et al.*, 1998). Although the S_I derived from the minimal model correlates well with glucose clamp measurements, identifying S_I from the minimal model in subjects with impaired insulin secretion is often problematic (Saad *et al.*, 1994). Some studies have demonstrated systematic errors in minimal model estimates of glucose effectiveness and insulin sensitivity that could be due to oversimplified model representation of physiology (Quon *et al.*, 1994; Finegood & Tzur, 1996; Cobelli *et al.*, 1998). Since the minimal model relies on the glucose disappearance rate in response to insulin, it is essential to have adequate endogenous insulin secretion (Wallace & Matthews, 2002). In subjects with diabetes the test is modified by giving a bolus of intravenous tolbutamide (a potassium channel blocker which stimulates the secretion of insulin) (Yang *et al.*, 1987) to augment second phase insulin secretion or by giving a bolus of insulin, 20 minutes into the test (Finegood *et al.*, 1990). Saad *et al.*, (1997), compared the two MINMOD protocols and showed that both the tolbutamide and insulin MINMOD protocols give insulin sensitivity measures that correlate strongly with each other and with those from the glucose clamp in nondiabetic individuals. However, when expressed in the same units, insulin sensitivity estimates from the tolbutamide protocol were only 13% lower than those calculated from the clamp, whilst insulin sensitivity measures from the insulin protocol were much lower than both. The authors proposed a saturation of insulin action or its transendothelial

transport to explain the lower S_I values from the insulin protocol, however, the results did not support this (Saad *et al.*, 1997).

After an overnight fast, an intravenous bolus of glucose (0.3g/kg body weight) is infused over 2 minutes starting a time 0 minutes. In the modified IVGTT, exogenous insulin (0.03U/kg body weight) is also infused 20 minutes after the intravenous glucose bolus (Finegood *et al.*, 1990; Saad *et al.*, 1997). Some studies use tolbutamide instead of insulin in the modified IVGTT to stimulate endogenous insulin secretion at this time (Saad *et al.*, 1997). Blood samples are taken for plasma glucose and insulin measurements over a 3 hour period. These data are then subjected to minimal model analysis using the computer program MINMOD to generate an index of insulin sensitivity (S_I). The minimal model is defined by two coupled differential equations with four parameters (*Figure 1.3*). The first equation describes plasma glucose dynamics in a single compartment. The second equation describes insulin dynamics in a "remote compartment". MINMOD uniquely identifies model parameters that determine a best fit to glucose disappearance during the modified IVGTT (Muniyappa *et al.*, 2008).

The modified protocol is now almost always used because it allows better estimation of the S_I index (Yang *et al.*, 1987). As well as enhancing the estimation of S_I and S_G, the minimal model also enables the estimation of a number of other parameters including the basal glucose concentration (G_b); the basal insulin concentration (I_b); a parameter describing the removal rate of insulin from the interstitial space (P2); a parameter that describes the movement of circulating insulin to the interstitial space (P3); the acute insulin response to glucose (AIRg); the Disposition Index (DI) and the glucose effectiveness at zero insulin (GEZI). S_I represents the summed ability of insulin to both promote the disposal of glucose and to inhibit the endogenous production of glucose. S_G represents the capacity of glucose to mediate its own disposal. Under conditions of reduced insulin action and subsequent hyperglycaemia, S_G may contribute to the efficient disposal of glucose (Ader *et al.*, 1997). Martin *et al.* (1992) reported that decreased minimal model derived S_G was an independent risk factor for the development of T2DM in high risk individuals (Martin *et al.*, 1992a). Quon *et al.*, (1994), argue that S_G derived from the minimal model is overestimated. They suggest that the minimal model attributes some of the effects of incremental insulin to the effects of glucose itself. Finegood and Tzur have argued that only with a blunted insulin response can reliable estimates of

S_G be achieved (Finegood & Tzur, 1996). Very little information exists about the actual physiological mechanisms which determine S_G. While it is known that glucose can enhance glucose uptake and inhibit hepatic glucose output, the importance of these actions to determining S_G is unclear (Ader *et al.*, 1997). AIRg is a measure of the acute ability of the β-cells of the pancreas to respond to rising glucose by secreting insulin. In one study the measured environmental and anthropometric factors could account for 10% of the variation in the acute insulin response with the female gender being the major determinant (Clausen *et al.*, 1996). Loss of AIRg has long been recognised as a good predictor of subsequent development of T2DM (Efendic *et al.*, 1988). However, it still remains unclear as to whether AIRg is influenced by habitual diet, physical activity or genetics (Martin *et al.*, 1992b). DI is the product of S_I and AIRg and is an integrated measure of the body's ability to respond to hyperglycaemia. Previous studies have suggested that DI, which reflects insulin secretion corrected for insulin resistance, can give a more accurate estimate of β-cell function (Bergman *et al.*, 1981).

Use of the minimal model for S_I determination is often preferred over the more invasive and labour-intensive glucose clamp technique, especially in large clinical trials and epidemiological studies (Saad *et al.*, 1994). However, the procedure focuses solely on glucose and not other insulin-sensitivity fuels. In addition the individual roles of peripheral and hepatic glucose metabolism are not determined. It is also a costly procedure due to the complexity of the sampling procedure, the time and effort involved and the sophisticated data analysis.

$$dG(t)/dt = -[p_1 + X(t)]\,G(t) + p_1 G_b$$

$$dX(t)/dt = -p_2 X(t) + p_3[I(t) - I_b]$$

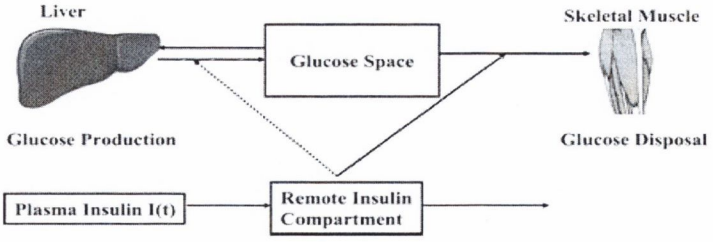


Figure 1.3: Schematic equations and parameters for the minimal model of glucose metabolism. Differential equations describing glucose dynamics [G(*t*)] in a monocompartmental "glucose space" and insulin dynamics in a "remote compartment" [X(*t*)] are shown above. Glucose leaves or enters its space at a rate

proportional to the difference between plasma glucose level, G(t), and the basal fasting level, G_b. In addition, glucose also disappears from its compartment at a rate proportional to insulin levels in the "remote" compartment [X(t)]. In this model, t = time; G(t) = plasma glucose at *time t*; I(t) = plasma insulin concentration at *time t*; X(t) = insulin concentration in "remote" compartment at *time t*; G_b = basal plasma glucose concentration; I_b = basal plasma insulin concentration; G(0) = G_0 (assuming instantaneous mixing of the iv glucose load); p_1, p_2, p_3, and G_0 = unknown parameters in the model that are uniquely identifiable from frequently sampled intravenous glucose tolerance test; glucose effectiveness = p_1; and insulin sensitivity = p_3/p_2 (Muniyappa *et al.*, 2008).

1.4.2 Fasting Indices-Non Intravenous Techniques

(i) Fasting Insulin

In healthy humans, the fasting condition represents a basal steady state where glucose is homeostatically maintained in the normal range such that insulin levels are not significantly changing and hepatic glucose production is constant (Muniyappa *et al.*, 2008).

In the European Group for the Study of Insulin Resistance (EGIR) definition of the MetS, hyperinsulinaemia is defined by the cutoff for the highest fasting plasma insulin quartiles and is used as a proxy for insulin resistance. With the introduction of laboratory methods that are specific for true (intact) insulin, the precision and standardisation of plasma insulin measurements have improved (Hu *et al.*, 2004). However, it must be considered that a single plasma insulin measurement does not always give a correct estimate of the "usual" plasma insulin level of an individual (Mooy *et al.*, 1996). Fasting plasma insulin concentration explains only about 40% of the variation in insulin resistance (Hu *et al.*, 2004) and therefore a single measurement used as a marker of insulin resistance in identifying persons with the MetS (Despres *et al.*, 1996) is far from ideal.

From a clinical perspective, taking a fasting plasma insulin sample is a quick, easy and practical means of assessing insulin resistance. There is a significant correlation between fasting insulin levels and insulin action as measured by the clamp technique (ADA, 1998). A limitation of using this method to determine insulin resistance is the lack of standardisation of the insulin assay procedure, as patient samples measured in different laboratories showed a high degree of variability. While

there is significant correlation between fasting insulin levels and M (refer to section 1.4.1 (i)) in normoglycaemic populations, this relationship deteriorates as diabetes develops, that is, as plasma glucose increases and insulin decreases. As a result, the plasma insulin level no longer reflects only insulin resistance, but also β-cell secretory capacity (Gutt *et al.*, 2000)

(ii) Oral Glucose Tolerance Test (OGTT)

The OGTT is the most commonly used method to evaluate whole body glucose tolerance *in vivo* (Matsuda & DeFronzo, 1999). Glucose tolerance is an expression of the efficiency with which homeostatic mechanisms restore glycaemia to basal levels after a perturbation (Radziuk, 2000). Clinically, the most common assessment is following an oral glucose load, a surrogate for a more physiological meal. The homeostasis response includes an increase in the insulin levels and therefore, also the insulin-dependent processes that lower glycaemia (Radziuk, 2000).

After an overnight fast, blood samples for determination of glucose and insulin concentrations are taken at 0, 30, 60 and 120 minutes following a standard oral glucose load (75g) or a standard meal (Dalla Man *et al.*, 2005). It is suggested that the OGTT mimics the glucose and insulin dynamics of physiological conditions more closely than conditions of the glucose clamp or IVGTT (Muniyappa *et al.*, 2008). However, glucose tolerance and insulin sensitivity are not equivalent concepts.

(iii) HOMA

Homeostasis Model Assessment (HOMA) of insulin sensitivity was proposed just over 20 years ago as a simple and inexpensive alternative to more sophisticated techniques (Matthews *et al.*, 1985). HOMA derives an estimate of insulin sensitivity from the mathematical modelling of fasting plasma glucose and insulin concentrations. It was found that the HOMA-based insulin resistance score was strongly correlated with insulin sensitivity as assessed by the glucose clamp technique in both nondiabetic and diabetic subjects (r=-0.83 and -0.92, respectively) (Matthews *et al.*, 1985). However, validation of the HOMA was carried out in few subjects and when compared with the glucose clamp technique in a relatively greater number of subjects (n=55), of which half had T2DM, weaker correlations were noted (r=-0.40) (Anderson *et al.*, 1995). Bonora *et al.*, (2000), however, found that HOMA scores and total glucose disposal (TGD) rates were strongly correlated and the results were

consistent in men versus women, younger subjects versus older subjects, obese versus nonobese and in diabetic versus nondiabetic subjects. The authors summised that the HOMA score is as good an indicator of clamp determined insulin sensitivity as the short insulin tolerance test (Bonora *et al.*, 1989) or the IVGTT (Beard *et al.*, 1986; Bergman *et al.*, 1987; Saad *et al.*, 1994; Saad *et al.*, 1997). Further support for the widespread use of HOMA comes from several studies. Insulin resistance estimated by HOMA predicts the development of T2DM in Mexicans (Haffner *et al.*, 1996). HOMA is based on measuring plasma glucose and serum/plasma insulin. Although the plasma glucose assay is highly reproducible across laboratories, in contrast the insulin assay can vary considerably (Robbins *et al.*, 1996). Therefore, comparing HOMA scores obtained in different studies cannot be done unless the insulin assay is standardised, which should precede the introduction of the HOMA in clinical practice (Bonora *et al.*, 2000). Since insulin secretion is pulsatile, the optimal sample should be the mean of three results at 5 minute intervals (0, 5 and 10 minute samples) (Matthews *et al.*, 1985). HOMA has proved to be a robust method for assessing insulin resistance in groups of patients over long periods of time because the sampling is simple, and the result is available without complex computing as soon as fasting glucose and insulin values are available (Wallace & Matthews, 2002). In contrast to other methods where estimates of stimulated insulin resistance are provided, HOMA gives an estimate of basal insulin resistance (Wallace & Matthews, 2002). The denominator of 22.5 is a normalising factor, that is, the product of normal fasting plasma insulin of 5μU/ml and normal fasting plasma glucose of 4.5mmol/L, typical ranges of a "normal" healthy individual = 22.5. Over wide ranges of insulin sensitivity/resistance, Log (HOMA-IR) which transforms the skewed distribution of fasting insulin values determined a much stronger linear correlation with glucose clamp estimates of insulin sensitivity (Katz *et al.*, 2000).

It has been suggested that HOMA-IR could overestimate the degree of insulin resistance in subjects with IFG, due simply to the way it is formulated. As people with IFG have higher than normal fasting glucose it follows that the HOMA-IR values will subsequently be higher than persons with normal glucose tolerance (Kim & Reaven, 2008).

(iv) Quantitative Insulin Sensitivity Check Index (QUICKI)

Katz *et al.* (2000) proposed an accurate index called "QUICKI", for the quantitative insulin sensitivity check index, defined from fasting plasma glucose and insulin as:

> QUICKI = 1/ [Log(I0) + Log(G0)], where I0 and G0 are the fasting insulin and glucose (Katz *et al.*, 2000)

QUICKI transforms the data by taking both the logarithm and the reciprocal of the glucose insulin product (Katz *et al.*, 2000). The authors explain the reasoning for these transformations in that the distribution of the fasting insulin value is skewed and as such, transformation of the data might be predicted to generate a better fit to glucose clamp measurements of insulin sensitivity (Katz *et al.*, 2000). As insulin levels are suggested to be indicators of insulin resistance (Reaven & Olefsky, 1977), rather than sensitivity, they should be therefore inversely related.

It has been shown that QUICKI is an accurate index of insulin sensitivity (Katz *et al.*, 2000; Bastard *et al.*, 2001), and better correlated to the gold standard SI_{CLAMP} than other indices, such as the minimal model index or HOMA (Matthews *et al.*, 1985; Bergman *et al.*, 1987). However, QUICKI is less correlated to the glucose clamp in non-obese non-diabetic control subjects than in obese T2DM patients (Katz *et al.*, 2000). The finding that QUICKI is less powerful when applied to non-obese subjects is probably related to the fact that this group's fasting glucose and insulin are both within narrow ranges (Perseghin *et al.*, 2001). Perseghin *et al.* (2000) improved its correlation to the SI_{CLAMP} by incorporating fasting plasma FFAs into the equation. This group explored the hypothesis that markers of lipolysis can add significant information to the estimate of insulin sensitivity, as lipolysis is more sensitive to insulin than glucose utilisation. Increased NEFA concentrations could reflect insulin resistance earlier than glycaemia (Stumvoll *et al.*, 2002).

$$RQUICKI = 1/[Log(I0) + Log(G0) + Log(FFA0)]$$

In addition, an experimental increment of plasma NEFA concentrations in healthy patients induces insulin resistance (Roden *et al.*, 1996) and in insulin resistant subjects the impaired regulation of lipolysis has been well-documented (Groop *et al.*, 1989). However, the proposed benefit of adding a marker of lipolysis to the QUICKI formula did not improve correlations for T2DM, obese subjects and first-degree relatives of diabetic patients (Rabasa-Lhoret *et al.*, 2003)

QUICKI is a simple, useful, inexpensive and minimally invasive surrogate index for glucose clamp derived measures of insulin sensitivity (Muniyappa *et al.*,

2008), making it an appropriate and effective tool for use in epidemiological and clinical research studies. Changes in QUICKI after therapeutic interventions are significantly correlated with change in SI_{CLAMP} (Katsuki *et al.*, 2002; Chen *et al.*, 2003).

Fasting based indices like HOMA, QUICKI and RQUICKI offer important advantages in estimating insulin sensitivity. They are obtained from a small number of fasting blood samples making them ideal for large epidemiological studies. However, it remains an important point to consider, whether the methods based on fasting concentrations present information similar to that exported from the more invasive techniques in which dynamic changes in glucose and insulin metabolism is investigated.

Table 1.3: Measures of Insulin Sensitivity

Technique	Sampling	Method	Outcomes
Intravenous techniques:			
Euglycaemic Hyperinsulinaemic Clamp	Blood Glucose measurements every 3-5min, in addition to plasma samples over 150-180min	Constant rate IV insulin infusion calculated as a dose per unit of surface area and variable rate IV glucose infusion	Insulin resistance estimated from the ratio of the mean glucose infusion to the mean insulin concentration over the last 20-30min
IVGTT with Minimal Modelling	12-35 samples taken over 180min	IV glucose bolus, IV insulin/tolbutamide at 20min	Analysis of results with MinMod computer programme: estimates key indices of glucose-insulin dynamics: SI,insulin sensitivity; Sg,glucose Effectiveness; AIRg,acute insulin response to glucose;
Insulin Suppression Test	Six samples at 0, 60, 120, 150, 160, 170, 180 min	Constant rate infusion of somatostatin, glucose and insulin for 150-180min	SSPG calculated from the mean of the glucose concentrations over the last 30min
Indices derived from data collected during OGTT data:			
Fasting Insulin	Average of 2 fasting samples		Logarithm usually best transformation
HOMA-IR	3 basal samples at 5min intervals		HOMA-IR= (fasting glucose (mmol/l) x fasting insulin (μU/ml))/22.5
QUICKI	1 sampling occasion		QUICKI= [1/(log fasting insulin + log fasting glucose)]

IV, intravenous; IVGTT, intravenous glucose tolerance test; IS, insulin sensitivity; HOMA-IR, Homeostasis model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index.

1.5 DYSREGULATION OF FATTY ACID METABOLISM IN THE AETIOLOGY OF THE METABOLIC SYNDROME

The cellular mechanisms involved in the development of insulin resistance and the role of diet are yet to be defined (Cefalu, 2001). Research is underway in exploring the effects of fatty acids on insulin signalling, on insulin secretion as well as on genetic determinants involved in the regulation of cellular glucose and lipid metabolism. An increased supply of non-esterified free fatty acids (NEFAs) has been identified as having an important role in the development of insulin resistance. Elevated NEFA levels are considered the norm in the fasted state. However in our Westernised societies where high fat diets are more typically consumed, overspill of fatty acids into the circulation following the breakdown of circulating fat results in elevated levels of NEFAs in the fed or postprandial state.

1.5.1 The Effects of Non-Esterified Free Fatty Acids (NEFAs) on insulin resistance

The mechanisms responsible for the differential effects of fatty acids on insulin action have not been fully elucidated. In obesity, adipose tissue reaches its maximum capacity to store lipids and as a result, the pool of circulating fatty acids increases and leads to ectopic storage of fat in non-adipose tissues such as muscle, liver and pancreas. An excessive lipid burden placed on the muscle cell in the form of NEFA or VLDL borne fatty acids can cause increased long-chain acyl CoA (LCACOA) levels. This leads to the metabolic abnormalities that are associated with insulin resistance (Hansen *et al.*, 2006). It is now recognised that the intracellular accumulation of fatty acids and their metabolites alter insulin signalling and impede insulin's ability to stimulate glucose uptake (Shulman, 2000).

Adipocytes are highly responsive to insulin. Insulin promotes adipocyte TAG stores by a number of mechanisms, including fostering the differentiation of pre-adipocytes to adipocytes and stimulating glucose transport and TAG synthesis aswell as inhibiting lipolysis in mature adipocytes. Insulin increases the uptake of fatty acids derived from circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue. Insulin's metabolic effects are mediated by a broad array of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as change in gene expression (Kahn & Flier, 2000). The initial molecular signal

for insulin action involves activation of insulin receptor tyrosine kinase. This results in phosphorylation of insulin receptor substrates (IRSs) on multiple tyrosine residues. These phosphotyrosine residues act as docking sites for many SH2 domain-containing proteins including phosphoinositide 3' kinase (PI3K) (Kahn & Flier, 2000). Binding of the p110 catalytic subunit of PI3K to p85 activates the lipid kinase that promotes glucose transport (White, 1998). Pathways downstream of PI3K may also mediate insulin's metabolic effects (Kitamura *et al.*, 1999). Of note, the anti-lipolytic effect of insulin requires a lower insulin concentration than needed for stimulation of glucose transport. Therefore, even in insulin resistant states where glucose transport may be somewhat impaired, sensitivity to insulin's antilipolytic effect is relatively well preserved (Kahn & Flier, 2000). There is overwhelming *in vitro* evidence that a 5-to-10 fold increase in pancreatic islet fat content that occurs *in vivo* in the prediabetic phase of the disease causes the compensatory β-cell hyperplasia and hyperinsulinaemia. However, a further increase in islet fat to ~50 times normal reverses the compensatory changes and causes β-cell dysfunction, a reduction in the number of β-cells and diabetes (Hirose *et al.*, 1996).

The regulation of insulin resistance has been defined, somewhat, by the discovery of peroxisome proliferators-activated receptor's (PPARs), their role in regulation of adipocyte differentiation and gene expression and chemicals that activate PPARs and reduce insulin resistance (Ryan *et al.*, 2000). Fatty acids are regulators of PPARs. Reports that the LC n-3 PUFAs and other eicosanoids bind and activate PPARγ are paramount in unravelling this relationship.

1.5.2 Insulin Signalling-Disruption with Fatty Acids

To understand the effects of free fatty acids on the disruption of insulin signalling, the pathways that regulate glucose metabolism in muscle cells and adipocytes must first be defined.

During fasting, most of the glucose in the blood is supplied by the liver and is used by the brain, independently of insulin. After a meal, a rise in blood glucose levels rapidly stimulates insulin secretion, which results in increased glucose transport, metabolism and storage by muscle and adipocytes. In addition, insulin both inhibits glucagon secretion and lowers free-fatty acid concentrations, contributing to the sharp decline in hepatic glucose production (Shepherd & Kahn, 1999). Muscle is the principle site of insulin stimulated glucose disposal *in vivo*; less glucose is

transported into adipose tissue (DeFronzo *et al.*, 1992). Resistance to the stimulatory effect of insulin on glucose utilisation is a key pathogenic feature of obesity and the metabolic syndrome (Shepherd & Kahn, 1999).

GLUT-4 is the main insulin-responsive glucose transporter and is located primarily in muscle cells and adipocytes (Shepherd & Kahn, 1999). In normal muscle cells and adipocytes, GLUT-4 is recycled between the plasma membrane and intracellular storage pools. Insulin-stimulated intracellular movement of GLUT-4 is initiated by the binding of insulin to the extracellular portion of the transmembrane insulin receptor (*Figure 1.4*). Its binding activates tyrosine kinase phosphorylation at the intracellular portion of the receptor. The chief substrates for this tyrosine kinase include insulin receptor substrate molecules (IRS-1, IRS-2, IRS-3 and IRS-4), Gab-1 and SHC (Shepherd & Kahn, 1999). In both adipocytes and skeletal muscle, subsequent activation of phosphoinositide-3 kinase (PI3K) is necessary for the stimulation of glucose transport by insulin (Holman & Kasuga, 1997; Shepherd *et al.*, 1998) and is sufficient to induce at least partial translocation of GLUT-4 to the plasma membrane (Tanti *et al.*, 1996; Frevert & Kahn, 1997). Activation of downstream protein serine-threonine kinases may also be involved. PI3K also activates these other kinases by generating phosphatidylinositol lipid products in the lipid bilayer of cellular mechanisms. These lipids, in turn, bring into proximity and thereby activate key signalling molecules. In this way, a serine-threonine kinase called protein kinase B (PKB or Akt) and phosphoinositide-dependent kinase 1 are brought together (Alessi *et al.*, 1997), allowing the latter to phosphorylate and activate protein kinase B (Shepherd & Kahn, 1999). Some isoforms of protein kinase C (PKC) are also activated by insulin and phosphoinositide-dependent protein kinase 1 may contribute to the activation of PKC because it phosphorylates a site in the activation loop of PKC (Le Good *et al.*, 1998). Intracellular translocation of GLUT-4 to the plasma membrane is stimulated by the expression of active forms of PKB or atypical isoforms of PKC in cultured cells (Shepherd & Kahn, 1999).

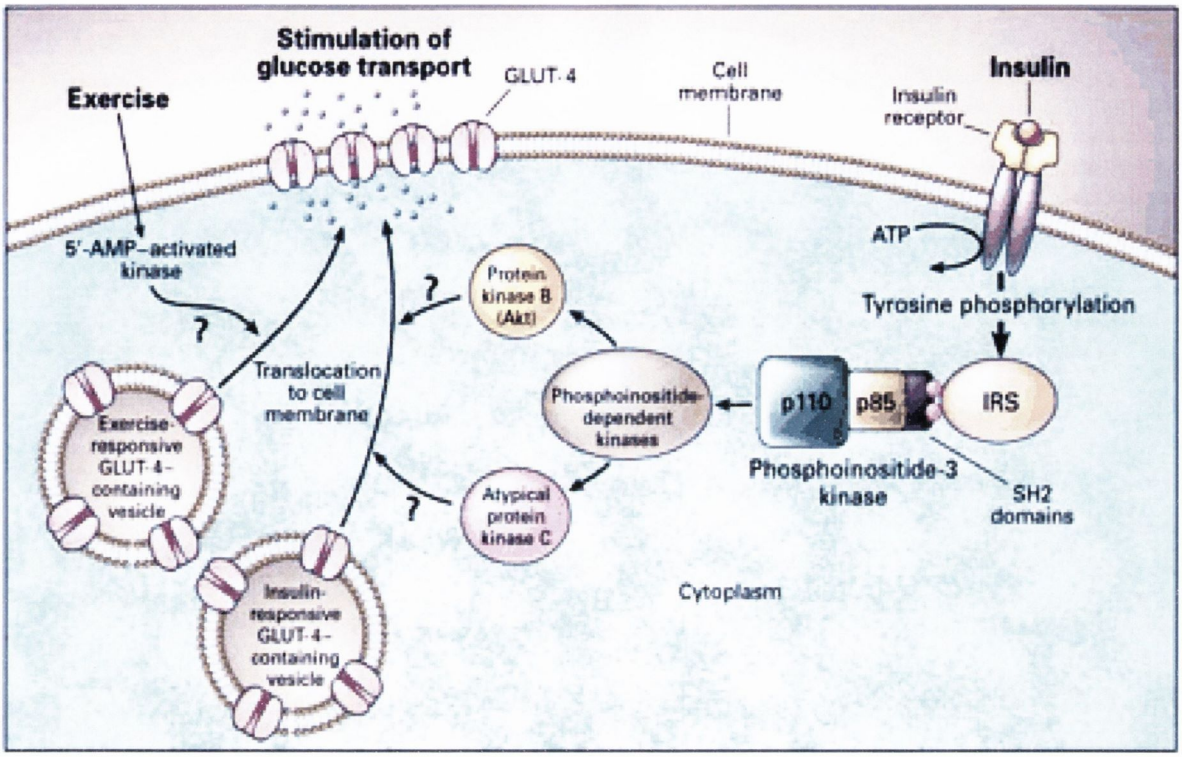
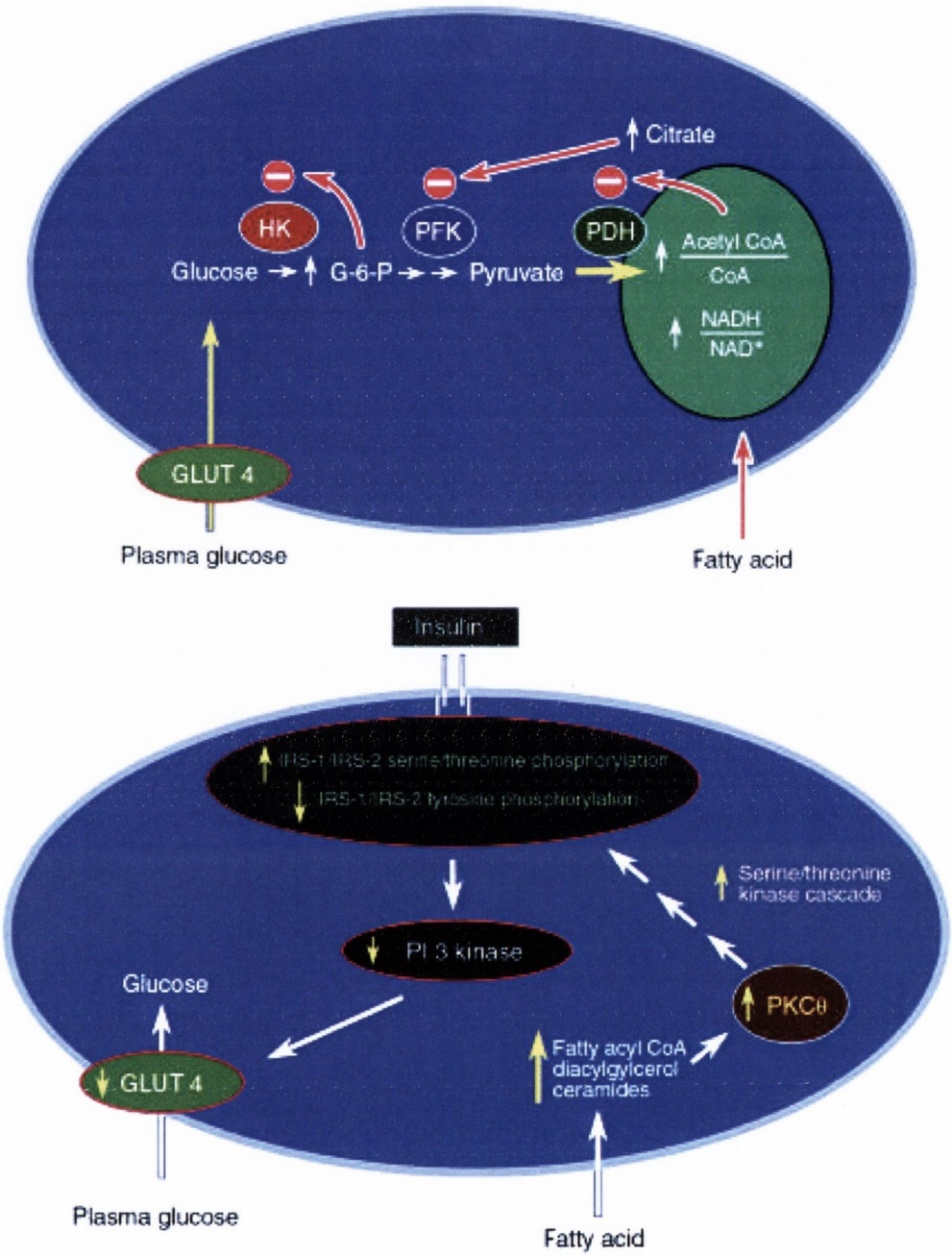


Figure 1.4: Insulin Signalling Pathways that Regulate Glucose Metabolism in Muscle Cells and Adipocytes

GLUT-4 is stored in intracellular vesicles. Insulin binds to its receptor in the plasma membrane, resulting in phosphorylation of the receptor and insulin-receptor substrates such as the IRS molecules. These substrates form complexes with docking proteins such as phosphoinositide-3 kinase at its 85-kd subunit (p85) by means of SH2 (Scr homology region 2) domains. Then p85 is constitutively bound to the catalytic subunit (p110). Activation of phosphoinositide-3 kinase is a major pathway in the mediation of insulin-stimulated glucose transport and metabolism. It activates phosphoinositide-dependent kinases that participate in the activation of protein kinase B (also known as Akt) and atypical forms of protein kinase C (PKC). Exercise stimulates glucose transport by pathways that are independent of phosphoinositide-3 kinase and that may involve 5'-AMP–activated kinase (Shepherd & Kahn, 1999).

Data exists linking IRS dysfunction in skeletal muscle to adipocyte biology and lipotoxicity (Schinner *et al.*, 2005). Circulating NEFAs may increase serine phosphorylation of IRS proteins causing impaired insulin signal transduction (White, 2002). There is strong evidence that dysfunction of adipose tissue plays a crucial role in the development of insulin resistance and T2DM through defects in the intracellular translocation of GLUT-4 from intracellular vesicles to the plasma membrane (Zierath *et al.*, 1996), defects in signalling pathways and impairment of insulin-stimulated glucose and glucose toxicity.

Randle and colleagues were the first to show that fatty acids inhibit glucose uptake, in the rat heart muscle *in vitro* (Randle *et al.*, 1964). They hypothesised that the crucial mechanism was increased fatty acid oxidation by the muscle, inhibiting pyruvate dehydrogenase activity, resulting in an increase in intracellular citrate concentrations which would in turn result in inhibition of phosphofructokinase (a key rate controlling enzyme in glycolysis) activity, leading to an increase in intracellular glucose-6-phosphate; this in turn would inhibit hexokinase activity, resulting in decreased glucose uptake and increased intracellular glucose concentrations. However, Shulman and colleagues performed *in vivo* studies and revealed a very different mechanism of fatty acid-induced insulin resistance to challenge the conventional Randle hypothesis. An increase in plasma fatty acids resulted in lower intramyocellular glucose-6-phosphate (Roden *et al.*, 1996) and glucose concentrations suggesting that fatty acids inhibit insulin-stimulated glucose transport activity (Dresner *et al.*, 1999). These changes were associated with reduced insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1 associated PI3K activity (Dresner *et al.*, 1999). Thus demonstrating that fatty acids cause insulin resistance through inhibition of insulin signalling thought to occur through activation of a serine kinase cascade involving PKC suggesting that glucose uptake rather than intracellular glucose metabolism is the rate limiting step for fatty acid induced insulin resistance in humans (Shulman, 2000) resulting in a reduced translocation of GLUT-4 into the plasma membrane. *Figure 1.5* illustrates the differences between the theories of Randle and Shulman.



HK: hexokinase II; PFK: phosphofructokinase; PDH: pyruvate dehydrogenase; PKCθ: protein kinase Cθ.

Figure 1.5: Top: Mechanism of fatty acid–induced insulin resistance in skeletal muscle as proposed by Randle et al., (1964). An increase in fatty acid concentration results in an elevation of the intramitochondrial acetyl CoA/CoA and NADH/NAD^+ ratios, with subsequent inactivation of pyruvate dehydrogenase. This in turn causes citrate concentrations to increase, leading to inhibition of phosphofructokinase.

Subsequent increases in intracellular glucose-6-phosphate concentration would inhibit hexokinase II activity, which would result in an increase in intracellular glucose concentration and a decrease in muscle glucose uptake.

Bottom: Proposed alternative mechanism for fatty acid–induced insulin resistance in human skeletal muscle. An increase in delivery of fatty acids to muscle or a decrease in intracellular metabolism of fatty acids leads to an increase in intracellular fatty acid metabolites such as diacylglycerol, fatty acyl CoA, and ceramides. These metabolites activate a serine/threonine kinase cascade (possibly initiated by protein kinase Cθ) leading to phosphorylation of serine/threonine sites on insulin receptor substrates (IRS-1 and IRS-2), which in turn reduces the ability of the insulin receptor substrates to activate PI 3-kinase. As a consequence, glucose transport activity and other events downstream of insulin receptor signalling are diminished (Shulman, 2000)

1.5.3 Insulin Secretion-Disruption with Fatty Acids

Free fatty acids or NEFA have an important stimulatory effect on glucose-stimulated insulin secretion (GSIS), by maintaining a basal rate of insulin secretion, thus regulating adipose tissue lipolysis. In the MetS and T2DM, increased NEFA contributes to hyperinsulinaemia and ultimately over-exposure results in β-cell damage. It has been proposed that an elevation of the basal glucose level produces an increase in glucose-derived malonyl CoA within the β-cell (Prentki & Corkey, 1996). Increased levels of intracellular malonyl CoA inhibit activity of carnitine palmitoyl transferase-1 (CPT-1) (McGarry *et al.*, 1977). This in turn causes an increase in long-chain acyl CoA levels. The malonyl-CoA/CPT-1 interaction is central to GSIS highlighting the role of fatty acids in this process (McGarry, 2002)

From studies in animals, it has been suggested that in the fasted state, were it not for elevated concentrations of circulating NEFAs, the pancreatic β-cell would be blind to glucose in terms of insulin secretion when the fast is terminated. Secondly, NEFA promote a fall in their own plasma concentration during the fasted to the fed state by virtue of their ability to act with glucose to promote insulin secretion, furthermore, exerting its anti-lipolytic effect on the fat cell (McGarry, 2002). In obese, insulin resistant states, elevated plasma FFA levels contribute to hyperinsulinaemia (McGarry, 2002).

1.5.4 Membrane phospholipid fatty acid composition and insulin action

Cell membranes are dynamic lipid bilayers which separate the cell from the extracellular milieu and surround intracellular organelles (Pan *et al.*, 1995). The fatty acid composition of cell membranes has also been suggested to be one mediating factor in the interplay of dietary fat and insulin sensitivity (Storlien *et al.*, 1996). In a study where polyunsaturated fatty acids (PUFAs) were replaced with monounsaturated fat (MUFA), a reduction in the fasting insulin and insulin resistance was observed (Ryan *et al.*, 2000). In the same study, adipocytes with oleic acid rich membranes had higher insulin stimulated glucose transport rates, related to the change in the oleic/linoleic acid ratio and proposed changes in membrane fluidity. It has been suggested that high fluidity of adipocyte membranes may impede conformational changes and aggregation of insulin receptors, therefore reducing insulin action (Tong *et al.*, 1995). Due to the presence of only one double bond in oleic acid, an oleic acid rich membrane would by default be less fluid than a membrane rich in linoleic acid which has two double bonds (Ryan *et al.*, 2000).

1.5.5 Adipokines

Increasing evidence indicates that adipose tissue is an important source of cytokines (Ahima & Flier, 2000) and that adiposity contributes to a pro-inflammatory environment (Yudkin *et al.*, 1999). The idea that adipose tissue functions solely as a storage vesicle has been replaced by the fact that it serves as both a dynamic endocrine organ, as well as a highly active metabolic tissue (Lyon *et al.*, 2003). Fat produces and secretes inflammatory factors collectively called adipocytokines or adipokines. These include TNFα, leptin, plasminogen activator inhibitor-1 (PAI-1), interleukin (IL)-6, resistin and angiotensinogen (Ahima & Flier, 2000). It has been demonstrated that levels of these adipokines are elevated in humans and animals with excess adiposity (Yudkin *et al.*, 1999), and to further corroborate this, a reduction in fat mass correlates with a decrease in the serum levels of these adipokines (Folsom *et al.*, 1993; Dandona *et al.*, 1998; Ziccardi *et al.*, 2002).

Cytokines produced by visceral adipose tissue are coming to the forefront in the insulin sensitivity story. TNFα is produced by adipose tissue and its expression is increased in the adipose tissue of obese states (Hotamisligil *et al.*, 1994b). TNFα enhances the release of other adipocyte-specific proteins including adiponectin (Ruan & Lodish, 2003). TNFα causes an inhibition of auto-phosphorylation of tyrosine

residues of the IR and an induction of serine phosphorylation of IRS-1 which in turn causes serine phosphorylation of the IR in adipocytes and inhibits tyrosine phosphorylation (Hotamisligil *et al.*, 1994a). It has been shown that neutralisation of TNFα improves insulin sensitivity by increasing the activity of insulin receptor tyrosine kinase in muscle and fat tissues (Sikaris, 2007).

Leptin is also produced by adipose tissue (although not exclusively as it is also produced in several other places e.g. bone marrow, placenta, stomach, muscle and brain) (El-Atat *et al.*, 2003). Leptin has been shown to enhance cellular immune responses (Lord *et al.*, 1998), as well as to increase BP (Correia *et al.*, 2001). Leptin markedly improves insulin sensitivity in patients with lipodystrophy with low circulating levels of leptin (Oral *et al.*, 2002).

Adiponectin is a 244-amino acid protein synthesised and secreted exclusively by the adipose tissue (Maeda *et al.*, 1996). Adiponectin expression and secretion is increased by insulin like growth factor-1 and decreased by glucocorticoids and β-adrenergic agonists (Sikaris, 2007). Adiponectin is distinct from other known adipokines in that it alone among then appears to improve insulin sensitivity. Animal studies have shown that adiponectin lowers blood glucose, prevents lipid accumulation in skeletal muscle and antagonises TNFα (Ouchi *et al.*, 1999; Berg *et al.*, 2001; Combs *et al.*, 2001). In humans, the degree of insulin resistance is inversely related to circulating levels of adiponectin (Arita *et al.*, 1999; Weyer *et al.*, 2001; Stefan *et al.*, 2002). Furthermore, a reduction in BMI is associated with an increase in circulating adiponectin, suggesting a long-term regulation of adiponectin levels by changes in insulin sensitivity (Yang *et al.*, 2001). Adiponectin is a potent anti-inflammatory cytokine which also exhibits anti-atherosclerotic effects (Goldstein & Scalia, 2004). Adiponectin concentrations have an inverse relationship with adiposity (WHR), insulin resistance, diastolic BP, TAG concentrations and TNFα receptor concentrations (Fernandez-Real *et al.*, 2003). Adiponectin exists in a wide range of multimer complexes in plasma and combines via its collagen domain to create 3 major oligmeric forms: a low molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer and a high molecular weight (HMW) 12-to-18-mer adiponectin (Pajvani *et al.*, 2003; Waki *et al.*, 2003). The hypothesis that HMW adiponectin is the more active form of the protein and has a more relevant role in insulin sensitivity and in protecting against diabetes has been supported (Kadowaki *et al.*, 2006). Increases in the ratio of plasma HMW adiponectin levels to total adiponectin levels correlate with

improvements in insulin sensitivity during treatment with the group of insulin–sensitising drugs, thiazolidinediones (TZDs), in mice and human diabetic patients, whereas increases in total serum adiponectin levels did not show good correlations (Pajvani *et al.*, 2004).

Another factor linking adipocyte hypertrophy with the secretion of pro-inflammatory compounds is believed to be endoplasmic reticulum (ER) stress (Ozcan *et al.*, 2004). Stress of the ER results from a number of factors, including glucose and nutrient deprivation, increased synthesis of secretory proteins and the presence of unfolded or misfolded proteins in the ER lumen (Ozcan *et al.*, 2004). Among the pathways activated by the unfolded protein response in adipocyte are the serine/threonine kinases c-Jun N-terminal kinase (JNK) and inhibitor of nuclear factor- kB (NF-kB) kinase (IKK), which induce the pro-inflammatory transcription factors activator protein-1 and NF-kB, respectively (Wellen & Hotamisligil, 2005).

1.6 DIETARY FAT AND INSULIN SENSITIVITY

Many facets of the composition of the diet (carbohydrate (CHO), fat, fibre, vitamins, minerals, alcohol) have been considered in the modulation of insulin resistance. However, more recently, attention has been focused on the ability of the quality and quantity of dietary fat and its influence on insulin sensitivity. In the 1930's Himsworth concluded that "the glucose tolerance of a healthy individual is determined by the composition of the diet which he is receiving" (Himsworth, 1939) and further, that "fat feeding retards and diminishes the action of insulin on blood sugar, prevents or delays the progressive improvement of sugar tolerance which occurs on injection of consecutive doses of glucose and impairs the ability of insulin to diminish the hyperglycaemia following intravenous injection of glucose" (Himsworth, 1934).

Fatty acids are hydrocarbon chains with a carboxyl group at one end and a methyl group at the other. The carboxyl group is reactive and readily reacts to form ester bonds. Fatty acids are usually straight chains with even numbers of C (2 to ≥30). Fatty acids in the human diet are typically between C10 and C30. Saturated fatty acids contain no double bonds and generally vary in a chain length from 12 to 18 carbons. The most prevalent saturated fatty acid in the diet is palmitic acid (C16:0), then stearic acid (C18:0), myristic acid (C14:0) and lauric acid (C12:0). Major sources of saturated fat include dairy and red meat products (Schaefer, 2002). Known for its

cholesterol raising effects, not all saturated fatty acids affect total cholesterol concentrations in the same manner wherein stearic acid has little impact (Grande *et al.*, 1970) and myrisitc and palmitic acids have the greatest cholesterol-raising potential (Schaefer, 2002). The proposed rapid conversion of stearic acid to oleic acid in the body could account for its neutral effects (Schaefer, 2002). Unsaturated fatty acids contain double bonds with MUFAs presenting with one double bond and PUFAs with two or more. The major MUFA in the diet is oleic acid (C18:1 n-9) and contains one double bond at position 9 carbon. The major sources are similar to those of SFA with the addition of canola oil (Schaefer, 2002).

The shape and physical properties of the fatty acid change with the presence of double bonds, their number, position and configuration (*cis* or *trans*). Fatty acids are substrates for energy expenditure by β-oxidation. Where energy intake exceeds expenditure, fatty acids may be stored in adipose tissue. In addition, their physiological roles include modulation of membrane fluidity (Stubbs & Smith, 1984), interaction with intracellular signalling pathways and transcription factors (Sampath & Ntambi, 2004) and act as substrates for the production of signalling molecules (Lewis *et al.*, 1990; Calder, 2003).

Both the amount and quality of dietary fat can modify glucose tolerance and insulin sensitivity (Storlien *et al.*, 1996). Both IGT and T2DM are increased in persons with a high amount of fat and especially saturated fat (Marshall *et al.*, 1994; Feskens *et al.*, 1995). The epidemiological data is less definitive for the role of monounsaturated fat and subsequent risk. It has been postulated that both MUFA and SFA may have undesirable effects (Maron *et al.*, 1991; Feskens *et al.*, 1995). However, in some experimental studies in T2DM patients (Garg *et al.*, 1992; Parillo *et al.*, 1992) and in a meta-analysis (Garg, 1998) it has been suggested that high–MUFA diets have beneficial effects on glycaemic control, but this effect has not been shown by all studies in this patient group (Lerman-Garber *et al.*, 1994). Population studies have shown that high intakes of MUFA are associated with high 2-hour postload glucose concentrations (Feskens *et al.*, 1995) and low S_I (Mayer-Davis *et al.*, 1997). Because our diets i.e. Western type diets, derive most MUFA from meat, milk and less from vegetable oils, it could be postulated that the negative effects observed are due to the strong intercorrelation with saturated fat intake. It is important in analysis to adjust one type of fatty acid for another.

Public health recommendations aim to reduce the intake of dietary fat in the adult population with particular emphasis on the reduction of saturated fat. However, the alternative low-fat diet is not without its negative attributes also, particularly on lipoprotein profiles (Krauss & Dreon, 1995). Results of the NHANES have indicated that the replacement of dietary fat with dietary CHO failed to reverse the trend of the increasing obesity rates as CHO increased blood glucose concentrations stimulating the release of insulin which in turn promotes the growth of fat tissue and thus can cause weight gain (German & Dillard, 2004).

1.6.1 Animal Data

Himsworth's observations were confirmed in animal models where fat feeding induced a reduction in glucose uptake, oxidation and conversion to fatty acids as a result of decreased insulin sensitivity (Lichtenstein & Schwab, 2000). In adipocytes isolated from rats fed a high fat diet, less insulin was bound and subsequently showed a decrease response to insulin (Ip *et al.*, 1976). In a similar study, Olefsky and Saekow found a decrease in the number of insulin receptors in adipocytes isolated from rats fed a high-fat diet, as well as a decrease in activity of the glucose transport system and intracellular glucose metabolism (Olefsky & Saekow, 1978). This led the authors to speculate that the type of fat and its effects on plasma membrane phospholipid composition and function have an important impact. With respect to muscle tissue which is the major site of insulin action, Grundleger and Thenen reported that both *in vivo* and *in vitro* measures of insulin resistance were enhanced by feeding a high-fat diet to lean Zucker rats (Grundleger & Thenen, 1982). These included decreased receptor number independent of a change in receptor affinity, decreased insulin stimulated glucose transport and pathway-specific alterations in basal and insulin-stimulated glucose metabolism (Grundleger & Thenen, 1982; Lichtenstein & Schwab, 2000). In a well-cited study, Storlien and colleagues assessed the effect of fat feeding on *in vivo* insulin resistance in rats (Storlien *et al.*, 1986). The high-fat diet resulted in decreased whole body insulin resistance with decreased glucose utilisation in skeletal muscle and brown adipose tissue and insulin resistance in the liver. To further explore this, one study fed Sprague-Dawley rats a high fat diet which resulted in hyperinsulinaemia and decreased response of adipocytes to insulin (Watarai *et al.*, 1988). Decreased insulin-stimulated glucose uptake was associated with a decrease in the insulin stimulated phosphorylation of the β-subunit of the insulin receptor

(Watarai *et al.*, 1988). To build on the initial reports and observations that the insulin resistance observed in rats as a result of high-fat feeding was due to increased TAG storage in the muscle and/or changes in membrane fluidity acid composition (Storlien *et al.*, 1986; Storlien *et al.*, 1991), Storlien assessed the effect of altering intracellular skeletal muscle TAG levels on insulin resistance (Storlien *et al.*, 1993). Feeding a high fat diet, resulted in no significant effects on plasma TAG levels however it was observed that TAG content in skeletal muscle was increased.

In summary, multiple mechanisms have been proposed for the effects of high-fat feeding on altered glucose and insulin metabolism in animal studies. A decrease in basal and insulin-stimulated glucose utilisation, a decrease in the binding of insulin to its receptor and decrease in the active form of glycogen synthase are among the proposed mechanisms.

With respect to the type of fatty acid and its effects on insulin resistance, the work to date in animals is not as abundant. Storlien and co-workers have also investigated these effects where long chain n-3 PUFAs replaced ~6% of linoleic acid in a high-fat diet for 24 days (Storlien *et al.*, 1987). They concluded that this subtle substitution prevented the development of insulin resistance and hypotheised that the potential mechanisms responsible for this effect included alterations in the production of various prostaglandins, thromboxanes and prostacyclins, changes in the membrane fluidity and inhibition of VLDL synthesis by the liver (Storlien *et al.*, 1987). In a later study, this same group assessed the effects of high-fat diets enriched in SFA, MUFA, PUFA, PUFA plus LC n-3 PUFA, PUFA plus short chain n-3 PUFA and SFA plus short chain n-3 PUFA on insulin resistance (Storlien *et al.*, 1991). Interestingly they found that regardless of the fatty acid profile, high-fat diets resulted in an increase in insulin resistance, however the degree of insulin resistance was greatest in the group of rats fed the SFA enriched diet relative to MUFA and PUFA fats. The addition of LC n-3 PUFA saw an improvement in insulin resistance. The addition of the short chain n-3 PUFA had no effect (Storlien *et al.*, 1991). Given such extreme results, the qualitative and quantitative effects of the various fatty acid groups would have to be proven in human studies to change health policy and clinical guidelines.

1.6.2 Human Studies

In a review detailing the relationship of dietary fat to glucose metabolism, a summary of the *in vivo* metabolic studies highlighted high-fat diets with favourable fatty acid composition (high PUFA:SFA ratio) appear to result in more desirable glycaemic control compared to high-CHO diets in T2DM patients (Lichtenstein & Schwab, 2000). For *in vitro* studies, fatty acid composition of the membranes of peripheral tissues affects insulin sensitivity (Lichtenstein & Schwab, 2000). Vessby *et al.*, (1994b) assessed the effect of skeletal muscle phospholipid fatty acid profile on insulin sensitivity in seventy-year-old men. This study demonstrated that peripheral insulin sensitivity was significantly and negatively correlated with the proportion of palmitic, palmitoleic and di-homo-γ-linolenic acids and positively correlated with linoleic acid. Similar findings were previously reported (Borkman *et al.*, 1993). Possible mechanisms include cell membrane fluidity and number and affinity of insulin receptors.

Epidemiological studies are perhaps the most appropriate way to study the relationship between diet, obesity, eventual T2DM and other degenerative diseases as an outcome. In the Zuptphen Elderly Study, it was demonstrated that in normal subjects, SFA intake was positively correlated with fasting glucose concentrations, and that insulin concentrations during an OGTT were inversely associated with PUFA intake and positively associated with SFA intake. They also investigated the role of diet as a predictor of the onset of T2DM and found that the intake of total, SFA and MUFA were higher in men with newly diagnosed T2DM (Feskens & Kromhout, 1990; Feskens *et al.*, 1994; Feskens *et al.*, 1995). In a cross-sectional study, it was reported that the consumption of butter (high in SFA) was positively associated with blood glucose concentrations, whereas consumption of olive oil (high in MUFA) was inversely associated with blood glucose concentrations (Trevisan *et al.*, 1990). In another study a positive association with the intake of SFA and cholesterol with fasting insulin concentrations, BMI and WHR was demonstrated (Maron *et al.*, 1991). From the Nurse's Health Study, a strong inverse relationship between the PUFA:SFA ratio of the diet and risk of developing T2DM was reported (Colditz *et al.*, 1992). The epidemiological data demonstrates that habitual diets high in fat are associated with a higher incidence of T2DM. In a cross-sectional study of >4000 healthy individuals, the percentage of SFA and MUFA in plasma phospholipids were positively and negatively associated with fasting insulin, respectively (Folsom *et al.*, 1996). Lovejoy

et al., (2001) have reported inverse associations between insulin sensitivity and serum concentrations of myristic acid (C14:0), palmitoleic acid (C16:1) and di-homo-γ-linolenic acid (C20:3 n-6).

The evidence points to high intakes of dietary fat (especially hard animal fats) being positively associated with glucose intolerance. Regarding the type of fatty acid and assigning blame in terms of insulin resistance, concrete clear evidence has still to emerge. No doubt other factors such as body weight, type of diet consumed and other environmental influences should be tightly controlled to draw on meaningful conclusions.

Intervention studies allow the investigation of particular amounts and types of fat on insulin sensitivity whilst controlling for body weight or changing isoeneregetic conditions, which could not be determined in observational studies (Riccardi *et al.*, 2004). In intervention studies, examining the effects of high- versus low-fat diets (total fat intake changed between 20% and 40% of total energy), no major effect is observed on insulin sensitivity (Riccardi *et al.*, 2004). The main intervention studies evaluating the effects of saturated versus unsaturated fat on insulin sensitivity have been reviewed and tabulated by Ricarrdi *et al.*, (2004). The majority saw no effect on insulin sensitivity with changes in dietary fat quality (Heine *et al.*, 1989; Uusitupa *et al.*, 1994; Schwab *et al.*, 1995; Fasching *et al.*, 1996; Lovejoy *et al.*, 2002). As Riccardi underlines, these studies were performed with small subject numbers and/or short study durations.

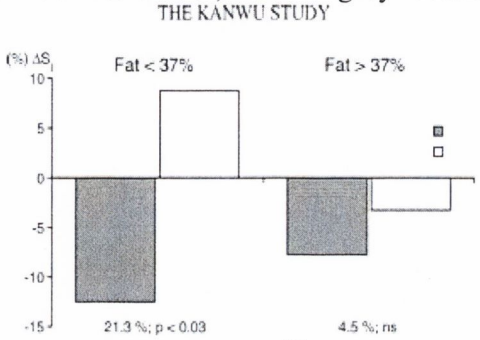
In a controlled feeding trial, Lovejoy and co-workers investigated the effects of saturated, monounsaturated and *trans* fat enriched diets on insulin sensitivity in a healthy cohort (Lovejoy *et al.*, 2002). No effect of high-SFA or *trans*-fatty acids on insulin action was observed. The authors concluded that it is the total dietary fat that impacts on insulin sensitivity and as the total fat in the study was 28% total energy, it is postulated that with <30% total dietary fat, a relative enrichment of saturated or *trans* does not significantly alter insulin sensitivity. When stratifying the group according to BMI, a greater decrease in S_I on the saturated diet was observed in overweight individuals. Lovejoy proposed that this response in overweight individuals could be due to the fact that "their relative baseline insulin resistance produces a diminished ability to respond to environmental challenges to glucose insulin homeostasis" (Lovejoy *et al.*, 2002). In the IRAS, higher dietary fat intakes were also associated with insulin resistance in obese but not lean individuals (Mayer-Davis *et*

al., 1997). In an intervention study performed in 162 healthy subjects, Vessby *et al.*, (2001) randomly assigned subjects to a high-SFA or a high-MUFA diet. A randomly selected subsample within each dietary group was also given 3.6g/day fish oil supplement or placebo. Insulin sensitivity as determined by the FSIVGTT was significantly impaired on the SFA diet but remained unchanged on the MUFA diet. On further inspection, when the group were stratified according to their habitual intake of total fat the effect of fat composition was clearly different between the high- and low-fat diet groups. In those consuming <37% energy from fat, the difference between the two diets in relation to their effects on insulin sensitivity was profound (20.3%, p<0.03) (*Figure 1.6*).

In the Insulin Resistance Atherosclerosis Study (IRAS), dietary fat was positively associated to insulin secretion in subjects with normal glucose tolerance but not in IGT states (Mayer-Davis *et al.*, 2001). Larsson *et al.*, (1999) did not however find any association between intake of specific dietary fatty acids and insulin secretion. In a crossover study of diabetic patients comparing the effects of isoenergetic diets with a high and low ratio of PUFA to SFA, an increased insulin response and an improved insulin sensitivity in the group that consumed the diet with a high ratio (Heine *et al.*, 1989) was observed. This effect could be derived from the fact that the fatty acid composition of cell membranes modulates insulin action.

A diet high in fibre, low in SFA and low in simple CHO is more often advised to T2DM patients. Debate exists over whether replacement of SFA with MUFA or CHO should be encouraged. An improvement in *in vivo* insulin sensitivity in a young healthy normoglycaemic population was demonstrated, when SFA was isocalorically substituted with CHO or MUFA, showing an equal effectiveness with both (Perez-Jimenez *et al.*, 2001). Of note, the fibre content in both diets was the same.

Figure 1.6: The KANWU study: effects of changing dietary fat composition (high SFA or high MUFA) on insulin sensitivity (S_I) in relation to total fat intake during treatment (total fat <37 vs. >37%). SFA: grey column; MUFA white column. $^{*}P<0.03$.



It was found that men who over a 4 year follow up developed IFG or DM had smaller proportions of the major dietary PUFA linoleate at baseline than men who remained normoglycaemic (Laaksonen *et al.*, 2002). Men with lower proportions of linoleate had more adverse changes in insulin and glucose concentrations during follow up which led the authors to suggest that diets low in vegetable fats relative to saturated fat predispose to impairment of insulin and glucose metabolism. The degree of serum esterified fatty acid saturation and a higher proportion of palmitate (an individual SFA which has by far the greatest concentration in the serum) at baseline were associated with unfavourable changes in fasting serum insulin concentration even when confounding factors were controlled for (Laaksonen *et al.*, 2002).

In a study of relatively short-duration, a decrease in total and LDL cholesterol concentration and an increase in insulin sensitivity following a PUFA-rich diet compared with a SFA-rich diet was noted (Summers *et al.*, 2002). Epidemiological and animal studies suggest that insulin sensitivity is decreased by a SFA-rich diet relative to a PUFA-rich diet (Storlien *et al.*, 1991; Lichtenstein & Schwab, 2000; Vessby, 2000). Short term dietary studies in humans have not confirmed this finding (Schwab *et al.*, 1995; Fasching *et al.*, 1996).

Table 1.4: Summary of Human studies investigating the role of fatty acids on glucose and insulin action

Study	Population	Type of Study	Type of Diet	Outcome
Vessby *et al.* 2001	162 healthy subjects	Randomised Intervention	Isoenergetic diet; High SFA *vs* High MUFA	MUFA diet improved IS (<37%energy from fat)
Parillo *et al.* 1992	10 NIDDM patients	Randomised Crossover	High MUFA/Low CHO *vs* High CHO/low MUFA	High MUFA/Low CHO- ↓in postprandial glucose & plasma insulin
Sarkkinen *et al.* 1996	31subjects IGT	Randomised Intervention	High Fat MUFA rich *vs* Low Fat PUFA enriched	MUFA rich diet-↓fasting plasma glucose; PUFA no change S_G higher in MUFA group than the PUFA group
Garg *et al.* 1988	10 NIDDM,receiving insulin therapy	Randomised Crossover	High CHO diet *vs* High MUFA	High MUFA diet-↓plasma glucose levels, ↓insulin requirements-improve glycaemic control
Perez-Jimenez *et al*. 2001	59 young healthy subjects	Randomised Crossover	Isoenergetic High MUFA *vs* Low Fat High CHO after a baseline saturated-fat	Improvement in IS with high-CHO& high-MUFA diet
Uusitupa *et al.* 1994	10 young healthy females	Randomised Crossover	High SFA *vs* High MUFA	MUFA diet-Glucose AUC lower;
Thomsen *et al.* 1999	16 healthy (6men and 10women) (first degree relatives of T2DM)	Randomised Crossover	Isocaloric CHO rich or MUFA rich (olive oil)	IS similar in both diets
Marshall *et al*. 1997	1069 individuals	Observational Study	Habitual dietary intake	No associations with fasting insulin conc. for MUFA or PUFA High total and SFA were associated with higher fasting insulin
Ryan *et al.* 2000	11 T2DM	Observational	MUFA (oleic acid rich) *vs* PUFA	MUFA ↓insulin resistance
Lovejoy *et al.* 2002	25 healthy men and women	Randomised Crossover	MUFA *vs* SFA *vs* *Trans*	MUFA-no impact on IS; S_I decreased on the SFA versus the MUFA diet in overweight subjects
Esposito *et al.* 2004	180 patients with MS (ATPIII)	Observational	Mediterranean style diet *vs* Prudent diet	Med diet ↓ insulin resistance
Thomsen *et al.* 1999	10 healthy people	Fat meals	High Fat, High MUFA, control	GLP-1 and GIP responses↑after the olive oil meal
Mayer Davis *et al.* 1997	1173 men&women, no hx of diabetes	Cross-sectional	Habitual dietary intake	Higher fat intake associated with lower IS among obese; results consistent for MUFA, PUFA and SFA
Mayer *et al.* 1993	544 non-diabetic twins	Cross-sectional	Habitual dietary intake	Higher intakes of SFA, oleic acid and linoleic acid- positively related to higher fasting insulin values
Rojo-Martinez *et al.* 2006	538 free-living subjects	Cross-sectional	Habitual dietary intake	Favourable relationship of MUFA with β-cell insulin secretion
Bonanome *et al.* 1991	19 NIDDM patients	Intervention	3 isocaloric diet changes; Phase 1&3 Rich in CHO, Phase 2 Rich in MUFA	No significant changes noted for plasma glucose, insulin, C-peptide between the diets
Soriguer *et al.* 2004	538 Pizarra Spian	Cross-sectional study	Habitual dietary intake; usual cooking oil used	IR less in people who used olive oil
Feskens *et al.* 1995	338 men (Seven Countries Study)	Longitudinal	Habitual dietary intake	Intake of total, SFA, mono,dietary cholesterol higher in men with newly diagnosed diabetes

Table 1.4 continued

Salmeron *et al.* 2001	84204 women (Nurses Health Study) no diabetes	Prospective study	Habitual dietary intake	No association between total fat intake and risk of T2DM. PUFA associated with a reduction in risk. Trans fat and dietary cholesterol associated with an increased risk
Trevisian *et al.* 1990	4903 Italian men and women	Cross-sectional	Habitual dietary intake	Consumption of olive oil- inversely associated with glucose levels; consumption of butter positively associated with glucose levels
Maron *et al.* 1991	215 non-diabetic men with CAD	Intervention	Habitual dietary intake	MUFA correlated positvely with fasting insulin SFA-positively correlated with fasting insulin
Soriguer *et al.* 2006	538 subjects from Pizarra Spain	Cross-sectional	Habitual dietary intake-high in oleic acid	Interaction between dietary MUFA and the PPARG2 Ala-12 allele
Louheranta *et al.* 2002	31 subjects with IGT	Intervention	High SFA run in diet(3wks), followed by a High MUFA (40%Energy) or High PUFA (34% Energy) for 8 weeks	Higher S_G during the MUFA diet
Brady *et al.* 2004	29 indian Asian Men (35-70years)	Intervention	Moderate or High n-6 PUFA background diet for 6wks Both groups supplemented with 4.0g fish oil/d for 6ks	No modulation of insulin resistance or insulin sensitivity
Feskens and Kromhout 1990	Healthy subjects	Longitudinal	Habitual dietary intake	SFA positively correlated with fasting glucose conc.
Feskens *et al* 1994	Healthy Subjects	Longitudinal	Habitual dietary intake	Insulin conc. during an OGTT were inversely associated with dietary PUFA and positively with SFA
Colditz *et al* 1992	Women in the Nurses Health Study	Prospective study	Habitual dietary intake	Linoleic acid inversely related to risk of T2DM. Animal fat weakly related to risk of T2DM. Strong inverse relationship between P:S ratio and risk of T2DM
Mayer *et al* 1993	Healthy women twins	Prospective	Habitual dietary intake	Increase in dietary fat associated with fasting insulin conc SFA associated with 2hour postglucose load
Folsom *et al* 1996	>4000 healthy individuals	Cross sectional	Habitual dietary intake	SFA positively associated with fasting insulin; MUFA inversely associated with fasting insulin in plasma phospholipids
Heine *et al* 1989	14 Diabetic subjects	Intervention	SFA vs PUFA	No effects on insulin sensitivity
Summers *et al* 2002	6 Diabetic, 6 Healthy, 5 Obese	Intervention	SFA vs PUFA	Insulin sesnitivity improved with PUFA (n-6)

T2DM Type 2 Diabetes Mellitus;SFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids; CHO carbohydrate; IS insulin sensitivity
NIDDM non-insulin dependent diabetes mellitus; ↓decrease; IGT impaired glucose tolerance;S_G glucose effectiveness; AUC area under curve; MS metabolic syndrome
ATP III Adult Treatment Panel III; ↑increase;hx history;IR insulin resistant;CAD coronary artery disease

1.6.3 n-3 PUFA

The essential fatty acid α-linolenic (C18:3n-3) is found in green leaves, some seeds, nuts and cooking oils (soybean oil and flaxseed oil). LC PUFA's EPA (C20:5n-3) and DHA (C22:6n-3) are primarily found in oily fish but the amount varies with different species (Childs *et al.*, 2008). In addition to dietary intakes, LC n-3 PUFA can be endogenously synthesised from their essential fatty acid precursor C18:3n-3 (α-linolenic acid) which predominantly occurs in the liver. Δ15 desaturase which is required to introduce the double bond into linoleic acid (C18:2n-6) in animals is absent and therefore α-linolenic acid is an essential n-3 PUFA. Once ingested, α-linolenic acid can be converted into LC n-3 PUFA via elongase, desaturase and β-oxidation steps. These enzymes are also involved in the metabolism of n-6 PUFA, linoleic acid, which is by far more abundant in the typical Western diet, into arachidonic acid (C20:4 n-6) and adrenic acid (C22:4n-6) and hence competition arises between the two for metabolism. Providing additional dietary α-linolenic acid results in dose dependent increase in EPA in plasma phospholipids, with the outcome on DHA less clear (Childs *et al.*, 2008). Overall more research is needed to define this relationship and produce more conclusive results.

Reviews of the evidence from epidemiological and human intervention studies of LC n-3 PUFA have summarised the significant benefits in disease states such as CVD, inflammatory conditions (e.g. rheumatoid arthritis), in early brain development and visual development and more recently in the areas of childhood behaviour and adult psychiatric disorders, dementia and Alzheimer's disease (Childs *et al.*, 2008). Epidemiological and case-control study data have demonstrated that the risk of CVD is lowest among those with the highest fish or LC n-3 PUFA intake (Kris-Etherton *et al.*, 2002; Calder, 2004; Wang *et al.*, 2006). The use of supplements containing LC n-3 PUFA have been shown to reduce the risk of CVD mortality in subjects who had previously suffered a myocardial infarction (MI) (Wang *et al.*, 2006).

The beneficial effects of fish oil on health were questioned in the light of evidence suggesting deleterious effects of high doses of fish oil on glucose control in subjects with T2DM (Borkman *et al.*, 1989; Friday *et al.*, 1989). However, further studies with moderate doses showed no adverse effects on plasma glucose control (Friedberg *et al.*, 1998; Montori *et al.*, 2000). Animal studies have reported an improvement in insulin sensitivity in insulin-resistant states (Storlien *et al.*, 1987; Rossi *et al.*, 2005) with reduced glucose oxidation rates (Luo *et al.*, 1996) and

lipolysis in isolated adipocytes (Peyron-Caso *et al.*, 2003). In a recent study with a double blind parallel design, 27 women with T2DM (without hypertriglyceridaemia) received 3g/day of either fish oil (1.8g n-3 PUFA) or placebo (paraffin oil) for 2 months (Kabir *et al.*, 2007). The results showed an improvement in adiposity markers (total fat mass and adipocyte size in subcutaneous and abdominal adipose tissue) and in atherogenic factors. A decrease in plasma TAG was observed, a finding only previously shown in subjects with an increased TAG at baseline (Friedberg *et al.*, 1998; Luo *et al.*, 1998; Montori *et al.*, 2000) and an increase in HDL-cholesterol also highlighted aswell as reductions in plasma PAI-1 concentrations (Kabir *et al.*, 2007). The authors proposed that decreased fat absorption, increased gastric emptying and gut hormone responses could be the mechanisms involved in the reduction in fat mass. However, insulin sensitivity was not affected in this study (Kabir *et al.*, 2007).

It has been suggested that an imbalance in dietary n-6 and n-3 PUFAs may be a contributory factor to the insulin resistance and related blood lipid abnormalities of the MetS (Brady *et al.*, 2004b). The current Western diet is very high in n-6 fatty acids relative to n-3 (ratio of n-6 to n-3 is 20-30:1) (Simopoulos, 1999). Intake of n-3 fatty acids has declined secondary to the decrease in fish consumption, and this coupled with increased intakes of n-6 fatty acids from vegetable oils and the increased use of this fatty acid in animal feeds leading to the subsequent ingestion of meat rich in the fatty acid, has led to this imbalance. Because of the increased amounts of n-6 fatty acids in the diet, the eicosanoid metabolites from arachidonic acid (prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acids, lipoxins) are produced in larger quantities than those formed from n-3 fatty acids (Simopoulos, 1999), contributing to the formation of thrombi and atheromas. This prothrombotic state increases the risk of atherosclerosis, a major complication in T2DM. Animal studies reported that feeding LC n-3 PUFAs results in improvements in insulin sensitivity (Storlien *et al.*, 1987; Somova *et al.*, 1999), whereas feeding n-6 PUFAs leads to a worsening of insulin sensitivity (Jucker *et al.*, 1999). Human studies are not as clear with some reporting beneficial effects (Popp-Snijders *et al.*, 1987; Fasching *et al.*, 1991; Feskens *et al.*, 1995) while others report a lack of effect of LC n-3 PUFAs (Toft *et al.*, 1995) on insulin sensitivity. A high dietary intake of n-6 PUFAs has not been shown to attenuate the beneficial effects of fish oil supplement on the plasma TAG response (Brady *et al.*, 2004b) or insulin resistance (HOMA-IR) or sensitivity

(minimal model) (Brady *et al.*, 2004b). Further clarification of this phenomenon/finding is warranted in patient groups with different states of glycaemia.

Recently, Riccardi reviewed the literature with respect to intervention studies examining the effects of n-3 PUFA supplementation on insulin sensitivity, (Riccardi *et al.*, 2004). In 6 studies (Borkman *et al.*, 1989; Annuzzi *et al.*, 1991; Boberg *et al.*, 1992; McManus *et al.*, 1996; Rivellese *et al.*, 1996; Luo *et al.*, 1998) there were no effects of n-3 PUFA observed on insulin sensitivity, including the previously mentioned KANWU study (Vessby *et al.*, 2001). Although intervention studies have shown no effect of n-3 PUFA on insulin sensitivity, prospective studies have demonstrated an inverse association between intakes and incidence of T2DM (Giacco *et al.*, 2007). In considering these data, Giacco put forward three theories of interest: 1) that fish oil may be able to modulate insulin action rather than reverting an established insulin resistance allowing the beneficial effects to be more evident in healthy people rather than diabetic patients; 2) fish oil may be more active as part of a diet rich in saturated fat, wholly or in part reverting the negative effects of the fatty acids on insulin action and 3) a different habitual intake of n-6 and n-3 PUFAs might condition the effects of fish oil on insulin sensitivity i.e. through competition of the same enzymes or transcriptional factors or production of eicosanoids (Giacco *et al.*, 2007). However, on further investigation, the author found no effect on insulin action, insulin secretion or β-cell function in healthy volunteers, independent of the fatty acid composition of the diet and the serum phospholipids n-6/n-3 ratio, indicative of the habitual dietary intake.

In muscle, n-3 PUFA might improve insulin sensitivity through a relative increase in unsaturation of membrane phospholipids and/or a decrease in muscle TAG (Storlien *et al.*, 1996). The alterations in membrane composition could affect insulin receptors (Liu *et al.*, 1994) and/or IRS-1 and PI3K expression and protein abundance (Kim *et al.*, 1996). In adipose tissue, the defect in glucose transport induced by a high fat diet is not ameliorated by n-3 PUFA, which may result from reduced insulin receptor number and tyrosine kinase activity (Storlien *et al.*, 1987).

1.6.4 Plasma Fatty Acids

The fatty acid composition of plasma lipids and adipose tissue may reflect the type and amount of dietary fat consumed, with plasma phospholipids and cholesterol esters estimating dietary intake over weeks and months and adipose tissue fatty acids

providing a longer term evaluation (Riboli *et al.*, 1987). However, due to time and effort required to obtain adipose tissue biopsy samples, it is often quicker, less labour intensive and more accepted by patients to obtain a blood sample for plasma fatty acid composition determination, especially in large scale epidemiological studies. In a prospective investigation study, strong correlations between diet and individual plasma fatty acids were found for PUFA and n-3 fatty acids (Ma *et al.*, 1995b). Plasma MUFA, however, did not reflect MUFA intake but rather SFA intake. Because of complex metabolic processes involved (desaturation, elongation, transport to target cells, acylation and deacylation reactions), simple relationships cannot be drawn between dietary fatty acids and their resulting concentrations in plasma, plasma lipids and tissues (Galli *et al.*, 1993). Furthermore, plasma fatty acids (expressed as a proportion of the total amount) include endogenously synthesised MUFA and SFA therefore skewing the desired interpretation of dietary intakes even more. Other factors to consider are measurement error, within-person variation over time, age, sex, body weight, smoking, alcohol intake or disease status (Holman *et al.*, 1979; Wood *et al.*, 1984; Moilanen *et al.*, 1985; Asciutti-Moura *et al.*, 1988).

Differences in serum lipid fatty acid composition have been reported between subject groups with glucose intolerance and T2DM (Salomaa *et al.*, 1990). This may be due to differences in fatty acid metabolism but also dietary intakes as different types of dietary fat exert different changes in serum fatty acid profiles (Dougherty *et al.*, 1987; Salomaa *et al.*, 1990; Lopes *et al.*, 1991; Clifton & Nestel, 1998). In one study (Sarkkinen *et al.*, 1996), investigating the effects of a high-fat MUFA enriched diet and a low-fat PUFA enriched diet on glucose and insulin metabolism in subjects with IGT, lower fasting plasma glucose concentration and an improved glucose effectiveness index (S_G) were observed following the high-fat MUFA rich diet. In a later, but similar study examining the relationship between serum lipid fatty acids and glucose metabolism after a diet-enriched in MUFA (40% of Total Energy) or PUFA (34% of Total Energy), the authors demonstrated that the MUFA diet also resulted in beneficial changes in fasting plasma glucose and in the S_G and that this was associated with alterations in the proportions of oleic, α-linolenic and arachidonic acids in phospholipids (Louheranta *et al.*, 2002).

Dietary fatty acids have a significant effect on glucose and insulin metabolism in subjects with altered glucose metabolism. This emphasises the importance of

dietary fat quality in the prevention and treatment of such patient groups. Although both epidemiological studies and clinical evidence indicates that dietary saturated fat is deleterious to overall health, debate still abounds as to what is the most beneficial and overall mixture of dietary fats. International guidelines for the nutritional management and prevention of T2DM (Mann *et al.*, 2004) which may be regarded as being appropriate for reducing insulin resistance (McAuley & Mann, 2006) are presented in *Table 1.5*.

Table 1.5: Optimal range of dietary macronutrients for weight-loss and maintenance in insulin-resistant states (McAuley & Mann, 2006)

Macronutrient	Percentage of Total Energy
Carbohydrates[a]	45–60%
Sugars	<10%
Protein	15–25%
Total fat[b]	25–30%
Saturated fat	<8%
Monounsaturated fat	10–20%
Polyunsaturated fat	5%
Cholesterol	<200 mg
Dietary fibre	30–40 g/day (half should be soluble fibre)

[a] Preferred sources of carbohydrate include vegetables, legumes, intact fruit, and whole-grain cereals that are high in dietary fibre.

[b] *Trans* fatty acids should be avoided.

1.7 EFFECTS OF DIETARY FAT ON OTHER FEATURES OF THE MetS

Changes in dietary fat composition have different effects on plasma lipoprotein levels (Riccardi *et al.*, 2004) (*Table 1.6*). Few studies have investigated the effects of dietary fat on lipoprotein metabolism in a MetS cohort. Replacing saturated fat with unsaturated fat lowers both LDL-cholesterol and VLDL TAGs (Swinburn *et al.*, 1991a). When dietary cholesterol is kept constant, SFA relative to MUFA or PUFAs or CHO, increased HDL-cholesterol concentration. This is primarily because of the

delayed clearance of HDL apoA1 from the plasma (Schaefer, 2002). However, when dietary cholesterol is reduced along with total and SFA, decreased HDL apoA1 production and hepatic apoA1 mRNA expression are observed (Brinton *et al.*, 1990; Velez-Carrasco *et al.*, 1999). So even though HDL decreased, when SFA decreased, LDL-cholesterol decreased to a greater extent. MUFA was considered neutral with respect to their effects on plasma total cholesterol concentrations (Schaefer, 2002). However, the overall data indicate that MUFA does not lower LDL or HDL cholesterol relative to SFA as compared to PUFA (Mensink & Katan, 1992; Valsta *et al.*, 1992; Schaefer, 2002). Linoleic acid clearly has a hypocholesterolaemic effect when substituted for dietary SFA, reducing LDL and HDL-cholesterol concentrations. n-3 PUFA lower TAG concentrations significantly, independently of their influence on lipoprotein concentration (Schaefer, 2002). n-3 fatty acids have also been shown to increase LDL cholesterol in both normolipidaemic and hyperlipidaemic individuals (Rivellese, 2000).

Table 1.6: Effects of dietary fat composition on plasma lipoprotein levels (Riccardi *et al.*, 2004)

	VLDL TG	LDL chol	HDL chol
SAFA	— ↑	↑↑	—
MUFA	—	—	— ↑
PUFA ω-6	↓	—	— ↓
PUFA ω-3	↓↓	↑	—

Some epidemiological studies have found significant associations between dietary fat intake and blood BP (Riccardi *et al.*, 2004). SFA is associated with higher BP levels while MUFA intakes are associated with lower BP (Trevisan *et al.*, 1990; Stamler *et al.*, 1997). In a study where associations were examined between the fatty acid content of TAG, which reflects the dietary fatty acid intake of the previous few days, and metabolic variables of the insulin resistance syndrome, C16:0 was strongly and positively correlated with BP (Tremblay *et al.*, 2004). They suggested that this association is a result of competing effects of nutrients on BP regulation, because plasma fatty acid composition also reflects a certain dietary pattern. In the KANWU study, a MUFA-rich diet in contrast with a SFA-rich diet reduced the diastolic BP in healthy normotensive subjects. Furthermore, a MUFA-rich diet caused a reduction in both systolic and diastolic BP at a fat intake below 37% of energy (Rasmussen *et al.*,

2006). This decrease coincides with an increase in S_I, therefore the effect may be mediated via an improvement in insulin sensitivity (Rasmussen *et al.*, 2006).

Fish oil and in particular LC n-3 PUFAs have been shown to reduce BP in normotensive (Mortensen *et al.*, 1983; Rogers *et al.*, 1987) and hypertensive subjects (Toft *et al.*, 1995; Knapp, 1996). The detailed mechanism underlying this anti-hypertensive effect are unknown, but have been attributed to a shift in eicosanoid production away from the two-series prostaglandins derived from arachidonic acid, which are potent vasoconstrictors (Howe, 1997). Evidence that links insulin resistance and hyperinsulinaemia to the development of hypertension shows that manipulations aimed at inducing a state of insulin resistance in experimental animals led to elevated BP and in a study where fish oil caused a significant reduction in serum insulin concentrations in rats, a subsequent lowering effect on BP was observed (Aguilera *et al.*, 2004). For associations between n-3 PUFA and BP, positive dose-related effects were observed in hypertensive people and in people with vascular diseases (Morris *et al.*, 1993). The DASH (Dietary Approaches to Stop Hypertension) feeding study found that a combination diet rich in fruit, vegetables and low fat dairy products and low in saturated fats could substantially lower systolic and diastolic BP levels (Appel *et al.*, 1997).

Dietary fatty acids have the ability to regulate prostacyclin production to some degree (Hornstra *et al.*, 1998). Studies in Eskimos (Fischer *et al.*, 1986) as well as intervention studies using fish or fish oil supplementation (Honstra *et al.*, 1990) have led to the conclusion that n-3 fatty acids of marine origin increase PGI_2 and PGI_3. In an intervention study, a low-fat, low SFA and low cholesterol diet for 3 years resulted in significantly lower plasma vWF levels than the control diets (Blann *et al.*, 1995).

In a cohort of healthy men and women, intake of n-3 PUFA was inversely associated with plasma markers of TNFα activity (Pischon *et al.*, 2003). From the Nurses' Health Study lower concentrations of many markers of inflammation and endothelial activation (E-selectin, CRP, IL-6) were seen in the highest quintile of n-3 fatty acids (Lopez-Garcia *et al.*, 2004). However clinical trials have not yet published these effects.

According to a number of studies, there is little effect of dietary fatty acids on fibrinogen (Hornstra *et al.*, 1998). One study showed increased plasma fibrinogen levels after an extremely high stearic acid diet compared with a diet high in myristic and lauric acids (Bladbjerg *et al.*, 1995). In the ARIC study, a negative association

between the intake of LC n-3 PUFAs and plasma fibrinogen levels was found (Shahar *et al.*, 1993). In a long-term study with a low-fat, high-fibre diet, tPA activity increased significantly in healthy subjects (Marckmann *et al.*, 1993). Effects of dietary composition on PAI-1 are not consistent. LC n-3 PUFAs mainly increase PAI-1 antigen and either increase or have no effect on PAI-1 activity. A high oleic acid diet was shown to decrease PAI-1 activity when compared to a high-CHO diet (Lopez-Segura *et al.*, 1996).

1.8 LOW-FAT, HIGH-CARBOHYDRATE DIETS

With the increased production, advertisement and availability of lower-fat foods as a means of reducing body weight and blood cholesterol along with the deemed 'bad effects' of higher fat foods by the food industry, the effects of alternative low-fat, high-carbohydrate diets need to be considered. Low-fat, high-CHO diets in overweight subjects with characteristics of the MetS prevented a further increase in weight, with modest weight loss observed when dietary fat was replaced by simple or complex-CHO, with the latter illustrating more significant outcomes (Poppitt *et al.*, 2002). However, when an increase in CHO content of the diet was not accompanied by weight loss or an improvement in total or LDL-cholesterol, a decline in HDL-cholesterol was associated with an increase in circulating TAG concentrations (Poppitt *et al.*, 2002). The most published negative attribute is the hypertriacylglycerolaemic effect of such diets (Parks & Hellerstein, 2000), depending on the concentration of the dietary CHO fed, the type (mono- or polysaccharides), the physical form (liquid or solid) and the presence of fibre. This outcome can occur with the replacement of dietary fat with as little as 5% CHO (Parks & Hellerstein, 2000). It is proposed that hypertriacylglycerolaemia results from an increase in hepatic TAG secretion rate as a result of hepatic insulin resistance (Reaven *et al.*, 1967). Reaven went on to illustrate that as the CHO content of the diets increased, the insulin response was heightened to a much greater extent (Reaven & Olefsky, 1974a). Reaven and others proposed that the impaired ability of insulin to lower adipose tissue lipolysis was the metabolic basis of CHO induced-hypertriacylglycerolaemia. This leads to higher NEFA concentrations and increased VLDL production (Kissebah *et al.*, 1976a; Kissebah *et al.*, 1976b; Reaven, 1997).

In a randomised crossover study comparing the effects of a high MUFA fat diet with a high-CHO diet on patients with T2DM, the hypertriacylglycerolaemic

effect of the high-CHO diet (2.97mmol/L versus 1.91mmol/L) was demonstrated (Garg *et al.*, 1994). In an extension of the study, where subjects continued to consume a high-CHO diet for an additional 8 weeks, CHO induced hypertriacylglycerolaemia did not resolve.

The type of CHO is an important factor in this phenomenon. The beneficial effects of fibre in moderating CHO induced hypertriacylglycerolaemia are well documented in key studies (Simpson *et al.*, 1981; Riccardi & Rivellese, 1991). However, more research is needed to probe the effects of fibre on CHO induced-hypertriacylglycerolaemia in the postprandial state (Parks & Hellerstein, 2000).

In terms of the effects of a high-CHO diet on changes in glucose metabolism or insulin sensitivity, Daly *et al.*, (1997) reported no worsening of blood glucose concentrations with high-CHO diets if the subjects were not overweight and in general good health. However, for insulin resistant and diabetic cohorts, high-CHO diets have the potential to worsen glucose and insulin profiles (Reaven, 1997).

The optimal diet to ameliorate insulin sensitivity and prevent the development of the MetS is still under debate and scrutiny. From the evidence presented above the diet for prevention/treatment should constitute low intakes of saturated fat and cholesterol (unfavourable effects on S_I, BP and plasma lipids), while monounsaturated fat in moderate quantities should be permitted. Total fat does not need to be drastically reduced as within certain limits (35-40%) it is the quality and not the overall quantity that has had untoward effects. Low fat diets as promoted in the past led to high CHO intakes which are not without blame in relation to exacerbation of some metabolic features.

1.9 IMPACT OF WEIGHT LOSS ON INSULIN SENSITIVITY

Intentional weight loss in obese patients is paramount in terms of diabetes-related outcomes as well as risk of developing the disease (Aucott, 2008). Weight loss resulting from lifestyle, pharmaceutical and surgical interventions reduce the risk of developing T2DM by approximately 32%, 10-74% and 63%, respectively (Aucott, 2008), with further benefits such as reduced clinical symptoms, reduced medications and a reduction in mortality risk. Weight loss with reductions in both fat mass and fat-free mass improves insulin sensitivity in obesity, irrespective of the presence of T2DM (Goodpaster *et al.*, 2000). A minor weight loss of 4-6% in obese individuals

with IGT is associated with a reduction of the risk of T2DM by 58% within 3-5years (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002). The effect of weight loss on insulin sensitivity and insulin secretion was addressed in a group of obese nondiabetic subjects who were studied before and after a hypocaloric diet that halved their excess body weight (Muscelli *et al.*, 1997). At baseline, insulin sensitivity was inversely related to BMI. Following weight loss, the obese subjects regressed well along the insulin sensitivity/BMI line. Therefore, it can be said that insulin resistance can be improved by reducing adiposity and is proportional to the amount of weight lost (Ferrannini & Camastra, 1998).

Observational studies have suggested that dietary fat is not a major determinant of body weight (Chaput *et al.*, 2008). The Women's Health Initiative reported that almost 50,000 women randomly assigned to a low-fat intervention lost a small (0.5kg) but significant amount of weight compared to controls (Howard *et al.*, 2006). However, although the mean weight loss in groups consuming a low-fat diet is relatively small, on an individual basis, some do lose a considerable amount of weight attributed to a difference in motivation, compliance and biological factors (Chaput *et al.*, 2008). The authors propose that one contributing biological factor that might affect weight loss during a low-fat diet is the early insulin response to CHO, which is increased in such diets. They hypothesised that individuals with high insulin secretion consuming a low-fat diet might be susceptible to weight gain, and when this was investigated in 276 individuals followed for 6 years, it was found that insulin secretion predicted weight gain and change in waist circumference, especially in those consuming lower fat diets (Chaput *et al.*, 2008).

1.10 VALIDITY OF DIETARY INTAKE DATA – UNDERREPORTING

It is an attractive assumption to presume that from performing a dietary survey we will categorically know what people eat. However, in reality, it still remains difficult to obtain a true picture of the habitual diets of people. Day-to-day variations in food intake, dietary habits and of course misreporting are all factors to take into consideration when conducting such surveys. Underestimation of total energy intake by 20% is common (Black *et al.*, 1993) and could be as much as 50% in obese subjects (Hill & Davies, 2001). Other factors include sex, age, smoking, educational level, dieting behaviour and physiological issues (Briefel *et al.*, 1997; Price *et al.*, 1997; Braam *et al.*, 1998; Johansson *et al.*, 2001). Selective under-reporting is also

common i.e. underreporting of CHO and fats, in-between meals and snacks and 'unhealthy' type foods (Price *et al.*, 1997; Poppitt *et al.*, 1998; Lafay *et al.*, 2000; Johansson *et al.*, 2001). Studies investigating how underreporting affects the association between dietary factors and the MetS are scarce. One study investigated such a phenomenon in 301 healthy men aged 63 years (Rosell *et al.*, 2003). Lower intakes of fats (especially saturated fat), higher intakes of protein and lower intakes of sugars were seen in underreporters. This group also reported a lower intake of butter and margarine, buns and pastry, chips and snacks and a higher intake of bread, potatoes, meat and poultry, fish and shellfish which altogether give an impression of a healthier diet (Rosell *et al.*, 2003). To substantiate this, energy intake, energy expenditure and biological markers verified that the validity of the dietary data was low. Further analysis related underreporting to the MetS and observed that a greater waist circumference and BP were seen in the underreporters (Rosell *et al.*, 2003). This study highlighted that with studies involving dietary intake data, the popular approach of excluding underreporters in analysis may in fact lead to exclusion of a subgroup of people with poor health and to whom further investigations would be beneficial.

1.11 STRATEGIES TO PREVENT THE METABOLIC SYNDROME

The aim of national health policies in most industrialised countries is to improve the health status of the population as a whole and to diminish the social differences in this respect (Bruce, 2000). Food and nutrition is one area where measures are taken. Each of the metabolic components has a dietary relation, whether it be energy balance with respect to obesity, salt intake with hypertension or intake of dietary fats and dyslipidaemia. Nutritional strategies for the prevention of the MetS in the community are similar to national food-based dietary guidelines for health, as promoted by most European countries. In addition, approaches necessary for the management of overweight and central obesity are warranted (Anderson, 2000).The most effective prevention is usually through secondary prevention measures and is usually individualised.

1.12 GENE-NUTRIENT INTERACTIONS: IMPLICATIONS FOR DIETARY FATTY ACIDS

In light of the Human Genome Project and the rapid advances in molecular biology, a wealth of genetic information is being generated, particularly with respect to the

common, polygenetic, diet-related diseases including obesity, insulin resistance and T2DM. It is becoming increasingly obvious that an individual's phenotype represents a complex interaction between the genetic background and environmental factors over the course of an individual's lifetime. Food intake and nutrient exposure are key environmental factors in the pathogenesis and progression of the common polygenic, diet-related diseases. Therefore it is time to identify the nutrient sensitive genotypes and to develop a "personalised nutrition" approach, whereby nutrient intake is manipulated/optimised based on an individual's genetic profile to reduce disease risk and/or improve the effectiveness of dietary guidelines/recommendations in general (Phillips *et al.*, 2006).

For example the "thrifty genotype" (Neel, 1962), which conferred a protective effect in times of food deprivation, promoting fat deposition, has now been proposed to explain the escalating incidence of obesity in recent times as food is more abundant and physical activity has decreased. Insulin affects the transcription of several genes involved in energy homeostasis, glucose and lipid metabolism. Therefore the interactions between genes, dietary fat composition and insulin sensitivity are the focus of many studies in order to define the molecular mechanisms that affect insulin responsive genes. An example of one gene-nutrient interaction is Peroxisome Proliferator-Activated Receptor gamma (PPARγ) which has shown strong association with the development of the MetS and T2DM. PPARs are members of the nuclear-hormone receptor superfamily of which there are three isoforms: PPARα, PPARβ/δ and PPARγ. Unsaturated fatty acids can bind to specific isoforms and activate PPARs, varying to the PPARs affinity depending on fatty acid chain length and degree of unsaturation. It has been demonstrated that several PUFAs (α-linoleic acid (LA) C18:2n-6; γ-linolenic C18:3n-6; arachidonic acid C20:4n-6 and eicosapentaenoic acid (EPA) (C20:5 n-3) activate PPARγ (Daynes & Jones, 2002). In the context of insulin resistance, PPARγ regulates adipogenesis and is involved in insulin sensitisation (Daynes & Jones, 2002). PPARγ promotes the storage of fat and increases adipocyte differentiation and enhances the transcription of genes important for lipogenesis (Desvergne & Wahli, 1999).

The PPARγ Pro12Ala polymorphism has also been studied in the context of gene-nutrient interactions as a candidate genetic marker relevant to insulin resistance and the metabolic syndrome. This variant results from a Proline to Alanine substitution at the codon 12, which modulates the transcriptional activity of the gene.

In a prospective population-based cohort study, Luan *et al.* (2001) demonstrated an important interaction between background dietary fat composition and the PPARγ Pro12Ala single nucleotide polymorphism (SNP). With an increase in the polyunsaturated to saturated (P:S) ratio, an inverse relationship for BMI and insulin concentrations in Ala carriers but not Pro homozygotes was seen (Luan *et al.*, 2001). This important interaction between dietary fatty acid intake and the Pro12Ala polymorphism of the PPARγ gene makes relevant the effects of diet on insulin resistance and other aspects of the complex metabolic syndrome. Luan's study is supported by a meta-analysis (Altshuler *et al.*, 2000) reporting an association between Ala12, insulin sensitivity and a decreased risk of T2DM. In the Quebec Family Study, total fat and SFA intakes correlated with BMI, abdominal adipose tissue area, waist circumference and fasting glucose levels in Pro12 homozygotes but not seen in carriers of the Ala allele. The PPARγ example demonstrates the significance of gene-nutrient interactions in modulating components of the metabolic syndrome. However, there is a need to further substantiate research in this area.

1.13 IRS-1 GENE

There has been much focus on proximal components of the insulin signalling pathway as functional variations of these molecules could in part explain the widespread disruption of post-receptor signalling that characterises T2DM (Zeggini *et al.*, 2004). IRS-1 plays a key role in proximal insulin signalling and many sequential variants of the IRS-1 gene have been identified (Zeggini *et al.*, 2004). The main focus has been on two relatively infrequent nonsynonymous variants P512A (rs1801276) and G971R (G972R, rs1801278). The combined prevalence of these variants was suggested to be increased in T2DM (Almind *et al.*, 1993). The G971R variant, however, is more common and the evidence to support direct effects on gene product formation is stronger (Almind *et al.*, 1996; Hribal *et al.*, 2000). When polymorphisms of IRS-1 were grouped together, the overall frequency of the codon 972 variant was 10.7% in T2DM patients versus 5.8% in control subjects ($p<0.02$), suggesting that this genetic variant is a potential contributor to the insulin resistance in T2DM (Hitman *et al.*, 1995). A recent augmented meta-analysis of 27 association studies indicated that carriage of the G971R allele conferred a significant increase in disease risk of ~15% (Zeggini *et al.*, 2004). To test the reproducibility of this association, Florez *et al.* (2004) recently attempted to replicate the same genetic model in a large sample of

9,000 white individuals. Despite >95% power to obtain a $p<0.05$ for the odds ratio of 1.25 that had been estimated in the meta-analysis, they did not observe an association of G971R with T2DM related traits or age of onset (Florez *et al.*, 2004). The R971 allele of the IRS-1 gene inhibits IRS-1 binding to PI3K owing to the defective interaction between IRS-1 and the p85 subunit of PI3K (Almind *et al.*, 1996).

Insulin stimulated glucose uptake is significantly decreased in skeletal muscle from the obese and in adipose tissue from rats fed a high-fat diet, with reduced protein levels of IRS-1 and PI3K (Goodyear *et al.*, 1995; Stevenson *et al.*, 1996). Mice made IRS-1 deficient by targeted gene knockout exhibit hyperinsulinaemia and glucose intolerance (Araki *et al.*, 1994; Tamemoto *et al.*, 1994). mRNA and protein levels of IRS-1 and PI3K were significantly lower in rats fed a beef tallow diet than those fed a safflower oil diet suggesting an impairment of the initial steps in insulin signal-transduction pathways contributing to the defect in insulin-stimulated glucose uptake in skeletal muscle (Kim *et al.*, 1996). In the Diabetes Prevention Study, the R971 allele predicted poor weight reduction in the intervention group and also predicted the development of T2DM (Laukkanen *et al.*, 2004).

1.14 RESEARCH OBJECTIVES

LIPGENE is an EU Integrated Project entitled "Diet, genomics and the metabolic syndrome (MetS): an integrated nutrition, agri-food, social and economic analysis" funded under the Framework 6 Food Safety and Quality Programme in 2003 (www.lipgene.tcd.ie). The primary objective of the Human Nutrition component of LIPGENE was to understand how dietary fatty acid composition interacts with an individual's genetic background in relation to the development and risk of the MetS and Type 2 Diabetes Mellitus (T2DM).

The LIPGENE Human Dietary Intervention Study was designed to determine the relative efficacy of reducing dietary SFA consumption, by altering the quality of dietary fat or reducing the quantity of dietary fat, on multiple metabolic and molecular risk factors of the MetS. The second objective of the LIPGENE study was to explore if common genetic polymorphisms associated with increased risk of the MetS play a causal role in determining an individual's responsiveness to dietary fat modification.

The aims of this Ph.D. thesis are as follows:

- To outline how a rich dataset of MetS volunteers were recruited and to present their screening characteristics before their participation in the 12-week dietary intervention study.
- To develop a food-exchange model to investigate the effects of four experimental diets, distinct in fat quantity and quality, suitable for use in free-living volunteers, with the MetS, and to evaluate the achievement of dietary targets.
- To assess, among MetS subjects, participating in the LIPGENE Dietary Intervention Study, the physiological, metabolic, biochemical and dietary characteristics between gender, age groups and BMI status and to examine the association of these characteristics with the number of metabolic disorders that define the MetS. To investigate the associations between both plasma and dietary fatty acids with insulin sensitivity.
- To determine the relative efficacy of reducing dietary SFA consumption, by altering the quality of dietary fat and reducing the quantity of dietary fat on multiple metabolic and molecular risk factors of the MetS; to determine insulin sensitivity and β-cell dysfunction using the frequently sampled intravenous glucose test (IVGTT) with minimal modelling and multivariate statistical approaches to identify determinants of increasing S_I after a 12-week intervention in four dietary treatment groups. A secondary aim of this chapter was to ascertain whether background total fat intake as a percentage of energy determined the effects of the four intervention diets on insulin sensitivity and other IVGTT parameters and measures of insulin resistance.
- To explore if the IRS-1 genetic polymorphism Gly972R associated with increased risk of the MetS plays a causal role in determining an individual's responsiveness to dietary fat modification.

Chapter Two

Subjects, Materials and Methods

2.1 RECRUITMENT

2.1.1 Subjects

The LIPGENE human dietary intervention study was a randomised, controlled trial and was registered with The US National Library of Medicine Clinical Trials registry. The study was approved by the local ethics committees at each of the 8 clinical intervention centres, all of which conformed to the Helsinki Declaration of 1975 as revised in 1983.

Free-living subjects with the Metabolic Syndrome (MetS), defined by a modified version of the NCEP criteria (2002), characterised by three or more of the following, participated in LIPGENE:

- Fasting glucose concentration 5.5 - 7.0 mmol/L

  (Whilst 7mmol/L was the ideal maximum fasting glucose concentration, individuals with concentrations of up to 8mmol/L were accepted onto the trial, provided that the person was not on any oral hypoglycaemic medication or diet therapy for elevated plasma glucose concentrations).

- Serum TAG concentrations ≥ 1.5 mmol/L
- Serum HDL cholesterol concentration < 1.0 mmol/L (men)
  < 1.3 mmol/L (women)
- Blood pressure systolic BP≥130
  diastolic BP≥85mmHg
  or treatment of previously diagnosed hypertension
- Waist girth > 102 cm (men)
  > 88 cm (women).

The intervention study was completed in 8 European centres: Trinity College Dublin (TCD); University of Reading, URREADNSI (UoR); University of Oslo (UO); INSERM U476 Marseille (INSERM); Maastricht University (UM); Hospital Universitario Reina Sofía-University of Córdoba (HURS-UCO); University of Krakow (JUMC); Uppsala University (UU).

2.1.2 Inclusion/Exclusion Criteria

Subjects with the MetS invited to participate in the LIPGENE dietary intervention study complied with the following criteria as in *Table 2.1*.

Table 2.1: Inclusion and Exclusion Criteria of the LIPGENE Dietary Intervention Study

Inclusion Criteria
• Age: 35 -70 years • Gender: males and females (not pregnant or lactating) • Body Mass Index (BMI) 20-40 kg/m^2 • Total cholesterol concentration equal to or < 8.0 mmol/L • Medications / nutritional supplements allowed on condition that the subjects adhered to the same regimen during the intervention: anti-hypertensive medication (including beta-blockers), oral contraceptives, hormone replacement therapy, multi-vitamin supplements, other non-fatty acid based nutritional supplements (e.g. garlic, anti-oxidants, etc) • Smokers and non-smokers • Persons who do not consume alcohol or regular consumers of alcohol, which is not excessive as defined by elevated liver enzymes (AST <41IU/L and ALT 7-56 IU/L) • Ethnicity: Intention to include white Europeans
Exclusion Criteria
• Age: < 35 or >70years • Diabetes or other endocrine disorders • Chronic inflammatory conditions • Kidney or liver dysfunction • Iron deficiency anaemia (haemoglobin < 12g/dl men, < 11g/dl women) • Prescribed hypolipidaemic medication • Prescribed anti-inflammatory medication • Prescribed weight loss drugs • Fatty acid supplements including fish oils, evening primrose oil, etc. • Consumers of high doses of antioxidant vitamins (A, C, E, β-carotene) • Red rice yeast (Monascus purpureus) supplement usage • High consumers of oily fish (> 2 serving of oily fish per week of herring, mackerel, kippers, pilchards, sardines, salmon, trout, tuna (fresh), crabmeat or marlin). One portion is defined as a small herring or mackerel, one can of salmon or sardines or one salmon or tuna steak. Tinned tuna is permitted as it contains only minor amounts of long chain n-3 PUFAs • Highly trained or endurance athletes or those who participate in more than 3 periods of intense exercise per week • Volunteers planning to start a special diet or lose weight (e.g. the Slimfast Plan, Atkins Diet etc). • Weight change equal or >3kg within the last 3 months • Alcohol or drug abuse (based on clinical judgement) • Pregnant / lactating females / women planning a pregnancy in the next 12 months. Women who became pregnant during the dietary intervention period were removed from the study.

Volunteers were excluded if their alcohol intake exceeded the recommended units per week and if their liver enzymes were above the stated ranges due to the hypertriacylglyerolaemic effect of high alcohol intakes Liver enzymes alone were not used to exclude subjects.

Volunteers brought their medication and nutritional supplement bottles, capsule packs etc. for inspection at the screening visit. If individuals were using fatty acid supplements and were willing to stop taking their supplements and participate in the study, a wash-out period of 8 weeks was required. Maximum intakes of Vitamin A was 800μg, Vitamin C 60mg, Vitamin E 10mg and β-carotene 400μg, equivalent to a daily dose present in a standard multivitamin capsule.

2.1.3 Recruitment Procedure

Initial contact was achieved using local advertisements (university e-mail, posters, newspaper article/adverts, flyers distributed to weight watcher clinics and taxi firms) and with established centre specific contacts (e.g. GP clinics, dietetic out-patient clinics, hospital records and other specific cohort databases).

Potential volunteers replied by telephone and a Subject Suitability Questionnaire (SSQ) was completed over the telephone. The purpose of this questionnaire was to exclude any subjects who did not meet the most basic criteria (e.g. age, health and dietary habits) for the study. If deemed suitable according to the SSQ, the volunteer was invited to attend the unit for a screening visit.

2.2 SCREENING VISIT

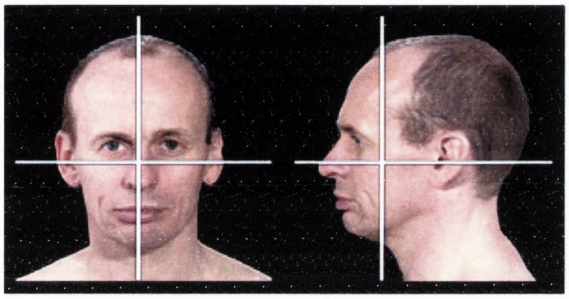
The study was explained in detail to the volunteer and any questions were answered. Each volunteer willing to participate signed the consent form. Each volunteer completed a Health and Lifestyle Questionnaire (HLQ), which was designed to assess a volunteer's health, lifestyle, physical activity and socio-demographic profile. This questionnaire was completed prior to the biochemical screening. Body weight, height, waist and hip circumference and blood pressure measurements were completed. A fasting (12 hours with no food or drink, except water) screening blood sample was taken. The volunteers that met all criteria were invited to take part in the study.

2.2.1 Body weight, Height, Waist and Hip Circumference and Blood Pressure Measurements

To minimise variability, all anthropometric measurements were taken by the same investigator throughout the study, as was practically feasible. Measurements were taken in the fasted state. Body weight was measured in duplicate on a centre specific, calibrated scale, to the nearest 0.1kg. Subjects were weighed in light clothing, without

shoes, belts removed and pockets emptied and after voiding. Height was measured in duplicate to the nearest 0.1cm, in the upright position using a stadiometer. The measurement was taken with the subject's head in the Franfurt Plane (*Figure 2.1).* This is a standard plane used for the correct orientation of the head. It is established by a line passing through the tragion (front of the ear) and the lowest point of the eye socket.

Figure 2.1: Frankfurt Plane position for measuring height



BMI was calculated using the equation: BMI = weight (kg)/height^2 (m^2)

Waist circumference was measured directly on the skin, at the mid-point between the supra iliac crest and lower ribs margin (*Figure 2.2)* at the end of a normal expiration, in duplicate to the nearest 0.1cm using a non-stretch tape measure. Hip circumference was measured in duplicate to the nearest 0.1cm, using a non-restrictive tape measure, at the level of the greater trochanter without compressing the skin (*Figure 2.2).* Blood pressure measurements were recorded in duplicate in the seated position after a 5 minute rest, using an appropriately sized cuff and recommended equipment before blood samples were taken and after the subject had been weighed.

Figure 2.2: Measurement of waist circumference and hip circumference

Waist Circumference Hip Circumference

2.2.2 Bio-electrical impedance (BIA) protocol

BIA was calculated with a Bodystat BIA machine (Bodystat 1500, Bodystat, UK). The subject was placed in a supine position with arms comfortably abducted from the body 15°, and legs comfortably separated (*Figure 2.3*). Two current-injection electrodes were placed at the right hand and foot on the dorsal surfaces proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively. The centres of two voltage-detector electrodes were placed on the midline between the prominent ends of the right radius and ulna of the wrist, and midline between the medial and lateral maleoli of the right ankle (*Figure 2.4*). The black current-injection and red voltage detector electrodes were at least 5cm apart, respectively. The black current-injection lead alligator clips and the red voltage-detector lead alligator clips were connected to the electrodes placed on the right hand and foot and right wrist and ankle, respectively.

Figure 2.3: Supine position for BIA measurement

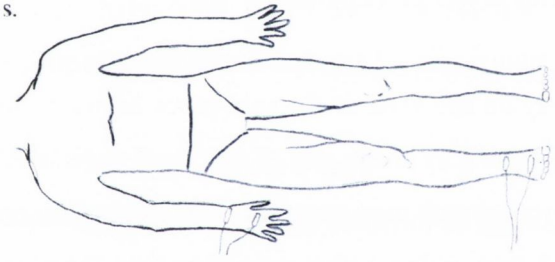
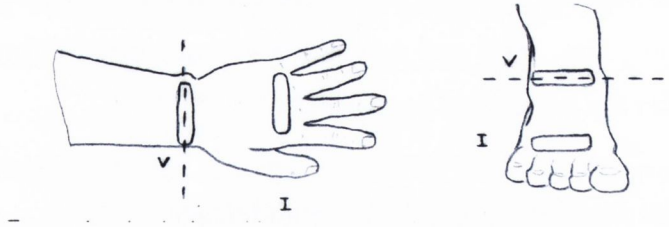


Figure 2.4: Positioning of electrodes for BIA measurement



2.2.3 Collection of Screening Blood Sample

Blood samples were taken by trained staff in phlebotomy or medics in each centre. Blood samples were obtained by venupuncture, following minimal compression with a tourniquet. Blood collected for the future determination of cholesterol, triacylglycerol (TAG) and high density lipoprotein (HDL) cholesterol concentrations were collected in EDTA-coated evacuated tubes and samples for glucose

determination were collected in tubes containing fluoride oxalate (Becton and Dickinson, Oxford, UK). Tubes were inverted eight to ten times to ensure that the coagulant was mixed throughout. Samples were centrifuged immediately at 3000rpm for 10min at 4°C. Plasma was immediately harvested in duplicate and frozen at -20° or -70° until subsequent analysis.

2.2.4 Determination of cholesterol concentration at screening

The quantitative determination of cholesterol in plasma was carried out using an enzymatic endpoint colorimetric method with reagents and standards supplied by Randox (Randox, Laboratories, Antrim, Ireland). The assay is based on the principle that cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_2O → cholesterol + fatty acids; cholesterol esterase

Cholesterol + O_2 → cholest-4-en-3-one + H_2O_2; cholesterol oxidase

$2H_2O_2$ + 4-aminoantipyrine + phenol → quinoneimine + 4 H_2O (red colour); peroxidase.

Quinoneimine production is proportional to the cholesterol concentration in the sample, and is quantified spectrophotometrically at 500nm and a blanking wavelength of 700nm.

2.2.5 Determination of TAG concentration at screening

The determination of TAG in plasma was measured by enzymatic endpoint analysis using a colourimetric assay (Randox, Laboratories, Antrim, Ireland). In this assay TAG is hydrolysed by lipoprotein lipase forming glycerol and fatty acids. In the presence of adenosine triphosphate (ATP), glycerol is converted to glycerol-3-phosphate and adenosine diphosphate (ADP) by glycerol kinase. Glycerol-3-phosphate is then oxidised by glycerophosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase, yielding quinoneimine and water. Quinoneimine production is proportional to the concentration of TAG in the sample, and is quantified spectrophotometrically at 500nm and a blanking wavelength of 700nm.

2.2.6 Determination of glucose concentration at screening

Plasma glucose was determined by endpoint bichromatic analysis with coupled hexokinase methodology using a colorimetric assay (Glucose RTU, Biomerieux, Lyon, France). In the presence of ATP, glucose is converted to glucose-6-phosphate and nicotinamide adenine dinucleotide (NAD)+ to 6-phosphogluconate and the reduced form of nicotinmide adenine, NADH. Although hexokinase catalyses the phosphorylation of hexoses other than glucose, the glucose-6-phosphate dehydrogenase is specific for glucose-6phosphate, and therefore glucose. The increased absorbance due to conversion of NAD+ to NADH is directly proportional to the glucose present in the sample. Absorbance is measured at a primary wavelength of 500nm.

2.2.7 HDL isolation and analysis at screening

Plasma total HDL was isolated using the Quantolip HDL Cholesterol Precipitation Reagents (Immuno Ag, Vienna, Austria). These reagents contain polyethylene glycol (PEG) of different concentrations and pH, dissolved in 0.1M phosphate buffer, and 0.9g/L sodium azide. These PEG solutions enable the selective precipitation of the different lipoprotein fractions. Reagent A precipitates the VLDL and LDL fractions, leaving HDL in the supernatant. The precipitation reagents were added to fresh plasma samples in a ratio of 2:1. The mixtures were vortexed, incubated at room temperature for 10 minutes and centrifuged for 15 minutes at 2500 x g. The supernatants were removed and frozen at -20°C for subsequent analysis. An immunolip control sample (Ommuno Ag, Vienna, Austria) was precipitated with every batch of samples and assayed to ensure the precipitation step was successful. Results were only accepted when the control value was within two standard deviations of the manufacturers expected HDL-cholesterol concentration. The supernatant from the HDL fraction was assayed for cholesterol as in Section 2.2.4.

2.3 RANDOMISATION AND STUDY DESIGN

Randomisation was completed by the coordinating centre (TCD), according to age, gender and fasting plasma glucose concentration. An equal number of subjects were allocated to each dietary group at each centre using the MINIM (Minimisation Programme for Allocating patients to Clinical Trials, Dept of Clinical Epidemiology, The London Hospital Medical College, UK) randomisation programme. Subjects were

randomly assigned to one of the four diets for 12 weeks. The rationale for the LIPGENE dietary intervention study was to determine the relative efficacy of replacing or reducing dietary SFA on insulin sensitivity, by comparing the effect of four different dietary treatments:

- High-fat (38% energy) SFA-rich diet (16% SFA, 12 % MUFA 6% PUFA)
- High-fat (38% energy), MUFA-rich diet (8% SFA, 20% MUFA, 6% PUFA)
- Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11%; MUFA; 6% PUFA), with 1.24 g/d high oleic sunflower oil supplement.
- Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g/d LC n-3 PUFA supplement.

2.4 DIETARY INTERVENTION

Subjects were recruited and assigned to one of the four dietary treatments. The intervention was conducted in two phases. In phase 1, approximately half of the subjects were recruited and the dietary intervention completed. In phase 2, the remaining subjects were recruited and phase 2 dietary intervention was completed. Details of the dietary strategy employed to achieve the intervention targets are presented in Chapter 4.

2.4.1 Dietary Assessments

Table 2.2 outlines the dietary assessments undertaken by the volunteers during the 12-week intervention period.

Pre-Intervention	Week- 0	Pre-Intervention Food Frequency Questionnaire Pre-Intervention 3-day weighed Food Dietary Assessment
Mid-Intervention	Week 1-2 (~ day 10)	24 hour recall to assess compliance Food Use Questionnaire, Body Weight
	Week 5 – 7	Mid-Intervention 3-day weighed Food Dietary Assessment
	Week 9 –10	24 hour recall, Body Weight
Post-Intervention	Week 12	Post-Intervention 3-day weighed Food Dietary Assessment, Weight Check

A 3-day weighed food diary, to include 2 weekdays and 1 weekend day was completed pre-, mid- and post-intervention. Pre-intervention, the investigator met the subject and demonstrated how to complete the diary and use the food scales. The

scales were calibrated at the beginning and end of each assessment to ensure accuracy of weighing. This was done by weighing standard known weights on 4 different points of the scales and making sure the weight was the same at each point as the weight being used. The investigator met with the subject after the food diaries were completed (pre, mid and post) and checked weights and types of foods consumed with the aid of a photo atlas. Volunteers were asked to include the food packaging and home made recipes where possible. Various dietary analysis programs were used across the 8 European centres involved to ensure culturally specific foods were included (Reading, UK: Food Base version 3.1; Dublin, Ireland: WISP version 3.0; Oslo, Norway: Kostberegningssystem (nutrient calculation software developed at the Department of Nutrition, University of Oslo); INSERM, France: Nutrilog; Maastricht, The Netherlands: Komeet to enter the dietary data and Orion to analyse the data; JUMC, Poland: "Dietitian software"; Cordoba, Spain: Dietsource version 2.0; Uppsala, Sweden: MATs connected to the Swedish Board of Food Administration database for foods).

2.4.2 Intervention Foods

Spreads, oils, mayonnaise/dressing, baking products and commercial fat were supplied by Unilever Best Foods (Unilever Health Institute, Unilever Bestfoods, Vlaardingen, The Netherlands). Cold storage (4°C) was required for the spreads and baking fats. Oils, mayonnaise and salad dressings were kept at low room temperature (in storage cupboards, out of direct sunlight). Commercial fat products of differing compositions were also provided and used in the manufacture of biscuit products (Fusco Foods, Ltd., Dublin, Ireland). Compositional details of the intervention foods are provided in Chapter 4.

2.5 CLINICAL INVESTIGATION DAY

A common Training Protocol and Standard Operating Procedures (SOP) were developed and adhered to in each centre to standardise collection of biological samples. At the clinical investigation day, pre- and post-intervention, urine, blood, adipose tissue and skeletal muscle (optional) were collected. Samples were collected after a 12 hour overnight fast, no food or drink was permitted except for water.

2.5.1 Collection of fasting blood samples

Blood sampling and the Intravenous Glucose Tolerance Test (IVGTT) were completed after an overnight fast (12 hours). Subjects refrained from vigorous exercise and alcohol the day before the IVGTT. The fasting samples were taken at the first sampling point (-5 minutes) of the IVGTT. The fasting blood samples were collected in the order presented in *Table 2.3*

Table 2.3: Amount, type and sample fasting analyte taken at pre- and post-clinical investigation day

Number	Volume	Colour	**Tube Type**	**Analytes**
2	10ml	RED	SERUM SEPERATOR	Leptin, resistin, adiponectin, CRP, IL-6 TNFα, sICAM, sVCAM
2	7ml	RED	SERUM	Insulin & c-peptide LDL isolation
3	10ml	PURPLE	K3-EDTA	Plasma cholesterol, TAG , NEFA Plasma apo A1, B, B48, CII, CIII, E, TRL, TRL Apo B, Total HDL, Plasma fatty acids, spare sample Buffy coat isolation
3	*4.5ml*	*BLUE*	SODIUM CITRATE*	PAI-1, t-PA, fibrinogen,
3	*2ml*	*GREY*	FLUORIDE OXALATE	Glucose

Every blood sample for plasma (K3 EDTA, sodium citrate and fluoride oxalate) was inverted 8-10 times to ensure the coagulant was mixed throughout. *The sodium citrate tube for PAI-1, tPA was pre-chilled on ice before collection. All samples, except for the serum samples, were put on ice after collection and processed within 1 hour.

2.5.2 Sample Preparation Protocols

The preparation of blood samples for analysis and centrifugation conditions are summarised in *Table 2.4* and *Table 2.5*, respectively. All blood samples were processed immediately after collection. Blood samples for plasma were placed on ice

after collection and processed within 1 hour. Blood samples for serum samples were not placed on ice after collection. The serum separator tube (SST) samples were allowed to clot for 30 minutes before centrifugation. TRL, LDL and HDL fractions were isolated within 24 hours. The buffy coat was isolated within 2 hours.

Table 2.4: Organisation of Blood Samples for Analysis – *(All samples aliquoted in duplicate)*

Analytical aliquots	Vaccutainer type	Sample type	Volume (ml)	Preparation	Analysing Centre	Temp storage (°C)
Glucose	Fluoride oxalate	Plasma	0.5	Centrifuge & freeze immediately	UoR	-20
Insulin, c-peptide	Serum	Serum	1.0	Allow to clot, centrifuge & freeze.	TCD	-20
TAG & cholesterol	EDTA	Plasma	0.5	Centrifuge & freeze immediately	UoR	-20
NEFA	EDTA	Plasma	0.5	Centrifuge & freeze immediately	UoR	-20
Plasma fatty acid composition	EDTA	Plasma	0.4	Centrifuge & freeze immediately	TCD	-20
Apo AI, B, CII, CIII & E, Apo B48	EDTA	Plasma	0.5	Centrifugation & Add protein preservative	INSERM	-20
TRL TRL Apo B	EDTA EDTA	Plasma Plasma	4.0	Ultra-centrifugation, separation and freeze	UoR INSERM	-20 -20
Total HDL	EDTA	Plasma	0.4	Precipitation and freeze	UoR	-20
LDL cholesterol	Serum	Serum	0.4	Precipitation and freeze	UoR	-20
DNA	EDTA	Buffy Coat	1.00	Centrifugation & isolation	TCD	-70
Leptin	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
Resistin	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
Adiponectin	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
CRP	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
IL-6	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
TNFα	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70
sICAM	Serum separator tube	Serum	0.20	Allow to clot, centrifuge &freeze.	TCD	-70
sVCAM	Serum separator tube	Serum	0.20	Allow to clot, centrifuge & freeze.	TCD	-70

PAI-1	Citrate	Plasma	0.2	Centrifuge immediately	TCD	-70
tPA	Citrate	Plasma	0.2	Centrifuge immediately	TCD	-70
Fibrinogen	Citrate	Plasma	0.2	Centrifugation	TCD	-70
Spare	Serum separator tube	Serum	0.5	Centrifugation	TCD/ UoR	-70
Spare	EDTA	Plasma	0.5	Centrifugation	UoR INSERM	-70
Spare	Citrate	Plasma	0.5	Centrifugation	TCD	-70

Table 2.5: Centrifugation and sample preparation details

Tube	Analyte	Conditions
2ml Fluoride oxalate	Glucose	1. 3000rpm 10 minutes @ 4°C. 2. Aliquot plasma samples. 3. Freeze immediately (-20°C).
6ml Serum	Insulin & c-peptide LDL isolation	1. Clot for 30min @ room temperature 2. 3000rpm 10 minutes @ 4°C. 3. Aliquot serum samples. 4. Freeze insulin and c-peptide samples immediately (-20°C). 5. Refrigerate remaining plasma for LDL isolation.
10ml K3-EDTA	Plasma cholesterol, TAG , NEFA Plasma A1, B, B48, CII, CIII, E TRL isolation HDL isolation Buffy coat isolation	1. 3000rpm 10 minutes @ 4°C. 2. Remove plasma. 3. Aliquot samples for plasma analysis & freeze immediately (-20°C). 4. Refrigerate remaining plasma samples for TRL, HDL & LDL isolation. 5. Pipette 1ml of intermediate white layer (buffy coat) into eppendorf & snap freeze & transfer to freezer immediately (-70°C).
10 ml serum	Leptin, resistin, adiponectin, CRP, IL-6, TNFα, sICAM, sVCAM,	1. Clot for 30min @ room temperature 2. 3000rpm 10 minutes @ 4°C 3. Aliquot plasma samples. 4. Freeze immediately (-70°C).
4.5ml Sodium citrate	PAI-1, t-PA,	1. 3000rpm 10 minutes @ 4°C 2. Aliquot plasma samples. 3. Snap freeze and transfer to freezer immediately (-70°C).

2.5.3 Intravenous Glucose Tolerance Test Protocol

Insulin sensitivity was assessed from a frequently sampled intravenous glucose tolerance test (IVGTT) with minimal model computer analyses of insulin sensitivity (Bergman *et al.*, 1985; Beard *et al.*, 1986; Pacini & Bergman, 1986). Volunteers attended the Clinical Investigation Unit after a 12 hour overnight fast, having refrained from vigorous exercise and alcohol on the day prior to the IVGTT. On the morning of the IVGTT two cannulae were inserted into the antecubital veins of both forearms. Fasting blood samples were taken from cannula 1. Then a bolus of 50% glucose solution (Phoenix Pharma Ltd, Gloucester, UK) (0.3g/kg body weight (BW)) was infused into cannula 2 over a 1-minute period followed by 20ml of saline. Twenty minutes later a dose of insulin (Novo Nordisk Pharmaceuticals Ltd, West Sussex, UK) (0.03U/kg BW) was infused into the same cannula (cannula 2). Meanwhile, blood was sampled through cannula 1 at frequent intervals over a 3 hour period (-5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, 180 minutes) after the start of the glucose injection. A total volume of 8ml of blood was collected at each time point (2ml into a paediatric fluoride oxalate tube for subsequent glucose analysis and 6ml into serum tube for subsequent analysis of insulin). At each time point real time blood glucose monitoring was performed using a gluco-trend machine to confirm that the volunteer's blood glucose was not excessively low (<2.0mmol/L). If the blood glucose dropped below 2.5mmol/L, the study was stopped and an isotonic high glucose drink was administered immediately. In addition, sterile 50% dextrose was on hand to give intravenously to the volunteers if their blood glucose dropped too low. Blood samples were spun at 3000rpm for 10 minutes after which the plasma/serum was aliquoted and stored at -20°C until it was required for analysis.

2.6 BIOCHEMICAL ANALYSIS

2.6.1 Triacylglycerol-rich lipoprotein (TRL) Isolation protocol

The TRL fraction was isolated from each patient's fasting blood sample and two aliquots of the TRL were harvested and stored (one for analysis, one spare). 25µl preservative was added to plasma. Polycarbonate tubes were labelled and weighed and the empty weight of each tube was recorded. 3.0ml of plasma was pipetted into a polycarbonate centrifuge tube and overlayed carefully with 3.0ml of sodium chloride solution (density <1.006 g/ml). The centrifuge tubes were weighed to ensure that

tubes opposite each other in the rotor were balanced. The rotor (Type 100.4 TLA) was placed into the ultracentrifuge (Ultra Optima TLX, Beckman Coulter) and spun at 50,000 rpm for 4 hours, 45 minutes at 24° C using maximum acceleration and deceleration. After the spin, the tubes were carefully removed from the rotor and placed into a rack. The top 1.5ml of solution (TRL) was isolated using a glass Pasteur pipette and placed into a pre-weighed plastic graduated tube. The tube was reweighed and the weight noted. 75µl preservative was added to the sample. TRL was aliquoted into 4 labelled graduated tubes. 0.5ml/tube was sent to UoR and 0.25ml/tube was sent to INSERM for future analysis. The samples were stored at -20° C. Following analysis, all TRL concentrations were corrected back to their respective concentration in plasma by multiplying by weight of TRL/3.0. This procedure was carried out by Jolene McMonagle, Nutrigenomics Research Group, Trinity College Dublin.

2.6.2 LDL Isolation Protocol and Analysis

LDL was isolated using a LDL Precipitation Reagent (Randox, Antrim, Ireland). The reagent contains heparin, which causes LDL to be precipitated at its isoelectric point (pH 5.04). LDL precipitating reagent was added to fresh serum (1ml/100µl serum), vortexed, and incubated at room temperature for 10 minutes. Samples were then centrifuged for 15 minutes at 2500 x g. After centrifugation, HDL and VLDL remained in the supernatant while LDL formed a pellet at the bottom of the tube. The supernatant was removed and stored at -20°C until cholesterol analysis was performed (Section 2.2.4). The concentration of LDL-cholesterol was calculated as the difference between the total cholesterol concentration of the plasma sample and cholesterol concentration of the supernatant. An immunolip control sample (Immuno Ag, Vienna, Austria) was precipitated with every batch of samples and analysed to ensure that the LDL precipitation was successful. Results were only accepted when the control value was within two standard deviations of the manufacturers expected LDL-cholesterol concentration. LDL and HDL-cholesterol isolation (Section 2.2.7) was carried out by Jolene McMonagle, Nutrigenomics Research Group, Trinity College Dublin and analysis by Danielle Shaw, University of Reading. CVs were less than 10% for internal controls.

2.6.3 Determination of Cholesterol, TRL Cholesterol, TAG, TRL TAG and HDL cholesterol concentrations

The quantitative determination of cholesterol, TRL cholesterol, TAG in plasma and TRL fraction was carried out using the IL Test™ Cholesterol kit and IL Test™ Triglycerides kit (Instrumentation Laboratories, Warrington, UK). The principles of the assays are described in Section 2.2.4 and 2.2.5 respectively. The IL Test™ HDL-C Kit (Instrumentation Laboratories, Warrington, UK) was used for direct quantification of HDL-cholesterol. A detergent specifically solubilises HDL-cholesterol and a polyanion assists in the selectivity by complexing LDL-cholesterol, VLDL and CM lipoproteins. All samples taken were stored and analysed at the end of the trial in a single batch to minimise inter-assay variability. This analysis was carried out by Danielle Shaw, University of Reading. CVs were less than 10% for internal controls for all analytes.

2.6.4 Determination of NEFA concentrations

The WAKO NEFA C enzymatic colour kit (Alpha Laboratories, Hampshire, UK) was used to quantitate plasma NEFA concentration. The intensity of coloured product (quinoneimine) is proportional to the concentration of free fatty acids and measured by absorbance using the ILAB. Control 01 and control 02 (Instrumental Laboratories, Warrington, UK) and a NEFA calibrator were used with each analysis (Alpha Laboratories, Hampshire, UK) and were within reference ranges. This analysis was carried out by Danielle Shaw, University of Reading.

2.6.5 Determination of Plasma Glucose

The IL Test™ Glucose Hexokinase Clinical Chemistry kit (Instrumentation Laboratories, Warrington, UK) was used to determine plasma glucose concentration. The principle of the assay is previously described in Section 2.2.6. This analysis was carried out by Danielle Shaw, University of Reading. CVs were less than 10% for internal controls.

2.6.6 Buffy Coat Isolation

The buffy coat is the small white layer between the plasma and the red blood cells. The buffy coat contains the white blood cells including the leucocytes, which contain DNA. 1ml of intermediate white layer (buffy coat) was pipetted into a 1.5ml

eppendorf tube using a plastic 3ml or 156mm (Starstedt) transfer pipette. Aliquoted samples were placed in liquid nitrogen and stored at -70°C.

2.6.7 Determination of Insulin and C-peptide

Serum insulin and C-peptide concentrations were measured using solid phase, two-site fluoroimmunometric assays (AutoDELFIA Insulin kit and AutoDELFIA C-peptide kit respectively, Wallac Oy, Turku, Finland). The assays are based on a direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants of the protein under investigation i.e. either the insulin or the C-peptide molecule. Samples are simultaneously reacted with immobilised monoclonal antibodies directed against the protein of interest and with europium-labelled monoclonal antibodies directed against a different specific antigenic site on the insulin or C-peptide molecule. Enhancement solution dissociates europium ions from the europium-labelled monoclonal antibody into solution, where they form highly fluorescent chelates with components of the enhancement solution. The fluorescence in each well is measured, the concentration being directly proportional to the concentration of insulin/C-peptide in the sample. This analysis was carried out by the author and Declan Gasparro in the Central Pathology Laboratory, St. James Hospital, Dublin, Ireland. CVs were less than 10% for all internal controls.

2.6.8 Plasma fatty acid compositional analysis

This analysis was completed at the Nutrition Laboratory, Department of Clinical Medicine, Trinity Centre for Health Sciences, Dublin 8, Ireland. Extraction and transmethylation was carried out by the author and Jolene McMonagle and the gas chromatographic analysis of the samples was carried out by Jane Ferguson.

Extraction: 400µl of plasma was transferred into 16ml borosilicate glass, screw-capped tubes and 400µl of milliQ water was added. Lipid extraction was achieved by the addition of 2ml methanol, 1ml chloroform, 40µl 5nM BHT in ethanol, 100µl stock solution of heptadecanoic acid (C17:0). Samples were vortexed for 1min and 1ml milliQ water and 1ml chloroform were immediately added. The tubes were closed and vortexted for a further 30 seconds and centrifuged in a Beckman Avanti G25 equipped with a JLA rotor. They were spun at 3000rpm (RCF=1673xg) for 15 minutes at 20°C. Infranatants (organic phase) were carefully removed with a pasteur glass pipette and transferred to new 16ml borosilicate glass

tubes. The supernatants (aqueous phase) were then supplemented with 2ml chloroform and vortexed for 30 seconds and then centrifuged at 3000rpm for 15 minutes at 20°C. Infranatants were removed as previously indicated and pooled with the previous infranatants and dried under nitrogen. The dried samples were flushed with nitrogen, prior to sealing to prevent lipid oxidation and stored at -20°C.

Transesterfication (OHTA Method): To each tube containing the dried lipid extract, 1ml of methanol and 1ml BF3-methanol (14%) were added and vortexed for 1 minute. The tubes were placed in a boiling bath (100°C) for 40 minutes and then cooled at room temperature. The resulting methyl esters were then extracted by adding 2ml hexane and 2ml milliQ water, vortexted for 10 seconds and centrifuged at 2000rpm (RCF=744xg) for 5 minutes at 10°C. Supernatants (hexane phase) were collected. For optimisation of the extraction, 2ml of hexane was added on the lower phase, vortexed for 30 seconds and centrifuged at 2000rpm for 5 minutes. The supernatants were removed and pooled with the first supernatants and dried under nitrogen. The resulting methyl esters were then dissolved in 150µl of hexane and stored at -20°C under nitrogen until their analysis by GC.

Gas chromatographic analysis of total plasma lipids: Fatty acid methyl esters (FAME) were analysed by gas chromatography (GC) on a Shimadzu GC 2010 (Shimadzu, Japan) using a fused silica capillary column Omegawax 250, (30 m x 0.25 mm inner diameter, 0.25µm film) (Supelco, Saint Quentin Fallavier, France).

GC was equipped with a flame ionisation detector and GC solutions software (Shimadzu, Japan). Heptadecanoic acid was used as an internal standard. Helium was used as the carrier gas with pressure of 0.8 psig. Injector and flame ionisation detector temperatures were 250°C. The following temperature program was used: 215°C (43 minutes) and 10°C/minute to 260°C (2.5 minutes). Fatty acids were identified by comparing the relative retention times of plasma FAME with FAME standards (SUPELCO, Saint Quentin Fallavier, France). PUFA 2 (from Menhaden oil) and PUFA 3 (from an animal source), (both from Supelco), were run every week to determine the relative retention times of the fatty acids. The retention time of C17 was obtained from the blank sample from each batch. Fatty acid mass was determined as a relative percentage of the total quantified fatty acids. Individual fatty acid types were determined as follows: SFA (C14:0, C16:0,C18:0), MUFA (C16:1, C18:1, C20:1), PUFA (n-3 + n-6 PUFAs), n-3 PUFAs (C18:3:3, C18:4, C20:4:3, C20:5, C22:5, C22:6) and n-6 PUFAs (C18:2, C18:3:6, C20:3, C20:4:6,

C22:4) The fatty acid masses were calculated based on the blank C17 value for each batch. The efficiency of the experiment was also calculated from the C17 value. Samples with an extraction efficiency of less than 30% were rejected. To ensure consistency between batches, a standard serum sample, to serve as an internal control, was extracted as part of each batch. Batches were considered to be acceptable if the standard serum had a variance below 10%.

2.6.9 Plasma Apolipoprotein (apo) A1, B and E

Protease inhibitor was added (10µl/ml plasma) to protect the samples. Plasma apoA1, B and E concentrations were measured using an immunonephelometric method on a BN ProSpec® System with commercial kits (Dade Behring, Deerfield, USA) at the Analytic Biochemistry Laboratory, St. Marguerite, Hospital, Marseille, France. CVs were less than 10% for internal controls.

2.6.10 Plasma apoCII and CIII

Protease inhibitor was added (10µl/ml plasma) to protect the samples. Plasma apoCII and CIII concentrations were measured by immuno-turbidimetry on a Hitachi 911 system with commercial kits (Diasys, Bouffemont, France) at the Analytic Biochemistry Laboratory, St. Marguerite, Hospital, Marseille, France. CVs were less than 10% for internal controls.

2.6.11 Plasma apoB48

This analysis was completed at the Human Nutrition and Lipids (UMR 476-INSERM) Laboratory, University of Medicine Timone, Marseille, France. Protease inhibitor was added (10µl/ml plasma) to protect the samples. Assessment of apoB-48 was by competitive ELISA with a specific apoB-48 antibody obtained in rabbits. The assays were performed after a modification of the method (Lovegrove *et al.*, 1996; Lorec *et al.*, 2000). CVs were less than 10% for internal controls.

2.6.12 Serum Cytokine analysis

Human CRP, IL-6, TNFα, total Adiponectin, Resistin sICAM-1, sVCAM-1 and Leptin concentrations were measured by solid phase sandwich enzyme linked immunosorbent assay (ELISA). CRP was determined by high sensitivity (hs) CRP

kits (BioCheck Inc, CA, USA), Human IL-6 ultrasensitive kits were purchased from Biosource International Inc, CA, USA. TNFα was determined using hs TNFα kits (R&D Systems, Abingdon, UK). Adiponectin, Resistin and Leptin were analysed using a DuoSet® ELISA Development System (R&D Systems, MN, USA) and human sICAM-1 and sVCAM-1 were determined using R&D Systems, Abington, UK. The methods were completed in accordance with manufacturer's instructions by Jolene McMonagle, Nutrigenomics Research Group, Trinity College Dublin. CVs were less than 10% for all internal controls.

2.6.13 Determination of PAI-1 and tPA

Plasma PAI-1 and tPA were determined using Asserachrom® kits (Diagnostica Stago, France) which are enzyme immunoassay procedures by the sandwich technique, also known as enzyme-linked immunosorbent assays (ELISAs). These kits provide a plastic support, coated with mouse monoclonal anti-human PAI-1 or tPA antibody which captures the PAI-1 and tPA, respectively, to be measured. Next, a second mouse monoclonal anti-human antibody coupled with peroxidase binds to another antigenic determinant distant from the first one forming the "sandwich". The bound enzyme peroxidase is then revealed by its activity in a predetermined time on the substrate orthophenylenediamine in the presence of hydrogen peroxide. After stopping the reaction with a strong acid, the intensity of the colour produced bears a direct relationship with the PAI-1 or tPA concentration initially present in the plasma sample. The intensity of the colour produced is measured spectrophotometrically at 492nm. Specific conditions and details of the assays are outlined in *Table 2.6.* A standard curve was constructed by plotting the absorbance values versus standard concentrations. All samples were run in duplicate and readings averaged. Concentrations of PAI-1 and tPA for each sample were extrapolated from the appropriate standard curve. Results for the patients 1:5 dilutions were read directly from the curve, those for the 1:10 dilutions had their values multiplied by 2. Samples with a concentration greater than the standard curve were diluted and re-assayed to obtain a precise concentration. All control values were ensured to be in the correct range. The inter-assay CV for PAI-1 was 6% and tPA was 3%

2.6.14 Determination of Fibrinogen

Quantitative determination of fibrinogen was performed using an automated assay (Fibriquik, Organon Teknika Corporation, NC, USA) using a Coag-A-Mate MTX (Oraganon Teknika Corporation, NC, USA). The assay is based on the principle that when thrombin is added to a sample of plasma, fibrinogen is converted enzymatically to fibrin. Fibrin, in turn, undergoes polymerisation to form a fibrin network. When activated by thrombin, Factor XIII catalyses the formation of stabilising cross links to produce a visible clot. The elapsed time from the addition of thrombin to the formation of a clot is inversely proportional to the fibrinogen level.

Verify Reference Plasma (Bio Merieux, NC, USA) was used to calibrate the assay prior to sample analysis. Two sets of coagulation reference plasma, a normal and an abnormal control sera (Universal Coagulation Reference Plasma and Abnormal Coagulation Reference Plasma, respectively, Pacific Haemostasis, Fischer Diagnostics, Middleton, VA, USA) were used to test assay performance. Duplicate measurements were recorded. The inter-assay CV for fibrinogen was 4.5% for the low controls and 4.8% for the high controls.

Table 2.6: Details of PAI-1 and tPA ELISAs

PAI-1			tPA		
Pipette into each precoated well:			Pipette into each precoated well:		
Antigen Immobilization	Test Sample	200µl	Antigen Immobilization	Test Sample	200µl
	Cover the wells and let incubate for 1 hour (room temp, 18-25°C)			Cover the wells and let incubate for 2 hours (room temp, 18-25°C)	
Wash all wells 5 times with washing solution			Wash all wells 5 times with washing solution		
Immobilization of immuno-conjugate	Mouse monoclonal anti-human PAI-1 antibody	200µl	Immobilization of immuno-conjugate	Mouse monoclonal anti-human tPA antibody	200µl
	Cover the wells and let incubate for 1 hour at room temp			Cover the wells and let incubate for 2 hours at room temp	
Wash all wells 5 times with washing solution, then immediately proceed:			Wash all wells 5 times with washing solution, then immediately proceed:		
	OPD/H_2O_2 Substrate	200µl		OPD/H_2O_2 Substrate	200µl

Colour Development	Let incubate at room temp for exactly 3 minutes, then add acid		Colour Development	Let incubate at room temp for exactly 6 minutes, then add acid	
	Either 3M H_2SO_4 or 1M HCL	50µl 100µl		Either 3M H_2SO_4 or 1M HCL	50µl 100µl
Wait 30 minutes, then measure the absorbance at 492nm within 2 hours			Wait 30 minutes, then measure the absorbance at 492nm within 2 hours		

2.7 IVGTT MODELLING

Glucose data was entered in mg/dl (to convert from mmol/l→ mg/dl: x18). Insulin data was provided in µIU/ml (equivalent to the mIU/L). Glucose and insulin values from the -5 and 0 time points were averaged and entered for the 0 minute time point in the model. If the 2 fasting levels were significantly different, haemolysis notes were referred to, to identify the real value. The peak insulin value should have occurred at 22 minutes, if the correct protocol was adhered to. If the peak value did not occur at 22 minutes the modelling was questioned. If the low value was explained by haemolysis then the modelling could be carried out but the fit was queried and the model was checked for outlier status. If no haemolysis occurred, sampling protocol/IVGTT protocol was highly questionable and therefore the model was rejected. *Table 2.7* details the standard operating procedure adhered to for the IVGTT modeling. *Figure 2.5* shows an ideal model from a set of IVGTT data.

Table 2.7: IVGTT Modelling Standard Operating Procedure

1. Open MinMod and select new data entry.
2. Enter timepoints 0,2,4,8,19,22,30,40,50,70,90,180. Select other automatically displayed timepoints and delete so only those used in the LIPGENE IVGTT are present in the data sheet.
3. Copy and paste glucose and insulin data from excel sheet into minmod datasheet, name sheet with subject number and intervention stage (pre or post-intervention) code i.e. 101L1. Save sheet.
4. Weight timepoints 0, 2, 4 as zero, weight timepoints 8-180 inclusive as 1.
5. Always consult completed IVGTT incident questionnaires when entering data a. Adjust timepoints as is relevant. If for example timepoint taken at 2

minutes 36 seconds then enter timepoint as 2.6 rather than 2.

b. If any timepoint that has incomplete data e.g. if glucose or insulin data is missing. Ensure cell is left blank in minmod datasheet, do not enter a zero.

6. Save input - programme does not automatically save changes when minmod data sheet is closed.

7. Complete data entry for subjects in one centre

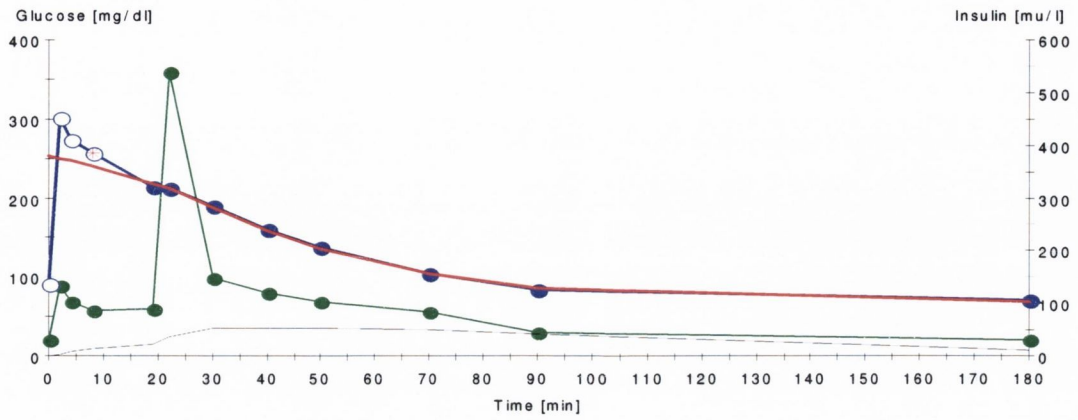
8. Select all files to be modelled and click Run MinMod

9. Data output will be produced with graph, analysis data and original data with fraction standard deviation (FSD).

10. Important: unweighting data ONLY applies to glucose; if insulin data is dubious it should be deleted.
    a. Unweight/ delete any data with an FSD >0.05 (written as 5.0000000 E-0.2), save datasheet and re-run minmod.
    b. Insulin data at time points 22 and 30 is critical and MUST be present if modelling is to be performed without query
    c. Check for carry over glucose which may have remained in cannula and administered alongside insulin - may need to unweight the glucose at timepoint 19 or 22 if this is the case. Decide by best fit to model.
    d. Check that insulin peak occurs at 22 minutes. Investigate data further if it does not.
    e. 0 to 8 minutes represents a mixing/settling period between the blood and extracellular fluid and the red line might not fall on the blue line for these timepoints - this is acceptable.
    f. The model often unweights the 8min glucose timepoint. This should be accepted.
    g. It is not ideal to unweight more than 3 points. Avoid this as the model won't be successful.
    h. If 90 minutes and 180 minutes samples are missing, delete last two rows and run data. MINMOD can't cope with these two points left blank.
    i. Make a note of any changes made to dataset in lab book and entered in comment cell in the excel sheet of exported data, alongside info from

the IVGTT questionnaires.
11. Note any inconsistencies in the modelling, e.g. unmatched blue and red line, S_I highlighted in red etc.
12. Export Minmod data into a notebook file and save as an excel file. Add comments where necessary e.g. any deviations noted in questionnaire that deviate from IVGTT SOP, any queries from modelling analysis
Notes: If extremely high insulin occurs at 22 minutes (~1000uIU/ml) then model should be accepted but queried. This value is much greater than the normal peak insulin ~500uIU/ml but still occurs at the correct time point therefore model not rejected but queried as pharmacologically high value is questionable If extremely high insulin occurs at 40 minutes or after, then the whole time point is removed i.e. both the glucose and insulin values. This is because the sampling at that time point is highly questionable as insulin is not within physiological range, a major question is raised for this entire time point and therefore model should not be based on either the glucose or insulin value from this sample. However if extremely high peak occurs at 30 minutes, it is unacceptable to remove the glucose and insulin data for the whole time point. This is because 30 minute data is essential to the model. Removal of it without reason is unacceptable because of importance of time point to model. In this instance the model is rejected.
If peak insulin occurs at 22 minutes but a steady decline in insulin after this point does not occur (and this is not explained by haemolysis) i.e. reduced at 30 minutes, again at 40 minutes and 50 minutes but the 70 minute is greater than 50 minutes. This is questionable but it is not known if this is a sampling query or indeed a physiological response. There is no known reason to delete insulin 70 value therefore all data is included in model but the model is queried and its status as an outlier investigated.

Figure 2.5 An Ideal IVGTT model



Blue dots: Glucose observations; *hollow blue dots*: unweighted glucose observations; *Green dots*: Insulin observations; *Red line*: Fit of minimal observations to glucose observations; *Black line*: Insulin action "X".

Parameters Exported from MINMOD Programme:

S_I:	Insulin Sensitivity
S_G:	Glucose Effectiveness
AIRg:	Acute Insulin Response to Glucose
DI:	Disposititon Index (AIRg*SI)
P2:	Removal rate of insulin from the interstitial space
P3:	Movement of circulating insulin to the interstitial space
G0:	Distributed glucose concentration at time 0

2.8 CANDIDATE GENE IDENTIFICATION SNP SELECTION AND GENOTYPING

A dual approach was used for candidate gene and SNP selection. SNPs identified in association, epidemiological and physiological studies as having an association with features of and/or risk of the MetS were included. Suitability of SNPs was determined in each candidate gene according to their allelic frequency and their putative involvement on function or expression of the gene product. The second SNP selection approach involved haplotype tagging. A number of recent studies have shown that this approach may be more advantageous than single SNP analysis. It is proposed that

haplotype tagging captures the majority of diversity within a region by typing those SNPs which cover the most diversity and uses the most informative SNPs to explain genetic variation.

Online databases such as dbSNP http://www.ncbi.nlm.nih.gov/, HapMap http://hapmap.org and Perlegen http://perlegen.com were used to source genotype data for each candidate gene. This information was uploaded into Hitagene https://web1.hitagene.com/lipgene/frame.php which enabled haplotype frequency estimation to be determined by implementation of the EM algorithm. Using a 5% cut-off for individual haplotype frequency and >70% for the sum of all haplotype frequencies, genotype data were then transferred from Hitagene to SNP Tagger http://www.broad.mit.edu/mpg/tagger/server.html an online tool which identifies haplotype tagged SNPs. When a choice of haplotype tagged (Ht) SNPs was available, HtSNPs were chosen based on their allelic frequency and type of variant, with priority given to SNPs in the following order; (i) non-synonymous SNPs (ii) synonymous SNPs, (iii) promoter/regulatory region, 5' UTR and 3'UTR SNPs, (iv) intronic SNPs.

Buffy coat samples were obtained from blood collected from participants of the dietary intervention study. The total number of samples was 464. The DNA was extracted using the Gentra Autopure LS system. Samples were thawed at 37°C and lysed using 1ml cell lysate solution. Each sample was placed in a bar-coded tube, and these were inputted into the system using a computer and scanner, and a standard protocol was selected. Further extraction was performed automatically. DNA was left to re-hydrate for ~24 hours prior to quantification and further use. The concentration of the extracted DNA was determined using Nanodrop. Samples with a concentration less than 60ng/ul were replaced by re-extracting another buffy coat sample. There were 55 low samples, i.e. ~12% of the total number. Where a replacement was not available, or the re-extraction also gave a low yield, the sample was amplified using the Qiagen Repli-g midi WGA kit. Altogether, 24 samples were amplified. An input amount of DNA of 10ng or more was used in all cases (this involved using 5ul in most cases, and 10ul in very low samples i.e. those below 5ng/ul to begin with). The resulting DNA quantified highly on Nanodrop (1000 – 2000ng/ul) but this is not a reliable indicator of actual concentration due to the presence of primers and other nucleotides that can mask an absence of amplified material. Performance was thus determined by Ilumina genotyping, and was generally good for the WGA samples.

2.9 STATISTICAL POWER OF STUDY

The statistical power of the LIPGENE dietary intervention study was based on insulin sensitivity as the primary biomarker of the MetS. Insulin sensitivity was assessed by an IVGTT wherein insulin sensitivity (S_I) was measured by the MINMOD minimal modeling programme. The power of the study was calculated with the ability to detect at least 1 unit difference at a SD of 2, between diets. The power calculation was based on the assumption that 15% difference in insulin sensitivity was physiologically important and that this difference can be detected at a significance level of 0.05 and a power of 0.8. Thus, a minimum of 98 subjects per dietary group would be required to detect significance differences. The group size was increased to 120 per group, to account for an anticipated 20% drop-out rate.

Power calculations for gene-environment interactions in a case-only population were determined using the QUANTO formula (Gauderman, 2002). This estimated a sample size requirement of 96 subjects per dietary group, assuming a minor allele frequency of 0.1; dominant inheritance, a power of 0.8 and type 1 error rate 0.05 (two-sided). This is based on the following criteria: Baseline disease risk: 1% Environmental relative risk: 1.0, Genetic relative risk: 2.2; Interaction effect: 3.4, the level of each being based on published data (Shen *et al.*, 2007) which determined the interaction between fatty acid composition and risk of the MetS.

Chapter Three

The LIPGENE Human Dietary Intervention Study: the interaction between dietary fatty acid modification and genetic risk of the metabolic syndrome in Europeans – Cohort Details and Study Design

---

3.1 ABSTRACT

Background: Evidence suggests that dietary fatty acid composition affects insulin sensitivity, wherein dietary saturated fatty acids (SFA) are associated with insulin resistance.

Objective: The LIPGENE Human Dietary Intervention Study is a multi-centre, Trans–European, randomised, controlled trial with two principal aims. Firstly, to determine the relative efficacy of reducing dietary SFA consumption, by altering quality of dietary fat and reducing the quantity of dietary fat, on metabolic and molecular risk factors of the metabolic syndrome (MetS). Secondly, to determine if common genetic polymorphisms affect an individual's responsiveness to dietary modification.

Design: A total of 486 free-living subjects with the MetS received one of four dietary treatments for 12 weeks: (1) High-fat (38% energy) SFA-rich diet; (2) High-fat (38% energy), monounsaturated fatty acid (MUFA)-rich diet; (3) Isoenergetic low-fat (28% energy), high-complex carbohydrate diet and (4) Isoenergetic low-fat (28% energy), high-complex carbohydrate diet, with 1.24 g/d LC n-3 PUFA. A 3-day weighed food intake diary assessed dietary compliance pre-, mid- and post-intervention. An IVGTT, lipoprotein analysis, cytokines, adhesion molecules, coagulation factors and isoprostane levels were determined pre- and post-intervention.

Results: Over 15,500 subjects were screened to recruit 486 subjects with the MetS who initiated the study. The average waist circumference for males and females were 111cm and 103cm, respectively, with average fasting plasma glucose (5.5mmol/L), triacylglycerol (TAG) (1.8mmol/L) and high-density lipoprotein (HDL) cholesterol (1.30mmol/L) concentrations, and blood pressure (143/89mmHg).

Conclusions: LIPGENE will provide important information in relation to dietary fatty acid modification, genetic determinants of dietary responsiveness and molecular markers of insulin sensitivity.

3.2 INTRODUCTION

LIPGENE is an EU Integrated Project entitled "Diet, genomics and the metabolic syndrome (MetS): an integrated nutrition, agri-food, social and economic analysis" funded under the Framework 6 Food Safety and Quality Programme in 2003 (www.lipgene.tcd.ie). The primary objective of the Human Nutrition component of LIPGENE was to understand how dietary fatty acid composition interacts with an individual's genetic background and is related to the development and risk of the MetS and Type 2 Diabetes Mellitus (T2DM).

The LIPGENE Human Dietary Intervention Study was designed to determine the relative efficacy of reducing dietary SFA consumption, by altering the quality of dietary fat or reducing the quantity of dietary fat, on multiple metabolic and molecular risk factors of the MetS. The second objective of the LIPGENE study was to explore if common genetic polymorphisms potentially associated with increased risk of the MetS play a causal role in determining an individual's responsiveness to dietary fat modification.

The MetS includes a constellation of interrelated factors of metabolic origin which is associated with increased risk of T2DM and cardiovascular disease (CVD) (Lakka *et al.*, 2002; Ninomiya *et al.*, 2004; Grundy, 2006; Sundstrom *et al.*, 2006). The growing prevalence of the MetS is linked with increasing incidence of obesity (Carr *et al.*, 2004). It is proposed that insulin resistance is the key metabolic perturbation of the MetS (Reaven, 2004), which is also characterised by central obesity, increased waist circumference, dyslipidaemia (increased triacylglycerol, (TAG) and non-esterified fatty acids (NEFA) and reduced high density lipoprotein (HDL)-cholesterol concentrations), hypertension, microalbuminuria and chronic, low-grade inflammation (NCEP 2002; Ghanim *et al.*, 2004; Hanley *et al.*, 2004; 2005). More recently, since the initiation of LIPGENE the International Diabetes Federation (IDF) has proposed a new definition of the MetS with central obesity as the main focus for the diagnosis in addition to at least two other factors, to include a raised TAG level, reduced HDL-cholesterol, raised blood pressure and raised fasting plasma glucose. Insulin resistance, which is not measured routinely in clinical practice, is not an essential requirement (IDF, 2005)(www.IDF.org).

Although diet is not listed specifically as a risk factor for the MetS, there is little doubt that metabolic stressors including energy dense, high-fat diets promote obesity, insulin resistance and the MetS (Roche, 2005). Furthermore there is evidence

to suggest that dietary fat composition may impede insulin signalling, wherein saturated fatty acids (SFA) are associated with reduced insulin sensitivity (Laaksonen *et al.*, 2002; Warensjo *et al.*, 2005; Warensjo *et al.*, 2006). Most dietary intervention studies have shown that isoenergetic substitution of SFA for monounsaturated fatty acids (MUFA) or carbohydrates improved insulin sensitivity (Perez-Jimenez *et al.*, 2001; Vessby *et al.*, 2001; Lovejoy *et al.*, 2002). Given recent evidence that dietary interventions have been more effective than pharmacological agents in preventing the development of the MetS and T2DM in high-risk individuals (Knowler *et al.*, 2002; Orchard *et al.*, 2005) and the notion that some pharmacological agents may even have adverse effects on T2DM patients (Nissen & Wolski, 2007) exploration of the concept that altered dietary fat intake may attenuate the impact of the MetS warrants investigation.

Genetic factors must also contribute to individual variation in the clinical presentation of the MetS. It is likely that the phenotypic expression of the diverse metabolic components associated with the syndrome is subject to genetic determinants. A variety of polymorphisms in genes involved in insulin signalling, glucose homeostasis, lipoprotein metabolism, TAG deposition and mobilisation in adipose tissue, vascular function, inflammation or coagulation could be adversely affected in subjects with the MetS (Phillips *et al.*, 2006). For example, the Pro^{12} PPARγ polymorphism has been associated with greater diabetes risk, which seems to be augmented by obesity (Altshuler *et al.*, 2000; Ghoussaini *et al.*, 2005; Meirhaeghe *et al.*, 2005). Interestingly, there is evidence for interaction between habitual dietary fat composition and PPARγ, whereby exposure to a SFA rich diet augments the insulin desensitising effect (Luan *et al.*, 2001). It is possible that the genetic determinants of some of the components of the MetS may be particularly sensitive to nutrient derived environmental exposures. For example, lipoprotein metabolism is sensitive to dietary fatty acid modification and there is considerable evidence that inter-individual variability in response to dietary modification is determined by genetic factors (Ordovas & Corella, 2004).

LIPGENE will provide important information in relation to the efficacy of dietary fat modification on risk factors for the MetS. It has the potential to identify the role of candidate genes in determining responsiveness to dietary fat intervention. It may provide insights in relation to the molecular interactions between dietary fat composition with adipose tissue biology, skeletal muscle metabolism and vascular

function that contribute to the MetS. This study design chapter outlines how this rich dataset of volunteers were recruited and presents their screening characteristics before their participation in the 12-week dietary intervention study. In effect, this chapter lays the foundation for the results and discussion presented in the remaining chapters.

3.3 SUBJECTS AND METHODS

3.3.1 Study design & Dietary Intervention Rationale

The LIPGENE human dietary intervention study was a randomised, controlled trial designed to determine the efficacy of changing dietary fatty acid consumption on risk factors of the MetS and to determine if common genetic polymorphisms associated with increased risk of the MetS affect an individual's responsiveness to dietary modification. The LIPGENE Dietary Intervention Study is registered with The US National Library of Medicine Clinical Trials registry. The study was approved by the local ethics committees at each of the 8 clinical intervention centres, all of which conform to the Helsinki Declaration of 1975 as revised in 1983.

Free-living subjects with the MetS, defined by a modified version of the NCEP criteria (2002), characterised by three or more of the following, participated in LIPGENE:

• Fasting glucose concentration	5.5 - 7.0 mmol/L
• Serum TAG concentrations	≥ 1.5 mmol/L
• Serum HDL cholesterol concentration	< 1.0 mmol/L (men) < 1.3 mmol/L (women)
• Blood pressure	systolic BP≥130 diastolic BP≥85mmHg **or** treatment of previously diagnosed hypertension
• Waist girth	> 102 cm (men) > 88 cm (women)

The rationale for the LIPGENE dietary intervention study was to determine the relative efficacy of replacing or reducing dietary SFA on insulin sensitivity, by comparing the effect of four different dietary treatments:

- High-fat (38% energy), SFA-rich diet (16% SFA, 12 % MUFA 6% PUFA) (HFSFA)
- High-fat (38% energy), MUFA-rich diet (8% SFA, 20% MUFA, 6% PUFA) (HFMUFA)
- Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11%; MUFA; 6% PUFA), with 1.24g/d high oleic sunflower oil supplement (LF)
- Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g/d LC n-3 PUFA supplement (LF n-3 PUFA)

Subjects were randomly assigned to one of the four diets for 12 weeks. The control diet was a high-fat, predominantly saturated fat diet. The second diet was to determine the efficacy of a high-fat, MUFA enriched diet. Two low-fat diets were included to evaluate the effectiveness of reducing total fat intake and within the low-fat groups the effect of LC n-3 PUFA supplementation was also determined. A range of fat-modified food products (margarines, cooking fats, baking fats, mayonnaise, dressings, biscuits, etc) were produced by Unilever Best Foods (Vlaardingen, The Netherlands) and provided to volunteers with specific dietetic counselling. Extensive details in relation to the dietary fatty acid modification and achievement of dietary goals is presented in Chapter 4. The LC n-3 PUFA supplement (Marinol ^{TM} C-38) and the comparator high-oleic acid sunflower seed oil (HOSO) supplement were provided by Lipid Nutrition, Loders Croklaan (Wormerveer, The Netherlands), the composition of which is presented in *Table 3.1*.

Table 3.1: Fatty Acid Composition and tocopherol content of the LC n-3 PUFA and comparator HOSO supplement

	LC n-3 PUFA	HOSO Supplement
Description:	Marinol™ C-38 is a concentrated natural fish oil with a high content of EPA and DHA	High Oleic Acid Sunflower Seed Oil supplement
Fatty Acid Composition	w/w %	w/w %
C14:0	5	0
C16:0	10.5	3.5
C16:1	4.5	0
C17:0	0.2	0
C18:0	2.5	3.4
C18:1	8.0	79.2
C18:2	1.0	11.8
C18:3	0.5	0.2
C20:0	0.2	0.3
C20:1	1.1	0.3
C22:0	0.2	0.9
C24:0	0.2	0.3
C20:5	24	-
C22:6	17.5	-
Total EPA and DHA	38*	-
Mixed Natural Tocopherol	3.0mg	3.0mg

*38% is the standard minimum value of EPA and DHA that is assured in all batches of the product.

The LIPGENE intervention study was completed in 8 European centres, in order to represent the heterogeneous nature of habitual European dietary fatty acid intake. Each centre adhered to a stringent common standard operating procedure. Approximately 60 subjects were recruited and randomly assigned to one of four dietary interventions per centre. Randomisation was completed by the coordinating centre, according to age, gender and fasting plasma glucose concentration. An equal number of subjects were allocated to each dietary group at each centre using the MINIM (Minimisation Programme for Allocating patients to Clinical Trials, Dept of Clinical Epidemiology, The London Hospital Medical College, UK) randomisation programme.

3.3.2 Recruitment and screening process

The LIPGENE screening process to recruit subjects was conducted in several stages, as illustrated in *Figure 3.1*. More than 15,500 subjects were screened to recruit 486

free-living subjects with the MetS. Each conformed to the LIPGENE inclusion and exclusion criteria (*Table 3.2*). Initial contact was attained by e-mail, posters in Universities and local hospitals, advertisements in local newspapers, GP clinics and dietetic out-patient clinics. Initially a subject suitability questionnaire was completed by telephone to determine if interested participants conformed with the LIPGENE criteria. Then an anthropometric and biochemical screening visit was scheduled and the nature of the LIPGENE study was explained. Written informed consent was provided by each potential volunteer before assessment of anthropometric indices, blood pressure and collection of a fasting blood sample. Weight, height, waist and hip circumference were measured at screening by the same trained investigator at each centre. Body weight was measured in duplicate on a centre specific, calibrated scale, to the nearest 0.1kg. Subjects were weighed in light clothing, without shoes and after voiding. Height was measured in duplicate to the nearest 0.1cm, in the upright position using a stadiometer. Waist circumference was measured directly on the skin, at the mid-point between the supra iliac crest and lower ribs margin at the end of a normal expiration, in duplicate to the nearest 0.1cm using a non-stretch tape measure. Hip circumference was measured in duplicate to the nearest 0.1cm, using a non-restrictive tape measure, at the level of the greater trochanter without compressing the skin. Blood pressure measurements were recorded in duplicate in the seated position after a 5 minute rest, using an appropriately sized cuff before blood samples were taken.

Blood samples were collected from subjects in the morning after a 12 hour fast and having avoided excessive alcohol and exercise during the previous 24 hours. Fasting plasma glucose, triacylglycerol, total cholesterol, high density lipoprotein, and haemoglobin levels were measured. Liver and kidney function tests were also measured. Biochemical analysis was completed at each centre, according to established and standardised in-house methods. A urine sample was taken in all female subjects to outrule pregnancy. A health and lifestyle questionnaire also determined each subject's level of physical activity, smoking habits, alcohol intake, menopausal state in females and demographics. Volunteers who attained 3 of the 5 MetS criteria listed above were offered participation in the LIPGENE dietary intervention study.

Figure 3.1: LIPGENE Dietary Intervention Study: Screening, Recruitment and Randomisation of subjects with the metabolic syndrome

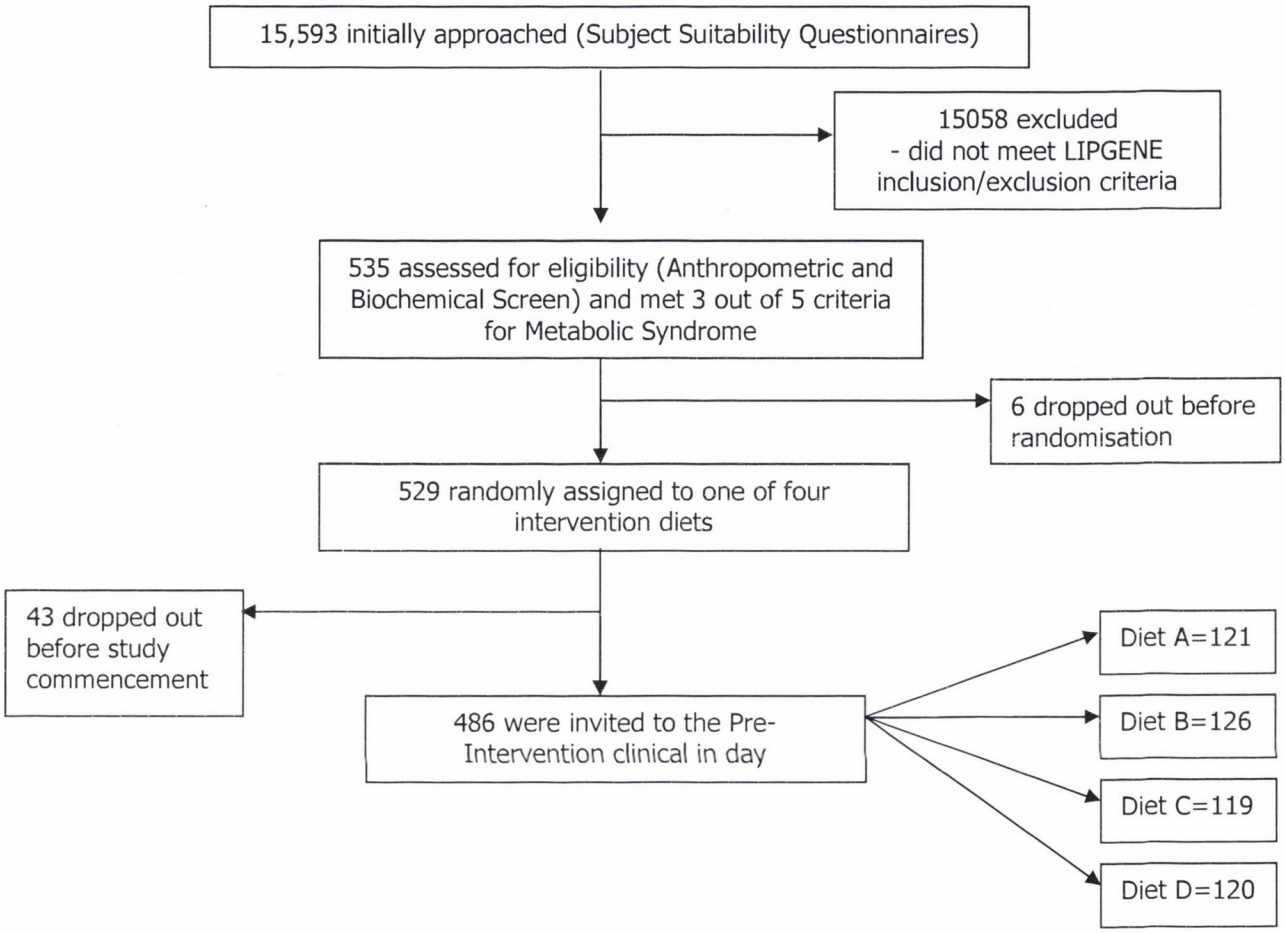


Table 3.2: Inclusion and Exclusion Criteria of the LIPGENE Dietary Intervention Study

Inclusion Criteria
• Age: 35-70years • Gender: males and females (not pregnant or lactating) • Body Mass Index (BMI) 20-40 kg/m^2 • Total cholesterol concentration equal to or < 8.0 mmol/L • Medications / nutritional supplements allowed, on condition that the subjects adhere to the same regimen during the intervention: anti-hypertensive medication (including beta-blockers), oral contraceptives, hormone replacement therapy, multi-vitamin supplements, other non-fatty acid based nutritional supplements (e.g. garlic, anti-oxidants, etc) • Smokers and non-smokers • Persons who do not consume alcohol or regular consumption of alcohol, which is not excessive as defined by elevated liver enzymes (AST <41IU/L and ALT 7-56IU/L) • Ethnicity: Intention to include white Europeans
Exclusion Criteria
• Age: < 35 or >70years • Diabetes or other endocrine disorders • Chronic inflammatory conditions • Kidney or liver dysfunction • Iron deficiency anaemia (haemoglobin < 12g/dl men, < 11g/dl women) • Prescribed hypolipidaemic medication • Prescribed anti-inflammatory medication • Fatty acid supplements including fish oils, evening primrose oil, etc. • Consumers of high doses of antioxidant vitamins (A, C, E, β-carotene) • Red rice yeast (Monascus purpureus) supplement usage • High consumers of oily fish (> 2 serving of oily fish per week of herring, mackerel, kippers, pilchards, sardines, salmon, trout, tuna (fresh), crabmeat or marlin). One portion is defined as a small herring or mackerel, one can of salmon or sardines or one salmon or tuna steak. Tinned tuna is permitted as it contains only minor amounts of long chain n-3 PUFAs • Highly trained or endurance athletes or those who participate in more than 3 periods of intense exercise per week • Volunteers planning to start a special diet or lose weight (e.g. the Slimfast Plan, Atkins Diet etc). • Weight change equal or >3kg within the last 3 months • Alcohol or drug abuse (based on clinical judgement) • Pregnant / lactating females / women planning a pregnancy in the next 12 months. Women who became pregnant during the dietary intervention period should be removed from the study

3.3.3 Clinical Investigations & Dietary Assessment Pre- and Post-intervention

A common Training Protocol and Standard Operating Procedures were developed and adhered to in each centre to standardise collection of biological samples and dietary data. The schedule of the clinical investigations and dietary assessment during the study are summarised in *Figure 3.2*. Pre- and post-intervention, subjects reported to one of the eight intervention centres following a 12 hour overnight fast. At each clinical investigation day subjects brought their fasting urine sample (first void), completed anthropometric measurements, donated a fasting blood sample and completed an insulin modified intravenous glucose tolerance test (IVGTT) as per

published protocols (Finegood *et al.*, 1990). Each subject had a cannula inserted into both forearms. Fasting samples were taken prior to the IVGTT. At 0 minutes a bolus of 50% glucose solution (0.3g/kg body weight) was infused within a 1 minute period followed by 20ml of saline. At 20 minutes an insulin dose (0.03U/kg body weight) was infused into the same cannula. Meanwhile, blood samples were drawn from the other cannula in the opposite arm at times -5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 minutes after the start of the glucose infusion. At each time point each subject's blood glucose was monitored using a gluco-trend machine to ensure the level did not drop below 2.0mmol/L. A select number of volunteer subjects also donated adipose tissue and skeletal muscle biopsies in the following days after the IVGTT assessment.

An intensive dietary assessment schedule was implemented. Volunteers kept a 3-day weighed food dietary record (two weekdays and one weekend day) to assess pre-intervention dietary habitual intake. This assessment formed the basis for dietary fat modification in order to achieve the dietary intervention targets using the intervention foods, without a significant (<2% body weight change) alteration in body weight. Dietary advice was given to each volunteer using the dietary intake data gained at the pre-intervention dietary assessment and modified in order to achieve their target intervention diet. Two further weighed 3-day food dietary records were completed at week 6 and 12 to assess dietary intake mid- and post-intervention. Compliance was promoted and assessed by regular contact with each volunteer by 24-hour recalls and food use questionnaires, every two weeks of the trial. Body weight was measured pre-, mid- and post-intervention at week 0, 6 and 12 to ensure isocaloric dietary compliance. Study foods were delivered by the investigators or subjects collected them from the study centre throughout the 12 week study period. Capsules were given to each participant on the pre-intervention clinical investigation day and a capsule count assessed. The level of physical activity, alcohol consumption and smoking habits did not deviate throughout the intervention period.

Figure 3.2: LIPGENE Dietary Intervention Study - Schedule of Dietary Assessments & Clinical Investigations

	Pre-Intervention	Intervention Period (weeks)										Post - Intervention
	0	1	2	3	4	5	6	7	8	9	10	11	12
Dietary Assessment													
3 day weighed food diary	✓						✓						✓
Food Frequency Questionnaire (FFQ)	✓												
Dietary Modification	✓												
Dietary Advice	✓						✓			✓			
24-recall compliance check		✓								✓			✓
Food Use Questionnaire		✓								✓			
Clinical Investigation Day	✓												✓
Urine Sample	✓												✓
Weight	✓						✓						✓
Height	✓												✓
Waist & Hip Circumference	✓												✓
Blood Pressure	✓												✓
Bioelectrical Impedance	✓												✓
Fasting blood sample	✓												✓
Intravenous glucose tolerance test (IVGTT)	✓												✓
Fat biopsy (optional)	✓												✓
Muscle biopsy (optional)	✓												✓

A list of the metabolic risk factors associated with the MetS measured in LIPGENE is presented in *Table 3.3*. Insulin and glucose concentrations were determined at each IVGTT time point and measures of insulin sensitivity, glucose effectiveness and the acute insulin response to glucose were determined with the MINMOD Millennium computer program (version 6.02, Richard N. Bergman). All blood samples were processed immediately after collection according to standard protocols.

Table 3.3: LIPGENE Metabolic Risk Markers of the Metabolic Syndrome

Insulin sensitivity	IVGTT, glucose & insulin C-peptide
Lipoprotein metabolism	Total plasma triacylglycerol (TAG), cholesterol & non-esterified fatty acids (NEFA). Apolipoprotein A1, B, CII, CIII, E & B48 TAG Rich Lipoprotein (TRL) TAG, cholesterol & apo B. Total High Density Lipoprotein (HDL), HDL3, HDL 2 and low density lipoprotein (LDL) cholesterol.
Oxidative injury & inflammation	Urinary 15-keto-dihydro-prostaglandin F_2 (15-keto-dihydro-PGF_2),8-iso-prostaglandin F_2 (8-iso-PGF_2) Tumour necrosis factor (TNF)-α, Interleukin (IL) 6, C-reactive protein (CRP), Leptin, resistin, adiponectin sICAM, sVCAM P-Selectin
Coagulation & fibrinolysis	Fibrinogen, plasminogen activator inhibitor (PAI-1), tissue plasminogen activator (tPA)
Biomarker of habitual dietary fat composition	Plasma fatty acid composition

3.3.4 Candidate gene identification, SNP Selection and Genotyping

A dual approach was used for candidate gene and SNP selection. SNPs identified in association, epidemiological and physiological studies as having an association with features of and/or risk of the MetS were included. Suitability of SNPs was determined in each candidate gene according to their allelic frequency and their putative involvement on function or expression of the gene product. The second SNP selection approach involved haplotype tagging. A number of recent studies have shown that this approach may be more advantageous than single SNP analysis. It is proposed that haplotype tagging captures the majority of diversity within a region by typing those SNPs which cover the most diversity and uses the most informative SNPs to explain genetic variation (Meirhaeghe *et al.*, 2005; Elosua *et al.*, 2006).

Online databases such as dbSNP http://www.ncbi.nlm.nih.gov/, HapMap http://hapmap.org and Perlegen http://perlegen.com were used to source genotype data for each candidate gene. This information was uploaded into Hitagene https://web1.hitagene.com/lipgene/frame.php which enabled haplotype frequency estimation to be determined by implementation of the expectation maximisation (EM) algorithm. Using a 5% cut-off for individual haplotype frequency and >70% for the sum of all haplotype frequencies, genotype data were then transferred from Hitagene to SNP Tagger http://www.broad.mit.edu/mpg/tagger/server.html an online tool which identifies haplotype tagged SNPs. When a choice of haplotype tagged (Ht) SNPs was available, HtSNPs were chosen based on their allelic frequency and type of variant, with priority given to SNPs in the following order; (i) non-synonymous SNPs (ii) synonymous SNPs, (iii) promoter/regulatory region, 5' UTR and 3'UTR SNPs, (iv) intronic SNPs.

DNA sample extraction (from stored buffy coat cell samples) was performed using the Puregene protocol for DNA extraction from buffy coats and samples were processed using the AutoPure LS automated system (Gentra Systems Inc., Minneapolis, MN, USA). Low yielding samples were subsequently subjected to whole genome amplification using the REPLI-g kit (Qiagen Ltd. West Sussex, UK). High-throughput genotyping using the GoldenGate assay was conducted by Illumina Inc. (San Diego, CA, USA). Additional genotyping was performed by KBiosciences (Herts, U.K.) using a competitive allele specific PCR system (KASPar) (available at http://www.kbioscience.co.uk).

3.3.5 Statistical power of study design

The statistical power of the LIPGENE dietary intervention study was based on insulin sensitivity as the primary biomarker of the MetS. Insulin sensitivity was assessed by an IVGTT wherein insulin sensitivity (S_I) was measured by the MINMOD minimal modeling programme. The power of the study was calculated with the ability to detect at least 1 unit difference at a standard deviation (SD) of 2, between diets. The power calculation was based on the assumption that 15% difference in insulin sensitivity was physiologically important and that this difference can be detected at a significance level of 0.05 and a power of 0.8. Thus, a minimum of 98 subjects per dietary group would be required to detect significance differences. The group size was increased to 120 per group, to account for an anticipated 20% drop-out rate.

Statistical Analysis was performed using SPSS® for Windows™ 14.0 software (SPSS Inc, Chicago, USA). All continuous variables were normalised when necessary. Results are presented as group means ± SEM. Differences in mean values between males and females were assessed using independent-samples t-test and Mann-Whitney Test (for normally distributed and skewed variables, respectively). One way ANOVA was used to calculate the statistical differences between the means of the continuous and normal variables across the diets. Non-parametric tests (Krukal-Wallis) were used for cases of non-normality. Significance was defined as $P<0.05$.

3.4 RESULTS

The results presented were obtained from the screening stage of the study before entry to the intervention. A total of 15,593 people completed the subject suitability questionnaire in all centres. Because of prescribed medications (mainly hypolipidaemic and anti inflammatory) nutritional supplements, medical conditions or other exclusion criteria, 15,058 people were eliminated at this stage. It was not recorded by each centre the exact reaon for exclusion or numbers given for each exclusion factor. Of the 535 volunteers invited to attend the relevant centre's clinical unit for a screening visit and were deemed suitable according to the LIPGENE criteria, 486 volunteers (91%; 220 males and 266 females), aged between 35-70 years went forward to take part in the study. *Table 3.4* presents the clinical characteristics indicative of the MetS and other demographics for the volunteers recruited to participate in the LIPGENE Dietary Intervention Study at screening of which 45% (n=220) were men and 55% (n=266) were women. Fasting plasma glucose, blood pressure, waist circumference, height and weight were significantly greater in males than females ($p<0.05$). In women, mean HDL cholesterol concentrations, age, total cholesterol, BMI and hip circumference were significantly greater than in males ($p<0.05$). Subjects were randomly assigned to one of four diets. *Table 3.5* illustrates there were no significant differences in the important clinical characteristics indicative of the MetS between the four dietary groups at screening. This demonstrates that the randomisation was successful as all groups began the intervention on a similar metabolic background. *Table 3.6* demonstrates the screening characteristics across the eight participating centres. Fasting glucose concentrations were highest in the UM cohort at screening and significantly higher compared to TCD, UoR, INSERM and UU. The highest TAG concentrations occurred in the UoR centre; however there were no statistical differences in total cholesterol concentrations across the eight centres. HDL-cholesterol concentrations were lowest in the UM cohort, compared to 5 other centres. Waist and Hip circumference were highest in the UM and JUMC cohorts, respectively. BMI was greatest in the HURS-UCO cohort which also saw the highest systolic blood pressure readings. A total of 486 subjects went forward to the pre-intervention clinical investigation day and received dietary intervention counselling for the study. 57%, 36% and 7% met 3, 4 and 5 criteria according to the LIPGENE criteria at screening.

Table 3.4: Screening characteristics of subjects who met the MetS according to the LIPGENE criteria and went forward to the intervention study n=486

	All n=486		Male n=220		Female n=266			
	Mean	SEM	Mean	SEM	Mean	SEM	*p value*	
Glucose (mmol/l)	5.54	0.03	5.62	0.05	5.46	0.05	*p=0.022*	§
TAG (mmol/L)	1.81	0.04	1.86	0.06	1.78	0.05	*ns*	§
HDL (mmol/L)	1.30	0.02	1.18	0.02	1.41	0.02	*p=0.0005*	*
Blood Pressure Systolic (mmHg)	142.80	0.73	144.79	1.01	141.14	1.02	*p=0.017*	§
Blood Pressure Diastolic (mmHg)	89.25	0.48	91.27	0.72	87.56	0.62	*p=0.0005*	§
Waist Circumference (cm)	106.42	0.50	110.60	0.68	102.97	0.65	*p=0.0005*	*
Age (y)	54.52	0.41	53.35	0.67	55.48	0.49	*p=0.039*	§
Total Cholesterol (mmol/L)	5.63	0.04	5.44	0.07	5.78	0.06	*p=0.0005*	*
Hip Circumference (cm)	114.27	0.55	110.28	0.73	117.90	0.72	*p=0.0005*	*
Weight (kg)	91.49	0.63	98.92	0.89	85.37	0.70	*p=0.0005*	*
Height (m)	1.68	0.00	1.76	0.00	1.62	0.00	*p=0.0005*	*
BMI (kg/m^2)	32.27	0.19	31.85	0.26	32.62	0.27	*p=0.036*	*

* *independent samples t-test significance p<0.05*

§ Mann-Whitney U Test p<0.05

Table 3.5: Screening characteristics of subjects that met the MetS according to the LIPGENE criteria and were assigned to one of four intervention diets and went forward to intervention study n=486

	High Fat SFA-rich n=121		High Fat MUFA-rich n=126		Low Fat High CHO n=119		Low Fat n-3 PUFA n=120	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mmol/L)	5.60	0.07	5.55	0.07	5.48	0.07	5.51	0.07
TAG (mmol/L)	1.94	0.08	1.70	0.06	1.84	0.08	1.77	0.06
HDL (mmol/L)	1.29	0.03	1.30	0.03	1.32	0.03	1.30	0.03
Blood Pressure Systolic (mmHg)	143.51	1.20	142.36	1.51	141.55	1.51	143.81	1.58
Blood Pressure Diastolic (mmHg)	89.16	0.84	88.67	0.97	89.21	0.91	89.99	1.11
Waist Circumference (cm)	104.89	0.91	106.16	1.01	107.68	1.10	106.97	0.98
Age (y)	54.45	0.78	54.88	0.76	54.07	0.85	54.65	0.88
Total Cholesterol (mmol/L)	5.69	0.08	5.58	0.09	5.58	0.10	5.66	0.09
Hip Circumference (cm)	114.08	1.07	113.77	1.23	115.50	1.01	113.74	1.07
Weight (kg)	90.16	1.17	91.47	1.26	92.61	1.37	91.75	1.28
Height (m)	1.69	0.01	1.68	0.01	1.68	0.01	1.68	0.01
BMI (kg/m^2)	31.79	0.39	32.38	0.37	32.59	0.37	32.32	0.39

no significance noted as assessed by one way anova and kruskal wallis tests between the diets

Table 3.6: Screening characteristics of subjects that met the LIPGENE MeS criteria across the 8 participating centres who went forward to intervention study n=486

	TCD, n=59			UoR, n=62			UO, n=64			INSERM, n=41			UM, n=59			HURS-UCO, n=77			JUMC, n=73			UU, n=51			
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	
Glucose (mmol/L)	5.64	ad	0.12	5.11	b	0.07	5.61	acd	0.09	5.16	ab	0.11	5.95	c	0.08	5.57	ac	0.09	5.60	cd	0.06	5.52	ad	0.11	¥
TAG (mmol/L)	1.60	ac	0.09	2.21	b	0.12	1.77	abc	0.10	2.00	ab	0.12	1.54	c	0.08	1.92	abc	0.11	1.79	abc	0.07	1.68	ac	0.11	¥
HDL (mmol/L)	1.23	acde	0.07	1.29	ade	0.04	1.37	ae	0.04	1.22	acd	0.05	1.07	c	0.03	1.42	ef	0.03	1.54	bf	0.03	1.13	cd	0.04	¶
Blood Pressure Systolic (mmHg)	143.44	ac	2.33	140.42	ac	1.68	142.97	acd	1.76	130.66	b	1.97	146.66	ac	2.10	149.01	c	1.95	144.46	a	1.85	138.37	abd	1.98	¥
Blood Pressure Diastolic (mmHg)	92.14	ac	1.55	87.06	ab	0.98	95.02	c	1.33	81.39	b	1.08	90.51	ac	1.25	90.49	a	1.07	89.99	ad	1.50	83.43	bd	0.91	¥
Waist Girth (cm)	108.71	a	2.07	105.28	ab	1.36	105.77	a	1.14	104.11	ab	1.70	109.37	a	1.14	108.74	a	0.95	107.54	a	1.32	99.29	b	1.37	¶
Age (y)	49.58	a	1.27	55.13	abd	1.20	53.80	ab	1.17	52.59	ab	1.43	57.37	bc	1.13	56.35	bc	0.88	51.64	a	0.77	59.96	c	1.06	¥
Total Chol (mmol/L)	5.36		0.13	5.75		0.13	5.96		0.13	5.89		0.15	5.47		0.11	5.52		0.11	5.56		0.10	5.60		0.11	¶
Hip (cm)	113.70	ad	1.74	114.15	ad	0.90	*			107.06	b	1.51	108.64	ab	1.35	117.67	de	0.93	119.92	ce	1.06	*			¶
Weight (kg)	92.80	a	1.97	91.60	a	1.63	93.18	a	1.71	87.00	ab	2.52	94.73	a	1.76	91.00	a	1.34	94.79	a	1.67	83.62	b	1.78	¶
Height (m)	1.67	ad	0.01	1.71	abd	0.01	1.74	b	0.01	1.68	abd	0.02	1.73	ab	0.01	1.61	c	0.01	1.67	d	0.01	1.68	abd	0.01	¶
BMI (kg/m^2)	33.03	acd	0.58	31.41	ab	0.47	30.66	b	0.39	30.82	ab	0.74	31.69	abd	0.60	34.99	cd	0.38	34.02	d	0.38	29.71	b	0.48	¶

*Hip circumference not measured at screening

abc... denotes significance across centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories)

¥ non parametric Kruskal Wallis Test p<0.0018

¶ One way anova p<0.05

3.5 DISCUSSION

The LIPGENE Dietary Intervention Study represents the largest European intervention study designed to determine the effect of dietary fat modification on insulin sensitivity and other metabolic markers associated with the MetS, including lipid metabolism, inflammation and coagulation. To date no study has addressed the relative efficacy of the removal of dietary SFA either by replacement with MUFA or reduction with a low-fat, high-complex carbohydrate diet whilst maintaining isoenergetic conditions or without a weight loss factor in a group of subjects with the MetS. The beneficial effects of MUFA and low-fat dietary interventions are highly controversial. Furthermore, the role of LC n-3 PUFA in terms of negating the hypertriacylglycerolaemic and HDL lowering effect of low-fat, high carbohydrate diets can be addressed in this very large cohort. The study involved individuals from 8 European countries, and will provide a wealth of data in terms of the effect of the diversity of habitual dietary fatty acid intake on insulin sensitivity. Also within LIPGENE, health and other lifestyle factors were collected which will give a broader view to cause and effect of the MetS in addition to diet.

It will be possible to ascertain the differences between the four intervention diets as there were no statistical differences at screening between the four arms. There were differences in the criteria between the centres which more than likely reflects the recruitment strategies employed in each centre. For example, based on their high glucose values at screening, perhaps UM recruited from impaired fasting glucose clinics, lipid clinics in UoR and perhaps weight loss or blood pressure clinics in HURS-UCO. These differences were taken into account when comparing effects of the diets in the follow-up analysis as was gender and age. An important justification for the investigation of the MetS is its ability to predict T2DM, therefore a study with adequate size and duration, comprehensive analysis and with an IVGTT to determine insulin sensitivity is of considerable importance. The modified version of the NCEP definition used detected more subjects at future risk for diabetes, supporting the notion of early detection for T2DM with prevalence rates rising to epidemic proportions. Impaired fasting glucose (IFG) is a strong predictor of T2DM. The NCEP definition has a clinical strategy with the objective of reducing the population burden of atherosclerosis (NCEP, 2002). In the San Antonio Heart Study, the prediction of MetS induced diabetes improved when using the fasting plasma glucose cutoff of ≥5.4mmol/L (Lorenzo *et al.*, 2003) and performed well as a predictor of diabetes. The LIPGENE study may also go towards determining the optimal cut-off point for fasting plasma glucose in further predictive models.

Furthermore, LIPGENE will address the hypothesis as to whether genetic markers associated with the MetS affect an individual's responsiveness to dietary alteration. A very comprehensive range of gene targets affecting the different metabolic components of the MetS have been determined including insulin signalling, glucose homeostasis, lipid metabolism, adipogenesis, inflammation, vascular function and coagulation (Phillips *et al.*, 2006). This will provide insight as to whether a personalised nutrition approach is appropriate for the treatment of the MetS.

Chapter Four

LIPGENE food exchange model for alteration of dietary fat quantity and quality, in free-living participants with the metabolic syndrome from eight European countries

---

4.1 ABSTRACT

Background: Human prospective studies support the hypothesis that dietary fat quality may influence insulin action, a central component of the metabolic syndrome. However, controlled human intervention trials are required to confirm this hypothesis and causality.

Objective: The aim was to develop a food-exchange model to investigate the effects of four experimental diets, distinct in fat quantity and quality, suitable for use in free-living volunteers, with the metabolic syndrome, participating in a human intervention study at eight centres across Europe.

Design: A theoretical food exchange model was successfully developed. The quantity of exchangeable fat in the average UK and Irish diet was calculated as the sum of fat provided by added fats (spreads and oils), milk, cheese, biscuits, cakes, buns and pastries as described in the National Diet and Nutrition Survey of UK adults and the North/South Ireland Food Consumption Survey of Irish adults. This exchangeable fat was replaced by specifically designed study foods including spreads, cooking oils, mayonnaises, baking fats and biscuits. Also critical to the food-exchange model were the use of carbohydrate exchanges to ensure the diets were isoenergetic. Volunteers completed 3-day weighed food diaries and a Food Frequency Questionnaire to assess habitual dietary intake. Based on these, nutritionists advised volunteers individually on food choices for their allocated diet. Fortnightly 24 h recalls were taken and the frequency of study food consumption monitored.

Results: Results indicated that overall compositional targets were largely achieved with significant differences in fat quantity and quality between the four study diets.

Conclusions: A robust, flexible food exchange model was developed and implemented successfully in the LIPGENE dietary intervention trial.

4.2 INTRODUCTION

The metabolic syndrome (MetS) was first proposed by Reaven (1988b) to describe a cluster of metabolic disorders linked with hyperinsulineamia. Although there is not yet a universally agreed definition, those proposed generally agree the five key features include insulin resistance, hyperglycaemia, dyslipidaemia, central obesity and hypertension, with attainment of three or more features indicative of the syndrome's presence (WHO., 1999; Expert Panel on detection evaluation and treatment of high blood cholesterol in adults, 2001; European Group for the study of Insulin Resistance (EGIR), 2002; Alberti *et al.*, 2006). With clear evidence to show the MetS is an important predictor of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD), it is a critical target for intervention (Shaw *et al.*, 2005).

Epidemiological evidence indicates that the incidence of the MetS increases with extent of insulin resistance (Bonora *et al.*, 2003; Resnick *et al.*, 2003; Weiss *et al.*, 2004) and central obesity (Ascaso *et al.*, 2003; Carr *et al.*, 2004; Palaniappan *et al.*, 2004). The incidence of obesity has risen exponentially over the past five decades and it is unlikely that this trend can be reversed in the near future (Parikh *et al.*, 2007). Moreover the full implications of the somewhat newer phenomenon of childhood obesity and young adult obesity, tracked from childhood, are not yet known. It therefore seems unlikely (even with extreme public health interventions) that the obesity epidemic can be reversed in the short term. Additional strategies must be considered to reduce the impact of the MetS in the short term, targeting insulin resistance represents a rational approach.

Mechanistic studies have shown that fatty acids may influence insulin action via effects on the insulin receptor, the insulin signalling cascade and insulin secretion (Boden, 1997; Boden & Shulman, 2002; Gravena *et al.*, 2002). Human prospective studies support the hypothesis that dietary fat quality may influence insulin action. For example, the Nurses' Health Study showed intakes of polyunsaturated fatty acids (PUFA) were negatively, and *trans* fatty acids were positively, associated with risk of T2DM, with monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) reported to have neutral effects (Salmeron *et al.*, 2001). However, controlled human intervention trials are required to confirm these suggested associations and to prove causality. To date, a very limited number have been carried out, mainly among those with diabetes or obesity and most have suffered from poor study design, often with low numbers of participants, short study duration and limited fatty acid subclass

comparison. The LIPGENE project, a large European, multi-centre human intervention trial (n=486 volunteers at baseline) has been conducted to compare the impact of substituting SFA for MUFA and the effects of low fat diets with and without additional long chain n-3 PUFA, on insulin sensitivity.

Although the achievement of target dietary intakes is essential to the successful outcome of any controlled dietary intervention study, detailed information on the strategies used to achieve target intakes is rarely provided (Knapper *et al.*, 1996; Nydahl *et al.*, 2003). In the present chapter, details of the approaches used to set up and complete the LIPGENE dietary intervention study are described. The aim was to create four isoenergetic diets which differed significantly in dietary fat composition. The approach was designed to cause minimal disruption to the normal dietary habits of the participants and should be appropriate for use in eight European countries with widely different habitual diets. The food exchange model used is described in detail, the achievement of dietary targets evaluated and the problems encountered discussed.

4.3 METHODS AND VOLUNTEERS

4.3.1 Study design

The dietary intervention study was a randomised, controlled study carried out at eight European centres (Trinity College Dublin, Ireland (TCD), University of Reading, URREADNSI, UK (UoR), University of Oslo, Norway (UO), INSERM U476 Marseille, France (INSERM), Maastricht University, The Netherlands (UM), Hospital Universitario Reina Sofía-University of Córdoba, Spain (HURS-UCO), University of Krakow, Poland (JUMC) and Uppsala University, Sweden (UU).

Volunteers with the MetS as defined by the presence of three of the following components; fasting glucose > 5.5 mmol/L, triacylglycerol ≥ 1.5 mmol/L, high density lipoprotein cholesterol (HDL-C) concentration < 1.0 mmol/L (men), < 1.3 mmol/L (women), blood pressure (BP) systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, or on prescribed BP medication, waist circumference > 102 cm (men), > 88 cm (women), were recruited for the study. The criteria were based on the National Cholesterol Education Programme (NCEP) definition (Expert Panel on detection evaluation and treatment of high blood cholesterol in adults, 2001) with minor refinements to aid recruitment (Tierney *et al.*, 2007). In total, 486 volunteers (220 male, 266 female) with an average age of 54.58y, started the study across the eight centres. Considered in the present analysis were volunteers who completed the 12-week intervention trial and provided satisfactorily completed food records (n=414).

Details of the recruitment strategies and volunteer details are presented in the previous chapter (Chapter 3). In brief, volunteers were recruited using various methods including use of general practitioner databases, poster and newspaper advertisements. Initially volunteers were screened over the telephone using a volunteer suitability questionnaire which assessed dairy food consumption, fish consumption and willingness to consume sandwiches during the study, to ensure potential alteration of diets. This was followed up in those meeting the inclusion criteria by completion of a health and lifestyle questionnaire, anthropometric and biochemical tests.

A central minimisation procedure was used to randomise volunteers to one of four study diets. The four diets differed in dietary fat quantity and quality whilst remaining isoenergetic. Two diets were designed to provide 38% energy intake from fat (%E from fat); diet HFSFA containing high saturated fatty acid levels should

provide ~16%E as SFA, diet HFMUFA containing high monounsaturated fatty acid levels was designed to provide ~20%E from MUFA. The other two diets (LF and LFn-3PUFA) were low fat and were designed to provide 28%E from fat with diet LFn-3PUFA including a 1.24g/day supplement of eicosapentaenoic and docosahexaenoic acids (EPA and DHA, ratio 1.4:1, 4 x 1g capsule/day) and diet LF including a comparator high-oleic acid sunflower seed oil capsule 4 x 1g capsule/day). The target fatty acid compositions of the four diets are included in *Table 4.1*. Diets were designed to differ only in SFA and MUFA, PUFA was to be maintained at the same level across all four diets.

4.3.2 Food Exchange Model

A food exchange model was developed based on the National Diet and Nutrition Survey (NDNS) of UK adults aged 19-64 yrs and the National Food Survey and the North South Ireland Food Consumption Survey (DEFRA. National Food Survey, 2000; Harrington et al., 2001; Henderson et al., 2003).

In the LIPGENE study, the food exchange model was designed to ensure the target dietary fatty acid compositions of the four diets were achievable in a free-living population, across Europe. In the present strategy, mean population intake data for total energy, total fat, SFA, MUFA and PUFA were tabulated separately. This included a breakdown of the major sources of exchangeable dietary fat (Table 4.2). Exchangeable fat was considered to be that which was not intrinsic within a food product; it could be removed from the diet and replaced with study foods. The amount of exchangeable fat in the average UK was calculated as the sum of fat provided by added fats (spreads and oils), milk, cheese, biscuits, cakes, buns and pastries. The total exchangeable fat was subtracted from the NDNS total fat intake to determine the non-modifiable fat intake, i.e. that which could not easily be altered without severely affecting common dietary habits of the volunteers. This formed the backbone of the food exchange model onto which specifically designed sources of exchangeable fat could be added to create diets of differing fat composition.

The re-introduction of fat involved the use of specifically designed study foods manufactured by Unilever R&D, Vlaardingen, The Netherlands. The compositions of the various study foods were designed alongside the food exchange model. Drawing from previous research (Knapper *et al.*, 1996), ideal profiles were established that allowed the theoretical alteration of dietary composition, through the use of food

products that were also aesthetically pleasing and without technological limitation. The study foods provided included spreads, cooking oils, baking fats and mayonnaises. Three types of spreads were available and formulated to be either high in SFA, high in MUFA or low in fat. Each type of spread had two varieties which differed only in salt content to account for the differences in acceptable sensorial properties by volunteers across Europe. Commercial fat products of differing compositions were also provided and used in the manufacture of biscuit products (Fusco Foods, Ltd., Dublin, Ireland). The average nutritional composition of the study foods is shown in *Table 4.3.* Looders-Croklaan, The Netherlands, supplied the study capsules used in diets LF and LFn-3PUFA.

Inclusion of these compositionally modified study foods in specified amounts allowed modification of overall dietary fat composition (*Table 4.4*). Also critical to the food exchange model was the use of carbohydrate (CHO) exchange to ensure all four diets were isoenergetic. One CHO exchange was considered as 35g complex carbohydrate, e.g. slice of brown bread, handful of rice, one potato. In diets HFSFA & HFMUFA, to ensure a higher %E from fat (38%) than the NDNS average, but without increased energy intake, one CHO exchange was removed. In diets LF and LF n-3 PUFA, fat intake was lower than the average NDNS level and therefore two CHO exchanges were added in the exchange model to ensure appropriate energy intake. *Table 4.4* illustrates that the food exchange model would enable the establishment of four diets, with distinct dietary fat compositions.

The LIPGENE food exchange model was verified using Irish food consumption survey data (Harrington *et al.*, 2001) and the North South Ireland Food Consumption databases (www.iuna.net). The food and nutrient intake database from this survey is stored in a disaggregated format with full compositional analyses for each food at every mealtime for 7 days presented in a relational database. Volunteers with a BMI of $\geq$ 25 kg/m^2 were randomly selected from the database. The modifiable foods they consumed were identified and removed from the diets for each volunteer. The removed foods were replaced with the proposed modified foods and nutrient composition was reanalysed for the modified diet. This procedure was repeated for each of the four diets. Reanalyses of the nutrient intakes for energy, total fat, SFA, MUFA and PUFA showed that the target diets were achievable in a random selection of Irish obese subjects without any net change in energy intake (data not shown).

4.3.3 Dietary advice

Prior to the start of the intervention period volunteers were asked to complete a 3-day weighed food diary and an extensive food frequency questionnaire (FFQ). The pre-intervention food diary was analysed using country-specific food analysis programmes to reveal the volunteer's current percentage of total fat, SFA, MUFA, PUFA, CHO and total energy intake. This gave an insight into the usual dietary habits and allowed identification of foods to be modified and appropriate snack substitution. At the start of the intervention period each volunteer was provided with a handbook specifically designed for the diet to which they had been randomised (available at http://www.ucd.ie/lipgene/internal/reports/wp_1_2_sop/intervention_download_documents/). Trained investigators read through the handbook with each volunteer individually explaining the food exchange model. Discussion was encouraged and considerable time was spent ensuring that the concept of food exchanges was understood. Key messages were highlighted in a summary sheet for the volunteers to refer to quickly and easily (available at http://www.ucd.ie/lipgene/internal/reports/wp_1_2_sop/intervention_download_documents/). This included a clear table stating foods to be removed and those that should be consumed instead. Amounts of food to be eaten were stated by weight and in general household measures such as teaspoons. Advice was also provided on foods to choose and those to avoid when eating outside the home. Volunteers were advised that other food components such as fruit and vegetables, meats, and drinks should be consumed as normal. The 3-day food diary completed at baseline was used to illustrate to the volunteer where their food exchanges should be made, taking into consideration the individual's habitual food patterns, meal timing and portion sizes. The investigator also consulted the detailed FFQ completed by each volunteer to assess in more detail the eating habits and areas that may not have been highlighted in the food diary that could be targeted as food exchanges during the intervention period. Included in the handbooks were sample recipes to illustrate how to incorporate the study foods within the habitual diet of the volunteers.

For all diets, volunteers were told to discontinue the use of any habitually used oils, fats or spreads and to use only those which had been provided by the investigators. Those volunteers randomised to diet HFSFA were asked to ensure all dairy foods consumed were of the full fat variety, e.g. whole milk, and to include cheese in their diets every day. They were asked to replace one snack product

normally consumed with their choice of either a muesli cookie or honey flapjack (diet HFSFA varieties) each day. These volunteers were also advised to eat less CHO (equivalent to one CHO exchange). It was recommended that they have two small handfuls less breakfast cereal or one less slice of bread, or one less potato than normal everyday (one CHO exchange equivalent to ~ 35 g portion of CHO).

Volunteers randomised to diets HFMUFA, LF & LFn-3PUFA were asked to only use low fat dairy products for the length of the study. They were encouraged to use only skimmed milk, and to choose low fat options of cheese and yogurts. Volunteers on diet HFMUFA were advised to consume the HFMUFA mayonnaise provided everyday. If they found this difficult, they were asked to ensure they consumed a handful of hazelnuts or cashew nuts (high in MUFA) when mayonnaise was not consumed, to ensure adequate intake of MUFA. Volunteers on this diet were also asked to replace one normally consumed snack product with their choice of biscuit provided.

In diets LF and LFn-3PUFA, in addition to the dairy recommendations described above, volunteers were asked to reduce their consumption of high fat snack options and encouraged only to have one study biscuit every other day. These volunteers were asked to ensure they consumed two extra portions of CHO daily (equivalent to ~ 70 g CHO) and to take supplied capsules daily.

Importantly, flexibility was possible with all diets; for example if spread could not be consumed at the required level every day then a greater consumption of oil was advised.

4.3.4 Food collection and compliance monitoring

At the beginning of the intervention period volunteers were provided with a supply of study foods to last for two weeks. Additional study foods were supplied if required, for example if they were used in meals given to other family members. Volunteers collected or were delivered additional study foods every fortnight or whenever needed. At these times a 24 h recall of the previous day's food intake and a short Food Use Questionnaire (FUQ) based on the study foods were completed. These were used to monitor and motivate volunteers to adhere to the dietary advice. A point system was used to assess the number of food exchanges achieved in the 24 h recall and additional advice was given if either the 24 h recall or FUQ showed inadequate intake of food exchange options. Those volunteers who met exchange requirements were

encouraged to continue in this manner. Body weight was measured at baseline, week 6 and 12, and additional weeks if required or requested for monitoring purposes. Further dietary advice was given if weight change from baseline had occurred. Usually this took the form of reducing or increasing CHO exchanges as appropriate.

4.3.5 Dietary assessment

Volunteers were asked to complete 3-day weighed food diaries at baseline, week 6 and week 12. Weighed food intake over two week days and one weekend day was recorded using scales provided by the investigators. Volunteers were asked to include the food packaging and home made recipes where possible. Various dietary analysis programs were used across the 8 European centres involved to ensure culturally specific foods were included (Dublin, Ireland: WISP version 3.0; Reading, UK: Food Base version 3.1; Oslo, Norway: Kostberegningssystem (nutrient calculation software developed at the Department of Nutrition, University of Oslo); INSERM, France: Nutrilog; Maastricht, The Netherlands: Komeet to enter the dietary data and Orion to analyse the data; JUMC, Poland: "Dietitian software"; Cordoba, Spain: Dietsource version 2.0; Uppsala, Sweden: MATs connected to the Swedish Board of Food Administration database for foods). Of note, analysis of individual fatty acid intakes was not available in all programs used, therefore the recorded intakes of EPA and DHA are based on intakes measured in a subgroup of volunteers.

4.3.6 Statistical Analysis

All data is presented as group means ± standard error of the mean unless stated otherwise. Data that was not normally distributed, as assessed by skewness and kurtosis, was normalised by log or square root transformation.

A one-way ANOVA was used to compare differences in dietary variables across the four diets at pre-, mid-, and post-intervention. A repeated measures ANOVA (RM ANOVA) was used to compare differences in outcome variables among the four diet groups between mid- and post-intervention and pre- and post-intervention. The analysis was adjusted for the between-subject factor diet. A significant time*diet group interaction effect, and a significant main effect of time were investigated using post-hoc analysis, paired t-tests and one-way ANOVA. A one-way ANOVA also determined statistical differences between the centres, pre-intervention and splitting the file by centre and running a one-way ANOVA

investigated if targets were met in each centre, post-intervention. Pearson chi-square determined statistical significance in the proportion of underreporters pre- and post-intervention. All statistical analysis was performed using SPSS® for Windows™ version 14.0 (SPSS Inc, Chicago, Ill). Statistical significance refers to a p value of at least <0.05.

4.4 RESULTS

4.4.1 Achievement of dietary targets

There were no significant differences in dietary composition at baseline between the four diet groups (*Table 4.1*). During the intervention period there were significant differences in various nutrient components between diet groups, with the exception of energy intake which did not differ significantly between diet groups. However, total energy intake at week 6 and 12 was significantly lower than baseline on diet LF, with similar differences on the LF n-3 PUFA diet which were of borderline significance.

The data presented in *Table 4.1* is from the week 0, week 6 and 12 dietary assessments. Over time there were no significant differences in any nutrient, in any of the diet groups, between the mid intervention (week six) and the end of the intervention period (week 12).

During the intervention period, %E from fat was significantly greater in diets HFSFA and HFMUFA compared to diets LF and LF n-3 PUFA. Conversely, %E from CHO was significantly greater in diets LF and LF n-3 PUFA compared to diets HFSFA and HFMUFA. %E from SFA was significantly greater in diet HFSFA than any of the other three diets. Diet HFMUFA also had significantly greater %E from SFA than diets LF and LF n-3 PUFA. Diet HFMUFA had significantly greater %E from MUFA than any of the other diets, whilst diet HFSFA had significantly greater %E from MUFA than diet LF *n*-3PUFA. Finally, %E from PUFA was significantly greater in diet HMUFA than in diet LF n-3 PUFA. Intake of EPA and DHA was significantly greater on LF n-3 PUFA diet compared to the other diets. Total energy intake did not differ significantly between the four study diets.

4.4.2 Achievement of dietary targets by each centre

Dietary intake at baseline differed significantly between centres (*Table 4.5*). Most notable were the significantly higher intakes of %E from fat in the HURS-UCO, INSERM and JUMC centres and with %E from MUFA also significantly higher in HURS-UCO and JUMC centres. In addition, significantly higher %E from CHO intake in the Uppsala centre was recorded.

Each centre successfully manipulated the habitual diets of their volunteers to achieve intervention diets that were distinct in fat composition. *Table 4.6* shows the composition of diets during the intervention period, as assessed at week 12, for each centre separately. In all centres %E from fat was greater in Diets HFSFA and

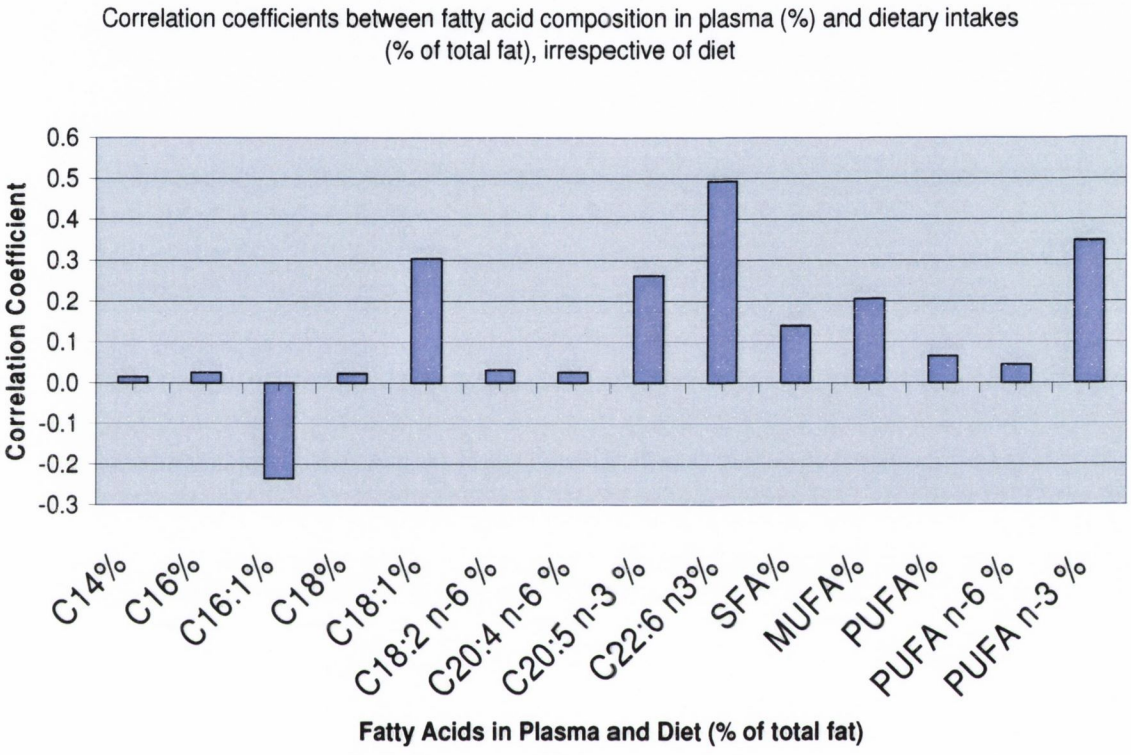
HFMUFA compared to Diets LF and LF n-3 PUFA, and this reached the level of statistical significance in five centres. In the other three centres although there was a distinct trend for reduced %E from fat levels in Diets LF and LF n-3 PUFA compared to Diets HFSFA and HFMUFA, one of the low fat diets was not distinct in %E from fat compared to one of the higher fat diets. In all centres %E from SFA was significantly greater in Diet HFSFA than the other 3 diets. In HURS-UCO centre, Diet HFMUFA was also significantly greater in %E from SFA than diets LF and LF n-3 PUFA. In all centres except JUMC, %E from MUFA was significantly greater in Diet HFMUFA compared to all other diets. In the JUMC centre, although %E from MUFA was greater in Diet HFMUFA than Diet HFSFA, this difference did not reach the level of statistical significance. In the TCD and HURS-UCO centres, %E from MUFA was significantly greater in Diet HFSFA compared to Diets LF and LF n-3 PUFA. %E from PUFA was similar across all four diets, with the exception of the UoR and UM centres, for which Diet HFMUFA had significantly greater %E from PUFA than Diet LF or LF n-3 PUFA, respectively. %E from CHO was consistently greater in Diets LF and LF n-3 PUFA compared to Diets HFSFA and HFMUFA and these differences were statistically significant in four out of the eight centres investigated. Finally, all centres achieved isoenergetic diets with the exception of the TCD centre, in which diets HFSFA and HFMUFA had significantly greater energy than diet LF n-3 PUFA.

4.4.3 Compliance and Under-reporting

All volunteers on Diets LF and LF n-3 PUFA reported ≥ 85% compliance to the capsules. *Figure 4.1* highlights the correlation coefficients between plasma and dietary fats, irrespective of diet, post-intervention. The relationship between these two datasets at post-intervention in the four diet groups are presented in *Table 4.7*. For plasma and dietary intakes, correlation coefficients for post-intervention palmitoleic acid (C16:1), saturated and monounsaturated fatty acids were weak (r<0.25). The only negative correlation was seen for palmitoleic acid (C16:1) where the results show that increasing levels of this fatty acid in the plasma at post-intervention is associated with decreasing concentrations in the diet. This was seen in Diets HFMUFA and LF only. The positive correlation observed with saturated fat was apparent in the HFMUFA diet. DHA (C22:6:3) had the strongest positive correlation (r=0.492) and was significantly correlated in all four diets. Oleic acid (C18:1) (Diets

HFMUFA, LF and LF n-3 PUFA), EPA (C20:5:3) (Diets HFSFA and HFMUFA) and n-3 PUFA (Diets HFMUFA and LF) were also significantly and positively correlated.

Figure 4.1 Correlation coefficients between fatty acid composition in plasma (w/w%) and dietary intakes (% of total fat), post-intervention, irrespective of diet.



Schofield equations were used to estimate BMR. The Goldberg cut off for Energy intake:BMR of 1.1 was used to identify under-reporters (Black, 2000). There were no significant differences in the level of under reporting between diet groups pre- and post-intervention. A one-way ANOVA assessed differences between mean EI:BMR between pre- and post-intervention and revealed significant differences for both low-fat diets with a reduction at post-intervention (LF diet: pre-Intervention 1.26, post-Intervention 1.58; LF n-3 PUFA: pre-intervention 1.26, post-intervention 1.17). RM-ANOVA demonstrated a significant within-subject time*diet group interaction (p=0.001) wherein body weight was significantly reduced post-intervention following both LF diets (p<0.0005).

Table 4.1:Composition of diets at baseline, mid and end of the intervention period, alongside dietary targets

Composition of diets pre-intervention over the four dietary groups	HFSFA, Diet A n=98		HFMUFA, Diet B n=110		LF Diet, Diet C n=106		LF n-3 PUFA, Diet D n=100		
Pre-Intervention	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Body weight (kg)	90.37	1.37	91.23	1.37	91.88	1.52	91.30	1.36	ns
Basal Metabolic Rate (MJ/day)	7.14	0.10	7.12	0.11	7.15	0.12	7.17	0.11	ns
Total Energy(MJ)	8.47	0.27	8.80	0.26	8.99	0.26	8.99	0.24	ns
Total fat (g)	82.82	3.17	83.70	2.74	87.57	3.50	87.25	3.00	ns
Total fat (%Energy)	36.46	0.75	35.60	0.61	35.86	0.75	36.23	0.81	ns
SFA (g/day)	28.91	1.28	29.16	1.19	29.30	1.34	30.60	1.10	ns
SFA (%Energy)	12.71	0.32	12.21	0.30	11.94	0.32	12.69	0.30	ns
MUFA (g/day)	29.92	1.39	31.53	1.22	32.64	1.46	32.60	1.54	ns
MUFA (%Energy)	13.23	0.48	13.60	0.44	13.50	0.46	13.58	0.56	ns
PUFA (g/day)	13.61	0.72	12.78	0.60	14.34	0.87	12.76	0.63	ns
PUFA (%Energy)	5.93	0.23	5.40	0.19	5.79	0.25	5.24	0.21	ns
n-3 (g/day)	1.35	0.11	1.43	0.12	1.63	0.18	1.46	0.12	ns
n-6 (g/day)	9.17	0.71	8.89	0.58	9.97	0.87	9.03	0.56	ns
CHO (g/day)	225.45	9.14	238.86	8.54	239.28	7.53	239.10	7.91	ns
CHO (%Energy)	43.29	0.83	44.19	0.67	43.89	0.76	43.70	0.79	ns
Protein (g/day)	87.22	2.79	88.55	2.61	94.59	3.25	92.40	3.02	ns
Protein (%Energy)	17.86	0.42	17.36	0.37	17.93	0.41	17.45	0.33	ns
Alcohol (g/day)	8.66	1.72	9.25	1.37	7.42	1.19	8.83	1.27	ns
Alcohol (%Energy)	2.49	0.47	2.81	0.41	2.29	0.34	2.62	0.35	ns

Composition of diets mid-intervention over the four dietary groups

Mid-Intervention	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	*ANOVA p*
Body weight (kg)	89.94		1.32	90.95		1.37	91.61		1.55	91.06		1.34	0.869
Basal Metabolic Rate (MJ/day)	7.13		0.11	7.11		0.11	7.18		0.12	7.15		0.11	0.973
Total Energy(MJ)	8.71		0.24	8.65		0.23	8.09		0.20	8.37		0.22	0.178
Total fat (g)	92.00	a	3.00	89.56	a	2.80	65.72	b	2.13	66.13	b	2.09	0.0005
Total fat (%Energy)	39.48	a	0.61	38.48	a	0.62	30.50	b	0.68	29.63	b	0.60	0.0005
SFA (g/day)	39.87	a	1.46	23.91	b	0.87	18.97	c	0.77	19.71	c	0.85	0.0005
SFA (%Energy)	17.06	a	0.34	10.21	b	0.21	8.67	c	0.23	8.78	c	0.29	0.0005
MUFA (g/day)	28.92	a	1.06	43.45	b	1.47	25.88	a	0.90	26.10	a	0.95	0.0005
MUFA (%Energy)	12.40	a	0.27	18.54	b	0.44	12.07	a	0.31	11.65	a	0.30	0.0005
PUFA (g/day)	14.32	ab	0.52	15.54	a	0.56	13.43	b	0.53	12.76	b	0.43	0.001
PUFA (%Energy)	6.24	ab	0.19	6.67	a	0.17	6.33	ab	0.26	5.77	b	0.16	0.002
n-3 (g/day)	1.94	a	0.17	1.97	a	0.15	1.83	a	0.13	2.92	b	0.11	0.0005
n-6 (g/day)	9.76		0.62	10.37		0.59	8.94		0.55	8.59		0.49	0.086
CHO (g/day)	220.38	a	7.34	224.33	a	6.52	241.27	ab	7.18	251.79	b	7.31	0.006
CHO (%Energy)	41.48	a	0.71	42.53	a	0.61	48.67	b	0.76	49.42	b	0.65	0.0005
Protein (g/day)	88.72		2.84	86.73		2.51	89.48		2.87	92.77		2.84	0.461
Protein (%Energy)	17.38	a	0.38	17.13	a	0.32	18.74	b	0.40	18.84	b	0.38	0.0005
Alcohol (g/day)	6.32		1.58	6.73		1.40	6.37		1.27	7.10		1.30	0.642
Alcohol (%Energy)	1.78		0.42	2.00		0.37	2.07		0.43	2.27		0.40	0.624

Composition of diets post-intervention over the four dietary groups

Post-Intervention	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	*ANOVA p*
Body weight (kg)	89.70		1.33	90.94		1.34	91.03		1.53	90.45		1.35	0.905
Basal Metabolic Rate (MJ/day)	7.09		0.10	7.08		0.11	7.14		0.12	7.14		0.11	0.975
Total Energy(MJ)	8.67		0.25	8.67		0.26	8.23		0.30	8.27		0.24	0.359
Total fat (g)	92.64	a	3.01	90.36	a	2.86	65.83	b	3.33	64.37	b	2.17	0.0005
Total fat (%Energy)	39.83	a	0.62	38.92	a	0.51	29.63	b	0.60	29.12	b	0.50	0.0005
SFA (g/day)	40.79	a	1.45	24.34	b	0.93	19.44	c	1.18	18.98	c	0.77	0.0005
SFA (%Energy)	17.50	a	0.33	10.38	b	0.20	8.56	c	0.26	8.65	c	0.26	0.0005
MUFA (g/day)	29.58	a	1.03	43.04	b	1.34	26.11	c	1.40	25.24	c	0.96	0.0005
MUFA (%Energy)	12.74	a	0.26	18.73	b	0.37	11.78	ac	0.30	11.40	c	0.28	0.0005
PUFA (g/day)	14.52	ac	0.55	15.54	a	0.59	13.13	bc	0.69	12.57	b	0.48	0.0005
PUFA (%Energy)	6.25	ab	0.16	6.66	b	0.16	6.00	a	0.17	5.67	a	0.14	0.0005
n-3 (g/day)	1.84	a	0.12	1.90	a	0.12	1.89	a	0.16	3.05	b	0.14	0.0005
n-6 (g/day)	9.96	ab	0.63	10.57	a	0.58	8.91	b	0.78	8.31	b	0.54	0.002
CHO (g/day)	218.70	a	7.90	222.72	a	7.50	245.92	ab	8.19	254.06	b	8.04	0.003
CHO (%Energy)	41.24	a	0.67	42.07	a	0.59	49.35	b	0.76	50.22	b	0.68	0.0005
Protein (g/day)	87.85		2.82	86.18		2.83	92.21		4.14	88.58		2.77	0.907
Protein (%Energy)	17.42	ac	0.41	16.87	a	0.34	19.18	bd	0.49	18.34	cd	0.39	0.0005
Alcohol (g/day)	5.96		1.14	7.31		1.36	6.31		1.36	7.05		1.27	0.439
Alcohol (%Energy)	1.59		0.26	2.20		0.37	1.78		0.37	2.29		0.40	0.365

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, carbohydrate;

[abc] Mean values within a row with dissimilar superscript letters were significantly different ($p < 0.05$, $p<0.01$ where bonferroni correction applied

Targets	HFSFA	HFMUFA	LF	LF n-3 PUFA
%E from fat	**38**	**38**	**28**	**28**
%E from SFA	**16**	**8**	**8**	**8**
%E from MUFA	**12**	**20**	**11**	**11**
%E from PUFA	**6**	**6**	**6**	**6**
				1.24 g/d total EPA and DHA (g/d)

Table 4.2: Exchangeable fat intake and its removal

		Total energy (MJ/day)		Total fat (g/day)		SFA (g/day)		MUFA (g/day)		PUFA (g/day)	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	NDNS total intake	**9.68**	**6.83**	**86.5**	**61.4**	**32.5**	**23.3**	**29.1**	**20.2**	**15.2**	**11.1**
Exchangeable fat intake	Added fats and oils	0.38	0.26	10.0	7.0	0.9	0.7	3.9	2.7	4.7	3.3
	Added fats- spreads	0.39	0.27	10.4	6.8	3.9	2.6	3.5	2.2	2.3	1.3
	Milk	0.48	0.41	4.3	3.7	2.6	2.1	1.2	0.8	0.9	0.8
	Cheese	0.29	0.21	5.2	3.7	3.3	2.3	1.2	0.8	0.3	0.2
	Biscuits, cakes, buns, pastries	0.58	0.48	6.1	4.3	2.6	1.9	2.0	1.4	1.1	0.8
Total exchangeable fat intake		**2.12**	**1.63**	**36.0**	**25.4**	**13.3**	**9.6**	**11.8**	**8.0**	**9.3**	**6.4**
NDNS intake minus exchangeable fat (Non-modifiable fat intake)		**7.56**	**5.20**	**50.6**	**36.0**	**19.3**	**13.7**	**17.3**	**12.2**	**5.9**	**4.7**

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Intake and contribution to total nutrient intake data extracted from NDNS and National food survey and the North/South Ireland Food Consumption Survey (DEFRA. National Food Survey, 2000; Henderson *et al.*, 2003)

Table 4.3: Nutrient composition of study foods

	Energy (MJ)	Fat (g)	Protein (g)	CHO (g)	Sugar (g)	SFA (g)	MUFA (g)	PUFA (g)	C8:0 (g)	C10:0 (g)	C12:0 (g)	C14:0 (g)	C16:0 (g)	C18:0 (g)	C18:1 (g)	C18:2 n-6 (g)
Diet HFSFA																
Honey flap jack	2.23	31.7	7.7	57.3	18.8	14.4	8.8	4.5	0.34	0.31	3.42	1.33	3.37	2.94	7.18	4.13
Muesli cookie	1.79	19.3	5.9	60.4	32.2	9.2	5.0	3.6	0.29	0.29	3.07	1.21	2.67	2.39	5.45	4.25
Spread	2.96	80	0	0	0	39.4	20.7	19.8	0.64	0.63	9.49	3.31	11.73	12.90	20.13	16.74
Spread (Low Salt)	2.96	80	0	0	0	39.4	20.7	19.8	0.67	0.65	9.58	3.34	11.71	12.79	20.11	16.76
Oil replacement	3.69	99.6	0	0	0	48.2	33.4	17.8	0.72	0.73	10.85	3.87	15.18	16.06	32.52	15.02
Baking fat	3.66	99	0	0	0	47.9	33.1	17.7	0.80	0.77	11.07	3.86	14.90	15.71	32.37	15.39
Diet HFMUFA																
Honey flap jack	2.23	31.7	7.7	57.3	18.8	7.2	15.2	5.3	0.25	0.25	3.09	1.21	3.39	3.06	7.39	4.45
Muesli cookie	1.79	19.3	5.9	60.4	32.2	5.3	8.5	4.0	0.17	0.15	1.62	0.70	1.79	0.89	7.77	4.16
Spread	2.96	80	0	0	0	15.3	47.8	16.7	0.13	0.13	1.78	0.65	7.92	4.01	46.72	14.13
Spread (Low Salt)	2.96	80	0	0	0	15.3	47.9	16.7	0.13	0.13	1.78	0.65	7.94	3.99	46.76	14.12
Oil	3.70	100	0	0	0	15.6	74.4	9.9	0	0	0.03	0	12.0	2.88	73.01	9.34
Baking fat	3.66	99	0	0	0	19.0	49.5	30.3	0.17	0.17	2.36	0.86	9.30	5.30	48.35	25.86
Mayonnaise (Cohort 1)	3.02	81.4	0.7	0	0	12.7	60.7	7.6	0	0	0	0	9.32	2.44	59.09	7.02
Mayonnaise (Cohort 2)	2.97	79.1	1.1	1.3	1.3	6.2	50.0	22.9	0	0	0	0	3.96	1.42	48.27	15.98
Diet LF and LFn-3																
Honey flap jack	2.23	31.7	7.7	57.3	18.8	7.2	15.2	5.3	0.25	0.25	3.09	1.21	3.39	3.06	7.39	4.45
Muesli cookie	1.79	19.3	5.9	60.4	32.2	5.3	8.5	4.0	0.17	0.15	1.62	0.70	1.79	0.89	7.77	4.16
Spread	1.03	27.9	0	0	0	6.1	14.4	7.5	0.07	0.06	0.91	0.33	2.73	1.74	14.01	6.29
Spread (Low Salt)	1.03	27.9	0	0	0	6.1	14.4	7.5	0.67	0.06	0.82	0.33	2.76	1.69	14.07	6.31
Oil	3.70	100	0	0	0	11.0	55.9	33.0	0	0	0.13	0.07	7.24	2.64	54.55	27.64
Mayonnaise (Cohort 1)	1.13	26.4	1.9	6.8	5.7	3.2	14.3	8.5	0	0	0.06	0.03	1.79	0.71	13.45	6.98
Mayonnaise (Cohort 2)	1.24	29.8	0.7	6.7	2.2	2.7	18.7	8.3	0	0.03	0.03	0.12	1.70	0.57	18.09	5.71

Table 4.4: Replacement of exchangeable fat with study foods

		Weight of replacement fat source (g/day)		Total energy (MJ/day)		Total fat (g/day)		SFA (g/day)		MUFA (g/day)		PUFA (g/day)	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
NDNS intake minus exchangeable fat				**7.56**	**5.20**	**50.6**	**36.0**	**19.3**	**13.7**	**17.3**	**12.2**	**5.9**	**4.7**
Diet HFSFA	Spread	20	15	0.59	0.44	16.0	12.0	7.9	5.9	4.1	3.1	4.0	3.0
	Oil replacement	10	6	0.37	0.22	10.0	6.0	4.8	2.9	3.3	2.0	1.8	1.1
	biscuit	45	45	0.80	0.80	8.7	8.7	4.1	4.1	2.3	2.3	1.6	1.6
	Cheese (full fat)	17	12	0.29	0.21	5.8	4.1	3.7	2.6	1.6	1.1	0.2	0.2
	Milk (whole)	230	230	0.64	0.64	9.0	9.0	5.5	5.5	2.5	2.5	0.2	0.2
	CHO portion	-35	-35	-0.34	-0.34	-0.7	-0.7	-0.1	-0.1	-0.1	-0.1	-0.2	-0.2
	Total intake			9.91	7.17	99.3	75.0	45.2	34.7	31.1	23.1	13.5	10.6
	% total energy					**37.8**	**39.5**	**17.2**	**18.2**	**11.8**	**12.2**	**5.1**	**5.6**
Diet HFMUFA	Spread	20	15	0.59	0.44	16.0	12.0	3.1	2.3	9.6	7.2	3.3	2.5
	Oil	10	6	0.37	0.22	10.0	6.0	1.6	0.9	7.4	4.5	1.0	0.6
	Mayonnaise C1	20	20	0.60	0.60	16.3	16.3	2.5	2.5	12.1	12.1	1.5	1.5
	Biscuit	45	45	0.80	0.80	8.7	8.7	2.4	2.4	3.8	3.8	1.8	1.8
	Cheese (low fat)	17	12	0.19	0.13	2.6	1.8	1.6	1.1	0.7	0.5	0.1	0.1
	Milk (skimmed)	230	230	0.32	0.32	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0
	CHO portion	-35	-35	-0.34	-0.34	-0.7	-0.7	-0.1	-0.1	-0.1	-0.1	-0.2	-0.2
	Total intake			10.09	7.37	103.6	80.2	30.5	23.1	50.9	40.2	13.5	11.0
	% total energy					**38.7**	**41.0**	**11.4**	**11.8**	**19.0**	**20.6**	**5.0**	**5.6**
Diet LF & LF n-3 PUFA	Spread	15	10	0.15	0.10	4.2	2.8	0.9	0.6	2.2	1.4	1.1	0.8
	Oil	6	4	0.22	0.15	6.0	4.0	0.7	0.4	3.4	2.2	2.0	1.3
	Biscuit	22.5	22.5	0.50	0.50	7.1	7.1	1.6	1.6	3.4	3.4	1.2	1.2
	Cheese (low fat)	17	12	0.19	0.13	2.6	1.8	1.6	1.1	0.7	0.5	0.1	0.1
	Milk (skimmed)	230	230	0.32	0.32	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0
	CHO portion	70	70	0.69	0.69	1.4	1.4	0.2	0.2	0.2	0.2	0.4	0.4
	Total intake			9.63	7.09	72.1	53.3	24.4	17.9	27.2	20.0	10.7	8.5
	% total energy					**28.2**	**28.3**	**9.6**	**9.5**	**10.6**	**10.7**	**4.2**	**4.5**

Table 4.5: Baseline Composition of diets across the 8 LIPGENE centres

	TCD, n=55		UoR, n=57		UO, n=54		INSERM, n=38		UM, n=49		HURS-UCO, n=74		JUMC, n=42		UU, n=45		
Pre-Intervention	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*ANOVA p*
Body weight (kg)	93.39 ^{a}	2.13	91.99 ^{ab}	1.84	92.89 ^{a}	1.98	87.42 ^{ab}	2.62	92.46 ^{ab}	1.99	90.95 ^{ab}	1.42	96.03 ^{a}	2.07	83.38 ^{b}	1.85	0.00
BMR (MJ/day)	7.34 ^{a}	0.17	7.31 ^{a}	0.15	7.24 ^{a}	0.17	7.11 ^{a}	0.19	7.50 ^{a}	0.14	7.03 ^{a}	0.11	7.21 ^{a}	0.16	6.33 ^{b}	0.14	0.00
Total Energy(MJ)	9.93 ^{a}	0.39	9.62 ^{ac}	0.39	8.84 ^{abc}	0.40	7.66 ^{b}	0.27	9.39 ^{abc}	0.39	8.55 ^{abc}	0.22	7.85 ^{b}	0.41	8.12 ^{bc}	0.35	0.00
Total fat (g)	89.97 ^{ab}	5.17	80.84 ^{a}	3.93	85.85 ^{ab}	4.95	77.16 ^{a}	3.83	89.88 ^{ab}	5.05	98.50 ^{b}	2.97	78.14 ^{a}	4.28	71.84 ^{a}	3.58	0.00
Total fat (%Energy)	33.34 ^{ac}	1.05	31.62 ^{a}	0.91	35.52 ^{bcf}	0.83	37.66 ^{be}	1.07	35.00 ^{ace}	1.00	43.27 ^{d}	0.67	36.86 ^{ce}	0.84	32.53 ^{af}	0.69	0.00
SFA (g/day)	32.37 ^{ab}	2.08	28.39 ^{ab}	1.73	33.10 ^{ab}	1.99	27.68 ^{ab}	1.63	33.22 ^{a}	1.96	25.61 ^{b}	0.97	27.27 ^{ab}	1.63	28.88 ^{ab}	1.62	0.01
SFA (%Energy)	11.99 ^{ab}	0.44	10.99 ^{a}	0.44	13.71 ^{b}	0.44	13.36 ^{b}	0.60	12.92 ^{b}	0.42	11.15 ^{a}	0.24	13.02 ^{b}	0.51	13.01 ^{b}	0.39	0.00
MUFA (g/day)	30.88 ^{a}	1.80	24.43 ^{a}	1.21	29.55 ^{a}	1.81	24.48 ^{a}	1.51	28.64 ^{a}	1.84	48.56 ^{b}	1.44	31.10 ^{a}	1.88	26.69 ^{a}	1.41	0.00
MUFA (%Energy)	11.44 ^{a}	0.43	9.55 ^{bc}	0.30	12.26 ^{a}	0.36	12.05 ^{a}	0.61	11.10 ^{ac}	0.39	21.47 ^{d}	0.43	14.60 ^{e}	0.43	12.07 ^{a}	0.33	0.00
PUFA (g/day)	15.13 ^{ac}	1.06	11.98 ^{ab}	0.73	16.38 ^{ce}	1.23	9.95 ^{b}	0.95	18.38 ^{c}	1.17	10.51 ^{bd}	0.40	13.86 ^{ade}	1.28	11.06 ^{ab}	0.67	0.00
PUFA (%Energy)	5.56 ^{ac}	0.29	4.73 ^{a}	0.25	6.72 ^{bd}	0.30	4.84 ^{a}	0.37	7.22 ^{bd}	0.36	4.59 ^{a}	0.11	6.45 ^{cd}	0.44	5.04 ^{ac}	0.19	0.00
n-3 (g/day)	0.73 ^{a}	0.07	1.67 ^{bc}	0.15	2.66 ^{c}	0.22	0.99 ^{ad}	0.11			1.13 ^{d}	0.06	1.68 ^{bd}	0.27			0.00
n-6 (g/day)	6.10 ^{a}	0.51	10.30 ^{bc}	0.67	13.23 ^{c}	1.02	7.70 ^{ab}	1.02	no data		7.99 ^{ab}	0.36	11.13 ^{abc}	1.92	no data		0.00
CHO (g/day)	281.72 ^{a}	11.58	281.94 ^{a}	13.47	227.08 ^{bc}	11.12	192.21 ^{bc}	8.94	242.72 ^{ac}	9.71	196.43 ^{b}	6.50	223.31 ^{bc}	13.58	237.83 ^{abc}	11.99	0.00
CHO (%Energy)	45.12 ^{ac}	1.17	45.85 ^{ac}	1.12	43.74 ^{a}	0.94	41.81 ^{ab}	1.08	44.55 ^{a}	0.95	38.32 ^{b}	0.70	43.58 ^{a}	0.97	49.58 ^{c}	0.89	0.00
Protein (g/day)	101.04 ^{ac}	3.93	110.58 ^{a}	6.00	93.68 ^{acd}	4.14	84.07 ^{cde}	2.62	87.55 ^{cde}	3.49	86.34 ^{cde}	1.96	80.07 ^{bd}	4.61	75.55 ^{be}	2.69	0.00
Protein (%Energy)	17.40 ^{ab}	0.47	19.47 ^{a}	0.78	18.34 ^{ab}	0.46	18.87 ^{a}	0.66	16.22 ^{b}	0.44	17.16 ^{ab}	0.30	17.60 ^{ab}	0.61	16.17 ^{b}	0.40	0.00
Alcohol (g/day)	13.76 ^{ac}	2.57	10.02 ^{abc}	2.12	7.12 ^{abc}	1.51	7.76 ^{abc}	2.47	15.17 ^{a}	2.68	5.59 ^{abc}	0.96	4.24 ^{b}	1.32	4.28 ^{bc}	1.06	0.00
Alcohol (%Energy)	3.92 ^{ab}	0.71	2.97 ^{ab}	0.60	2.39 ^{ab}	0.53	1.92 ^{ab}	0.49	4.15 ^{a}	0.72	1.74 ^{ab}	0.30	1.59 ^{b}	0.48	1.62 ^{ab}	0.40	0.00

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, carbohydrate;

^{abc} Mean values within a row with dissimilar superscript letters were significantly different ($p < 0.05$, $p<0.0018$ where bonferroni correction applied).

Table 4.6: Composition of diets at end of intervention period at each centre

	TCD																UoR																UO																INSERM															
	HFSFA n=13				HFMUFA n=14				LF n=13				LF n-3 PUFA n=13				HFSFA n=14				HFMUFA n=15				LF n=15				LFn-3 PUFA n=14				HFSFA n=10				HFMUFA n=14				LF n=14				LFn-3PUFA n=13				HFSFA n=8				HFMUFA n=11				LF n=9				LFn-3PUFA n=10			
	Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM	
Body weight (kg)	88.93		4.08		92.37		3.36		100.91		5.03		88.22		3.74		87.17		2.17		92.94		3.38		93.27		5.14		94.55		3.55		93.87		3.32		92.23		3.64		92.50		4.45		90.87		4.45		86.80		5.95		80.41		5.25		84.61		4.23		90.87		5.40	
Total Energy(MJ)	9.80	a	0.69		10.95	a	0.87		8.59	ab	0.89		6.48	b	0.49		9.14		0.66		10.30		0.71		7.89		0.48		8.87		0.74		9.41		0.85		8.41		0.72		9.51		1.61		8.79		0.68		7.98		0.43		6.28		0.42		7.98		0.78		7.99		0.64	
Total fat (%Energy)	38.50	a	0.80		36.85	a	1.28		28.57	b	1.43		29.08	b	1.53		37.25	a	2.24		36.51	a	1.40		24.83	b	2.32		28.24	b	1.44		40.87	a	2.83		40.40	a	1.88		33.39	ab	1.52		31.39	b	1.63		40.88	a	2.19		36.93	a	1.19		29.07	b	0.57		28.93	b	0.91	
SFA (%Energy)	15.39	a	0.55		11.15	b	0.37		8.95	b	0.52		9.60	b	0.85		16.56	a	1.07		9.50	b	0.54		7.18	b	0.87		8.83	b	0.61		19.04	a	1.48		11.29	b	0.75		10.38	b	0.63		10.22	b	0.79		18.19	a	1.16		8.60	b	0.30		7.09	b	0.37		7.56	b	0.54	
MUFA (%Energy)	12.58	a	0.35		15.44	b	0.64		9.76	c	0.50		10.05	c	0.67		10.68	a	0.64		16.19	b	0.75		8.21	a	0.86		9.52	a	0.54		12.84	a	0.93		19.28	b	1.22		12.97	a	0.64		12.54	a	0.85		12.75	a	1.17		18.78	b	1.11		12.30	a	0.51		11.91	a	0.41	
PUFA (%Energy)	6.54		0.38		6.58		0.37		5.72		0.49		5.30		0.26		5.41	ab	0.61		6.25	a	0.46		4.05	b	0.30		4.69	ab	0.31		6.41		0.58		7.28		0.47		7.22		0.43		6.16		0.40		5.85		0.34		6.21		0.44		6.30		0.34		6.48		0.17	
n-3 (g/day)	0.76	a	0.12		0.93	a	0.14		0.69	a	0.13		1.68	b	0.07		1.88	a	0.20		2.39	ac	0.20		1.22	b	0.11		2.86	c	0.23		2.50	a	0.24		2.71	a	0.32		3.36	ab	0.47		4.23	b	0.44		2.16	a	0.37		1.71	a	0.20		2.19	a	0.22		3.49	b	0.29	
CHO (%Energy)	42.71		1.53		42.93		1.95		48.38		2.41		48.23		2.05		40.34		2.28		41.42		1.89		48.99		3.73		48.37		1.81		41.70	ab	2.42		39.51	a	1.83		46.75	b	1.66		49.35	b	1.79		37.95	a	2.54		44.47	a	1.56		52.91	b	1.56		53.29	b	1.12	

	UM																HURS-UCO																JUMC																UU															
	HFSFA n=14				HFMUFA n=12				LF n=11				LF n-3 PUFA n=12				HFSFA n-17				HFMUFA n=17				LF n=20				LFn-3 PUFA n=20				HFSFA n=9				HFMUFA n=14				LF n=11				LF n-3 PUFA n=8				HFSFA n=12				HFMUFA n=13				LF n=11				LFn-3PUFA n=9			
	Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM		Mean		SEM	
Body weight (kg)	91.08		3.87		99.41		4.05		87.20		3.45		90.03		4.10		92.46		3.06		88.40		3.13		89.37		2.63		91.05		2.64		96.10		3.29		97.30		2.91		91.73		4.70		94.08		4.44		81.24		4.16		83.18		3.30		84.76		3.98		83.13		3.50	
Total Energy(MJ)	8.68		0.58		9.31		0.53		8.85		0.68		8.94		0.56		8.16		0.44		7.76		0.43		7.68		0.36		9.21		0.54		5.77		0.84		7.36		0.53		7.16		0.93		6.30		0.60		9.60		0.74		8.60		0.64		8.31		0.55		8.29		0.47	
Total fat (%Energy)	40.58	a	1.52		40.24	a	1.37		29.88	b	1.56		28.22	b	1.02		40.33	a	0.50		40.29	a	0.69		27.05	b	0.50		26.49	b	0.49		38.44	a	2.54		38.93	a	1.90		33.21	ab	1.42		29.01	b	1.90		42.18	a	1.62		41.02	ab	1.36		33.93	c	1.67		34.57	bc	2.08	
SFA (%Energy)	17.88	a	0.64		10.75	b	0.53		9.13	b	0.57		8.82	b	0.44		17.92	a	0.29		9.13	b	0.43		6.62	c	0.25		6.35	c	0.28		14.95	a	0.86		11.70	b	0.65		10.50	b	0.90		9.21	b	1.08		20.03	a	0.93		10.95	b	0.19		9.89	b	0.67		10.37	b	0.54	
MUFA (%Energy)	12.60	a	0.69		18.62	b	0.73		11.80	a	0.84		10.55	a	0.47		12.84	a	0.34		21.06	b	0.37		11.54	c	0.25		11.07	c	0.26		14.92	ab	1.25		19.03	a	1.36		14.14	b	0.69		11.61	b	1.05		13.65	a	0.65		21.28	b	0.99		15.20	a	0.82		15.74	a	1.14	
PUFA (%Energy)	6.99	ab	0.44		8.47	a	0.52		6.51	b	0.37		6.30	b	0.44		6.11	a	0.27		5.68	ab	0.17		5.32	ab	0.24		4.96	b	0.22		6.23		0.56		5.87		0.30		6.33		0.69		5.77		0.56		6.41		0.34		7.43		0.54		7.53		0.35		6.78		0.54	
n-3 (g/day)	no data																2.13	a	0.15		1.73	a	0.19		1.96	a	0.27		3.22	b	0.15		1.84		0.75		1.78		0.34		2.52		0.68		1.28		0.04		no data																	
CHO (%Energy)	43.02	ab	1.60		41.69	a	1.73		49.70	bd	1.81		50.58	cd	2.02		38.24	a	0.99		40.60	a	1.07		51.20	b	1.09		54.05	b	0.92		43.23		2.62		42.69		2.01		48.27		1.94		45.73		3.42		43.18		1.76		44.21		0.99		48.70		1.21		48.80		2.47	

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, carbohydrate;

[abc] Mean values within a row with dissimilar superscript letters were significantly different ($p < 0.01$, bonferroni corrected).

Table 4.7: Correlation coefficients between fatty acid composition in plasma (%) and dietary intakes (% of total fat) measured at post-intervention of the 12 week dietary interventon study in the four diets

Post-Intervention Plasma Fatty Acids(w/w%)	Post-Intervention Dietary Fat intakes							
	HFSFA diet (Diet A)		**HFMUFA diet (Diet B)**		**LF diet (Diet C)**		**LF n3PUFA diet (Diet D)**	
	C16:1%Total Fat		C16:1%Total Fat		C16:1%Total Fat		C16:1%Total Fat	
	r		*r*		*r*		*r*	
Post C16:1%	-0.173	ns	-0.318	*	-0.277	*	-0.158	0.160
	C18:1 %Total Fat		C18:1 %Total Fat		C18:1 %Total Fat		C18:1 %Total Fat	
	r		*r*		*r*		*r*	
Post C18:1%	0.130	ns	0.287	*	0.218	*	0.368	**
	C20:5 n-3 %Total Fat		C20:5 n-3 %Total Fat		C20:5 n-3 %Total Fat		C20:5 n-3 %Total Fat	
	r		*r*		*r*		*r*	
Post C20:5 n-3%	0.343	*	0.421	***	0.163	ns	0.105	ns
	C22:6 n-3 %Total Fat		C22:6 n-3 %Total Fat		C22:6 n-3 %Total Fat		C22:6 n-3 %Total Fat	
	r		*r*		*r*		*r*	
Post C22:6 n-3%	0.592	***	0.462	***	0.467	***	0.438	***
	SAFA %Total Fat		SAFA %Total Fat		SAFA %Total Fat		SAFA %Total Fat	
	r		*r*		*r*		*r*	
Post SFA%	0.081	ns	0.208	*	0.114	ns	0.088	ns
	MUFA %Total Fat		MUFA %Total Fat		MUFA %Total Fat		MUFA %Total Fat	
	r		*r*		*r*		*r*	
Post MUFA%	0.115	ns	0.143	ns	0.077	ns	0.151	ns
	PUFA n-3 %TotalFat		PUFA n-3 %TotalFat		PUFA n-3 %TotalFat		PUFA n-3 %TotalFat	
	r		*r*		*r*		*r*	
Post PUFA n-3%	0.217	ns	0.409	***	0.469	***	0.143	ns

4.5 DISCUSSION

The purpose of this chapter was to describe and evaluate the methods employed in the design and implementation of four experimental diets, differing in saturated, monounsaturated and total fat composition in a dietary intervention study across eight European centres with the aim of assessing the effects of the quantity and quality of dietary fat on multiple metabolic and molecular risk factors on the metabolic syndrome. The key objectives of this strategy were to develop diets that were i) significantly different in overall fat intake, ii) one diet was to be significantly higher in its SFA content compared to all other diets, another significantly higher in its MUFA content compared with the three other diets and iii) the effect of long chain n-3 PUFA supplementation was tested, based on a low fat background diet.

The results indicated that the food exchange model used was successful. The compositional objectives were largely achieved. This food exchange model enabled the creation of two diets (HFSFA and HFMUFA) that were significantly different in terms of %E from fat than the other two diets (LF and LF n-3 PUFA), thus fulfilling the first objective of the model. This was achieved by the use of CHO exchanges, which significantly increased %E from CHO in the low fat diets compared with the higher fat diets. The second objective was also fulfilled as diet HFSFA had significantly greater %E from SFA compared to the other three diets whereas diet HFMUFA had significantly greater %E from MUFA than the other diets. Similarly, the third objective was met with significantly greater LC n-3 PUFA intakes in the LF n-3 PUFA diet group.

At baseline, there were no significant differences in the nutrient composition of the four groups, indicating successful randomisation. In contrast, at both the mid and end of the intervention periods these four diets were distinct in their dietary fat composition. The dietary change instigated during the intervention period was maintained throughout as no significant differences in the levels of key nutrients were found between the mid and end of intervention periods.

The distinct differences in baseline dietary intake between centres highlight the challenge which was set; to change the habitual diets of volunteers at all centres with the use of a single food exchange model. This highlights the novel aspect of the present work. Previous reports have shown replacement of SFA for MUFA is possible, when at least one meal per day is provided alongside additional study foods (Knapper *et al.*, 1996; Nydahl *et al.*, 2003). The current work has shown dietary fat

alteration can also be achieved through the sole use of study foods when incorporated by volunteers themselves into desirable meal formats. Furthermore, these results show that this strategy can be used across populations with variations in habitual dietary intakes.

Overall the %E from fat was significantly different between the high and low fat diets. However, in three centres this difference did not reach the level of statistical significance at the week 12 dietary assessment (UO, JUMC, UU). Notably, at mid-intervention the UU and UO centres did achieve significant distinction in %E from fat between these diets. The UU data provides an important insight into the usefulness of the food exchange model and highlights the challenges encountered when habitual diets differed across the intervention population. Volunteers from the UU cohort had significantly greater %E from CHO at baseline compared to all other centres except TCD and UoR. The food exchange model described depended on the ability of volunteers to undertake fat-CHO exchanges in order to achieve %E from fat targets in the low fat diets. As CHO formed a large part of the habitual diets of volunteers in the UU centre (49.6 %E) before intervention, further increases in this macronutrient proved difficult to achieve. Thus, at the end of the intervention period, although %E from fat was greater in diet HFMUFA than the LF n-3 PUFA diet, these differences in %E from fat did not reach the level of statistical significance.

Importantly however, a statistically significant distinction in %E from CHO between the high-fat and low-fat diets was not an essential output of the model. In the TCD and UoR centres, differences in %E from CHO between the high and low fat diets were observed but these too did not reach statistical significance. However, these centres achieved target differences in %E from fat between diet groups. Key to this was the ability of these centres, in contrast to UU, to further increase CHO intake in the low fat diets above their baseline levels.

All centres achieved the second objective (%E from SFA in diet HFSFA was significantly greater than the other three diets), irrespective of baseline intake. In addition, in the HURS-UCO and TCD cohorts, diet HFMUFA had significantly greater %E from SFA than the low fat diets. Although this did not reach statistical significance in the other centres, most displayed a similar trend. This highlights a difficulty in the targeted reduction of SFA alongside the maintenance of a high fat, high MUFA diet, which has been reported previously (Nydahl *et al.*, 2003). In the

HURS-UCO centre however, the data suggests the significant difference in %E from SFA reported between the HFMUFA and low fat diets was due instead to low %E from SFA intakes within the low fat diet groups, reflective of habitual dietary intake, rather than a problem with reducing SFA in the HFMUFA diet. Critically, these data do not indicate a fault in the model as %E from SFA remained significantly greater in diet HFSFA compared to the other diets.

All centres also achieved a level of %E from MUFA in diet HFMUFA that was greater than the other diets. This reached statistical significance in all centres except the JUMC centre, where although %E from MUFA was higher in HFMUFA compared to diet HFSFA the difference was not statistically significant. This difficulty again is likely to be associated with habitual dietary intake. The %E from MUFA intake at baseline for this centre was significantly greater than all centres except HURS-UCO. This highlights the difficulties associated with the targeted substitution of a specific fatty acid subclass with another, whilst maintaining a high fat intake. Furthermore, in the TCD and HURS-UCO centres, %E from MUFA was significantly greater in diet HFSFA compared to the lower fat diets, once again underlining the difficulty of targeted subclass manipulation alongside control of fat quantity.

PUFA levels were maintained at similar levels across all diets, although diet HFMUFA tended to have greater levels compared to the low fat diets, reaching statistical significance for the UoR and UM centres. At baseline, these centres had the lowest intake of %E from MUFA and therefore were set the greatest challenge in reaching target %E from MUFA levels for diet HFMUFA. To substantially increase %E from MUFA, the use of food sources e.g. hazelnuts, in addition to the study foods provided, were actively encouraged at these centres. This allowed the successful distinction of diet HFMUFA in terms of MUFA content but concurrently is likely to have increased PUFA intake. An unavoidable change in the composition of the mayonnaise product used in diet HFMUFA, as detailed below, is also likely to explain the increased %E from PUFA in the HFMUFA diet.

The mean ratio of energy intakes to estimated basal metabolic rate (EI/BMR) is used to assess the validity of reported energy intakes (Black *et al.*, 1991; Goldberg *et al.*, 1991) . Mean EI/BMR values in the low fat diet groups were lower than the high fat diets groups, suggesting that underreporting of energy intake may have occurred. Further research to look at the percentage of overweight and obese

underreporters in these diet groups is warranted as it has been well documented that these groups of people are more likely to underreport energy intakes than those of normal weight (Briefel *et al.*, 1997). When interpreting the influence of underreporting, consideration must be given to the cut-off criteria used to define underreporters. Numerous studies have shown that it is difficult to obtain a representative picture of what people usually eat, mainly because of large day-to-day variations in food intake, misreporting and changes in dietary habits during a given study.

Although underreporting is more common in subjects with a high body mass index, other factors e.g. sex, age, smoking, educational level and dieting behaviour have also been found to be related to underreporting (Price *et al.*, 1997; Braam *et al.*, 1998; Johansson *et al.*, 2001). A crude estimate of 1.1 EI/BMR was used to define underreporters. However, much is to be gained if participants are categorised differently based on their physical activity level.

Logistic challenges

Whereas the results show the strategy for dietary manipulation used in the present study was successful, certain problems were encountered. It is important these are disseminated as they may be valuable for those planning similar dietary intervention studies in the future.

Provision of adequate refrigerated storage for a period of up to 18 months was required for volumes of up to 180 kg spread, 270 kg baking fat and room temp storage for 1254 kg biscuits, 208 kg oil, 100 kg mayonnaises per centre. Logistics for the distribution of the various products to all centres was highly complex. Required food volumes had to be individually calculated per centre, per cohort, based on their number of volunteers (and their study food preferences).

Food production challenges

Difficulties also arose in the design of the study foods, in terms of their ideal theoretical nutritional composition versus a useable composition with satisfactory organoleptic properties. A major difficulty arose in the formulation of an oil product for diet HFSFA. To fulfil the requirements of the model this product had to be based largely on SFA. Originally a suitable bottled liquid product was produced, however this could not be repeated as seasonal temperature changes resulted in this product

solidifying during production of subsequent batches. Thus the product had to be packaged in a tub format, with improvements in colour and flavour to improve volunteer acceptability. Another unavoidable occurrence was a change in supplier of the mayonnaise product, as the factory responsible for production of the original products went into liquidation. Unfortunately the second supplier could not produce products to match the exact nutrient composition of the first. As a result the mayonnaise used in cohort 2, for diet HFMUFA, had lower levels of MUFA and higher levels of PUFA than originally planned (*Table 4.3*).

Key to the overall success of this food exchange model was the flexibility incorporated within its design. Provision of foods other than those which were essential within the food exchange model enabled this achievement. For example, a mayonnaise product was provided for the low fat diets. This was not a specific tool within the model to alter dietary composition, but as the intervention study was completed in summer months when salad cream and mayonnaise can be consumed in large amounts, and therefore contribute to fat intake, a suitable alternative was provided. Baking fat was also provided for diets HFSFA and HFMUFA (to allow volunteers to prepare their own cakes or biscuits if desired).

In conclusion, a robust, flexible food exchange model was developed and implemented successfully in the LIPGENE pan European study. The main objective, to design four diets distinct in fat quality and quantity, was achieved. This food exchange model has the flexibility to be implemented in future studies designed to investigate the effects of diet in free-living populations with varying habitual dietary intakes.

ACKNOWLEDGEMENTS

- The Food Exchange Model was developed by Dr. Julie Lovegrove (University of Reading) and Dr. Sinead McCarthy (Trinity College Dublin).
- All analysis for this chapter was carried out by the author.

Chapter Five

Determining age, gender and BMI differences in a Metabolic Syndrome cohort and the associations between their plasma and dietary fatty acids and insulin sensitivity

---

5.1 ABSTRACT

Background: Costly efforts are now being employed to detect individuals and thus treat the MetS, reducing the risks and attempting to prevent the continuing surge of obesity, T2DM and CVD. Dietary fat composition has been implicated in the development of impaired fasting glucose and T2DM.

Objective: To assess, among MetS subjects, participating in the LIPGENE Dietary Intervention Study, the physiological, metabolic, biochemical and dietary characteristics between gender, age groups and BMI status. To examine the association of these characteristics with the number of metabolic disorders that defines the MetS. To investigate the associations between both plasma fatty acids and dietary fatty acids with insulin sensitivity.

Design: The LIPGENE human dietary intervention study was a randomised, controlled trial. 486 free-living subjects with the MetS attended one of the eight intervention centres, pre-intervention where anthropometry and fasting blood samples were taken. Subjects underwent an IVGTT and completed a pre-intervention 3-day weighed food diary.

Results: Females had greater insulin sensitivity at baseline ($p<0.05$) with no differences across the age categories. Body weight, BMI and waist circumference significantly decreased with increasing insulin sensitivity ($p<0.05$). Insulin sensitivity measured by QUICKI decreased with increasing number of MetS criteria and increasing body weight determined both S_I and HOMA-IR in linear regression analysis ($p<0.05$).

Conclusion: Body weight significantly decreased from low to high insulin sensitivity tertiles as did BMI, confirming its underlying causality in the MetS. Increasing body weight also increased the likelihood of having "5 versus 3" criteria of the MetS.

5.2 INTRODUCTION

Insulin sensitivity is the overall measure of the ability of insulin to regulate the uptake of glucose and glucose metabolism (DeFronzo *et al.*, 1979). Individuals with insulin resistance require higher than normal amounts of insulin to maintain normal levels of glycaemia. Insulin resistance, however, varies among normal, overweight and obese individuals (Ferrannini *et al.*, 1997; Yeni-Komshian *et al.*, 2000). An advocate of the metabolic syndrome (MetS), Gerald Reaven stated that insulin resistance is the underlying component of the different metabolic abnormalities that cluster and define the MetS (Reaven, 2006). The core components include hypertension, hypertriglyceridemia, low HDL-cholesterol, abdominal obesity and impaired glucose tolerance. These main features are accompanied by pro-thrombotic and pro-inflammatory states (Beckman *et al.*, 2002; Scheffer *et al.*, 2004). Usual traits of elevated triglycerides and reduced HDL-cholesterol concentrations are now accompanied by apolipoproteins as markers of vascular risk (Sniderman & Faraj, 2007). Apolipoprotein (Apo) CIII, recognised as a useful marker of triglyceride rich lipoprotein metabolism (Wang *et al.*, 1985) has also been proposed as an indicator of coronary risk in healthy subjects (Luc *et al.*, 1996; Sacks *et al.*, 2000). In a MetS cohort, total and non HDL apoCIII were determinants of the MetS (Onat *et al.*, 2003).

As yet there is no internationally agreed definition of the MetS with 5 definitions being proposed in the last 8 years. In the absence of a true definition, it is, however, agreed that these highly intercorrelated disturbed metabolic features predict the future development of Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Indeed, it has been reported that persons with the MetS are more likely to have a CVD event and are twice as likely to die (Isomaa *et al.*, 2001). The aim of identifying subjects with the MetS is to distinguish those with an elevated risk of developing CVD. Costly efforts are now being employed to detect and treat the MetS thus reducing the risks and attempting to prevent the continuing surge of obesity, T2DM and CVD, which many populations are now experiencing.

Health and lifestyle factors are also associated with the MetS including physical activity, smoking and diet (Pekkanen *et al.*, 1995; Hu *et al.*, 2001; Liu & Manson, 2001). Zhu *et al.*, (2004) demonstrated that the level of physical activity was most strongly associated with a lower risk of having the MetS. This has also been observed in epidemiologic studies (Carroll *et al.*, 2000; Irwin *et al.*, 2002; Kullo *et al.*, 2002). This could be a result of the favourable effects of physical activity on HDL-

cholesterol concentrations (Reaven *et al.*, 1990; Krummel *et al.*, 1993; Leon & Sanchez, 2001), TAG levels (Fahlman *et al.*, 2002), blood pressure (Katzmarzyk *et al.*, 2003), improving glucose tolerance (DiPietro *et al.*, 1998) and insulin sensitivity (Mayer-Davis *et al.*, 1998).

Different definitions lead to different prevalence rates and perhaps identification of high risk individuals as opposed to low risk persons in the pre-diabetic stage, should be first targeted for prevention and treatment to better utilise our already economically challenged health systems and budgets. For example central adiposity for men as defined by the World Health Organisation (WHO) requires a BMI > 30kg/m^2 or waist-hip-ratio of >0.90 (Alberti & Zimmet, 1998). The NCEP ATPIII (2001) definition defines a cut-off of >102cm and the European Group for the study of Insulin Resistance (EGIR) and International Diabetes Federation definitions (IDF) (2005) request that >94cm be defined as the waist circumference level, for identification of MetS male subjects. It is estimated that approximately 30% of people are diagnosed by most definitions (Day, 2007). As well as including lower cut-off values for central obesity with ethnic specific considerations, the IDF also lowered the fasting plasma glucose and included patients receiving appropriate treatments for previously diagnosed events (www.idf.org). On the other hand lowering the threshold levels of the core components helps identify low risk individuals in clinical practice and allows effective intervention at this preliminary stage. The prevalence of T2DM in adults is continuing to rise with figures quoted of 8% in 2000 (International Diabetes Federation, 2001) to 9.4% in 2007, projected to increase to 10.3% in 2025 (International Diabetes Federation, 2006). Using the NCEP ATPIII definition, the prevalence of the MetS is estimated to be 25% of the general population, varying with genetic background (Ford *et al.*, 2002). A consistent finding is the highly age dependent prevalence of the condition (Ford *et al.*, 2002), increasing from 7% in 20-29year olds to 40% in over 60years (Meigs, 2002). In the family heart study, the MetS increased substantially over a 7 year period (Kraja *et al.*, 2006) with central adiposity as the main driver for the longitudinal increase. However, the syndrome is not exclusive to middle and older age groups. Overweight and obese states have nearly risen 9% in 2-5 year olds, 12% in 6-11 year olds and 12.4% in 12-19 year olds (National Centre for Health Statistics , 2004) . The prevalence of the MetS has been calculated for 12-19 year olds and has doubled in the last 10 years (Jolliffe & Janssen, 2007). Age, therefore, could be an adjunct factor in the development of the MetS. In

European populations, the MetS is found to be more frequent in males, with estimates of 50% higher prevalence in men according to the WHO criteria compared to the EGIR (Balkau *et al.*, 2002). This could in part be explained by the different cut-off values used for abdominal obesity in the WHO definition. Recently, it was suggested that women present earlier than men with the first isolated component of the MetS (Hwang *et al.*, 2007) but present later as cases. With increasing rates of the MetS, it should be noted and of concern that the prevalence is now higher in women than in men, which coincides with the rise in obesity in women (Steinbaum, 2004). Data in the literature also indicates that elevated TAG is more of an important risk factor for women than men (Regitz-Zagrosek *et al.*, 2006). In middle aged men, dyslipidaemia remains a priority whereas in older men, elevated fasting plasma glucose was of greater concern (Kobayashi *et al.*, 2007). To further understand the characteristics of the MetS and population groups most at risk, attention needs to be paid to age groups and gender differences in the various abnormalities to prevent the multicomponent disorder.

As a multifactorial disorder, diet is sure to play an important role in the development of the MetS, where a dietary pattern characterised by high consumption of fruit, vegetables, poultry and legumes termed the "healthy dietary pattern" was associated with reduced risk of insulin resistance and the MetS (Esmaillzadeh *et al.*, 2007). In another study, poor dietary quality was associated with the development of abdominal obesity and MetS (Millen *et al.*, 2006). Dietary fat composition has been implicated in the development of IFG and T2DM (Colditz *et al.*, 1992; Feskens *et al.*, 1994; Vessby *et al.*, 1994a; Storlien *et al.*, 1996). Non-esterified fatty acids (NEFAs) may induce long term hyperinsulinaemia and ultimately β-cell toxicity and damage (McGarry & Dobbins, 1999). In one study, serum fatty acids such as linoleate were associated with the risk for development of IFG or T2DM and unfavourable changes in insulin and glucose metabolism (Laaksonen *et al.*, 2002). Fat composition attained from dietary records was not, however, associated with hyperglycaemia. Warensjo *et al.*, (2005) suggest that altered fatty acid metabolism could be a potentially significant predictor of MetS with the high levels of saturated fatty acids such as palmitic acid (C16:0) and low levels of linoleic acid (C18:2:6) observed in subjects with the syndrome. Cross-sectional and human intervention studies have suggested that a high intake of fat contributes to the development of obesity (Astrup *et al.*, 2000). Obesity is one of the most important determinants of the risk of developing T2DM (Ohlson *et*

al., 1985). And in the context of the MetS, with impaired insulin sensitivity as the underlying component, some studies have demonstrated that a high intake of fat is associated with this underlying feature (Mayer *et al.*, 1993; Parker *et al.*, 1993; Marshall *et al.*, 1997; Mayer-Davis *et al.*, 1997). Furthermore, the risk of developing insulin resistance and T2DM may be altered with the type of predominant fatty acid in the diet (Tsunehara CH *et al.*, 1991; Colditz *et al.*, 1992). In animal studies, omega-3 fatty acids improved the insulin action, saturated fat had a detrimental effect and monounsaturated and omega-6 fatty acids had little influence (Storlien *et al.*, 1991). In a recent review, Riccardi *et al.*, (2004) summarised from the available literature, that higher intakes of saturated fat is associated with impaired insulin action, including hyperinsulinaemia, and increasing intakes of unsaturated fat is associated with an improved insulin sensitivity. The amount and type of fat is also associated with changes in plasma lipoprotein levels. The effects of dietary fat on blood pressure are few, especially in MetS subjects.

Fatty acid composition of serum lipid esters reflects proportions of dietary fatty acids, especially polyunsaturated, essential fatty acids and some saturated fatty acids (Vessby, 2000). Insulin sensitivity is associated with low proportions of palmitic and palmitoleic acid and a high proportion of linoleic acid (Vessby *et al.*, 1994b). This led to the suggestion of a decreased activity of the enzyme delta-5 desaturase and increased activities of delta-6 and delta-9 desaturase. Positive associations between the incidence of diabetes and SFA in plasma phospholipids and diet have recently been reported (Hodge *et al.*, 2007) while lower diabetes incidences are associated with increased proportions of linoleic acid in plasma phospholipids (Wang *et al.*, 2003). Lovejoy *et al.*, (2001) demonstrated significant relationships between fasting insulin and myristic and palmitoleic acid in the serum cholesterol of patients with IGT and T2DM. Vessby concluded that the typical plasma fatty acid pattern observed with the MetS is characterised by increasing SFA, especially palmitic acid with a reduced content of linoleic acid (Vessby, 2003). Diets rich in monounsaturated fat have been associated with an improved lipoprotein profile, a lowering of plasma glucose concentrations, fasting and postprandial reductions in daytime blood pressure and did not include weight gain in a calorie controlled environment (Garg *et al.*, 1988). The low rates of total mortality and CHD in the Mediterranean region where oleic acid is the predominant dietary fat further substantiates this.

Investigations of fasting insulin, glucose and C-peptide concentrations and intakes of dietary fat are scarce. Overall adiposity is found to be positively associated with both fasting and postprandial insulin concentrations (Parker *et al.*, 1993). Obesity is an important determinant of insulin resistance, MetS and T2DM. Dietary factors such as energy and total fat intake are also significantly associated with increasing body weights (Riccardi *et al.*, 2004). Although obesity is probably the major lifestyle determinant of the MetS, examining a persons education, work and living status as well as physical activity, cigarette smoking, alcohol intake and age enables public health strategists to target specific groups of the population deemed at risk.

Lovejoy and DiGirolamo demonstrated that habitual fat intake is associated with reduced insulin sensitivity (Lovejoy & DiGirolamo, 1992). However, few have determined the effect of habitual diet on MetS subjects with distinctions on the type of fat or the effect of insulin sensitivity levels, which is essential to draw conclusions on a cohort of these disease susceptible people. A noticeable strength of this study is that it was carried out in 8 European groups, living in comparable socio-economic conditions. Centre/country differences cannot be due to genetics of a population alone but more indicative of environmental influences.

Characterising a group of MetS subjects in terms of differences between countries, age, BMI and intakes of total amount and type of fat will give a better understanding of isolated components and combined risk factors that can be better targeted. The objective of this study was to assess, among MetS subjects, participating in the LIPGENE Dietary Intervention Study, the physiological, metabolic, biochemical and dietary characteristics between gender, age groups and BMI status. This study offers a unique opportunity to address the objective as the dataset contains information on directly measured insulin sensitivity and insulin secretion determined from an IVGTT. We also examined the association of these characteristics with the number of metabolic disorders that defined the MetS. Our aim was also to investigate the associations between both plasma fatty acids and dietary fatty acids with insulin sensitivity. We identified independent (univariate) predictors of insulin sensitivity as measured by an IVGTT and insulin resistance estimated by the Homeostasis Model Assessment (HOMA-IR). The data is obtained from free-living subjects with the MetS using natural foods which will increase the generalisation of the findings.

5.3 SUBJECTS AND METHODS

The LIPGENE human dietary intervention study was a randomised, controlled trial approved by the local ethics committees at each of the 8 clinical intervention centres, all of which conformed to the Helsinki Declaration of 1975 as revised in 1983. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195). The nature of the LIPGENE study was explained to each subject and written informed consent was attained before assessment of anthropometric indices, blood pressure, and collection of a fasting blood sample at the screening visit. The statistical power of the LIPGENE dietary intervention study was based on insulin sensitivity as the primary biomarker of the MetS and was calculated with the ability to detect at least 1 unit difference at a SD of 2, between diets. The power calculation was based on the assumption that 15% difference in insulin sensitivity was physiologically important and that this difference can be detected at a significance level of 0.05 and a power of 0.8. Thus, a minimum of 98 subjects per dietary group would be required to detect significance differences. The group size was increased to 120 per group, to account for an anticipated 20% drop-out rate.

486 free-living subjects with the MetS, defined by a modified version of the NCEP criteria (2001), characterised by three or more of the following criteria, initiated the LIPGENE dietary intervention programme:

- Fasting glucose concentration — 5.5 - 7.0 mmol/L
- Serum TAG concentrations — ≥ 1.5 mmol/L
- Serum HDL cholesterol concentration — < 1.0 mmol/L (men); < 1.3 mmol/L (women)
- Blood pressure — systolic BP≥130; diastolic BP≥85mmHg; **or** treatment of previously diagnosed hypertension
- Waist girth — > 102 cm (men); > 88 cm (women).

Each conformed to the LIPGENE inclusion and exclusion criteria. None of the subjects were receiving pharmacological treatment for glycaemic control, hyperlipidaemic medication, anti-inflammatory treatment or weight loss drugs. Chapter 3 provides a more detailed description of the inclusion and exclusion criteria,

as well as information on the recruitment procedures and screening process. The intervention study was completed in 8 European centres: Trinity College Dublin (TCD), University of Reading, URREADNSI (UoR), University of Oslo (UO), INSERM U476 Marseille (INSERM), Maastricht University (UM), Hospital Universitario Reina Sofía-University of Córdoba (HURS-UCO), University of Krakow (JUMC) and Uppsala University (UU).

5.3.1 Dietary Assessment

Subjects completed a 3-day weighed food dietary record (two weekdays and one weekend day, not necessarily consecutive) to assess pre-intervention habitual dietary intake. The nutrient composition of the subject's diets was assessed using centre specific dietary analysis programmes to ensure a comprehensive assessment of culturally specific foods (Ireland: WISP version 3.0; UK: Food Base version 3.1; Norway: Kostberegningssystem (nutrient calculation software developed at the Department of Nutrition, University of Oslo); France: Nutrilog; The Netherlands: Komeet to enter the dietary data and Orion to analyse the data; Poland: "Dietitian software"; Spain: Dietsource version 2.0; Sweden: MATs connected to the Swedish Board of Food Administration database for foods).

5.3.2 Clinical Investigations

Pre-intervention, subjects reported to one of the eight intervention centres following a 12-hour overnight fast. Subjects abstained from strenuous exercise and alcohol intake for 24 hrs before the examination. Subjects were asked to provide details of their last meal eaten i.e. time and amounts in household quantities.

5.3.3 Collection of fasting blood samples

Fasting blood samples were taken at the first sampling (-5 minutes) of the intravenous glucose tolerance test (IVGTT). Blood for insulin, C-peptide and low density lipoprotein (LDL) isolation was collected in serum tubes (Becton Dickinson, Oxford, UK). Samples for plasma cholesterol, triacylgycerol (TAG), non-esterified fatty acids (NEFA), total high density lipoprotein (HDL), triacylglyceride rich lipoproteins (TRL), plasma apolipoproteins (Apo) A1, B, B48, CII, CIII, E, TRL apoB and for plasma fatty acid analysis were collected in K3-EDTA tubes (Becton Dickinson, Oxford, UK). Samples for glucose analysis were collected in tubes containing sodium

fluoride. Every blood sample for plasma (K3 EDTA, sodium citrate and fluoride oxalate) was inverted 8-10 times to ensure the coagulant was mixed throughout. All samples, except for the serum samples were put on ice after collection and processed within 1 hour. The serum separator tubes were allowed to clot for 30 minutes before centrifugation. Most blood samples were centrifuged and the plasma was harvested and stored at the appropriate temperature. TRL, LDL and HDL fractions were isolated within 24 hours, with the buffy coat isolated within 2 hours. In Chapter 2, *Table 2.4* details the organisation of blood samples for analysis (vaccutainer type, plasma or serum harvested, volume, preparation, analysing centre and temperature storage). *Table 2.5* shows the centrifugation details.

5.3.4 Intravenous Glucose Tolerance Test (IVGTT)

On the morning of the IVGTT two cannulae were inserted into the antecubital veins of both forearms. Following donation of the fasting blood samples from cannula 1, a bolus of 50% glucose solution (Phoenix Pharma Ltd, Gloucester, UK) (0.3g/kg body weight) was infused into cannula 2 over a 1 minute period followed by 20ml of saline. Twenty minutes later a dose of insulin (Novo Nordisk Pharmaceuticals Ltd, West Sussex, UK) (0.03U/kg body weight) was infused into the same cannula (cannula 2). Meanwhile, blood was sampled through cannula 1 at frequent intervals over a 3 hour period (-5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 minutes after the start of the glucose injection). A total of 8ml of blood was collected at each time point (2ml into paediatric fluoride oxalate tubes for subsequent glucose analysis and 6ml into serum tube for subsequent analysis of insulin). At each time point real time blood glucose monitoring was performed using a gluco-trend machine to confirm that the volunteer's blood glucose was not excessively low (<2.0mmol/l). If blood glucose dropped below this level, the IVGTT was discontinued and an isotonic high glucose drink was administered immediately. Blood samples were spun at 3000rpm for 10 minutes after which the plasma/serum was aliquoted and stored at -20°C for future analysis. After analysis, glucose and insulin values at each timepoint were entered into the MINMOD computer programme (version 6.02, Richard N. Bergman) to determine insulin sensitivity (S_I), glucose effectiveness (S_G), acute insulin response to glucose (AIRg), disposition index (DI), removal rate of insulin from the interstitial space (P2), movement of circulating insulin to the interstitial space (P3) and distributed glucose concentration at time 0 minutes (G0) (Bergman *et al.*, 1985; Beard

et al., 1986; Pacini & Bergman, 1986), further described in section 2.7. Homeostasis model assessment-insulin resistance (HOMA-IR) (fasting glucose x fasting insulin/22.5)(Matthews *et al.*, 1985) and quantitative insulin sensitivity check index (QUICKI) (1/(log fasting insulin concentration + log fasting glucose concentration) (Katz *et al.*, 2000) were calculated from fasting data also.

5.3.5 Biochemical Analysis

Serum insulin and C-peptide concentrations were measured by solid-phase, two-site fluoroimmunometric assays (AutoDELFIA Insulin kit and AutoDELFIA C-peptide kit, respectively; Wallac Oy, Turku, Finland) on a 1235 AutoDELFIA automatic immunoassay system (Wallac Oy, Turku, Finland) at the Central Pathology laboratory, St. James's Hospital, Dublin 8, Ireland (Section 2.6.7). The IL Test™ Glucose Hexokinase Clinical Chemistry kit (Instrumentation Laboratories, Warrington, UK) was used to determine plasma glucose concentration (Section 2.6.5).

The TRL fraction was isolated form each patient's fasting blood sample. The preparation of the TRL fraction is described in Section 2.6.1. Total HDL concentrations were determined after precipitation with Quantolip total HDL precipitation Reagent (Immuno Ag, Vienna, Austria) as described in section 2.2.7 and 2.6.3. LDL-cholesterol concentrations were measured after precipitation of LDL using Randox Precipitation Reagent (Randox CH1350, Antrim, Ireland) as outlined in section 2.6.2. The IL Test™ HDL-C Kit (Instrumentation Laboratories, Warrington, UK) was used for direct quantification of HDL-C. Quantification of total cholesterol, LDL cholesterol and TRL cholesterol from defrosted plasma and supernatant samples was performed using the IL Test™ Cholesterol Kit (Instrumentation Laboratories, Warrington, UK) on the ILAB 600 clinical chemistry analyzer (Instrumentation Laboratory, Warrington, UK). Quantification of plasma and TRL TAG was performed using the IL Test™ Triglycerides Kit (Instrumentation Laboratories, Warrington, UK). The WAKO NEFA C enzymatic colour kit (Alpha Laboratories, Hampshire, UK) was used to quantify plasma NEFA concentration. The intensity of coloured product (quinoneimine) is proportional to the concentration of free fatty acids and measured by absorbance using the ILAB. Control 01 and control 02 (Instrumental Laboratories, Warrington, UK) and a NEFA calibrator were used with each analysis (Alpha Laboratories, Hampshire, UK). Plasma apoA1, B and E concentrations were measured using an immunonephelometric methods on a BN Prospec® System with

commercial kits (Dade, Behring, Deerfield, USA) at the Analytic Biochemistry Laboratory, Ste Marguerite Hospital, Marseille, France. Plasma apoCII and CIII concentrations were measured by immuno-turbidimetry on a Hitachi 911 system with commercial kits (Diasys, Bouffemont, France) at the Analytic Biochemistry Laboratory, Ste Marguerite Hospital, Marseille, France. Assessment of apoB-48 was by a competitive ELISA with a specific apoB-48 antibody obtained in rabbits. The assays were performed after a modification of the method (Lovegrove *et al.*, 1996; Lorec *et al.*, 2000).

5.3.6 Fatty acid compositional analysis

Plasma fatty acid composition was measured with the use of gas liquid chromatography. Lipid extraction (Bligh and Dyer Method, Bligh & Dyer, 1959) and transesterfication (OHTA Method) of samples was performed (Section 2.6.8) before analysis of the fatty acid methyl esters (FAME) of plasma fatty acids by gas chromatography (GC) on a Shimadzu GC 2010 (Shimadzu, Japan) using a fused silica capillary column Omegawax 250, (30 m x 0.25 mm inner diameter, 0.25µm film) (Supelco). GC was equipped with a flame ionisation detector and GC solutions software (Shimadzu, Japan). Fatty acids were identified by comparing the relative retention times of fatty acids with a FAME standard (PUFA n-3, PUFA n-2 and C17 (standard)). The fatty acid composition was represented as a relative percentage of the total of the quantified fatty acids (Section 2.6.8).

5.3.7 Anthropometric measurements

Body weight was measured in duplicate on a centre specific, calibrated scale, to the nearest 0.1kg. Subjects were weighed in light clothing, without shoes and after voiding. Height was measured in duplicate to the nearest 0.1cm, in the upright position using a stadiometer. Waist circumference was measured directly on the skin, at the mid-point between the supra iliac crest and lower ribs margin at the end of a normal expiration, in duplicate to the nearest 0.1cm using a non-stretch tape measure. Hip circumference was measured in duplicate to the nearest 0.1cm, using a non-restrictive tape measure, at the level of the greater trochanter without compressing the skin. Blood pressure measurements were recorded in duplicate in the seated position after a 5 minute rest, using an appropriately sized cuff before blood samples were taken. Bioelectric impedance analysis was measured in subjects in the supine position,

using a body fat analyser (TBF-300; Tanita Corporation, Arlington Heights, IL, USA).

5.3.8 Data preparation and statistical analysis

All data is presented as group means ± standard error of the mean unless stated otherwise. Data that was not normally distributed, as assessed by skewness and kurtosis, was normalised by log, square root, inverse or sin transformation.

Descriptive statistics were generated for baseline measures of IVGTT parameters, measures of insulin resistance, anthropometric measurements, lipid and apolipoprotein concentrations, plasma fatty acid concentrations and dietary variables measured from the 3-day weighed food diaries pre-intervention. Independent samples t-test (non-parametric: Mann-Whitney) was used to compare differences between males and females and a one-way ANOVA (non parametric: Kruskal Wallis) was used to compare differences between age-categories, BMI-categories and centre differences. Insulin sensitivity was ranked into three tertiles and a one-way ANOVA (non parametric: Kruskal Wallis) tested statistical differences between the low, medium and high rankings. Quartiles were generated for percentage energy total fat and tertiles for percentage energy saturated and monounsaturated fat. The same statistical tests were applied. The Bonferroni and Tamhane post-hoc tests were used to identify significant differences ($p<0.05$) between the groups as appropriate (Coakes & Steed, 1999). A Bonferroni correction applied if multiple testing occurred in order to account for the increase in type 1 error. Age categories were defined into three groups: 35-49y, 50-64y and 65-70y. BMI categories were also defined into three categories: normal weight (20-24kg/m^2); overweight (25-29kg/m^2) and obese (>30kg/m^2). Correlation analysis was used to describe the strength and direction of the linear relationship between two variables. Pearson product-moment coefficient was used for continuous variables. Spearman rank order correlation was used for variables not normalised by transformation. Interrelationships among the pre-intervention variables were explored (IVGTT with IVGTT; IVGTT with anthropometry; IVGTT with lipids and lipoproteins; IVGTT with plasma fatty acids and IVGTT with dietary factors). All correlations reported below are significant at $p<0.05$.

Linear regression analysis was used to determine the best fitting model to describe the relationship between the dependent variables S_I and HOMA-IR measured

at pre-intervention and a set of explanatory variables. When constructing the complex models with several predictors, care was taken in selecting the type of regression analysis to perform as the predictors included and the way in which they are entered into the model can have a great impact. The forced entry linear regression analysis was chosen as all known and suspected predictors are forced into the model simultaneously based on theory, logic and past research. The stepwise method was not used for this analysis as predictors may be entered (and others removed) based on slight differences in their semi-partial correlations rather than on their theoretical importance (Field, 2005). Three separate regression analysis steps were carried out in the final model development for S_I and HOMA-IR. Firstly, each of the biochemical parameters and dietary variables were entered individually in a univariate regression analysis against the dependent variable measured (Enter method). Secondly, multivariate regression analysis was performed for each of the individual variables, controlling for the volunteer's age, sex and body weight. These models were then examined to identify the variables that would be used in further multiple linear regression modelling. Two approaches were taken to decide which variables to include; variables that were significant ($p<0.1$) in both models were included and variables that were significant ($p<0.1$) in only one of the models for which there was strong literature support for their inclusion. At this early stage the cut-off used to denote significance was $p<0.1$, as the customary cut-off of $p<0.05$ may fail to identify variables that are influential in the final model (Hosmer & Lemeshow, 2000). The explanatory variables to be included were then grouped into six further models: 1. Centre and IVGTT parameters, 2. Anthropometry, 3. Lipid and Lipoproteins, 4. Plasma Fatty Acids, 5. Dietary Variables and 6. Health and Lifestyle data. Multiple linear regression analysis, controlling for the volunteer's age, sex and pre-intervention body weight, was performed for each of the six different models. Finally, the variables that were significant ($p<0.05$) in each of the six different models were included in a final model controlling for age, sex and body weight. The strength of each explanatory variable was determined using the beta co-efficient value. In the final multiple linear regression model significance was taken at $p<0.05$. Multicollinearity and singularity were checked at the end of each model. Tolerance and Variance Inflation Factor (VIF) values were noted to identify highly intercorrelated independent variables that would confound the model. If

multicollinearity was present, one predictor was removed as it restricted the accuracy and predictive power of the model.

In an attempt to discriminate patterns of having 3, 4 or 5 criteria of the MetS defined by the modified version of the NCEP ATPIII criteria, binary logistic regression was used to find the best fitting model to describe the relationship between the dependent dichotomous variable "3 versus 4", "4 versus 5" and 3 versus 5" and a set of explanatory variables for each. IVGTT parameters, measures of insulin sensitivity and insulin resistance, centre, anthropometrics, lipids and apolipoproteins, plasma fatty acids, dietary factors and health and lifestyle variables taken from the database were analysed to see which of these factors predict the number of criteria. The model development was approached using three separate regression analysis steps as done so in the linear regression analysis. Firstly, the independent influence of each of the variables on the binary variable was assessed. Multivariate logistic regression analysis was then performed for each of the individual variables, controlling for the subject's sex, age and body weight. These models were then examined to identify the variables that would be used in further multiple binary logistic regression modelling. The models were then examined to identify significance ($p<0.02$) (Hosmer & Lemeshow, 2000). The variables significant in either or both models were then grouped into six further models: 1 Centre and IVGTT parameters, 2. anthropometry, 3. Lipids and lipoproteins, 4. Plasma fatty acids, 5. Dietary variables and 6. Health and lifestyle factors. Multiple binary logistic regression analysis, controlling for the volunteer's age, sex and body weight was performed for the six models. Finally, the variables that were significant ($p<0.05$) in each of the six separate models were included in a final model controlling for the individual's sex, age and body weight. In the final model, significance was taken at $p<0.05$. The model fit for the data was assessed by measuring the -2 log likelihood (-2LL) ratio. Smaller -2LL values indicate better model fit.

All statistical analysis was performed using SPSS® for Windows™ version 14.0 (SPSS Inc, Chicago, Ill).

5.4 RESULTS

5.4.1 Characteristics of the cohort at baseline

Baseline characteristics were determined for 486 volunteers who donated a pre-fasting blood sample or an IVGTT or both at the pre-intervention clinical investigation day.

Table 5.1 demonstrates the characteristics of the group as a whole, whose mean age was 54.58y. Looking at the criteria of the MetS, mean fasting glucose at pre-intervention was 6.0mmol/L, TAG and HDL cholesterol were 1.80mmol/L and 1.10mmol/L, respectively. Waist circumference was 106.31cm and blood pressure, 138/86mmHg. BMI for the cohort was 32.36kg/m^2. Pre-intervention insulin sensitivity (S_I) and HOMA-IR was 2.849 (mU/L)-1min-1 and 2.673, respectively.

Plasma palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2:6), total SFA, MUFA, PUFA, n-6 PUFA and n-3 PUFA were 26.48%, 26.41%, 27.46%, 32.54%, 28.02%, 39.44%, 35.44% and 4%, respectively.

Table 5.2 illustrates the differences in the characteristics between males (n= 220, 45.3%) and females (n=266, 54.7%), at baseline. Females were significantly older, had a greater hip circumference, body fat (% and kg) and impedance values ($p<0.05$). Females also had greater insulin sensitivity at baseline. Total cholesterol, NEFA, LDL- and HDL-cholesterol, apoA1, apoCIII and apoE concentrations were greater in females whilst TRL TAG and TRL cholesterol were lower in females compared to males, pre-intervention ($p<0.05$). Males were significantly taller, heavier, had a greater waist circumference and diastolic blood pressure, lean body mass (% and kg) and body water (% and L) ($p<0.05$). Males had a significantly greater acute insulin response to glucose (AIRg), revised QUICKI and C-peptide concentrations. Males had significantly greater concentrations of plasma myristic acid (C14:0), palmitic acid (C16:0), SFA and lower concentrations of palmitoleic acid (C16:1), stearic acid (C18:0) and plasma EPA (C20:5:3), compared to females ($p<0.05$). With respect to dietary information generated, males had a greater BMR, higher total energy and dietary fat intakes including SFA, MUFA, PUFA and *trans* fatty acids and also had greater intakes of CHO and alcohol compared to females ($p<0.05$).

Table 5.3 highlights the differences in the metabolic markers associated with the MetS across three age categories; 35-49y, 50-64y and 65-70y. The younger age group (35-49y) were significantly heavier, had a greater BMI, greater waist circumference, lean body mass (% and kg) and body water (% and L), $p<0.05$. With

the IVGTT parameters, AIRg and DI were significantly higher in the 35-49 age group compared to the other age groups, $p<0.05$. There were no differences in insulin sensitivity across the three age-categories. From the dietary variables exported BMR, total energy, total fat (g), SFA (g), CHO (g) and Protein (g) were significantly higher in the 35-49 year olds than volunteers aged between 50-64y and 65-70y whilst MUFA (g) was significantly higher in the younger age group compared to the oldest age category only, $p<0.05$. The older age groups (50-64y and 65-70y) were significantly smaller, had higher systolic blood pressure, and higher impedance values, $p<0.05$. Interestingly, fasting plasma glucose was also highest in the older age groups ($p<0.05$). Compared to the oldest age group the 35-49y and the 50-64 year age categories had a greater hip circumference, fasting insulin and HOMA-IR was significantly higher, QUICKI and Revised QUICKI were lower and plasma concentrations of EPA (C20:5:3) were also lower, $p<0.05$. The 50-6y age group had higher cholesterol, HDL-cholesterol, apoA1 and apoB as compared to those aged between 35-49y. The plasma concentrations of myristic acid (C14:0) was lower in this age group as compared to the younger age-category, $p<0.05$.

The volunteers were stratified according to their BMI status at baseline into one of three groups: normal weight (20-24kg/m^2), overweight (25-29kg/m^2) and obese (>30kg/m^2). A one way ANOVA demonstrated significant differences between the groups for anthropometric and biochemical markers (*Table 5.4*). Obese volunteers were significantly older, had the greatest body fat (%), the lowest lean body mass (% and kg), greater acute insulin response to glucose (AIRg), lower P3 (movement of circulating insulin to the interstitial space), C-peptide and insulin to C-peptide was significantly higher, as compared to the normal weight and overweight categories, $p<0.05$. Compared to the obese volunteers, the overweight volunteers were statistically taller, had significantly higher impedance values and lower G0 (distributed glucose at time 0 minutes). HOMA-IR was considerably lower in the overweight volunteers as compared to the obese subjects, $p<0.05$. The normal weight and overweight volunteers also had lower intakes of total fat (% energy), MUFA (% energy), dietary palmitoleic acid (C16:1 g/day), oleic acid (C18:1 g/day) and alcohol (g/day) but statistically lower in the overweight subjects as compared to the obese, $p<0.05$. The normal weight and overweight subjects had statistically significant and obviously lower body weights, BMI, waist and hip circumference, considerable lower

fasting insulin values and higher QUICKI and Revised QUICKI values. BMR was also statistically lower as compared to the obese volunteers, $p<0.05$.

There were many differences in the baseline anthropometrics, IVGTT parameters, measures of insulin resistance, lipids and lipoprotein concentrations, plasma fatty acids and dietary measures between the 8 European centres as demonstrated in *Table 5.5*. Some of these differences were as a result of the different recruitment strategies employed at the screening stage. UU volunteers were significantly older compared to volunteers in TCD, UoR, INSERM and HURS-UCO with the TCD volunteers being the youngest, $p<0.05$. JUMC subjects were significantly heavier compared to UU volunteers and HURS-UCO participants had the greatest BMI compared to subjects in UoR, UO, INSERM, UM and UU who had the lowest BMI index, $p<0.05$. UM volunteers had the greatest waist circumference compared to Uppsala, $p<0.05$. Both systolic and diastolic blood pressure were significantly higher in the JUMC cohort and significantly higher compared to INSERM, $p<0.05$. Interestingly, there were no significant differences in insulin sensitivity, AIRg and P2 (removal rate of insulin from the interstitial space) between the centres. Fasting glucose, however, was significantly higher in the HURS-UCO cohort compared to TCD, UoR, INSERM, UM and JUMC, $p<0.05$. Fasting insulin was highest in the JUMC cohort and significantly higher than UU, $p<0.05$. HOMA-IR was also significantly higher in the HURS-UCO volunteers but only significantly higher compared to UU volunteers, $p<0.05$. Accordingly, UU had the highest QUICKI values and significantly higher than UoR, UO, UM, HURS-UCO and JUMC cohorts, $p<0.05$. Triglyceride and HDL-cholesterol concentrations were highest in the UoR and UU cohorts, respectively, $p<0.05$. Body weight, BMI, waist circumference, blood pressure and lipid concentrations were targets at the screening stage to recruit volunteers onto the LIPGENE Intervention study, through weight management clinics, blood pressure clinics and lipid clinics etc. Of the plasma fatty acid concentrations, UU had the highest plasma oleic acid (C18:1) and plasma MUFA concentrations, significantly higher than plasma concentrations in TCD, UO and UM, $p<0.05$. Plasma EPA (C20:5:3) and DHA (C22:6:3) were highest in the UO centre as was plasma PUFA and plasma n-3 PUFA, $p<0.05$. Plasma SFA was highest in the UM cohort, significantly higher than UO, INSERM, HURS-UCO, JUMC and UU cohorts, $p<0.05$. Plasma n-6 PUFA was significantly higher in INSERM volunteers compared to UoR and UU centres, $p<0.05$. From the dietary information exported and

analysed from the dietary programmes, total energy (MJ/day) was highest in the TCD cohort, and significantly higher than energy intakes in INSERM, JUMC and UU cohorts, $p<0.05$. Intakes of total fat (% Energy) and MUFA (% Energy) were significantly higher in the HURS-UCO cohort compared to the other seven centres, $p<0.05$. UO had the highest intakes of SFA (% Energy) compared to UoR and HURS-UCO cohorts, $p<0.05$. Intake of PUFA (%Energy) was highest in the UM cohort compared to all other centres, except UO and JUMC, $p<0.05$. Dietary n-3 PUFA g/day, EPA (C20:5:3 g/day) and DHA (C22:6:3 g/day) were significantly higher in the UO cohort which showed good agreement with the plasma fatty acid concentrations.

Table 5.1: Baseline characteristics of the LIPGENE Cohort n=486

Anthropometry	n	Mean	SEM
Age(y)	486	54.58	0.406
Height(m)	485	1.68	0.004
BodyWeight(kg)	485	91.63	0.649
BMI (kg/m^2)	485	32.36	0.190
Waist circumference(cm)	485	106.31	0.500
Hip circumference (cm)	485	112.89	0.455
Diastolic BP(mmHg)	484	86.18	0.456
Systolic BP(mmHg)	484	138.47	0.716
Body Fat (%)	464	36.55	0.404
Body Fat(kg)	464	33.27	0.428
Lean Body Mass(%)	464	61.80	0.446
Lean Mass Body(kg)	464	56.65	0.636
Body Water(%)	398	44.64	0.346
Body Water (L)	380	40.89	0.523
Impedance(KHz)	339	520.82	5.076
IVGTT Parameters	n	Mean	SEM
$S_I (mU/L)^{-1}min-1$	432	2.849	0.083
$S_G min-1$	434	0.016	0.000
AIRg mUL^{-1} min^{-1}	434	358.178	16.170
DI	434	879.701	34.286
G0 mg/dl^{-1}	434	298.308	1.927
P2 min^{-1}	434	0.044	0.001
P3 (mU/L) min^{-2}	434	0.000013	0.000001
Fasting Glucose (mmol/l)	475	5.956	0.039
Fasting Insulin(ulU/ml)	475	10.106	0.253
HOMA-IR	480	2.673	0.074
QUICKI	475	0.598	0.017
Revised QUICKI	474	0.225	0.001
C-Peptide ng/ml	472	2.638	0.041
Insulin to C-peptide Ratio	472	3.801	0.070
Lipids and Lipoproteins	n	Mean	SEM
TAG (mmol/L)	484	1.80	0.04
Cholesterol (mmol/L)	484	5.34	0.04
NEFA (mmol/L)	484	616.55	10.49
TRL TAG (mmol/L)	476	0.88	0.03
TRL Cholesterol (mmol/L)	476	0.39	0.01
LDL Cholesterol (mmol/L)	479	3.27	0.05
HDL (mmol/L)	484	1.10	0.01
Apo A1 (g/L)	475	1.40	0.01
Apo B (g/L)	475	1.02	0.01
Apo CII (mg/L)	474	45.59	0.81
Apo CIII (mg/L)	474	157.98	2.37
Apo E (mg/L)	473	41.57	0.76
Apo B48 (mg/L)	374	0.79	0.04
TRL ApoB (mg/L)	461	50.38	2.10
Plasma Fatty Acids (w/w%)	n	Mean	SEM
C14:0	475	1.90	0.04
C16:0	475	26.48	0.22
C16:1	475	1.39	0.05
C18:0	475	4.16	0.10
C18:1	475	26.41	0.20
C18:2:6	475	27.46	0.25
C20:4:6	475	6.88	0.11
C20:5:3	475	0.97	0.04
C22:6:3	475	2.19	0.06
SFA	475	32.54	0.29
MUFA	475	28.02	0.22
PUFA	475	39.44	0.32
n-6 PUFA	475	35.44	0.29
n-3 PUFA	475	4.00	0.09
Dietary Measures	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	451	7.20	0.05
Total Energy (MJ)	453	8.81	0.12
Total fat (g/day)	453	85.55	1.47
Total fat (%Energy)	453	36.12	0.35
SFA (g/day)	453	29.57	0.58
SFA (%Energy)	453	12.43	0.15
MUFA (g/day)	453	31.85	0.66
MUFA (%Energy)	453	13.54	0.23
PUFA (g/day)	453	13.51	0.34
PUFA (%Energy)	453	5.66	0.10
n-3 (g/day)	314	1.50	0.07
n-6 (g/day)	314	9.39	0.34
C8 (g/day)	176	0.22	0.01
C10 (g/day)	237	0.53	0.02
C12 (g/day)	283	1.11	0.05
C14 (g/day)	360	2.53	0.09
C16 (g/day)	360	14.06	0.32
C16:1 (g/day)	360	1.33	0.04
C18 (g/day)	360	6.52	0.20
C18:1 (g/day)	360	26.23	0.75
C18:2:6 (g/day)	419	9.91	0.30
C18:3:3 (g/day)	419	1.16	0.04
C20:4:6 (g/day)	237	0.12	0.01
C20:5:3 (g/day)	373	0.14	0.01
C22:6:3 (g/day)	373	0.22	0.02
Trans fatty acids (g/day)	204	2.48	0.14
CHO (g/day)	453	234.32	3.85
CHO (%Energy)	453	43.56	0.36
Starch (g/day)	245	142.01	3.60
Total Sugars (g/day)	260	102.72	3.13
Protein (g/day)	453	90.97	1.38
Protein (%Energy)	453	17.71	0.18
Alcohol (g/day)	453	8.74	0.67
Alcohol (%Energy)	453	2.62	0.19
Fibre (g)	443	19.48	0.39
Vitamin E (mg/day)	453	10.21	0.25

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

Table 5.2: Baseline characteristics of male and females volunteers of the LIPGENE Dietary Intervention Study (pre-intervention clincial investigation day, n=486)

	Male			Female			
Anthropometry	n	Mean	SEM	n	Mean	SEM	
Age(y)	220	53.41	0.66	266	55.54	0.49	*
Height(m)	219	1.76	0.00	266	1.62	0.00	*
BodyWeight(kg)	219	99.15	0.92	266	85.45	0.71	*
BMI (kg/m^2)	219	31.96	0.26	266	32.70	0.27	
Waist circumference(cm)	219	110.83	0.67	266	102.59	0.64	*
Hip circumference (cm)	219	109.97	0.61	266	115.30	0.62	*
Diastolic BP(mmHg)	219	87.85	0.69	265	84.80	0.59	*
Systolic BP(mmHg)	219	138.89	1.01	265	138.12	1.01	
Body Fat (%)	207	29.89	0.39	257	41.91	0.42	*
Body Fat(kg)	207	29.91	0.59	257	35.98	0.55	*
Lean Body Mass(%)	207	69.29	0.44	257	55.77	0.45	*
Lean Mass Body(kg)	207	68.29	0.71	257	47.28	0.48	*
Body Water(%)	165	50.14	0.38	233	40.75	0.35	*
Body Water (L)	160	49.82	0.64	220	34.39	0.38	*
Impedance(KHz)	170	474.28	5.17	169	567.63	7.14	*
	Male			**Female**			
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	
S_I (mU/L)$^{-1}$min-1	202	2.424	0.094	230	3.222	0.128	*
S_G min-1	203	0.015	0.000	231	0.016	0.000	
AIRg mUL^{-1} min^{-1}	203	400.598	28.143	231	320.900	17.340	*
DI	203	875.192	52.943	231	883.663	44.660	
G0 mg/dl^{-1}	203	295.659	2.467	231	300.635	2.895	
P2 min^{-1}	203	0.041	0.002	231	0.046	0.002	*
P3 (mU/L) min^{-2}	203	0.000010	0.000001	231	0.000015	0.000001	*
Fasting Glucose (mmol/L)	218	6.025	0.059	257	5.897	0.052	
Fasting Insulin(uIU/ml)	218	10.207	0.357	257	10.021	0.357	
HOMA-IR	219	2.734	0.105	261	2.622	0.103	
QUICKI	218	0.594	0.037	257	0.602	0.007	
Revised QUICKI	218	0.227	0.001	256	0.223	0.001	*
C-Peptide ng/ml	218	2.737	0.060	254	2.554	0.056	*
Insulin to C-peptide Ratio	218	3.712	0.107	254	3.877	0.093	
	Male			**Female**			
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	
TAG (mmol/L)	219	1.85	0.07	265	1.75	0.05	
Cholesterol (mmol/L)	219	5.08	0.06	265	5.56	0.06	*
NEFA (mmol/L)	219	557.84	15.39	265	665.07	13.65	*
TRL TAG (mmol/L)	215	0.95	0.04	261	0.82	0.04	*
TRL Cholesterol (mmol/L)	215	0.42	0.02	261	0.38	0.02	*
LDL Cholesterol (mmol/L)	217	3.11	0.07	262	3.39	0.07	*
HDL (mmol/L)	219	0.98	0.02	265	1.20	0.02	*
Apo A1 (g/L)	216	1.31	0.01	259	1.47	0.02	*
Apo B (g/L)	216	1.00	0.02	259	1.04	0.01	
Apo CII (mg/L)	215	45.18	1.28	259	45.94	1.04	
Apo CIII (mg/L)	215	151.06	3.43	259	163.72	3.23	*
Apo E (mg/L)	216	39.68	1.04	257	43.16	1.07	*
Apo B48 (mg/L)	167	0.73	0.06	207	0.83	0.06	
TRL ApoB (mg/L)	209	56.23	3.13	252	45.54	2.81	*
	Male			**Female**			
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	
C14:0	214	2.00	0.07	261	1.82	0.06	*
C16:0	214	27.22	0.35	261	25.87	0.28	*
C16:1	214	1.20	0.06	261	1.55	0.07	*
C18:0	214	4.07	0.15	261	4.24	0.12	
C18:1	214	26.22	0.31	261	26.56	0.26	
C18:2:6	214	27.15	0.38	261	27.71	0.33	
C20:4:6	214	6.98	0.18	261	6.79	0.14	
C20:5:3	214	0.90	0.06	261	1.03	0.06	*
C22:6:3	214	2.12	0.09	261	2.25	0.07	
SFA	214	33.29	0.45	261	31.93	0.37	*
MUFA	214	27.66	0.33	261	28.31	0.29	
PUFA	214	39.05	0.49	261	39.77	0.41	
n-6 PUFA	214	35.17	0.44	261	35.66	0.38	
n-3 PUFA	214	3.88	0.14	261	4.11	0.12	
	Male			**Female**			
Dietary Measures	n	Mean	SEM	n	Mean	SEM	
Basal Metabolic Rate (MJ/day)	210	8.20	0.06	241	6.34	0.03	*
Total Energy (MJ)	210	10.10	0.18	243	7.70	0.12	*
Total fat (g/day)	210	97.05	2.34	243	75.62	1.59	*
Total fat (%Energy)	210	35.76	0.52	243	36.43	0.46	
SAFA (g/day)	210	33.96	0.96	243	25.78	0.61	*
SAFA (%Energy)	210	12.39	0.21	243	12.46	0.21	
MUFA (g/day)	210	35.52	1.02	243	28.67	0.82	*
MUFA (%Energy)	210	13.20	0.32	243	13.84	0.33	
PUFA (g/day)	210	15.85	0.54	243	11.50	0.37	*
PUFA (%Energy)	210	5.85	0.16	243	5.48	0.13	
n-3 (g/day)	138	1.71	0.12	176	1.33	0.07	*
n-6 (g/day)	138	10.45	0.53	176	8.56	0.42	*
C8 (g/day)	79	0.25	0.02	97	0.19	0.01	
C10 (g/day)	108	0.59	0.04	129	0.48	0.03	
C12 (g/day)	118	1.19	0.09	165	1.05	0.07	
C14 (g/day)	148	2.85	0.14	212	2.31	0.11	*
C16 (g/day)	148	16.21	0.54	212	12.56	0.36	*
C16:1 (g/day)	148	1.51	0.06	212	1.21	0.05	*
C18 (g/day)	148	7.61	0.31	212	5.76	0.25	*
C18:1 (g/day)	148	29.47	1.24	212	23.96	0.91	*
C18:2:6 (g/day)	190	11.62	0.49	229	8.50	0.35	*
C18:3:3 (g/day)	190	1.31	0.07	229	1.04	0.04	*
C20:4:6 (g/day)	108	0.14	0.01	129	0.10	0.01	*
C20:5:3 (g/day)	180	0.15	0.02	193	0.13	0.01	
C22:6:3 (g/day)	180	0.22	0.03	193	0.21	0.02	
Trans fatty acids (g/day)	114	2.72	0.21	90	2.17	0.18	*
CHO (g/day)	210	263.30	5.93	243	209.27	4.44	*
CHO (%Energy)	210	42.74	0.52	243	44.27	0.48	*
Starch (g/day)	128	164.74	4.87	117	117.13	4.29	*
Total Sugars (g/day)	131	109.55	4.66	129	95.79	4.09	*
Protein (g/day)	210	103.54	2.26	243	80.11	1.33	*
Protein (%Energy)	210	17.53	0.28	243	17.87	0.24	
Alcohol (g/day)	210	14.39	1.26	243	3.87	0.43	*
Alcohol (%Energy)	210	3.97	0.34	243	1.46	0.16	*
Fibre (g)	203	20.75	0.60	240	18.40	0.49	*
Vitamin E (mg/day)	210	11.04	0.42	243	9.50	0.29	*

** p value <0.05 independent samples t test*

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

Table 5.3: Baseline characteristics of volunteers in three different age categories that participated in LIPGENE pre-intervention clinical investigation day n=486

	35-49y			50-64y			65-70y		
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Height(m)	130	1.71 a	0.01	290	1.67 b	0.01	65	1.68 b	0.01
BodyWeight(kg)	130	98.33 a	1.30	290	90.06 b	0.78	65	85.29 c	1.60
BMI (kg/m^2)	130	33.41 a	0.35	290	32.35 b	0.25	65	30.35 c	0.49
Waist circumference(cm)	130	108.29 a	0.97	290	105.98 ab	0.65	65	103.83 b	1.21
Hip circumference (cm)	130	114.72 a	0.87	290	112.93 a	0.60	65	109.06 b	0.99
Diastolic BP(mmHg)	130	86.06	1.01	289	86.26	0.57	65	86.05	1.01
Systolic BP(mmHg)	130	134.25 a	1.32	289	138.74 b	0.91	65	145.68 c	1.92
Body Fat (%)	125	34.98	0.72	276	37.13	0.53	63	37.16	1.12
Body Fat(kg)	125	34.26	0.82	276	33.26	0.57	63	31.35	1.02
Lean Body Mass(%)	125	65.09 a	0.74	276	60.58 b	0.59	63	60.65 b	1.22
Lean Mass Body(kg)	125	63.94 a	1.17	276	54.48 b	0.78	63	51.71 b	1.60
Body Water(%)	111	47.18 a	0.55	234	43.68 b	0.47	53	43.58 b	0.91
Body Water (L)	99	47.18 a	1.00	230	39.17 b	0.63	51	36.42 b	1.14
Impedance(KHz)	97	494.39 a	6.99	199	530.02 b	6.73	43	537.85 b	18.48
	35-49y			**50-64y**			**65-70y**		
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I (mU/L)$^{-1}$min-1	118	2.59	0.13	257	2.97	0.12	57	2.84	0.21
$S_G$min-1	118	0.016	0.001	259	0.016	0.000	57	0.015	0.001
AIRg mUL^{-1} min^{-1}	118	513.26 a	43.33	259	296.31 b	15.33	57	318.23 b	33.57
DI	118	1130.42 a	74.22	259	778.11 b	41.01	57	822.26 b	83.41
G0 mg/dl^{-1}	118	295.62	4.27	259	300.82	2.36	57	292.48	4.70
P2 min^{-1}	118	0.046 a	0.002	259	0.043 b	0.002	57	0.043 ab	0.005
P3 (mU/L) min^{-2}	118	0.0000125	0.0000010	259	0.0000130	0.0000007	57	0.0000123	0.0000015
Fasting Glucose (mmol/L)	127	5.68 a	0.07	284	6.07 b	0.05	64	5.99 b	0.10
Fasting Insulin(ulU/ml)	127	11.34 a	0.50	284	10.04 a	0.33	64	7.94 b	0.54
HOMA-IR	129	2.82 a	0.14	287	2.72 a	0.10	64	2.14 b	0.16
QUICKI	127	0.58 a	0.01	284	0.60 a	0.03	64	0.64 b	0.01
Revised QUICKI	127	0.22 a	0.001	283	0.23 a	0.001	64	0.23 b	0.002
C-Peptide ng/ml	127	2.63	0.07	281	2.66	0.05	64	2.56	0.12
Insulin to C-peptide Ratio	127	4.30 a	0.15	281	3.75 b	0.09	64	3.03 c	0.12
	35-49y			**50-64y**			**65-70y**		
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	130	1.90	0.09	289	1.77	0.05	65	1.73	0.10
Cholesterol (mmol/L)	130	5.12 a	0.08	289	5.47 b	0.06	65	5.22 ab	0.11
NEFA (mmol/L)	130	610.63	20.60	289	614.23	13.42	65	638.68	29.33
TRL TAG (mmol/L)	129	0.97	0.06	282	0.84	0.03	65	0.85	0.07
TRL Cholesterol (mmol/L)	129	0.42	0.03	282	0.37	0.02	65	0.44	0.05
LDL Cholesterol (mmol/L)	129	3.14	0.09	286	3.35	0.06	64	3.13	0.13
HDL (mmol/L)	130	1.03 a	0.02	289	1.13 b	0.02	65	1.12 ab	0.04
Apo A1 (g/L)	128	1.34 a	0.02	282	1.42 b	0.02	65	1.38 ab	0.03
Apo B (g/L)	128	0.98 a	0.02	282	1.04 b	0.01	65	0.99 ab	0.02
Apo CII (mg/L)	127	44.78	1.59	282	46.19	1.05	65	44.59	2.17
Apo CIII (mg/L)	127	154.21	4.57	282	158.87	3.07	65	161.45	6.45
Apo E (mg/L)	128	41.25	1.51	280	40.76	0.74	65	45.69	3.33
Apo B48 (mg/L)	100	0.79	0.09	224	0.80	0.06	50	0.74	0.09
TRL ApoB (mg/L)	128	51.22	3.84	269	49.15	2.84	64	53.87	5.39
	35-49y			**50-64y**			**65-70y**		
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	126	2.08 a	0.09	285	1.83 b	0.06	64	1.90 ab	0.12
C16:0	126	27.07	0.47	285	26.28	0.28	64	26.21	0.52
C16:1	126	1.39	0.09	285	1.37	0.06	64	1.49	0.14
C18:0	126	4.23	0.21	285	4.23	0.11	64	3.73	0.26
C18:1	126	26.45	0.37	285	26.19	0.25	64	27.27	0.62
C18:2:6	126	26.69	0.48	285	27.83	0.32	64	27.33	0.62
C20:4:6	126	7.07	0.25	285	6.92	0.13	64	6.32	0.26
C20:5:3	126	0.81 a	0.08	285	0.98 a	0.05	64	1.22 b	0.14
C22:6:3	126	2.03	0.11	285	2.24	0.07	64	2.31	0.14
SFA	126	33.38	0.64	285	32.33	0.36	64	31.84	0.71
MUFA	126	28.07	0.41	285	27.77	0.27	64	29.02	0.67
PUFA	126	38.55	0.68	285	39.91	0.40	64	39.15	0.72
n-6 PUFA	126	34.86	0.60	285	35.87	0.37	64	34.64	0.66
n-3 PUFA	126	3.68 a	0.19	285	4.03 ab	0.12	64	4.50 b	0.25
	35-49y			**50-64y**			**65-70y**		
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	118	7.93 a	0.11	268	7.01 b	0.06	65	6.67 b	0.12
Total Energy (MJ)	119	9.82 a	0.27	269	8.46 b	0.15	65	8.40 b	0.25
Total fat (g/day)	119	94.56 a	3.37	269	83.15 b	1.76	65	79.01 b	3.23
Total fat (%Energy)	119	35.80	0.68	269	36.56	0.45	65	34.85	0.88
SAFA (g/day)	119	32.75 a	1.38	269	28.75 b	0.71	65	27.17 b	1.15
SAFA (%Energy)	119	12.26	0.29	269	12.60	0.20	65	12.06	0.38
MUFA (g/day)	119	34.33 a	1.42	269	31.45 ab	0.85	65	28.94 b	1.44
MUFA (%Energy)	119	13.04	0.41	269	13.93	0.32	65	12.86	0.52
PUFA (g/day)	119	14.87	0.76	269	12.95	0.40	65	13.35	0.87
PUFA (%Energy)	119	5.58	0.21	269	5.63	0.13	65	5.89	0.32
n-3 (g/day)	95	1.44	0.13	186	1.52	0.08	33	1.53	0.20
n-6 (g/day)	95	9.61	0.72	186	9.27	0.40	33	9.44	0.94
C8 (g/day)	66	0.22	0.02	93	0.22	0.02	17	0.15	0.02
C10 (g/day)	81	0.54	0.05	133	0.53	0.03	23	0.47	0.07
C12 (g/day)	86	1.04	0.10	157	1.12	0.07	40	1.21	0.12
C14 (g/day)	100	2.80	0.21	210	2.44	0.10	50	2.41	0.15
C16 (g/day)	100	14.93	0.74	210	13.78	0.39	50	13.54	0.68
C16:1 (g/day)	100	1.41	0.09	210	1.29	0.04	50	1.35	0.09
C18 (g/day)	100	7.60	0.55	210	6.16	0.20	50	5.88	0.33
C18:1 (g/day)	100	25.51	1.51	210	26.74	0.99	50	25.54	1.73
C18:2:6 (g/day)	109	10.07	0.66	248	9.75	0.37	62	10.32	0.79
C18:3:3 (g/day)	109	1.18	0.10	248	1.15	0.05	62	1.20	0.09
C20:4:6 (g/day)	81	0.12	0.01	133	0.12	0.01	23	0.14	0.02
C20:5:3 (g/day)	104	0.11	0.02	224	0.14	0.01	45	0.17	0.04
C22:6:3 (g/day)	104	0.18	0.03	224	0.23	0.02	45	0.23	0.04
Trans fatty acids (g/day)	65	2.48	0.23	110	2.64	0.21	29	1.86	0.24
CHO (g/day)	119	264.88 a	8.57	269	221.58 b	4.64	65	231.08 b	8.36
CHO (%Energy)	119	43.75 ab	0.73	269	42.96 a	0.45	65	45.72 b	0.90
Starch (g/day)	78	153.43	7.40	138	135.79	4.36	29	140.84	9.32
Total Sugars (g/day)	73	109.65	6.80	141	98.36	4.01	46	105.12	6.67
Protein (g/day)	119	100.79 a	3.12	269	88.07 b	1.69	65	84.99 b	2.76
Protein (%Energy)	119	17.56	0.37	269	17.88	0.24	65	17.27	0.42
Alcohol (g/day)	119	9.94	1.59	269	8.71	0.84	65	6.68	1.16
Alcohol (%Energy)	119	2.82	0.44	269	2.66	0.24	65	2.09	0.35
Fibre (g)	116	18.42	0.82	262	19.96	0.50	65	19.44	0.83
Vitamin E (mg/day)	119	9.82	0.48	269	10.41	0.33	65	10.09	0.62

[abc] denotes significance across age categories (when two superscripts contain the same letter there is no significant difference between two of the three age categories) p<0.05, One Way ANOVA, p<0.017 when bonferroni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Table 5.4: Baseline characteristics of volunteers across three BMI categories that participated in LIPGENE pre-intervention clinical investigation day n=486

	20-24Kg/m^2			25-29Kg/m2			>30Kg/m2		
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	13	62.06 a	1.92	135	56.68 a	0.76	337	53.43 b	0.48
Height(m)	13	1.67 ab	0.02	135	1.70 a	0.01	337	1.68 b	0.01
BodyWeight(kg)	13	65.64 a	1.94	135	81.03 b	0.83	337	96.88 c	0.68
BMI (kg/m^2)	13	23.50 a	0.30	135	27.90 b	0.11	337	34.49 c	0.16
Waist circumference(cm)	13	94.96 a	6.73	135	97.85 b	0.60	337	110.14 c	0.50
Hip circumference (cm)	13	97.19 a	1.44	135	104.78 b	0.63	337	116.75 c	0.46
Diastolic BP(mmHg)	13	78.88 a	1.54	135	85.22 ab	0.77	336	86.85 b	0.57
Systolic BP(mmHg)	13	131.77	4.31	135	137.85	1.35	336	138.97	0.86
Body Fat (%)	13	31.78 a	2.40	128	33.66 a	0.85	323	37.89 b	0.44
Body Fat(kg)	13	20.84 a	1.58	128	26.78 b	0.62	323	36.35 c	0.46
Lean Body Mass(%)	13	68.24 a	2.39	128	64.40 a	0.87	323	60.52 b	0.51
Lean Mass Body(kg)	13	44.95 a	2.27	128	52.20 a	1.04	323	58.88 b	0.78
Body Water(%)	10	50.49 a	2.04	106	46.52 a	0.69	282	43.73 b	0.40
Body Water (L)	11	33.58 a	1.97	104	37.72 a	0.83	265	42.43 b	0.65
Impedance(KHz)	9	566.54 ab	27.31	98	568.22 a	11.52	232	499.03 b	4.90
	20-24Kg/m^2			25-29Kg/m2			>30Kg/m2		
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I (mU/L)$^{-1}$min-1	10	4.94	1.18	120	3.14	0.15	302	2.66	0.09
$S_G$min-1	10	0.02	0.0018	121	0.02	0.0006	303	0.02	0.0004
AIRg mUL^{-1} min^{-1}	10	148.21 a	28.61	121	299.09 a	22.76	303	388.70 b	21.01
DI	10	818.64	287.63	121	879.85	65.96	303	881.66	40.52
G0 mg/dl^{-1}	10	281.56 ab	9.71	121	288.14 a	3.08	303	302.92 b	2.41
P2 min^{-1}	10	0.0424	0.0041	121	0.0488	0.0032	303	0.0417	0.0016
P3 (mU/L) min^{-2}	10	0.000020 a	0.000005	121	0.000015 a	0.000001	303	0.000012 b	0.000001
Fasting Glucose (mmol/L)	12	5.82	0.24	133	5.91	0.07	330	5.98	0.05
Fasting Insulin(uIU/ml)	12	5.22 a	0.88	133	7.51 b	0.27	330	11.33 c	0.32
HOMA-IR	12	1.38 ab	0.26	135	1.95 a	0.08	332	3.02 b	0.09
QUICKI	12	0.73 a	0.04	133	0.64 b	0.01	330	0.58 c	0.02
Revised QUICKI	12	0.24 a	0.004	133	0.23 b	0.001	329	0.22 c	0.001
C-Peptide ng/ml	12	1.82 a	0.22	132	2.33 a	0.06	328	2.79 b	0.05
Insulin to C-peptide Ratio	12	2.77 a	0.18	132	3.26 a	0.09	328	4.06 b	0.09
	20-24Kg/m^2			25-29Kg/m2			>30Kg/m2		
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	13	1.79	0.19	135	1.83	0.09	335	1.78	0.05
Cholesterol (mmol/L)	13	5.24 ab	0.24	135	5.51 a	0.08	335	5.28 b	0.05
NEFA (mmol/L)	13	545.37	30.59	135	618.59	20.35	335	618.38	12.69
TRL TAG (mmol/L)	12	0.86	0.14	132	0.89	0.06	331	0.88	0.03
TRL Cholesterol (mmol/L)	12	0.45	0.07	132	0.44	0.03	331	0.37	0.02
LDL Cholesterol (mmol/L)	12	3.03	0.30	135	3.41	0.09	331	3.21	0.06
HDL (mmol/L)	13	1.17	0.07	135	1.11	0.02	335	1.09	0.02
Apo A1 (g/L)	13	1.37	0.07	132	1.36	0.02	329	1.41	0.01
Apo B (g/L)	13	0.96	0.07	132	1.04	0.02	329	1.01	0.01
Apo CII (mg/L)	13	43.49	3.54	132	47.95	1.57	328	44.71	0.97
Apo CIII (mg/L)	13	149.10	12.54	132	163.64	4.70	328	156.03	2.81
Apo E (mg/L)	13	43.98	5.27	132	41.14	1.38	327	41.65	0.92
Apo B48 (mg/L)	12	0.92	0.23	105	0.71	0.07	256	0.81	0.05
TRL ApoB (mg/L)	12	58.63	10.78	129	59.17	4.90	319	46.31	2.24
	20-24Kg/m^2			25-29Kg/m2			>30Kg/m2		
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	13	1.84	0.28	131	1.99	0.08	330	1.87	0.05
C16:0	13	24.99	0.90	131	26.86	0.42	330	26.39	0.27
C16:1	13	1.88	0.29	131	1.41	0.09	330	1.37	0.05
C18:0	13	4.00	0.42	131	4.04	0.18	330	4.21	0.12
C18:1	13	27.14	0.64	131	25.83	0.37	330	26.61	0.24
C18:2:6	13	28.29	1.37	131	27.91	0.43	330	27.25	0.31
C20:4:6	13	6.52	0.43	131	6.54	0.22	330	7.02	0.13
C20:5:3	13	1.17	0.29	131	1.06	0.09	330	0.92	0.05
C22:6:3	13	2.07	0.41	131	2.22	0.12	330	2.18	0.06
SFA	13	30.84	1.23	131	32.89	0.55	330	32.48	0.35
MUFA	13	29.15	0.85	131	27.45	0.41	330	28.20	0.26
PUFA	13	40.01	1.24	131	39.65	0.60	330	39.33	0.38
n-6 PUFA	13	36.00	1.28	131	35.49	0.54	330	35.39	0.35
n-3 PUFA	13	4.01	0.75	131	4.16	0.20	330	3.93	0.10
	20-24Kg/m^2			25-29Kg/m2			>30Kg/m2		
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	13	5.64 a	0.17	128	6.78 b	0.08	310	7.44 c	0.06
Total Energy (MJ)	13	7.88	0.52	128	8.91	0.22	312	8.81	0.15
Total fat (g/day)	13	69.81	5.86	128	82.65	2.63	312	87.40	1.81
Total fat (%Energy)	13	33.14 ab	1.73	128	34.62 a	0.68	312	36.85 b	0.41
SAFA (g/day)	13	25.84	2.19	128	30.45	1.12	312	29.37	0.71
SAFA (%Energy)	13	12.48	0.98	128	12.73	0.32	312	12.31	0.17
MUFA (g/day)	13	23.47 a	2.18	128	28.31 a	1.05	312	33.65 b	0.84
MUFA (%Energy)	13	11.17 ab	0.81	128	11.96 a	0.36	312	14.29 b	0.29
PUFA (g/day)	13	11.61	1.92	128	13.51	0.66	312	13.60	0.40
PUFA (%Energy)	13	5.34	0.60	128	5.62	0.23	312	5.68	0.12
n-3 (g/day)	6	1.16	0.44	80	1.54	0.13	228	1.49	0.08
n-6 (g/day)	6	7.24	1.96	80	9.61	0.72	228	9.37	0.39
C8 (g/day)	5	0.22	0.05	47	0.22	0.02	124	0.22	0.02
C10 (g/day)	6	0.46	0.08	71	0.55	0.04	160	0.52	0.03
C12 (g/day)	10	1.17	0.39	91	1.16	0.09	182	1.08	0.07
C14 (g/day)	10	2.32	0.31	100	2.68	0.15	250	2.48	0.11
C16 (g/day)	10	11.22	1.34	100	14.00	0.61	250	14.20	0.39
C16:1 (g/day)	10	0.92 ab	0.16	100	1.19 a	0.06	250	1.41 b	0.05
C18 (g/day)	10	5.03	0.45	100	6.57	0.34	250	6.56	0.25
C18:1 (g/day)	10	18.24 ab	2.69	100	22.93 a	1.16	250	27.87 b	0.96
C18:2:6 (g/day)	12	8.67	1.87	123	10.41	0.61	284	9.75	0.35
C18:3:3 (g/day)	12	1.10	0.22	123	1.26	0.07	284	1.12	0.05
C20:4:6 (g/day)	6	0.07	0.03	71	0.13	0.01	160	0.12	0.01
C20:5:3 (g/day)	8	0.06	0.03	103	0.12	0.02	262	0.15	0.02
C22:6:3 (g/day)	8	0.10	0.04	103	0.19	0.03	262	0.23	0.02
Trans fatty acids (g/day)	7	1.66	0.60	66	2.46	0.25	131	2.53	0.18
CHO (g/day)	13	217.92	16.16	128	242.09	7.55	312	231.81	4.60
CHO (%Energy)	13	46.11	1.79	128	44.71	0.71	312	42.99	0.42
Starch (g/day)	8	117.91	14.09	75	139.33	6.44	162	144.43	4.49
Total Sugars (g/day)	12	91.07	9.20	91	108.43	5.06	157	100.30	4.20
Protein (g/day)	13	84.53	8.21	128	91.65	2.66	312	90.96	1.65
Protein (%Energy)	13	18.45	1.58	128	17.60	0.36	312	17.72	0.21
Alcohol (g/day)	13	6.81 ab	3.32	128	10.76 a	1.42	312	7.99 b	0.77
Alcohol (%Energy)	13	2.19	0.98	128	3.08	0.37	312	2.45	0.23
Fibre (g)	12	19.77	2.12	123	19.69	0.74	308	19.38	0.47
Vitamin E (mg/day)	13	10.41	1.52	128	9.94	0.47	312	10.32	0.30

^{abc} denotes significance across BMI categories (when two superscripts contain the same letter there is no significant difference between two of the three BMI categories) p<0.05, One Way ANOVA, p<0.017 when bonerroni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

Table 5.5: Baseline characteristics of volunteers across the 8 participating centres

	TCD			UoR			UO			INSERM			UM			HURS-UCO			JUMC			UU	
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	59	49.58 ad	1.27	62	56.13 bcd	1.20	64	53.81 abd	1.17	41	52.29 abd	1.41	59	57.39 bc	1.14	77	56.35 bd	0.88	73	52.08 d	0.76	51	60.12 c	1.00
Height(m)	59	1.68 a	0.01	62	1.71 ab	0.01	64	1.74 b	0.01	41	1.68 ad	0.02	59	1.73 bd	0.01	77	1.61 c	0.01	72	1.67 a	0.01	51	1.67 a	0.01
BodyWeight(kg)	59	93.37 a	2.01	62	91.50 ab	1.75	64	94.14 a	1.74	41	86.73 ab	2.52	59	94.49 a	1.78	77	90.82 ab	1.39	72	94.79 a	1.67	51	90.87 b	1.88
BMI (kg/m^2)	59	33.18 ac	0.57	62	31.46 ab	0.49	64	31.18 ab	0.39	41	30.78 ab	0.75	59	31.60 ab	0.61	77	34.92 c	0.40	72	34.02 c	0.38	51	29.95 b	0.51
Waist circumference(cm	59	107.10 a	1.54	62	106.62 a	1.76	64	106.01 a	1.10	41	103.40 ab	1.77	59	109.37 a	1.14	77	108.36 a	1.00	72	107.56 a	1.36	51	99.34 b	1.43
Hip circumference (cm)	59	114.19 ac	1.64	62	114.62 a	0.89	64	108.57 b	0.75	41	107.06 b	1.56	59	108.64 b	1.35	77	116.99 ac	0.97	72	119.92 c	1.06	51	106.20 b	1.05
Diastolic BP(mmHg)	59	85.25 a	1.12	62	90.25 a	1.29	64	89.91 a	1.08	41	79.51 b	1.40	58	85.48 a	1.23	77	85.64 a	1.11	72	89.69 a	1.50	51	84.07 b	0.96
Systolic BP(mmHg)	59	138.15 ab	1.89	62	139.33 a	1.82	64	136.52 ab	1.87	41	128.71 b	2.56	58	138.50 ab	2.11	77	139.88 a	1.78	72	144.46 a	1.85	51	137.44 ab	2.23
Body Fat (%)	57	38.64 ac	1.24	62	35.72 ab	1.01	64	36.05 ab	0.85	39	34.30 ab	1.42	48	31.55 b	1.25	74	35.37 ab	0.87	69	37.40 a	1.08	51	42.87 c	1.01
Body Fat(kg)	57	35.86 a	1.32	62	32.23 ab	1.03	64	33.12 ab	0.85	39	30.21 ab	1.71	48	29.94 b	1.41	74	32.46 ab	1.11	69	35.30 a	1.21	51	35.76 a	0.99
Lean Body Mass(%)	57	61.44 ad	1.24	62	64.32 ab	1.02	64	63.29 ab	0.95	39	65.51 ab	1.39	48	68.45 b	1.25	74	64.87 c	1.20	69	62.62 a	1.08	51	57.17 cd	1.01
Lean Mass Body(kg)	57	57.44 ac	1.78	62	58.41 a	1.52	64	59.57 a	1.63	39	56.11 abc	1.73	48	64.64 a	1.67	74	59.64 bc	1.85	69	59.07 a	1.53	51	48.31 b	1.62
Body Water(%)	56	45.19 ac	0.79	62	47.09 a	0.75	64	46.80 a	0.70	30	47.28 ac	1.21		not measured		74	39.59 b	0.91	61	45.72 ac	0.88	51	42.86 bc	0.72
Body Water (L)	31	41.67 ab	1.81	62	42.76 a	1.11	64	43.84 a	1.22	38	41.94 ab	1.27		not measured		74	36.57 b	1.37	60	43.87 a	1.29	51	36.38 b	1.19
Impedance(KHz)	57	507.23 a	10.85	62	567.19 bcd	8.71	64	581.84 b	14.28	34	499.16 a	11.43	48	442.94 c	11.69	74	500.14 a	7.34		not measured			not measured	
	TCD			UoR			UO			INSERM			UM			HURS-UCO			JUMC			UU	
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I (ml/UL)^{1}min-1	52	3.24	0.27	61	2.94	0.20	54	2.80	0.26	31	2.83	0.31	57	2.42	0.21	67	2.58	0.19	63	2.93	0.19	47	3.15	0.30
S_G min-1	53	0.02 ab	0.0009	61	0.02 ab	0.0007	55	0.02 a	0.0014	31	0.01 ab	0.0012	57	0.01 b	0.0006	67	0.01 b	0.0006	63	0.01 ab	0.0006	47	0.02 ab	0.0007
AIRg mUL^{-1} min^{-1}	53	426.68	81.10	61	393.19	40.64	55	326.97	37.38	31	372.86	51.65	57	464.20	43.85	67	297.60	29.80	63	311.71	32.46	47	282.39	33.89
DI	53	1096.06 a	105.70	61	969.67 ab	92.98	55	864.86 ab	104.43	31	913.60 ab	128.09	57	974.92 ab	106.95	67	645.90 ab	64.60	63	920.15 ab	80.87	47	785.64 ab	101.43
G0 mg/dl $^{-1}$	53	304.13 ac	6.41	61	295.54 abc	4.52	56	320.28 a	7.66	31	273.61 b	6.07	57	285.19 bc	3.67	67	300.97 ac	4.54	63	293.95 abc	3.83	47	302.58 ac	4.52
P2 min^{-1}	53	0.04	0.003	61	0.04	0.003	55	0.05	0.004	31	0.04	0.003	57	0.04	0.003	67	0.05	0.006	63	0.04	0.003	47	0.05	0.006
P3 (ml/UL) min^{-2}	53	0.000015 a	0.000002	61	0.000012 ab	0.000001	55	0.000015 ab	0.000002	31	0.000011 ab	0.000002	57	0.000009 b	0.000001	67	0.000012 ab	0.000001	63	0.000014 ab	0.000001	47	0.000014 ab	0.000002
Fasting Glucose (mmol/	59	5.85 ac	0.12	62	5.76 ac	0.08	64	6.12 ab	0.11	35	5.67 c	0.15	59	5.87 ac	0.08	77	6.33 b	0.12	68	5.83 ac	0.08	51	5.95 abc	0.10
Fasting Insulin(uU/ml)	59	9.61 ab	0.82	62	11.22 a	0.77	64	10.51 a	0.67	35	8.68 ab	0.68	59	10.34 a	0.78	77	10.78 a	0.61	68	11.43 a	0.63	51	6.76 b	0.49
HOMA-IR	59	2.47 ab	0.21	62	2.94 a	0.22	64	2.93 ab	0.22	35	2.14 ab	0.16	59	2.72 a	0.21	77	3.07 a	0.19	73	2.78 a	0.18	51	1.82 b	0.15
QUICKI	59	0.54 abc	0.08	62	0.59 ac	0.01	64	0.58 ac	0.01	35	0.62 ab	0.02	59	0.59 ac	0.01	77	0.57 ac	0.01	68	0.57 c	0.01	51	0.78 b	0.12
Revised QUICKI	59	0.23 abc	0.004	62	0.23 abc	0.002	64	0.22 ab	0.002	35	0.23 ac	0.002	59	0.23 abc	0.002	77	0.22 b	0.002	67	0.22 ab	0.001	51	0.23 c	0.003
C-Peptide ng/ml	59	2.40 acd	0.11	61	2.81 ab	0.10	64	3.22 b	0.12	34	2.19 cd	0.13	59	2.84 ab	0.11	76	2.51 acd	0.11	68	2.73 ac	0.09	51	2.12 d	0.10
Insulin to C-peptide Rati	59	3.84 abc	0.24	61	3.85 acd	0.17	64	3.16 b	0.11	34	3.88 abc	0.20	58	3.54 ab	0.16	76	4.52 abc	0.24	65	4.14 c	0.15	51	3.21 bd	0.17
	TCD			UoR			UO			INSERM			UM			HURS-UCO			JUMC			UU	
Lipids and Lipoproteir	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	59	1.65 ab	0.10	62	2.15 a	0.16	64	1.77 ab	0.10	40	1.71 ab	0.15	59	1.91 ab	0.15	77	1.68 ab	0.09	72	1.91 ab	0.09	51	1.55 b	0.09
Cholesterol (mmol/L)	59	5.07 a	0.12	62	5.47 abc	0.12	64	5.63 bc	0.11	40	5.07 ab	0.14	59	5.03 a	0.10	77	5.41 abc	0.12	72	5.64 c	0.10	51	5.19 abc	0.11
NEFA (mmol/L)	59	662.12	34.11	62	568.60	30.25	64	667.93	32.30	40	630.64	34.32	59	578.46	29.54	77	654.86	23.00	72	563.20	26.82	51	619.05	24.88
TRL TAG (mmol/L)	59	0.80 abc	0.06	60	1.19 a	0.10	63	0.86 abc	0.06	40	0.64 bc	0.07	58	0.99 a	0.10	76	0.59 c	0.04	69	1.09 a	0.07	51	0.84 abc	0.08
TRL Cholesterol (mmol/	59	0.34 ac	0.03	60	0.61 b	0.06	63	0.41 abc	0.04	40	0.33 acd	0.04	58	0.44 abc	0.04	76	0.24 d	0.02	69	0.39 a	0.03	51	0.42 ac	0.06
LDL Cholesterol (mmol/	58	2.79 a	0.15	61	3.30 ab	0.12	63	3.51 bc	0.12	38	3.42 ab	0.12	59	2.88 a	0.11	77	3.27 ab	0.14	72	3.76 b	0.11	51	3.10 ac	0.12
HDL (mmol/L)	59	1.16 ac	0.04	62	1.03 ab	0.04	64	1.11 ac	0.03	40	1.02 ab	0.04	59	0.96 b	0.03	77	1.08 abc	0.03	72	1.21 c	0.04	51	1.22 c	0.04
Apo A1 (g/L)	56	1.40 ac	0.03	61	1.37 a	0.03	63	1.32 a	0.02	38	1.27 a	0.03	59	1.29 a	0.02	76	1.50 bc	0.03	71	1.58 b	0.04	51	1.33 a	0.03
Apo B (g/L)	56	0.93 a	0.03	61	1.07 b	0.03	63	1.06 b	0.03	38	1.01 ab	0.03	59	1.00 ab	0.03	76	1.02 ab	0.03	71	1.06 b	0.03	51	0.97 ab	0.03
Apo CII (mg/L)	56	40.18 a	2.09	61	43.84 ab	2.59	62	46.93 ab	2.21	38	44.32 ab	2.36	59	46.01 ab	2.34	76	43.67 ab	1.86	71	50.42 b	2.16	51	41.66 ab	2.45
Apo CIII (mg/L)	56	151.72 abc	2.28	61	160.26 a	1.91	62	160.57 abc	5.38	38	143.87 bc	6.84	59	151.11 bc	7.56	76	138.70 c	5.36	71	164.80 ab	5.33	51	171.85 ab	8.01
Apo E (mg/L)	56	37.56 a	1.73	61	41.69 ab	1.98	62	40.94 a	2.34	38	41.58 ab	2.36	59	37.51 a	1.65	76	47.27 b	2.28	70	43.78 ab	1.85	51	39.73 a	2.43
Apo B48 (mg/L)	50	0.57	0.05	47	0.89	0.15	47	0.68	0.08	32	0.63	0.10	44	0.67	0.10	68	0.79	0.11	47	1.20	0.17	39	0.85	0.15
TRL ApoB (mg/L)	56	25.72 a	1.76	58	36.08 b	8.93	63	56.93 ce	5.81	38	54.70 ce	4.76	56	63.12 ce	5.93	74	12.80 d	1.27	70	58.49 c	3.78	47	40.03 ce	3.35
	TCD			UoR			UO			INSERM			UM			HURS-UCO			JUMC			UU	
Plasma Fatty Acids (w%	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	56	2.21 ac	0.13	60	2.70 a	0.16	64	1.96 bc	0.09	41	1.63 be	0.08	58	2.11 ce	0.12	74	1.13 d	0.07	72	1.90 ce	0.13	50	1.56 be	0.09
C16:0	56	29.29 a	0.73	60	28.77 ab	0.84	64	26.55 bc	0.38	41	24.40 cd	0.37	58	30.50 a	0.61	74	24.46 cd	0.50	72	23.59 d	0.23	50	24.66 cd	0.39
C16:1	56	1.68 ac	0.13	60	2.20 a	0.21	64	1.23 be	0.11	41	1.50 ab	0.14	58	0.97 de	0.10	74	0.73 d	0.05	72	1.25 bc	0.07	50	1.88 a	0.09
C18:0	56	4.53 ab	0.45	60	4.46 ab	0.39	64	3.84 ab	0.18	41	4.01 ab	0.09	58	4.56 ab	0.38	74	3.50 a	0.15	72	4.59 b	0.07	50	3.80 ab	0.08
C18:1	56	24.38 a	0.42	60	26.65 b	0.48	64	23.58 a	0.46	41	27.48 bc	0.67	58	23.25 a	0.51	74	29.04 c	0.49	72	27.42 bc	0.44	50	29.38 c	0.46
C18:2 6	56	26.91 ab	0.70	60	25.34 a	0.63	64	29.53 b	0.70	41	28.55 ab	0.84	58	27.75 ab	0.82	74	28.63 bc	0.69	72	26.32 ac	0.50	50	26.64 ab	0.57
C20:4 6	56	6.80 ac	0.45	60	5.40 b	0.29	64	6.49 ab	0.22	41	7.94 cd	0.32	58	6.33 ab	0.36	74	7.66 cd	0.18	72	8.31 d	0.23	50	5.78 ab	0.17
C20:5 3	56	0.64 a	0.08	60	0.69 a	0.08	64	1.72 b	0.15	41	0.65 a	0.06	58	0.67 ac	0.09	74	0.47 c	0.06	72	1.30 b	0.10	50	1.59 b	0.13
C22:6 3	56	1.70 ac	0.16	60	1.16 a	0.08	64	3.11 b	0.18	41	1.60 c	0.10	58	1.60 c	0.12	74	2.59 b	0.09	72	2.82 b	0.13	50	2.51 b	0.13
SFA	56	36.03 ac	1.11	60	35.93 ac	1.14	64	32.35 ab	0.40	41	30.04 bd	0.41	58	37.17 c	0.96	74	29.14 d	0.45	72	30.06 bd	0.31	50	30.06 bd	0.39
MUFA	56	26.31 a	0.50	60	29.20 bc	0.62	64	24.93 a	0.52	41	29.15 bc	0.75	58	24.57 a	0.56	74	29.98 bc	0.49	72	28.79 b	0.45	50	31.46 c	0.49
PUFA	56	37.66 a	1.10	60	34.87 a	0.89	64	42.72 b	0.77	41	40.80 bc	0.98	58	38.26 ac	1.12	74	40.87 bc	0.67	72	41.12 bc	0.56	50	38.49 bc	0.66
n-6 PUFA	56	34.62 abc	0.97	60	31.89 ac	0.79	64	37.06 bc	0.73	41	37.86 b	0.97	58	34.97 abc	0.99	74	37.31 c	0.70	72	36.00 bc	0.53	50	33.53 c	0.60
n-3 PUFA	56	3.04 a	0.24	60	3.01 ac	0.17	64	5.63 b	0.33	41	2.94 ac	0.17	58	3.29 ac	0.20	74	3.56 c	0.14	72	5.12 b	0.22	50	4.96 b	0.26
	TCD			UoR			UO			INSERM			UM			HURS-UCO			JUMC			UU	
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (M	55	7.35 a	0.17	58	7.31 a	0.15	61	7.36 a	0.16	40	7.13 a	0.18	59	7.59 a	0.12	77	7.03 a	0.11	55	7.36 a	0.16	46	6.34 b	0.14
Total Energy (MJ)	58	9.82 a	0.37	57	9.62 ac	0.39	61	9.00 ab	0.36	40	7.78 b	0.27	59	9.15 ab	0.35	77	8.56 ab	0.21	55	7.86 b	0.33	46	8.15 bc	0.34
Total fat (g/day)	58	90.66 ab	4.93	57	86.84 a	4.93	61	87.73 ab	4.54	40	77.40 a	3.71	59	87.20 ab	4.52	77	96.75 b	2.89	55	79.82 a	3.68	46	71.92 a	3.51
Total fat (%Energy)	58	33.67 ac	1.03	57	31.62 a	0.91	61	35.65 cde	0.77	40	37.29 ce	1.08	59	34.72 ace	0.89	77	43.28 b	0.65	55	37.57 e	0.81	46	32.42 ad	0.68
SAFA (g/day)	58	32.32 ab	1.98	57	28.39 ab	1.73	61	33.78 a	1.86	40	27.48 ab	1.59	59	32.18 ab	1.77	77	25.67 b	0.95	55	27.92 ab	1.41	46	28.99 ab	1.59
SAFA (%Energy)	58	12.00 ab	0.42	57	10.99 a	0.44	61	13.72 b	0.40	40	13.29 b	0.61	59	12.78 bc	0.38	77	11.14 ac	0.24	55	13.26 b	0.44	46	13.02 b	0.38
MUFA (g/day)	58	31.43 ad	1.74	57	24.43 b	1.21	61	30.43 abd	1.67	40	24.42 ab	1.44	59	27.70 abd	1.62	77	48.64 c	1.40	55	32.22 d	1.67	46	26.63 abd	1.38
MUFA (%Energy)	58	11.67 a	0.43	57	9.56 b	0.30	61	12.40 ac	0.35	40	11.88 a	0.59	59	10.96 ab	0.35	77	21.45 c	0.41	55	15.08 d	0.43	46	12.61 a	0.33
PUFA (g/day)	58	15.35 ac	1.02	57	11.98 ab	0.73	61	16.56 cd	1.12	40	10.28 b	0.94	59	17.86 c	1.04	77	10.61 b	0.39	55	13.66 abd	1.04	46	10.98 ab	0.66
PUFA (%Energy)	58	5.66 ac	0.28	57	4.73 a	0.25	61	6.69 bc	0.28	40	4.90 a	0.35	59	7.17 b	0.31	77	4.63 a	0.11	55	6.39 bcd	0.37	46	4.99 ad	0.20
n-3 (g/day)	58	0.73 ad	0.07	57	1.67 b	0.15	61	2.68 c	0.20	30	0.96 de	0.10		not measured		77	1.13 e	0.06	31	1.70 be	0.21		not measured	
n-6 (g/day)	58	6.05 ac	0.49	57	10.30 bd	0.67	61	13.41 b	0.93	30	7.47 cd	0.96		not measured		77	8.09 d	0.35	31	11.16 bd	1.51		not measured	
C8:0 (g/day)	58	0.20 a	0.02	57	0.21 a	0.02		not measured		30	0.32 b	0.03		not measured			not measured		31	0.17 a	0.01		not measured	
C10:0 (g/day)	58	0.39 a	0.05	57	0.48 a	0.06	61	0.75 b	0.05	30	0.55 ab	0.05		not measured			not measured		31	0.42 a	0.04		not measured	
C12:0 (g/day)	58	0.64 a	0.08	57	0.91 a	0.09	61	1.60 b	0.13	30	0.69 a	0.07		not measured			not measured		31	0.77 a	0.09	46	1.80 b	0.15
C14:0 (g/day)	58	1.90 a	0.19	57	3.04 bc	0.26	61	3.28 b	0.19	30	2.28 ac	0.22		not measured		77	1.87 a	0.20	31	2.69 bc	0.22	46	2.87 bc	0.18
C16:0 (g/day)	58	11.01 a	0.76	57	14.61 b	0.80	61	16.51 b	0.91	30	9.75 a	0.91		not measured		77	14.76 b	0.56	31	14.69 b	0.96	46	15.20 b	0.83
C16:1 (g/day)	58	0.83 a	0.07	57	1.21 b	0.07	61	1.52 bd	0.08	30	0.72 a	0.07		not measured		77	1.86 c	0.08	31	1.61 cd	0.12	46	1.19 b	0.07
C18 (g/day)	58	5.18 ad	0.42	57	6.94 bc	0.43	61	8.47 b	0.53	30	5.00 d	0.77		not measured		77	6.47 c	0.51	31	6.49 abc	0.54	46	6.20 abc	0.35
C18:1 (g/day)	58	15.58 a	1.12	57	21.43 b	1.06	61	27.11 b	1.53	30	12.53 a	1.24		not measured		77	43.75 c	1.40	31	25.89 b	1.91	46	24.26 b	1.27
C18:2 6 (g/day)	58	6.27 a	0.49	57	9.94 bd	0.66	61	13.27 bc	0.93	30	6.90 ae	1.23	59	14.25 cf	0.88	77	8.15 dg	0.35	31	11.00 befg	1.50	46	8.65 dg	0.57
C18:3 3 (g/day)	58	0.58 a	0.05	57	1.08 be	0.07	61	1.96 c	0.16	30	0.87 abd	0.10	59	1.29 ef	0.10	77	0.77 d	0.05	31	1.41 cef	0.14	46	1.46 cf	0.08
C20:4 6 (g/day)	58	0.06 a	0.01	57	0.20 b	0.02	61	0.14 c	0.01	30	0.01 d	0.00		not measured			not measured		31	0.16 bc	0.02		not measured	
C20:5 3 (g/day)	58	0.05 ac	0.02	57	0.19 b	0.05	61	0.24 b	0.03	30	0.10 ab	0.04	59	0.08 c	0.03	77	0.15 b	0.02	31	0.10 ab	0.04		not measured	
C22:6 3 (g/day)	58	0.08 a	0.03	57	0.25 bd	0.06	61	0.42 c	0.05	30	0.17 ab	0.06	59	0.11 a	0.03	77	0.24 cd	0.02	31	0.19 ab	0.07		not measured	
Trans fatty acids (g/day'	58	1.98 ac	0.17	57	1.93 a	0.18		not measured		30	3.51 cd	0.59	59	2.97 bd	0.25		not measured			not measured			not measured	
CHO (g/day)	58	279.70 a	11.12	57	281.94 a	13.47	61	228.05 b	10.11	40	194.33 b	8.66	59	237.46 ab	8.72	77	197.31 b	6.27	55	217.86 b	10.69	46	238.74 ab	11.77
CHO (%Energy)	58	44.78 a	1.14	57	45.85 ab	1.12	61	43.19 a	0.90	40	41.66 ac	1.04	59	44.64 a	0.84	77	38.44 c	0.69	55	42.70 a	0.83	46	49.58 b	0.87
Starch (g/day)	58	162.77 a	7.26	57	153.25 ab	8.80		not measured		40	108.23 c	6.82	59	142.17 ab	4.89		not measured		31	125.74 bc	10.88		not measured	
Total Sugars (g/day)	58	112.96 ac	6.83	57	126.70 a	8.23		not measured		40	70.25 b	4.51	59	96.23 bc	5.59		not measured			not measured		46	97.96 ac	5.59
Protein (g/day)	58	101.90 ac	3.77	57	110.58 a	6.00	61	96.90 ac	3.75	40	85.04 bc	2.93	59	87.24 bc	3.08	77	85.94 bc	1.93	55	81.54 b	3.98	46	75.99 b	2.67
Protein (%Energy)	58	17.54 ab	0.46	57	19.47 a	0.78	61	18.43 ab	0.43	40	18.78 ab	0.63	59	16.67 b	0.46	77	17.04 ab	0.30	55	17.82 ab	0.56	46	16.19 b	0.39
Alcohol (g/day)	58	13.33 a	2.46	57	10.02 ab	2.12	61	8.46 ab	1.67	40	9.78 ab	2.74	59	13.85 a	2.32	77	5.62 ab	0.94	55	4.52 b	1.28	46	4.58 ab	1.08
Alcohol (%Energy)	58	3.81 ab	0.68	57	2.97 ab	0.60	61	2.71 ab	0.54	40	2.52 ab	0.63	59	3.91 a	0.63	77	1.75 ab	0.29	55	1.59 b	0.42	46	1.71 ab	0.40
Fibre (g)	58	16.03 ac	1.00	57	14.12 a	0.86	61	24.08 b	1.15	30	18.17 acd	1.52	59	23.22 b	1.15	77	18.24 cd	0.60	55	19.63 bc	1.04	46	22.32 bd	1.01
Vitamin E (mg/day)	58	9.23 ac	0.64	57	7.21 a	0.46	61	10.48 cd	0.63	40	9.54 ac	0.93	59	14.96 b	1.04	77	11.68 bd	0.32	55	8.30 ac	0.56	46	9.15 ac	0.53

abc denotes significance across BMI categories (when two superscripts contain the same letter there is no significant difference between two of the three BMI categories) p<0.05, One Way ANOVA, p<0.0018 when bonferoni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

5.4.2 Tertiles of Insulin Sensitivity (Low: 0.186 to 1.963; Medium: 1.966 to 3.114; High: 3.135 to 13.645)

The continuous variable S_I was ranked into three tertiles: low (0.186 to 1.963), medium (1.966 to 3.114) and high (3.135 to 13.645) and a one-way ANOVA investigated differences between the tertiles. This was to investigate if differences existed in the anthropometric and metabolic variables between the respective groups. These differences are illustrated in *Table 5.6.* Body weight, BMI and waist circumference significantly decreased with increasing insulin sensitivity, $p<0.05$. Lean body mass (kg) was significantly higher in the 'low' and 'medium' tertiles as compared to the highest tertile of insulin sensitivity. AIRg was significantly higher in the lowest tertile compared to the highest tertile of S_I. DI, P3, QUICKI and Revised QUICKI were shown to significantly increase with increasing insulin sensitivity, whilst fasting insulin and HOMA-IR significantly decreased with increasing insulin sensitivity, $p<0.05$. Fasting glucose concentrations were highest in the 'low' tertile and significantly higher compared to the 'high' tertile. C-peptide concentrations were significantly higher in the lowest tertile of S_I compared to the 'medium' and 'high' tertiles and Insulin to C-peptide ratio significantly decreased with increasing S_I, $p<0.05$. TAG and TRL TAG concentrations were significantly higher in the lowest tertile, however cholesterol concentrations were lowest in the 'low' tertile compared to the highest tertile, $p<0.05$. There were no statistical differences in plasma fatty acids, with the exception of plasma myristic acid (C14:0). BMR was significantly higher in the 'low' and 'medium' tertiles compared to the 'high' tertile. Total fat (g/day), MUFA (g/day), dietary caprylic acid (C8:0 g/day) and *trans* fat (g/day) were higher in the lowest tertile of S_I compared to the highest tertile, $p<0.05$.

Table 5.6: Tertiles (Low, Medium and High) of Insulin Sensitivity at baseline

	Low			Medium			High		
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	144	53.57	0.77	144	54.97	0.79	144	54.61	0.69
Height(m)	144	1.70 a	0.01	144	1.69 ab	0.01	144	1.67 b	0.01
BodyWeight(kg)	144	96.86 a	1.23	144	91.24 b	1.09	144	87.31 c	1.09
BMI (kg/m^2)	144	33.60 a	0.36	144	31.99 b	0.31	144	31.47 b	0.34
Waist circumference(cm)	144	110.35 a	0.86	144	106.08 b	0.79	144	102.37 c	0.96
Hip circumference (cm)	144	113.76	0.79	144	112.36	0.74	144	112.47	0.91
Diastolic BP(mmHg)	143	86.70	0.85	144	87.23	0.89	144	84.44	0.76
Systolic BP(mmHg)	143	138.70	1.29	144	140.13	1.33	144	137.21	1.37
Body Fat (%)	134	36.07	0.69	140	35.89	0.79	140	36.97	0.74
Body Fat(kg)	134	34.59	0.80	140	32.50	0.76	140	32.27	0.76
Lean Body Mass(%)	134	62.29	0.85	140	62.63	0.82	140	60.91	0.83
Lean Mass Body(kg)	134	60.26 a	1.28	140	57.28 a	1.15	140	53.35 b	1.05
Body Water(%)	112	45.11	0.69	117	45.14	0.65	123	43.73	0.63
Body Water (L)	109	43.37 a	1.10	112	41.12 ab	0.96	114	38.45 b	0.85
Impedance(KHz)	105	502.30 a	9.71	96	519.52 ab	9.99	101	541.05 b	8.46
	Low			**Medium**			**High**		
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I (mU/L)$^{-1}$min-1	144	1.32 a	0.04	144	2.53 b	0.03	144	4.69 c	0.14
S_G min-1	144	0.0149	0.0004	144	0.0155	0.0005	144	0.0165	0.0006
AIRg mUL^{-1} min^{-1}	144	470.74 a	38.84	144	350.65 ab	21.83	144	256.65 b	15.30
DI	144	608.24 a	47.92	144	881.59 b	54.85	144	1140.27 c	66.21
G0 mg/dl $^{-1}$	144	299.09	2.74	144	300.37	3.40	144	295.24	3.83
P2 min^{-1}	144	0.039 a	0.003	144	0.046 b	0.002	144	0.047 b	0.002
P3 (mU/L) min^{-2}	144	0.0000051 a	0.0000004	144	0.0000116 b	0.0000006	144	0.0000218 c	0.0000011
Fasting Glucose (mmol/L)	144	6.00 a	0.07	144	6.00 ab	0.07	144	5.79 b	0.07
Fasting Insulin(uIU/ml)	144	13.63 a	0.53	144	9.58 b	0.38	144	7.20 c	0.26
HOMA-IR	144	3.63 a	0.15	144	2.56 b	0.11	144	1.88 c	0.08
QUICKI	144	0.54 a	0.01	144	0.60 b	0.06	144	0.65 c	0.01
Revised QUICKI	144	0.22 a	0.001	143	0.23 b	0.002	144	0.23 c	0.001
C-Peptide ng/ml	144	4.53 a	0.14	143	3.60 b	0.12	142	3.19 b	0.08
Insulin to C-peptide ratio	144	3.04 a	0.07	143	2.66 b	0.07	142	2.25 c	0.06
	Low			**Medium**			**High**		
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	144	2.00 a	0.09	143	1.81 ab	0.08	144	1.67 b	0.06
Cholesterol (mmol/L)	144	5.21 a	0.07	143	5.32 ab	0.08	144	5.50 b	0.08
NEFA (mmol/L)	144	614.46	19.85	143	617.79	18.48	144	597.85	20.01
TRL TAG (mmol/L)	141	1.01 a	0.06	143	0.90 ab	0.05	139	0.79 b	0.04
TRL Cholesterol (mmol/L)	141	0.43	0.03	143	0.41	0.03	139	0.37	0.02
LDL Cholesterol (mmol/L)	143	3.18	0.08	143	3.16	0.09	140	3.40	0.09
HDL (mmol/L)	144	1.02 a	0.02	143	1.08 a	0.02	144	1.19 b	0.03
Apo A1 (g/L)	143	1.36 a	0.02	140	1.37 ab	0.02	140	1.45 b	0.02
Apo B (g/L)	143	1.03	0.02	140	1.01	0.02	140	1.03	0.02
Apo CII (mg/L)	142	45.93	1.59	140	47.25	1.64	140	44.77	1.30
Apo CIII (mg/L)	142	161.35	4.39	140	159.44	4.83	140	157.23	4.03
Apo E (mg/L)	143	42.75	1.42	139	42.85	1.69	139	39.31	0.99
Apo B48 (mg/L)	105	0.85	0.09	105	0.78	0.09	123	0.77	0.07
TRL ApoB (mg/L)	139	52.70	3.78	138	49.49	3.66	134	51.26	4.42
	Low			**Medium**			**High**		
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	142	2.10 a	0.09	138	1.77 b	0.07	142	1.86 ab	0.08
C16:0	142	27.32	0.41	138	26.32	0.42	142	26.31	0.42
C16:1	142	1.37	0.07	138	1.29	0.07	142	1.52	0.11
C18:0	142	4.03	0.16	138	4.39	0.19	142	4.27	0.19
C18:1	142	26.70	0.35	138	26.79	0.40	142	25.90	0.36
C18:2:6	142	26.38	0.46	138	27.44	0.44	142	27.89	0.44
C20:4:6	142	6.89	0.20	138	6.76	0.20	142	6.79	0.20
C20:5:3	142	0.85	0.07	138	0.99	0.07	142	1.01	0.08
C22:6:3	142	2.17	0.09	138	2.12	0.10	142	2.20	0.11
SFA	142	33.45	0.51	138	32.48	0.57	142	32.44	0.56
MUFA	142	28.30	0.37	138	28.27	0.42	142	27.66	0.42
PUFA	142	38.25	0.59	138	39.24	0.57	142	39.91	0.59
n-6 PUFA	142	34.38	0.54	138	35.31	0.52	142	35.79	0.52
n-3 PUFA	142	3.86	0.15	138	3.93	0.16	142	4.12	0.19
	Low			**Medium**			**High**		
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	135	7.55 a	0.10	136	7.26 a	0.10	131	6.90 b	0.09
Total Energy (MJ)	135	9.18	0.23	136	8.83	0.23	133	8.55	0.22
Total fat (g/day)	135	89.02 a	2.74	136	86.78 ab	2.84	133	80.03 b	2.37
Total fat (%Energy)	135	36.26	0.66	136	36.31	0.64	133	35.04	0.58
SFA (g/day)	135	30.45	1.06	136	30.44	1.18	133	28.08	0.98
SFA (%Energy)	135	12.29	0.25	136	12.67	0.29	133	12.27	0.28
MUFA (g/day)	135	33.13 a	1.27	136	32.92 a	1.25	133	28.75 b	1.00
MUFA (%Energy)	135	13.62	0.45	136	13.85	0.41	133	12.76	0.38
PUFA (g/day)	135	13.93	0.65	136	13.49	0.58	133	13.08	0.63
PUFA (%Energy)	135	5.63	0.19	136	5.65	0.19	133	5.61	0.20
n-3 (g/day)	86	1.50	0.15	87	1.54	0.12	102	1.43	0.11
n-6 (g/day)	86	9.80	0.66	87	9.21	0.57	102	9.24	0.63
C8 (g/day)	43	0.27 a	0.03	46	0.20 ab	0.03	68	0.19 b	0.02
C10 (g/day)	59	0.59	0.05	64	0.53	0.05	85	0.50	0.04
C12 (g/day)	69	1.18	0.10	84	1.23	0.11	97	1.04	0.10
C14 (g/day)	96	2.59	0.17	107	2.69	0.19	114	2.47	0.14
C16 (g/day)	96	14.77	0.62	107	14.78	0.63	114	13.27	0.51
C16:1 (g/day)	96	1.31	0.06	107	1.44	0.08	114	1.25	0.06
C18 (g/day)	96	6.82	0.36	107	6.92	0.45	114	6.20	0.31
C18:1 (g/day)	96	27.90 ab	1.52	107	28.09 a	1.41	114	23.17 b	1.10
C18:2:6 (g/day)	120	10.64	0.60	127	9.91	0.51	127	9.55	0.57
C18:3:3 (g/day)	120	1.20	0.09	127	1.21	0.07	127	1.08	0.06
C20:4:6 (g/day)	59	0.13	0.02	64	0.14	0.01	85	0.11	0.01
C20:5:3 (g/day)	110	0.13	0.02	107	0.13	0.02	115	0.14	0.02
C22:6:3 (g/day)	110	0.20	0.03	107	0.22	0.03	115	0.21	0.03
Trans fatty acids (g/day)	66	2.90 a	0.24	55	2.44 ab	0.31	64	2.05 b	0.20
CHO (g/day)	135	247.18	7.22	136	233.64	6.86	133	230.71	7.31
CHO (%Energy)	135	44.01	0.59	136	43.76	0.67	133	43.73	0.67
Starch (g/day)	70	159.74 a	6.90	68	137.48 ab	6.16	85	136.96 b	6.30
Total Sugars (g/day)	79	103.01	5.69	77	106.60	6.05	80	101.57	5.34
Protein (g/day)	135	93.50	2.70	136	90.64	2.58	133	88.74	2.49
Protein (%Energy)	135	17.36	0.34	136	17.63	0.33	133	17.86	0.36
Alcohol (g/day)	135	7.92	1.15	136	8.62	1.24	133	10.64	1.41
Alcohol (%Energy)	135	2.37	0.35	136	2.36	0.30	133	3.34	0.42
Fibre (g)	132	20.41	0.67	134	19.10	0.66	129	18.52	0.74
Vitamin E (mg/day)	135	10.87 a	0.53	136	10.28 ab	0.41	133	9.32 b	0.42

$^{abc...}$ denotes significance across tertiles (when two superscripts contain the same letter there is no significant difference between two of the three tertiles) $p<0.05$, One Way ANOVA, $p<0.017$ where bonferroni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

5.4.3 Ranking according to total fat, saturated fat and monounsaturated fat (%Energy).

This analysis was carried out to investigate differences between the subjects who have a low, medium or high dietary fat intake.

Quartiles of Total Fat (%Energy) (Low: 11.12% to 31.20%; Low/Medium: 31.2% to 36%; Medium/High: 36.02% to 41.7%; High: 41.8% to 57.3%; *Table 5.7*).

Anthropometry

BMI increased with increasing intakes of total dietary fat (%Energy). With 'medium/high' and 'high' intakes of dietary fat, BMI was significantly greater than BMI of volunteers with the lowest intakes of total dietary fat (%Energy), $p<0.05$. Those in the highest quartile of total fat (% Energy) had higher systolic blood pressure compared to the other three quartiles and significantly higher than the 'low/medium' and 'medium/high' quartiles, $p<0.05$.

IVGTT Parameters, HOMA-IR and QUICKI

There were no significant differences in S_I between the quartiles of total dietary fat (%Energy) intakes. Fasting insulin concentrations and HOMA-IR values were highest in the 'medium/high' quartile of dietary fat and lowest in the 'low' and 'low/medium' quartiles, respectively, $p<0.05$. QUICKI values were significantly higher in the 'low' and the 'low/medium' quartiles compared to the 'medium/high' and 'high' intakes of total dietary fat (%Energy).

Lipids and apolipoproteins

Interestingly, although cholesterol concentrations were not significantly different between the quartiles, TAG concentrations were significantly lower in the quartile of highest total fat intake, as was also seen for TRL TAG and TRL cholesterol concentrations, $p<0.05$. ApoA1 and apoCIII concentrations were also highest in this quartile, $p<0.05$.

Plasma Fatty Acids (w/w%)

There were differences in the plasma fatty acids between the lowest and the highest intakes of total fat (%Energy). Plasma myristic acid (C14:0) concentrations were lowest in the highest quartile of total fat intakes compared to the other three quartiles, $p<0.05$. Plasma palmitic acid (C16:0), palmitoleic acid (C16:1), EPA (C20:5:3) and SFA were also significantly lower in this quartile i.e. 'high', $p<0.05$. However, concentrations of plasma linoleic acid (C18:2:6), arachidonic acid (C20:4:6), total

PUFA and n-6PUFA were highest in the quartile representing the highest intake of total fat (%Energy), $p<0.05$.

Dietary Variables

From the dietary information analysed from the pre-intervention food diaries, SFA (g/day and %Energy) and MUFA (g/day and %Energy) were greatest in the 'high' quartile of total fat compared to the other three quartiles. Dietary caprylic acid (C8:0 g/day), dietary capric acid (C10:0 g/day), lauric acid (C12:0 g/day), palmitic acid (C16:0 g/day), stearic acid (C18:0 g/day), oleic acid (C18:1g/day) and *trans* fats were shown to be in greater amounts in the highest quartile of total dietary fat intakes (%Energy). CHO (g/day and %Energy), Starch (g/day), total sugars (g/day), Protein (%Energy) and Alcohol (g/day and %Energy) were greatest in the lowest quartile of total dietary fat (%Energy).

Tertiles of Saturated Fat (% Energy) (Low: 4.05% to 10.86%; Medium: 10.88% to 13.40%; High: 13.5% to 22.04%, *Table 5.8*)

There were no significant differences in the anthropometric measurements between the low, medium and high tertiles of saturated fat (%Energy) intakes. Insulin sensitivity was also not significantly affected by the amount of SFA (%Energy) in the diet. However, fasting insulin concentrations significantly increased with increasing intakes of SFA (%Energy), as was also seen for HOMA-IR. Revised QUICKI values significantly decreased between the low and high tertiles of dietary SFA (%Energy), $p<0.05$. Interestingly, there were no significant increases in plasma SFA (%) concentrations between the tertiles. There was however, a significant decrease in plasma MUFA concentrations and an increase in plasma PUFA and n-6 PUFA concentrations between the low and high tertiles of SFA (%Energy), $p<0.05$. A number of significant differences were seen with the dietary information, with total energy, total fat (%Energy and g/day), MUFA (%Energy and g/day), dietary PUFA, n-3 PUFA, n-6 PUFA and most dietary fatty acids increasing significantly between the low and high tertiles, $p<0.05$. Dietary EPA (C20:5:3) and DHA (C22:6:3) were significantly decreased, $p<0.05$. This is in contrast to the increase observed with plasma concentrations of EPA between medium and high tertiles of dietary SFA (%Energy). With increasing intakes of dietary SFA (%Energy), dietary CHO (%Energy), total sugars, Protein (%Energy) and Alcohol (%Energy) significantly decreased, $p<0.05$.

Tertiles of Monounsaturated Fat (% Energy) (Low: 3.14% to 10.97%; Medium: 10.98% to 14.44%; High: 14.46% to 29.99%, *Table 5.9*)

Most significant differences were seen between the low and the high tertiles of dietary MUFA intakes (%Energy). BMI and hip circumference increased and lean body mass (%) decreased significantly between the low and the high tertiles of dietary MUFA (%Energy), $p<0.05$. There were no significant differences in insulin sensitivity (S_I) across the tertiles. However, DI and QUICKI decreased significantly between the low and the high tertiles of dietary MUFA (%Energy). Fasting glucose increased significantly from the low and the medium and high tertiles, $p<0.05$. No significant differences were observed for TAG and Cholesterol concentrations, however, TRL TAG, TRL Cholesterol, apoCIII and TRL apoB decreased significantly from low to high intakes of dietary MUFA (%Energy), $p<0.05$. ApoAI and apoE increased significantly between the low and the high tertiles of dietary MUFA (%Energy), $p<0.05$. With the plasma fatty acids, concentrations of plasma myristic acid (C14:0 %), palmitic acid (C16:0 %) and total SFA (%) decreased from low to the high tertiles, whilst C16:1(%) decreased across all tertiles, $p<0.05$. Plasma oleic acid (C18:1 %) significantly increased from low to high intakes of dietary MUFA (%Energy) and plasma concentrations of MUFA increased significantly from medium to high intakes of dietary MUFA (%Energy), $p<0.05$. Dietary total fat (g and %Energy) significantly increased with increasing intakes of dietary MUFA (%Energy) as did dietary palmitoleic acid (C16:1) and oleic acid (C18:1). Dietary CHO (%Energy), total sugars, Protein (%Energy) and Alcohol (%Energy) decreased significantly from low to high intakes of MUFA (%Energy), $p<0.05$.

Table 5.7: Quartiles of Total Fat (% Energy)

	Low			Low/Medium			Medium/High			High		
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	113	55.16	0.93	114	54.87	0.82	113	53.79	0.84	113	55.02	0.82
Height(m)	113	1.70 a	0.01	114	1.68 ab	0.01	113	1.69 ab	0.01	113	1.66 b	0.01
BodyWeight(kg)	113	90.74	1.20	114	91.31	1.34	113	93.41	1.29	113	91.53	1.51
BMI (kg/m^2)	113	31.30 a	0.37	114	32.19 ab	0.40	113	32.88 b	0.41	113	33.22 b	0.40
Waist circumference(cm)	113	105.56	1.09	114	105.88	1.01	113	107.49	1.01	113	107.21	0.96
Hip circumference (cm)	113	111.09	0.92	114	112.28	0.92	113	113.61	0.95	113	113.91	0.99
Diastolic BP(mmHg)	113	85.25	0.81	114	85.46	0.88	112	85.09	0.72	113	88.10	1.14
Systolic BP(mmHg)	113	139.84 ab	1.32	114	135.80 a	1.45	112	136.03 a	1.39	113	141.79 b	1.60
Body Fat (%)	110	35.94 ab	0.85	110	38.25 a	0.86	108	36.95 ab	0.74	108	35.06 b	0.87
Body Fat(kg)	110	32.06	0.78	110	34.56	0.91	108	34.34	0.83	108	32.25	1.01
Lean Body Mass(%)	110	63.74 a	0.83	110	61.54 ab	0.86	108	61.81 ab	0.83	108	59.68 b	1.13
Lean Mass Body(kg)	110	57.48	1.20	110	56.03	1.19	108	57.74	1.21	108	55.18	1.64
Body Water(%)	88	46.23 a	0.66	98	45.11 ab	0.63	97	44.47 ab	0.60	94	42.51 b	0.90
Body Water (L)	81	41.20	0.98	90	40.91	0.97	95	41.17	0.93	94	39.68	1.34
Impedance(KHz)	86	518.09	9.76	75	518.58	9.37	81	528.01	12.04	88	515.41	10.05
	Low			**Low/Medium**			**Medium/High**			**High**		
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I (mU/L)$^{-1}$min-1	104	2.81	0.15	102	3.02	0.18	101	2.69	0.17	97	2.80	0.18
S_G min-1	105	0.0156	0.0007	102	0.0157	0.0006	102	0.0156	0.0006	97	0.0160	0.0007
AIRg mUL^{-1} min^{-1}	105	357.29	29.07	102	377.35	32.81	102	373.04	45.46	97	340.18	25.51
DI	105	970.73	77.99	102	923.65	75.70	102	828.02	69.89	97	818.07	60.73
G0 mg/dl^{-1}	105	298.68	3.31	102	299.74	4.46	102	296.15	4.06	97	300.62	4.27
P2 min^{-1}	105	0.0438	0.0027	102	0.0411	0.0019	102	0.0444	0.0041	97	0.0435	0.0029
P3 (mU/L) min^{-2}	105	0.000013	0.000001	102	0.000013	0.000001	102	0.000012	0.000001	97	0.000013	0.000001
Fasting Glucose (mmol/L)	113	5.88	0.08	110	5.94	0.07	111	6.01	0.08	113	6.03	0.09
Fasting Insulin(uIU/ml)	113	9.02 a	0.41	110	9.86 ab	0.60	111	11.30 b	0.55	113	10.35 ab	0.48
HOMA-IR	113	2.38 a	0.12	110	2.61 a	0.16	111	3.06 b	0.17	113	2.80 ab	0.15
QUICKI	113	0.61 a	0.01	110	0.66 a	0.06	111	0.53 b	0.04	113	0.59 b	0.01
Revised QUICKI	113	0.23	0.001	110	0.23	0.002	111	0.22	0.002	113	0.22	0.002
C-Peptide ng/ml	112	2.45 a	0.07	110	2.74 ab	0.09	109	2.82 b	0.09	113	2.58 ab	0.08
Insulin to C-peptide ratio	112	3.63	0.12	110	3.45	0.12	109	3.98	0.16	113	4.15	0.18
	Low			**Low/Medium**			**Medium/High**			**High**		
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	113	1.82 ab	0.10	114	1.82 ab	0.08	112	1.92 a	0.09	113	1.62 b	0.07
Cholesterol (mmol/L)	113	5.22	0.09	114	5.31	0.09	112	5.39	0.08	113	5.37	0.09
NEFA (mmol/L)	113	632.46	25.09	114	616.37	19.87	112	626.63	21.24	113	604.81	21.17
TRL TAG (mmol/L)	112	0.92 a	0.06	114	0.91 a	0.05	111	0.95 a	0.06	111	0.69 b	0.05
TRL Cholesterol (mmol/L)	112	0.42 a	0.03	114	0.43 a	0.03	111	0.42 a	0.03	111	0.30 b	0.02
LDL Cholesterol (mmol/L)	113	3.17	0.09	112	3.24	0.10	111	3.35	0.10	112	3.20	0.10
HDL (mmol/L)	113	1.07	0.03	114	1.09	0.03	112	1.08	0.02	113	1.11	0.03
Apo A1 (g/L)	112	1.34 a	0.02	114	1.37 ab	0.02	110	1.41 ab	0.02	111	1.44 b	0.02
Apo B (g/L)	112	1.01	0.02	114	1.03	0.02	110	1.03	0.02	111	1.02	0.02
Apo CII (mg/L)	112	44.70	1.80	114	46.59	1.63	110	47.61	1.75	110	42.32	1.48
Apo CIII (mg/L)	112	161.19 a	5.37	114	161.31 a	4.30	110	161.96 a	4.84	110	142.46 b	4.81
Apo E (mg/L)	111	38.07 a	1.34	113	42.07 ab	1.59	110	43.62 b	1.67	111	43.14 b	1.71
Apo B48 (mg/L)	92	0.73 ab	0.09	89	0.79 ab	0.08	81	0.97 a	0.10	99	0.72 b	0.09
TRL ApoB (mg/L)	110	57.14 a	5.16	112	53.00 a	4.14	107	55.91 a	4.29	109	32.70 b	3.16
	Low			**Low/Medium**			**Medium/High**			**High**		
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	113	2.09 a	0.09	113	1.96 a	0.10	110	1.99 a	0.08	109	1.56 b	0.09
C16:0	113	28.19 a	0.48	113	26.98 ab	0.47	110	25.78 bc	0.37	109	25.22 c	0.49
C16:1	113	1.68 a	0.11	113	1.48 ab	0.09	110	1.40 b	0.10	109	0.87 c	0.05
C18:0	113	4.12	0.22	113	4.45	0.22	110	3.88	0.17	109	4.12	0.18
C18:1	113	26.11	0.35	113	26.41	0.44	110	26.33	0.42	109	26.69	0.45
C18:2:6	113	26.22 a	0.48	113	27.06 a	0.51	110	27.78 ab	0.47	109	29.19 b	0.55
C20:4:6	113	6.35 a	0.25	113	6.32 a	0.21	110	7.34 b	0.21	109	7.43 b	0.22
C20:5:3	113	1.09 a	0.08	113	1.03 a	0.09	110	0.92 a	0.08	109	0.71 b	0.07
C22:6:3	113	1.98	0.11	113	2.14	0.12	110	2.33	0.12	109	2.25	0.10
SFA	113	34.40 a	0.65	113	33.38 ab	0.63	110	31.65 bc	0.45	109	30.89 c	0.61
MUFA	113	28.04	0.40	113	28.12	0.48	110	27.92	0.46	109	27.77	0.45
PUFA	113	37.56 a	0.67	113	38.49 ab	0.65	110	40.43 bc	0.57	109	41.34 c	0.67
n-6 PUFA	113	33.60 a	0.59	113	34.48 ab	0.58	110	36.28 bc	0.52	109	37.69 c	0.64
n-3 PUFA	113	3.96	0.18	113	4.01	0.21	110	4.16	0.20	109	3.65	0.16
	Low			**Low/Medium**			**Medium/High**			**High**		
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	112	7.21	0.11	113	7.11	0.11	113	7.30	0.10	112	7.18	0.11
Total Energy (MJ)	113	8.61 ab	0.25	114	8.40 a	0.23	113	9.30 b	0.25	113	8.94 ab	0.24
Total fat (g/day)	113	61.79 a	1.97	114	75.47 b	2.06	113	96.65 c	2.61	113	108.39 d	2.86
Total fat (%Energy)	113	26.76 a	0.33	114	33.49 b	0.13	113	38.74 c	0.16	113	45.49 d	0.31
SAFA (g/day)	113	22.77 a	0.83	114	27.29 b	0.84	113	33.95 cd	1.31	113	34.31 d	1.25
SAFA (%Energy)	113	9.84 a	0.20	114	12.15 b	0.20	113	13.43 cd	0.29	113	14.31 d	0.31
MUFA (g/day)	113	20.66 a	0.74	114	26.45 b	0.72	113	35.13 c	1.09	113	45.19 d	1.34
MUFA (%Energy)	113	8.93 a	0.17	114	11.86 b	0.19	113	14.22 c	0.32	113	19.16 d	0.43
PUFA (g/day)	113	10.81 a	0.51	114	12.17 a	0.51	113	15.74 b	0.72	113	15.34 b	0.80
PUFA (%Energy)	113	4.64 a	0.15	114	5.38 b	0.17	113	6.34 cd	0.25	113	6.26 d	0.22
n-3 (g/day)	70	1.10 a	0.09	74	1.49 ab	0.13	80	1.84 b	0.15	90	1.50 b	0.14
n-6 (g/day)	70	7.28 a	0.64	74	8.92 ab	0.58	80	10.79 b	0.71	90	10.17 b	0.66
C8 (g/day)	55	0.16 a	0.02	51	0.19 ab	0.01	41	0.28 b	0.04	29	0.29 b	0.03
C10 (g/day)	68	0.39 a	0.03	68	0.46 ab	0.03	63	0.64 bc	0.06	38	0.71 c	0.06
C12 (g/day)	86	0.93 a	0.09	87	1.02 ab	0.08	71	1.30 b	0.12	39	1.36 b	0.17
C14 (g/day)	88	2.08 a	0.12	93	2.37 ab	0.12	88	2.98 b	0.21	91	2.72 ab	0.20
C16 (g/day)	88	10.61 a	0.53	93	12.70 b	0.49	88	16.29 cd	0.72	91	16.64 d	0.59
C16:1 (g/day)	88	0.90 a	0.05	93	1.17 b	0.05	88	1.44 c	0.06	91	1.82 d	0.08
C18 (g/day)	88	4.93 a	0.28	93	6.02 b	0.34	88	7.26 cd	0.37	91	7.86 d	0.50
C18:1 (g/day)	88	16.28 a	0.92	93	21.00 b	0.88	88	28.75 c	1.31	91	38.76 d	1.60
C18:2:6 (g/day)	110	8.13 a	0.53	106	9.09 ac	0.48	101	11.49 bd	0.64	102	11.15 cd	0.70
C18:3:3 (g/day)	110	0.90 a	0.06	106	1.15 b	0.06	101	1.43 b	0.09	102	1.21 b	0.10
C20:4:6 (g/day)	68	0.12	0.01	68	0.12	0.01	63	0.13	0.01	38	0.12	0.02
C20:5:3 (g/day)	92	0.09 a	0.02	87	0.13 ab	0.03	93	0.19 b	0.03	101	0.14 b	0.02
C22:6:3 (g/day)	92	0.15	0.02	87	0.22	0.04	93	0.28	0.04	101	0.22	0.03
Trans fatty acids (g/day)	73	1.56 a	0.11	53	2.53 b	0.25	46	3.16 b	0.35	32	3.51 b	0.48
CHO (g/day)	113	267.44 a	8.92	114	237.59 b	7.20	113	235.81 b	7.07	113	196.39 c	5.87
CHO (%Energy)	113	50.57 a	0.67	114	46.13 b	0.45	113	41.43 c	0.43	113	36.09 d	0.44
Starch (g/day)	79	149.73 a	6.77	67	148.54 a	6.32	56	139.02 ab	7.34	43	121.51 b	8.25
Total Sugars (g/day)	93	115.02 a	6.08	75	103.04 a	4.96	56	100.23 a	6.12	36	74.18 b	6.11
Protein (g/day)	113	93.14	2.93	114	88.31	2.82	113	92.84	2.59	113	89.62	2.68
Protein (%Energy)	113	18.64 a	0.43	114	18.04 ab	0.37	113	17.14 b	0.33	113	17.02 b	0.31
Alcohol (g/day)	113	12.08 a	1.73	114	7.76 ab	1.23	113	9.53 ab	1.31	113	5.61 b	0.93
Alcohol (%Energy)	113	3.93 a	0.54	114	2.29 ab	0.30	113	2.62 ab	0.34	113	1.65 b	0.26
Fibre (g)	111	20.47 ab	0.88	111	19.39 ab	0.70	111	20.68 a	0.85	110	17.36 b	0.60
Vitamin E (mg/day)	113	8.95 a	0.48	114	9.46 ab	0.55	113	11.07 bc	0.50	113	11.38 c	0.44

$^{abc...}$ denotes significance across quartiles (when two superscripts contain the same letter there is no significant difference between two of the four quartiles) p<0.05, One Way ANOVA, p<0.01 where bonferonni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

Table 5.8: Tertiles of SFA (% Energy)

	Low			Medium			High		
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	151	54.13	0.80	151	55.47	0.69	151	54.59	0.72
Height(m)	151	1.68	0.01	151	1.68	0.01	151	1.69	0.01
BodyWeight(kg)	151	91.12	1.09	151	91.26	1.07	151	92.78	1.31
BMI (kg/m^2)	151	32.36	0.34	151	32.35	0.34	151	32.48	0.36
Waist circumference(cm)	151	106.62	0.90	151	105.92	0.82	151	106.98	0.93
Hip circumference (cm)	151	113.32	0.73	151	112.19	0.89	151	112.64	0.84
Diastolic BP(mmHg)	151	84.76	0.71	150	86.44	0.86	151	86.71	0.77
Systolic BP(mmHg)	151	139.32	1.12	150	138.82	1.33	151	137.01	1.33
Body Fat (%)	144	36.14	0.71	143	36.75	0.72	149	36.84	0.75
Body Fat(kg)	144	32.65	0.73	143	33.28	0.76	149	33.99	0.81
Lean Body Mass(%)	144	61.55	0.82	143	61.24	0.84	149	62.21	0.76
Lean Mass Body(kg)	144	55.96	1.12	143	55.81	1.15	149	57.90	1.16
Body Water(%)	129	44.55	0.63	122	43.81	0.67	126	45.21	0.57
Body Water (L)	119	39.96	0.88	117	39.92	0.95	124	42.11	0.95
Impedance(KHz)	118	515.63	6.94	112	518.77	7.90	100	526.28	12.13
	Low			**Medium**			**High**		
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I $(mU/L)^{-1}min^{-1}$	139	2.95	0.14	133	2.84	0.14	132	2.73	0.16
S_G min^{-1}	140	0.015	0.001	134	0.015	0.001	132	0.016	0.001
AIRg mUL^{-1} min^{-1}	140	346.44	25.36	134	354.07	24.03	132	384.77	37.99
DI	140	925.17	65.70	134	885.98	60.36	132	843.26	60.26
G0 mg/dl^{-1}	140	294.97	3.08	134	300.75	3.91	132	300.41	3.46
P2 min^{-1}	140	0.046	0.003	134	0.041	0.002	132	0.044	0.003
P3 (mU/L) min^{-2}	140	0.000013	0.000001	134	0.000012	0.000001	132	0.000012	0.000001
Fasting Glucose (mmol/L)	150	5.91	0.07	147	6.04	0.06	150	5.93	0.07
Fasting Insulin(uIU/ml)	150	8.87 a	0.39	147	10.22 b	0.39	150	11.28 b	0.54
HOMA-IR	150	2.33 a	0.10	147	2.79 ab	0.12	150	3.01 b	0.15
QUICKI	150	0.58 a	0.03	147	0.59 b	0.01	150	0.63 b	0.04
Revised QUICKI	150	0.23 a	0.002	147	0.22 ab	0.001	150	0.22 b	0.002
C-Peptide ng/ml	149	2.44 a	0.07	147	2.65 ab	0.07	148	2.84 b	0.08
Insulin to C-peptide ratio	149	3.54	0.10	147	3.92	0.13	148	3.94	0.14
	Low			**Medium**			**High**		
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	151	1.76	0.08	151	1.86	0.07	150	1.75	0.07
Cholesterol (mmol/L)	151	5.25	0.08	151	5.36	0.07	150	5.36	0.08
NEFA (mmol/L)	151	626.47	18.83	151	631.00	19.54	150	607.10	18.70
TRL TAG (mmol/L)	149	0.82	0.05	151	0.89	0.05	148	0.88	0.05
TRL Cholesterol (mmol/L)	149	0.39	0.03	151	0.40	0.02	148	0.38	0.02
LDL Cholesterol (mmol/L)	149	3.16	0.09	151	3.27	0.08	148	3.30	0.08
HDL (mmol/L)	151	1.10	0.02	151	1.06	0.02	150	1.11	0.02
Apo A1 (g/L)	149	1.41	0.02	150	1.38	0.02	148	1.39	0.02
Apo B (g/L)	149	1.00	0.02	150	1.05	0.02	148	1.02	0.02
Apo CII (mg/L)	149	44.21	1.49	150	47.21	1.49	147	44.55	1.37
Apo CIII (mg/L)	149	154.74	4.18	150	159.37	4.25	147	156.11	4.28
Apo E (mg/L)	148	41.14	1.56	149	42.40	1.48	148	41.63	1.04
Apo B48 (mg/L)	124	0.75	0.08	116	0.89	0.09	120	0.75	0.07
TRL ApoB (mg/L)	146	46.17	4.14	145	53.31	3.97	147	49.12	3.06
	Low			**Medium**			**High**		
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	150	1.85	0.08	147	1.86	0.08	148	1.98	0.08
C16:0	150	26.91	0.45	147	26.67	0.39	148	26.05	0.36
C16:1	150	1.49	0.10	147	1.31	0.07	148	1.29	0.07
C18:0	150	4.01	0.18	147	4.22	0.18	148	4.20	0.16
C18:1	150	26.94	0.34	147	26.31	0.37	148	25.85	0.36
C18:2:6	150	26.72	0.49	147	27.79	0.41	148	28.20	0.41
C20:4:6	150	6.83	0.20	147	6.71	0.20	148	7.03	0.18
C20:5:3	150	0.96 ab	0.07	147	0.83 a	0.07	148	1.02 b	0.07
C22:6:3	150	2.13	0.09	147	2.22	0.10	148	2.18	0.10
SFA	150	32.77	0.58	147	32.75	0.51	148	32.22	0.47
MUFA	150	28.71 a	0.37	147	27.82 ab	0.40	148	27.33 b	0.39
PUFA	150	38.51 a	0.61	147	39.44 ab	0.53	148	40.45 b	0.54
n-6 PUFA	150	34.61 a	0.57	147	35.58 ab	0.48	148	36.37 b	0.49
n-3 PUFA	150	3.91	0.15	147	3.86	0.17	148	4.08	0.17
	Low			**Medium**			**High**		
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	149	7.17	0.09	151	7.17	0.09	150	7.25	0.10
Total Energy (MJ)	151	8.47 a	0.20	151	8.73 ab	0.19	151	9.23 b	0.23
Total fat (g/day)	151	71.12 a	2.02	151	85.09 b	2.17	151	100.45 c	2.79
Total fat (%Energy)	151	31.50 a	0.60	151	36.39 b	0.51	151	40.46 c	0.44
SAFA (g/day)	151	20.89 a	0.56	151	28.34 b	0.65	151	39.50 c	1.08
SAFA (%Energy)	151	9.20 a	0.12	151	12.10 b	0.06	151	15.99 c	0.17
MUFA (g/day)	151	27.54 a	1.07	151	32.38 b	1.12	151	35.63 b	1.17
MUFA (%Energy)	151	12.37 a	0.45	151	13.90 b	0.40	151	14.36 b	0.32
PUFA (g/day)	151	12.14 a	0.54	151	13.80 b	0.51	151	14.60 b	0.67
PUFA (%Energy)	151	5.34 a	0.19	151	5.86 b	0.16	151	5.76 ab	0.19
n-3 (g/day)	117	1.29 a	0.09	101	1.50 ab	0.10	96	1.74 b	0.15
n-6 (g/day)	117	8.32 a	0.55	101	9.98 b	0.50	96	10.07 b	0.68
C8 (g/day)	73	0.15 a	0.01	46	0.19 a	0.02	57	0.33 b	0.03
C10 (g/day)	83	0.31 a	0.02	69	0.47 b	0.03	85	0.79 c	0.05
C12 (g/day)	95	0.63 a	0.05	85	1.10 b	0.09	103	1.56 c	0.10
C14 (g/day)	129	1.61 a	0.08	117	2.47 b	0.14	114	3.65 c	0.17
C16 (g/day)	129	10.55 a	0.36	117	14.23 b	0.42	114	17.87 c	0.66
C16:1 (g/day)	129	1.08 a	0.05	117	1.46 b	0.07	114	1.49 b	0.07
C18 (g/day)	129	4.57 a	0.22	117	6.85 b	0.36	114	8.38 c	0.37
C18:1 (g/day)	129	22.85 a	1.23	117	28.17 b	1.31	114	28.07 b	1.34
C18:2:6 (g/day)	144	8.64 a	0.50	142	10.46 b	0.46	133	10.71 b	0.60
C18:3:3 (g/day)	144	0.90 a	0.05	142	1.22 b	0.06	133	1.38 b	0.09
C20:4:6 (g/day)	83	0.13	0.01	69	0.13	0.01	85	0.10	0.01
C20:5:3 (g/day)	132	0.16 a	0.02	126	0.12 ab	0.02	115	0.13 b	0.02
C22:6:3 (g/day)	132	0.23 a	0.03	126	0.22 ab	0.03	115	0.20 b	0.03
Trans fatty acids (g/day)	80	1.71 a	0.16	62	2.29 a	0.19	62	3.65 b	0.32
CHO (g/day)	151	242.60	7.04	151	232.23	6.52	151	228.12	6.41
CHO (%Energy)	151	46.44 a	0.66	151	43.70 b	0.57	151	40.55 c	0.52
Starch (g/day)	90	148.38	6.42	74	138.74	5.97	81	137.91	6.16
Total Sugars (g/day)	94	111.25 a	5.93	81	104.52 ab	4.90	85	91.58 b	5.00
Protein (g/day)	151	92.01	2.59	151	88.50	2.01	151	92.41	2.53
Protein (%Energy)	151	18.56 a	0.37	151	17.44 ab	0.29	151	17.14 b	0.28
Alcohol (g/day)	151	10.94	1.38	151	8.94	1.15	151	6.35	0.88
Alcohol (%Energy)	151	3.47 a	0.42	151	2.60 ab	0.29	151	1.80 b	0.23
Fibre (g)	149	19.81	0.73	148	19.69	0.63	146	18.92	0.65
Vitamin E (mg/day)	151	9.98	0.40	151	10.70	0.49	151	9.96	0.42

$^{abc...}$ denotes significance across tertiles (when two superscripts contain the same letter there is no significant difference between two of the three tertiles) $p<0.05$, One Way ANOVA, $p<0.017$ where bonferroni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

Table 5.9: Tertiles of MUFA (% Energy)

	Low			Medium			High	
Anthropometry	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Age (y)	151	54.84	0.78	151	54.67	0.74	151	54.63	0.69
Height(m)	151	1.70 a	0.01	151	1.69 a	0.01	151	1.65 b	0.01
BodyWeight(kg)	151	90.71	1.16	151	92.30	1.10	151	92.24	1.22
BMI (kg/m^2)	151	31.28 a	0.33	151	32.18 a	0.35	151	33.73 b	0.33
Waist circumference(cm)	151	105.64	0.93	151	106.05	0.88	151	107.91	0.84
Hip circumference (cm)	151	111.17 a	0.70	151	111.78 a	0.88	151	115.22 b	0.83
Diastolic BP(mmHg)	151	84.99	0.71	150	85.86	0.70	151	87.07	0.91
Systolic BP(mmHg)	151	138.13	1.23	150	136.77	1.23	151	140.19	1.32
Body Fat (%)	146	35.92	0.70	147	37.36	0.75	143	36.36	0.72
Body Fat(kg)	146	32.15	0.70	147	34.04	0.76	143	33.72	0.83
Lean Body Mass(%)	146	63.96 a	0.70	147	62.46 a	0.75	143	58.61 b	0.89
Lean Mass Body(kg)	146	57.68	1.05	147	57.39	1.01	143	54.71	1.34
Body Water(%)	118	46.60 a	0.53	125	45.18 a	0.55	134	42.18 b	0.69
Body Water (L)	113	41.65	0.84	115	41.06	0.83	132	39.64	1.05
Impedance(KHz)	127	523.80	7.90	102	525.00	10.29	101	509.92	8.89
	Low			**Medium**			**High**	
IVGTT Parameters	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I $(mU/L)^{-1}min-1$	137	2.85	0.14	137	2.76	0.13	130	2.88	0.18
S_G min-1	138	0.0157	0.0006	138	0.0159	0.0006	130	0.0154	0.0005
AIRg mUL^{-1} min^{-1}	138	387.45	27.75	138	373.29	35.64	130	323.62	23.01
DI	138	981.92 a	66.39	138	912.87 ab	64.43	130	757.45 b	52.65
G0 mg/dl^{-1}	138	296.24	3.57	138	300.47	3.43	130	299.66	3.46
P2 min^{-1}	138	0.042	0.002	138	0.040	0.002	130	0.047	0.004
P3 (mU/L) min^{-2}	138	0.000013	0.000001	138	0.000012	0.000001	130	0.000014	0.000001
Fasting Glucose (mmol/L)	146	5.79 a	0.07	150	6.05 b	0.06	151	6.04 b	0.07
Fasting Insulin(uIU/ml)	146	9.56	0.45	150	10.32	0.48	151	10.48	0.42
HOMA-IR	146	2.49	0.12	150	2.82	0.14	151	2.83	0.12
QUICKI	146	0.61 a	0.01	150	0.60 ab	0.05	151	0.58 b	0.01
Revised QUICKI	146	0.226	0.001	150	0.226	0.002	151	0.223	0.001
C-Peptide ng/ml	144	2.57 a	0.06	150	2.83 b	0.08	150	2.54 a	0.07
Insulin to C-peptide ratio	144	3.61 a	0.10	150	3.57 a	0.12	150	4.22 b	0.15
	Low			**Medium**			**High**	
Lipids and Lipoproteins	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
TAG (mmol/L)	151	1.87	0.08	150	1.83	0.08	151	1.68	0.06
Cholesterol (mmol/L)	151	5.37	0.08	150	5.28	0.07	151	5.31	0.08
NEFA (mmol/L)	151	630.33	20.29	150	619.89	19.16	151	609.91	17.36
TRL TAG (mmol/L)	150	0.94 a	0.05	151	0.94 a	0.05	147	0.72 b	0.04
TRL Cholesterol (mmol/L)	150	0.45 a	0.03	151	0.42 a	0.03	147	0.30 b	0.02
LDL Cholesterol (mmol/L)	149	3.35	0.08	148	3.18	0.08	151	3.20	0.09
HDL (mmol/L)	151	1.08	0.02	150	1.08	0.02	151	1.10	0.02
Apo A1 (g/L)	149	1.35 a	0.02	149	1.36 a	0.02	149	1.46 b	0.02
Apo B (g/L)	149	1.03	0.02	149	1.02	0.02	149	1.01	0.02
Apo CII (mg/L)	149	46.11	1.48	149	45.96	1.54	148	43.86	1.33
Apo CIII (mg/L)	149	161.61 a	4.32	149	161.66 a	4.24	148	147.04 b	4.04
Apo E (mg/L)	148	39.43 a	1.17	149	41.66 ab	1.41	148	44.08 b	1.51
Apo B48 (mg/L)	119	0.74	0.07	116	0.76	0.07	126	0.87	0.09
TRL ApoB (mg/L)	147	63.52 a	4.51	147	52.98 a	3.44	144	32.25 b	2.59
	Low			**Medium**			**High**	
Plasma Fatty Acids (w/w%)	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
C14:0	150	2.19 a	0.09	148	2.02 a	0.07	147	1.49 b	0.07
C16:0	150	28.00 a	0.41	148	26.83 a	0.39	147	24.81 b	0.36
C16:1	150	1.75 a	0.10	148	1.37 b	0.06	147	0.95 c	0.05
C18:0	150	4.14	0.20	148	4.32	0.18	147	3.97	0.13
C18:1	150	25.82 a	0.33	148	25.57 a	0.36	147	27.77 b	0.37
C18:2:6	150	26.81	0.44	148	27.65	0.44	147	28.19	0.44
C20:4:6	150	6.36 a	0.21	148	6.60 a	0.19	147	7.61 b	0.17
C20:5:3	150	0.93 a	0.06	148	1.13 a	0.08	147	0.75 b	0.06
C22:6:3	150	1.78 a	0.09	148	2.30 b	0.11	147	2.45 b	0.08
SFA	150	34.33 a	0.57	148	33.16 a	0.52	147	30.27 b	0.41
MUFA	150	27.84 ab	0.40	148	27.15 a	0.39	147	28.91 b	0.37
PUFA	150	37.83 a	0.60	148	39.68 ab	0.58	147	40.81 b	0.49
n-6 PUFA	150	34.23 a	0.53	148	35.35 ab	0.52	147	36.91 b	0.48
n-3 PUFA	150	3.60 a	0.14	148	4.33 b	0.20	147	3.90 ab	0.14
	Low			**Medium**			**High**	
Dietary Measures	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
Basal Metabolic Rate (MJ/day)	150	7.20	0.09	151	7.21	0.09	149	7.19	0.10
Total Energy (MJ)	151	8.73	0.22	151	9.06	0.22	151	8.64	0.19
Total fat (g/day)	151	68.57 a	2.01	151	89.12 b	2.51	151	98.97 c	2.43
Total fat (%Energy)	151	29.30 a	0.44	151	36.24 b	0.35	151	42.81 c	0.40
SAFA (g/day)	151	25.17 a	0.79	151	33.73 b	1.14	151	29.83 c	0.95
SAFA (%Energy)	151	10.80 a	0.21	151	13.67 b	0.25	151	12.82 c	0.25
MUFA (g/day)	151	20.90 a	0.60	151	30.44 b	0.78	151	44.20 c	1.09
MUFA (%Energy)	151	8.92 a	0.12	151	12.46 b	0.08	151	19.24 c	0.30
PUFA (g/day)	151	11.70 a	0.53	151	15.24 b	0.59	151	13.60 b	0.59
PUFA (%Energy)	151	4.96 a	0.17	151	6.19 b	0.18	151	5.82 b	0.17
n-3 (g/day)	105	1.30 a	0.10	93	1.86 b	0.13	116	1.38 ab	0.11
n-6 (g/day)	105	8.36 a	0.62	93	10.98 b	0.60	116	9.05 a	0.51
C8 (g/day)	86	0.20	0.01	57	0.26	0.03	33	0.19	0.02
C10 (g/day)	104	0.45 a	0.03	92	0.63 b	0.05	41	0.49 ab	0.05
C12 (g/day)	118	0.94 a	0.07	116	1.35 b	0.09	49	0.96 ab	0.11
C14 (g/day)	119	2.33 a	0.12	117	3.02 b	0.16	124	2.27 a	0.15
C16 (g/day)	119	11.20 a	0.49	117	15.58 b	0.60	124	15.38 b	0.49
C16:1 (g/day)	119	0.92 a	0.05	117	1.33 b	0.05	124	1.74 c	0.06
C18 (g/day)	119	5.27 a	0.28	117	7.40 b	0.33	124	6.90 b	0.40
C18:1 (g/day)	119	16.09 a	0.74	117	23.67 b	0.79	124	38.38 c	1.30
C18:2:6 (g/day)	149	8.90 a	0.52	139	11.11 b	0.51	131	9.80 ab	0.53
C18:3:3 (g/day)	149	0.95 a	0.05	139	1.43 b	0.07	131	1.12 a	0.08
C20:4:6 (g/day)	104	0.12	0.01	92	0.12	0.01	41	0.14	0.02
C20:5:3 (g/day)	135	0.13 a	0.02	115	0.14 ab	0.02	123	0.14 b	0.02
C22:6:3 (g/day)	135	0.20	0.03	115	0.23	0.03	123	0.22	0.02
Trans fatty acids (g/day)	111	1.90 a	0.13	69	3.24 b	0.29	24	2.96 ab	0.53
CHO (g/day)	151	258.45 a	7.37	151	241.52 a	6.48	151	202.97 b	5.18
CHO (%Energy)	151	48.25 a	0.61	151	44.03 b	0.50	151	38.41 c	0.46
Starch (g/day)	118	143.71 a	5.57	82	149.81 a	5.53	45	123.33 b	7.84
Total Sugars (g/day)	127	109.04 a	4.80	96	106.29 a	4.83	37	71.81 b	5.17
Protein (g/day)	151	94.83	2.79	151	90.83	2.24	151	87.25	2.06
Protein (%Energy)	151	18.57 a	0.36	151	17.33 b	0.31	151	17.23 b	0.27
Alcohol (g/day)	151	11.99 a	1.42	151	8.55 ab	1.15	151	5.69 b	0.79
Alcohol (%Energy)	151	3.79 a	0.43	151	2.34 b	0.27	151	1.74 b	0.23
Fibre (g)	149	19.25	0.73	148	20.20	0.69	146	18.99	0.59
Vitamin E (mg/day)	151	9.48 a	0.52	151	10.06 a	0.42	151	11.09 b	0.34

$^{abc...}$ denotes significance across tertiles (when two superscripts contain the same letter there is no significant difference between two of the three tertiles) $p<0.05$, One Way ANOVA, $p<0.017$ where bonferroni correction applied

SEM: standard error of the mean

not all IVGTT data modelled sucessfully, therefore different n numbers

BMI (kg/m2), Body Mass Index; BP, blood pressure

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CHO, Carbohydrate

5.4.4 Correlation Analysis

One of the objectives of the study was to assess the relationship between plasma and dietary fatty acids with insulin sensitivity. Correlations were deemed suitable as an investigative statistical technique to demonstrate this. Further in-depth correlational analysis was also carried out on this rich dataset.

The parameters exported from the IVGTT as well as other measures of insulin sensitivity and insulin resistance were correlated with each other and also correlated with anthropometric measures, lipid and apolipoproteins, plasma fatty acids and dietary variables measured at baseline. Correlation analysis set out to determine the extent to which values of two variables are "proportional" or linearly related to each other. While correlation analysis assumes no causal relationship between variables, regression analysis assumes that one variable is dependent upon: A) another single independent variable (Simple Regression), or B) multiple independent variables (Multiple Regression). However, correlational analysis gives a better understanding of the relationship between the two variables as a prelude to the regression analysis. *Table 5.10* presents the correlation coefficients of the IVGTT parameters when correlated with each other. Insulin sensitivity (S_I) correlated significantly with all other IVGTT parameters with the exception of S_G and G0. S_I also correlated significantly with HOMA-IR, QUICKI, Revised QUICKI, C-peptide and insulin to C-peptide ratio. It was most strongly and positively correlated with P3, which represents the movement of circulating insulin to the interstitial space (0.723, $p<0.0005$). S_I was also strongly and negatively correlated with fasting insulin (-0.522, $p<0.0005$), HOMA-IR (-0.332, $p<0.0005$), C-peptide concentrations (-0.407, $p<0.0005$) and insulin to C-peptide ratio (-0.373, $p<0.0005$) and positively correlated with DI (0.364, $p<0.0005$), QUICKI (0.516, $p<0.0005$) and Revised QUICKI (0.461, $p<0.0005$). Glucose effectiveness (S_G) was strongly correlated with G0 (distributed glucose at time 0 minutes) only (0.589, $p<0.0005$). G0 and fasting glucose were strongly and positively correlated as expected (0.425, $p<0.0005$). Strong positive correlations were observed for fasting insulin and HOMA-IR, C-peptide concentrations and insulin to C-peptide ratio (0.967, $p<0.0005$; 0.713, $p<0.0005$ and 0.753, $p<0.0005$, respectively). QUICKI and Revised QUICKI were inversely and strongly correlated with fasting insulin (-0.954, $p<0.0005$ and -0.786, $p<0.0005$, respectively).

IVGTT with anthropometry

Baseline IVGTT parameters and measures of insulin sensitivity and resistance were correlated with the anthropometric measures taken on pre-intervention clinical investigation day and the results presented in *Table 5.11.* There were significant, albeit moderate correlations between S_I and baseline weight (-0.306, $p<0.0005$), BMI (-0.218, $p<0.0005$) and waist circumference (-0.340, $p<0.0005$) with increasing insulin sensitivity values associated with lower body weight, BMI index and a lower waist circumference. AIRg (acute insulin response to glucose) correlated negatively with age (-0.223, $p<0.0005$), but was positively correlated with body weight (0.225, $p<0.0005$) and waist circumference (0.191, $p<0.0005$). P2 and P3 were both inversely correlated with waist circumference (-0.172, $p<0.0005$ ad -0.348, $p<0.0005$, respectively) with P3 also negatively correlated with body weight and BMI (-0.256, $p<0.0005$ and -0.235, $p<0.0005$, respectively). There were weak and non-significant correlations between fasting glucose and anthropometric measures. A weak and positive correlation was observed for fasting glucose concentration at baseline and age (0.186, $p<0.0005$). Fasting insulin concentrations were strongly and significantly correlated with weight (0.342, $p<0.0005$), BMI (0.417, $p<0.0005$), waist and hip circumference (0.402 and 0.289, $p<0.0005$, respectively), body fat and lean body fat (kg) (0.271 and 0.201, $p<0.0005$, respectively). The same correlational trends were observed for HOMA-IR and C-peptide concentrations and inversely for QUICKI and Revised QUICKI.

IVGTT with lipids and apolipoproteins

IVGTT parameters and measures of insulin sensitivity and insulin resistance were correlated with fasting lipid and apolipoprotein concentrations and the results are presented in *Table 5.12.* Insulin sensitivity (S_I) was negatively and weakly, however, significantly correlated with fasting plasma TAG (-0.124, $p<0.05$) and TRL TAG (-0.114, $p<0.05$) and positively correlated with cholesterol concentrations (0.164, $p<0.001$), LDL cholesterol (0.118, $p<0.05$) and more strongly correlated with HDL cholesterol concentrations (0.245, $p<0.0005$). Fasting HDL cholesterol concentrations were also strongly and positively correlated with P3 and QUICKI (0.272 and 0.262, $p<0.0005$, respectively) and negatively correlated with fasting insulin, HOMA-IR and C-peptide concentrations (-0.276, -0.256, -0.269, $p<0.0005$, respectively). Fasting TAG and TRL TAG concentrations were also positively correlated with fasting

insulin, HOMA-IR and C-peptide concentrations (0.259, 0.236 and 0.289, $p<0.0005$, respectively) and negatively correlated with QUICKI and Revised QUICKI values (-0.250 and -0.246, $p<0.0005$, respectively). NEFA concentrations were strongly and negatively correlated with Revised QUICKI (-0.536, $p<0.0005$), which was as expected as it is an inherent feature of the calculation.

IVGTT with Plasma fatty acids (w/w%)

Table 5.13 presents the correlation coefficients between total plasma fatty acid composition (w/w %) and IVGTT parameters and measures of insulin resistance and insulin sensitivity. S_I correlated weakly and negatively with plasma myristic (C14:0), palmitic (C16:0) and saturated fatty acids (-0.122, -0.119 and -0.121, $p<0.05$, respectively) and positively with plasma linoleic acid (C18:2:6) (0.118, $p<0.05$). Plasma myristic (C14:0) acid was more strongly and positively correlated with fasting insulin, HOMA-IR and C-peptide concentrations (0.178, 0.168, 0.204, $p<0.0005$, respectively) and negatively correlated with QUICKI and Revised QUICKI (-0.160, -0.160 $p<0.0005$). Plasma palmitic acid had similar correlational trends but not as significant. Plasma linoleic acid concentrations (C18:2:6) were moderately correlated with glucose effectiveness (S_G) and P2 (0.129, 0.157, respectively, $p<0.05$). It was more significantly and positively correlated with P3, QUICKI and Revised QUICKI (0.171, 0.177 and 0.184, respectively, $p<0.0005$) and negatively correlated with fasting insulin concentrations, HOMA-IR, C-peptide concentrations and insulin to C-peptide ratio (-0.174, -0.171, -0.127 and -0.121, respectively, $p<0.05$). Plasma total SFA concentrations were weakly and negatively correlated with P2, P3 and Revised QUICKI (-0.133, -0.119, -0.138, respectively, $p<0.05$) and more strongly correlated with QUICKI (-0.193, $p<0.0005$). It was positively correlated with fasting insulin, HOMA-IR and C-peptide concentrations (0.184, 0.193, 0.242, respectively, $p<0.0005$). Plasma MUFA concentrations were negatively and weakly correlated to P2 and Revised QUICKI (-0.099 and -0.104, respectively, $p<0.05$). Plasma PUFA concentrations were negatively and also weakly correlated with AIRg, fasting insulin, HOMA-IR and C-peptide concentrations (-0.101, -0.152, -0.143, -0.129, respectively, $p<0.05$). Positive correlations were observed for plasma PUFA concentrations and P2, P3, QUICKI and Revised QUICKI (0.166, 0.151, 0.150, 0.154, respectively, $p<0.05$). Similar correlational relationships were observed for plasma n-6 PUFA

concentrations. Plasma n-3 PUFA, however, were negatively and weakly correlated with AIRg and insulin to c-peptide ratio only (-0.167, -0.136, $p<0.05$).

IVGTT with dietary data

Table 5.14 illustrates the correlation coefficients of the IVGTT parameters and measures of insulin resistance and sensitivity measured at baseline with the dietary data collected at week 0 of the intervention study from the pre-intervention 3-day weighed food diary. Few significant and strong correlations were observed from this analysis. Insulin sensitivity (S_I) was negatively correlated with Basal Metabolic Rate (BMR) (-0.272, $p<0.0005$), dietary *trans* fats (-0.218, $p<0.05$), dietary starch (-0.189, $p<0.05$) and fibre intakes (-0.125, $p<0.05$). Glucose effectiveness (S_G), G0 and P2 did not correlate significantly with any of the dietary variables. AIRg was positively correlated with BMR (0.200, $p<0.0005$), total energy (0.129, $p<0.05$), total dietary fat intake (0.105, $p<0.05$), dietary SFA intake (0.118, $p<0.05$), *trans* fat intake (0.160, $p<0.05$) and total dietary CHO intakes (0.113, $p<0.05$). DI negatively correlated with MUFA (%Energy) and dietary fibre intake (-0.118, -0.130, respectively, $p<0.05$) and positively correlated with dietary alcohol intake (g/day and %Energy) (0.110. 0.112, respectively, $p<0.05$). P3 was also negatively correlated with BMR as were QUICKI values (-0.212, -0.222, respectively, $p<0.0005$). Fasting glucose concentrations were positively correlated with dietary MUFA (%Energy) (0.163, $p<0.0005$). Fasting insulin concentrations were positively correlated with BMR (0.214, $p<0.0005$), total and saturated fat (0.144, 0.143, respectively, $p<0.05$), MUFA (g/day and %Energy) (0.153, 0.116, $p<0.05$), dietary n-6 PUFA (0.122, $p<0.05$) and negatively with alcohol (g/day and %Energy) (-0.155, -0.158, $p<0.001$). HOMA-IR index showed comparable correlation results.

Table 5.10: Correlation coefficients between IVGTT parameters at baseline for n=486 volunteers who took part in the LIPGENE dietary intervention study

	S_G		AIRg		DI		G0		P2		P3		Fasting Glucose		Fasting Insulin		HOMA-IR		QUICKI		Revised QUICKI		C-Peptide		Insulin to C-peptide Ratio	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
S_I	0.061	ns	-0.238	***	0.364	***	-0.071	ns	0.243	***	0.723	***	0.113	*	-0.522	***	-0.332	***	0.516	***	0.461	***	-0.407	***	-0.373	***
S_G			0.043	ns	0.072	ns	0.589	***	-0.113	*	-0.034	ns	-0.068	ns	-0.093	*	-0.105	*	0.092	*	0.119	*	-0.029	ns	-0.102	*
AIRg					0.759	***	-0.156	**	0.085	ns	-0.066	ns	-0.421	***	0.256	***	0.145	*	-0.147	*	-0.105	*	0.140	*	0.235	***
DI							-0.169	***	0.225	***	0.379	***	-0.480	***	-0.085	ns	-0.185	***	0.182	***	0.201	***	-0.123	*	-0.016	ns
G0									-0.123	*	-0.130	*	0.424	***	0.083	ns	0.168	***	-0.181	***	-0.106	*	0.125	*	0.015	ns
P2											0.793	***	-0.179	***	-0.271	***	-0.275	***	0.282	***	0.276	***	-0.252	***	-0.163	**
P3													-0.235	***	-0.509	***	-0.518	***	0.521	***	0.479	***	-0.441	***	-0.332	***
Fasting Glucose															0.197	***	-0.312	***	-0.410	***	-0.350	***	0.229	***	0.080	ns
Fasting Insulin																	0.967	***	-0.954	***	-0.786	***	0.712	***	0.753	***
HOMA-IR																			-0.987	***	-0.814	***	0.709	***	0.715	***
QUICKI																					0.801	***	-0.698	***	-0.702	***
Revised QUICKI																							-0.585	***	-0.595	***
C-Peptide																									0.124	*

Table 5.11: Correlation coefficients between IVGTT parameters and anthropometrics at baseline for n=486 volunteers who took part in the LIPGENE dietary intervention study

	Age		Height		Body Weight		BMI		Waist		Hip		Diastolic BP		Systolic BP		Body Fat %		Body Fat Kg		Lean Body Mass %		Lean Body Mass Kg		Body Water %		Body Water Litres		Impedance	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
S_I	0.042	ns	-0.159	**	-0.306	***	-0.218	***	-0.340	***	-0.048	ns	-0.139	*	-0.060	ns	0.070	ns	-0.097	*	-0.087	ns	-0.239	***	-0.139	*	-0.238	***	0.215	***
S_G	0.017	ns	0.021	ns	-0.015	ns	-0.027	ns	-0.049	ns	0.049	ns	0.058	ns	0.087	ns	0.023	ns	0.018	ns	-0.021	ns	-0.018	ns	0.027	ns	0.024	ns	0.162	*
AIRg	-0.223	***	0.116	*	0.225	***	0.147	*	0.191	***	0.088	ns	0.086	ns	0.011	ns	-0.050	ns	0.060	ns	0.093	ns	0.191	***	0.040	ns	0.155	*	-0.176	*
DI	-0.186	***	0.023	ns	0.016	ns	-0.017	ns	-0.022	ns	0.047	ns	0.012	ns	-0.031	ns	-0.015	ns	-0.016	ns	0.057	ns	0.045	ns	-0.019	ns	0.014	ns	-0.005	ns
G0	0.031	ns	0.020	ns	0.174	***	0.200	***	0.175	***	0.240	***	0.064	ns	0.082	ns	0.182	***	0.249	***	-0.162	**	0.012	ns	-0.086	ns	0.045	ns	0.114	*
P2	-0.145	*	-0.002	ns	-0.070	ns	-0.097	*	-0.172	***	0.008	ns	0.038	ns	0.024	ns	-0.031	ns	-0.072	ns	0.031	ns	-0.021	ns	0.030	ns	-0.037	ns	0.057	ns
P3	-0.062	ns	-0.087	ns	-0.256	***	-0.235	***	-0.348	***	-0.056	ns	-0.082	ns	-0.036	ns	0.003	ns	-0.137	*	-0.018	ns	-0.169	***	-0.062	ns	-0.195	***	0.178	**
Fasting Glucose	0.186	***	-0.025	ns	0.049	ns	0.091	*	0.099	*	0.007	ns	0.030	ns	0.099	*	0.007	ns	0.032	ns	-0.067	ns	-0.001	ns	-0.044	ns	-0.017	ns	-0.013	ns
Fasting Insulin	-0.174	***	-0.006	ns	0.342	***	0.417	***	0.402	***	0.289	***	0.113	*	0.049	ns	0.086	ns	0.271	***	-0.051	ns	0.201	***	-0.016	ns	0.239	***	-0.137	*
HOMA-IR	-0.110	*	-0.001	ns	0.332	***	0.401	***	0.399	***	0.245	***	0.109	*	0.066	ns	0.072	ns	0.251	***	-0.058	ns	0.190	***	-0.030	ns	0.213	***	-0.127	*
QUICKI	0.120	*	0.002	ns	-0.341	***	-0.415	***	-0.410	***	-0.266	***	-0.116	*	-0.070	ns	-0.079	ns	-0.264	***	0.060	ns	-0.195	***	0.024	ns	-0.216	***	0.139	*
Revised QUICKI	0.062	ns	0.144	**	-0.191	***	-0.368	***	-0.269	***	-0.245	***	-0.085	ns	-0.068	ns	-0.201	***	-0.290	***	0.220	***	0.002	ns	0.161	**	-0.029	ns	0.027	ns
C-Peptide	-0.016	ns	0.088	*	0.306	***	0.293	***	0.360	***	0.149	**	0.121	*	0.024	ns	0.068	ns	0.224	***	0.006	ns	0.202	***	0.049	ns	0.228	***	0.003	ns
Insulin to C-peptide Ratic	-0.236	***	-0.105	*	0.208	***	0.342	***	0.236	***	0.291	***	0.034	ns	0.041	ns	0.095	*	0.208	***	-0.111	*	0.067	ns	-0.095	ns	0.087	ns	-0.164	*

r correlation coefficient
***$p < 0.0005$
**$p < 0.001$
*$p < 0.05$
ns non significant

Table 5.12: Correlation coefficients between IVGTT parameters, lipids and apolipoproteins at baseline for n=486 volunteers who took part in the LIPGENE dietary intervention study

	Lipids and Apolipoproteins																							
	TAG		Cholesterol		NEFA		TRL TAG		TRL Cholesterol		LDL		Total HDL		Apo A1		Apo B		Apo CII		Apo CIII		Apo E		Apo B48		TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
S_I	-0.124	*	0.164	**	-0.058	ns	-0.114	*	-0.042	ns	0.118	*	0.245	***	0.143	*	0.009	ns	0.004	ns	0.003	ns	-0.054	ns	0.016	ns	-0.001	ns
S_G	-0.044	ns	0.062	ns	-0.080	ns	-0.040	ns	-0.057	ns	0.008	ns	0.121	*	0.047	ns	-0.012	ns	-0.047	ns	0.035	ns	-0.011	ns	0.138	*	0.030	ns
AIRg	0.053	ns	-0.086	ns	-0.056	ns	0.063	ns	0.037	ns	-0.063	ns	-0.123	*	-0.116	*	-0.045	ns	-0.062	ns	0.002	ns	-0.027	ns	-0.058	ns	0.063	ns
DI	-0.037	ns	0.011	ns	-0.114	*	-0.015	ns	0.008	ns	0.010	ns	0.035	ns	-0.029	ns	-0.053	ns	-0.060	ns	-0.008	ns	-0.076	ns	-0.027	ns	0.050	ns
G0	-0.059	ns	-0.018	ns	-0.042	ns	-0.054	ns	-0.062	ns	-0.105	*	0.049	ns	0.026	ns	-0.042	ns	-0.056	ns	0.017	ns	-0.012	ns	0.098	ns	-0.058	ns
P2	-0.127	*	0.061	ns	-0.086	ns	-0.099	*	-0.036	ns	0.023	ns	0.174	**	0.144	*	-0.024	ns	-0.010	ns	-0.011	ns	-0.005	ns	-0.068	ns	-0.057	ns
P3	-0.148	*	0.155	**	-0.093	*	-0.121	*	-0.045	ns	0.086	ns	0.272	***	0.207	***	-0.002	ns	0.012	ns	-0.016	ns	-0.043	ns	-0.023	ns	-0.040	ns
Fasting Glucose	0.052	ns	0.059	ns	0.038	ns	0.004	ns	-0.026	ns	-0.016	ns	-0.037	ns	0.078	ns	0.108	*	0.033	ns	0.065	ns	0.115	*	0.090	ns	-0.091	*
Fasting Insulin	0.259	***	-0.049	ns	-0.016	ns	0.230	***	0.146	*	-0.018	ns	-0.276	***	-0.031	ns	0.105	*	0.059	ns	0.079	ns	0.151	**	0.130	*	0.095	*
HOMA-IR	0.236	***	-0.038	ns	-0.004	ns	0.189	***	0.106	*	-0.029	ns	-0.256	***	-0.002	ns	0.117	*	0.055	ns	0.080	ns	0.165	***	0.133	*	0.043	ns
QUICKI	-0.250	***	0.030	ns	0.015	ns	-0.211	***	-0.122	*	0.026	ns	0.262	***	0.000	ns	-0.122	*	-0.063	ns	-0.089	*	-0.169	***	-0.140	*	-0.067	ns
Revised QUICKI	-0.246	***	-0.033	ns	-0.536	***	-0.174	**	-0.091	*	-0.063	ns	0.090	*	-0.103	*	-0.082	ns	-0.083	ns	-0.103	*	-0.166	***	-0.085	ns	0.002	ns
C-Peptide	0.289	***	-0.017	ns	0.016	ns	0.290	***	0.225	***	0.033	ns	-0.268	***	-0.082	ns	0.120	*	0.110	*	0.154	**	0.068	ns	0.161	*	0.227	***
Insulin to C-peptide Ratio	0.115	*	-0.058	ns	-0.005	ns	0.070	ns	0.001	ns	-0.057	ns	-0.139	*	0.033	ns	0.006	ns	-0.016	ns	-0.002	ns	0.162	***	0.063	ns	-0.078	ns

Table 5.13: Correlation coefficients between IVGTT parameters and plasma fatty acids (%) at baseline for n=486 volunteers who took part in the LIPGENE dietary intervention study

	Plasma Fatty Acids (%)																							
	C14:0		C16:0		C16:1		C18:0		C18:1		C18:2:6		C20:4:6		C20:5:3		C22:6:3		SFA		MUFA		PUFA		n-6		n-3	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
S_I	-0.122	*	-0.119	*	0.020	ns	0.049	ns	-0.061	ns	0.118	*	-0.027	ns	0.079	ns	-0.019	ns	-0.121	*	-0.043	ns	0.089	ns	0.086	ns	0.023	ns
S_G	-0.014	ns	-0.014	ns	0.048	ns	0.024	ns	-0.091	ns	0.129	*	-0.081	ns	0.099	*	0.007	ns	-0.012	ns	-0.080	ns	0.080	ns	0.081	ns	0.021	ns
AIRg	0.047	ns	0.098	*	-0.017	ns	0.007	ns	0.016	ns	-0.033	ns	-0.037	ns	-0.157	**	-0.181	***	0.094	*	0.012	ns	-0.101	*	-0.053	ns	-0.167	**
DI	-0.024	ns	0.012	ns	0.003	ns	0.031	ns	-0.036	ns	0.053	ns	-0.044	ns	-0.077	ns	-0.170	***	0.005	ns	-0.028	ns	-0.023	ns	0.014	ns	-0.131	ns
G0	-0.055	ns	0.041	ns	0.004	ns	0.175	***	-0.040	ns	0.002	ns	-0.131	*	0.075	ns	0.030	ns	0.057	ns	-0.052	ns	-0.029	ns	-0.050	ns	0.009	ns
P2	-0.090	ns	-0.131	*	-0.028	ns	0.002	ns	-0.103	*	0.157	**	0.035	ns	0.079	ns	0.062	ns	-0.133	*	-0.099	*	0.166	**	0.145	*	0.051	ns
P3	-0.134	*	-0.157	**	0.013	ns	0.011	ns	-0.088	ns	0.171	***	0.000	ns	0.127	*	0.000	ns	-0.119	*	-0.071	ns	0.151	*	0.138	*	0.049	ns
Fasting Glucose	-0.025	ns	0.069	ns	-0.084	ns	0.108	*	-0.011	ns	-0.083	ns	-0.015	ns	0.033	ns	0.154	**	0.086	ns	-0.041	ns	-0.043	ns	-0.080	ns	0.085	ns
Fasting Insulin	0.178	***	0.137	*	-0.016	ns	0.111	*	0.043	ns	-0.174	***	-0.032	ns	-0.131	*	-0.036	ns	0.184	***	0.020	ns	-0.152	**	-0.144	*	-0.082	ns
HOMA-IR	0.168	***	0.150	**	-0.036	ns	0.117	*	0.019	ns	-0.171	***	-0.025	ns	-0.118	*	-0.007	ns	0.193	***	-0.008	ns	-0.143	*	-0.142	*	-0.060	ns
QUICKI	-0.160	**	-0.145	*	0.027	ns	-0.125	*	-0.035	ns	0.177	***	0.036	ns	0.112	*	-0.006	ns	-0.193	***	-0.008	ns	0.150	**	0.150	**	0.050	ns
Revised QUICKI	-0.160	***	-0.147	**	-0.151	**	0.090	*	-0.094	*	0.184	***	-0.012	ns	0.077	ns	-0.016	ns	-0.138	*	-0.104	*	0.154	**	0.150	**	0.026	ns
C-Peptide	0.204	***	0.187	***	-0.003	ns	0.032	ns	-0.041	ns	-0.127	*	-0.085	ns	-0.024	ns	0.015	ns	0.242	***	-0.053	ns	-0.129	*	-0.142	*	0.014	ns
Insulin to C-peptide Ratio	0.072	ns	0.016	ns	-0.008	ns	0.129	*	0.105	*	-0.121	*	0.004	ns	-0.160	***	-0.072	ns	0.059	ns	0.083	ns	-0.116	*	-0.086	*	-0.136	*

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant

Table 5.14: Correlation coefficients between IVGTT parameters and dietary variables at baseline for n=486 volunteers who took part in the LIPGENE dietary intervention study

	Dietary Variables																																			
	BMR		**Total Energy MJ**		**Total fat(g)**		**Total fat%Energy**		**SAFA(g)**		**SAFA%Energy**		**MUFA(g)**		**MUFA%Energy**		**PUFA%Energy**		**n-3(g)**		**n-6(g)**		**Trans fat (g)**		**CHO(g)**		**Starch(g)**		**Alcohol(g)**		**Alcohol%Energy**		**Fibre(g)**		**Vitamin E**	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
S_I	-0.272	***	-0.073	ns	-0.086	ns	-0.067	ns	-0.055	ns	-0.027	ns	-0.091	ns	-0.060	ns	-0.011	ns	-0.067	ns	-0.061	ns	-0.218	*	-0.068	ns	-0.189	*	0.068	ns	0.080	ns	-0.125	*	-0.090	ns
S_G	-0.077	ns	-0.011	ns	-0.011	ns	0.025	ns	0.022	ns	0.073	ns	-0.021	ns	-0.003	ns	0.017	ns	0.118	*	0.108	ns	0.017	ns	-0.020	ns	-0.033	ns	0.016	ns	0.013	ns	0.006	ns	0.017	ns
AIRg	0.200	***	0.129	*	0.105	*	-0.004	ns	0.118	*	0.039	ns	0.046	ns	-0.069	ns	-0.013	ns	-0.005	ns	0.046	ns	0.160	*	0.113	*	0.107	ns	0.059	ns	0.057	ns	-0.078	ns	0.044	ns
DI	0.016	ns	0.078	ns	0.029	ns	-0.071	ns	0.062	ns	-0.001	ns	-0.026	ns	-0.118	*	-0.017	ns	-0.058	ns	-0.010	ns	0.024	ns	0.085	ns	0.011	ns	0.110	*	0.112	*	-0.130	*	-0.023	ns
G0	0.034	ns	-0.057	ns	-0.024	ns	0.029	ns	-0.014	ns	0.062	ns	0.014	ns	0.068	ns	0.012	ns	0.062	ns	0.050	ns	-0.023	ns	-0.072	ns	0.022	ns	-0.002	ns	0.004	ns	-0.021	ns	-0.007	ns
P2	-0.050	ns	0.084	ns	0.050	ns	-0.008	ns	0.053	ns	-0.015	ns	0.058	ns	0.010	ns	0.006	ns	0.037	ns	0.011	ns	0.004	ns	0.089	ns	0.007	ns	0.075	ns	0.071	ns	-0.007	ns	0.034	ns
P3	-0.212	***	0.005	ns	-0.040	ns	-0.065	ns	-0.035	ns	-0.043	ns	-0.032	ns	-0.040	ns	-0.020	ns	-0.060	ns	-0.028	ns	-0.162	*	0.017	ns	-0.116	ns	0.103	*	0.110	*	-0.074	ns	-0.059	ns
Fasting Glucose	0.046	ns	-0.013	ns	0.046	ns	0.078	ns	-0.018	ns	-0.004	ns	0.125	*	0.163	***	0.059	ns	0.033	ns	0.058	ns	0.073	ns	-0.073	ns	0.096	ns	0.021	ns	0.016	ns	0.084	ns	0.115	*
Fasting Insulin	0.214	***	0.078	ns	0.157	**	0.144	*	0.136	*	0.143	*	0.153	**	0.116	*	0.018	ns	0.084	ns	0.122	*	0.130	ns	0.058	ns	0.098	ns	-0.155	**	-0.158	**	-0.021	ns	0.036	ns
HOMA-IR	0.210	***	0.063	ns	0.148	**	0.144	*	0.117	*	0.128	*	0.162	***	0.139	*	0.028	ns	0.086	ns	0.116	*	0.131	ns	0.033	ns	0.114	ns	-0.138	*	-0.142	*	-0.010	ns	0.050	ns
QUICKI	-0.222	***	-0.073	ns	-0.158	**	-0.147	*	-0.123	*	-0.123	*	-0.171	***	-0.143	*	-0.032	ns	-0.085	ns	-0.111	*	-0.114	ns	-0.034	ns	-0.116	ns	0.124	*	0.129	*	0.022	ns	-0.043	ns
Revised QUICKI	-0.038	ns	0.040	ns	-0.051	ns	-0.111	*	-0.017	ns	-0.081	ns	-0.080	ns	-0.106	*	0.010	ns	-0.056	ns	-0.012	ns	-0.103	ns	0.044	ns	0.009	ns	0.134	*	0.128	*	0.004	ns	-0.045	ns
C-Peptide	0.221	***	0.042	ns	0.076	ns	0.055	ns	0.083	ns	0.137	*	0.019	ns	-0.019	ns	0.111	*	0.220	***	0.163	*	0.134	ns	-0.005	ns	0.049	ns	-0.050	ns	-0.051	ns	0.005	ns	0.005	ns
Insulin to C-peptide Ratio	0.087	ns	0.095	*	0.168	***	0.142	*	0.106	*	0.056	ns	0.193	***	0.147	**	-0.091	*	-0.103	ns	-0.004	ns	0.085	ns	0.088	ns	0.087	ns	-0.180	***	-0.181	***	-0.036	ns	0.046	ns

r correlation coefficient
***$p < 0.0005$
**$p < 0.001$
*$p < 0.05$
ns non significant

5.4.5 Linear Regression Analysis

Table 5.15 presents the independent relationship of each variable to S_I measured pre-intervention, using univariate and multivariate regression analysis. Although many variables were significant predictors when entered individually into the model, some failed to be significant in subsequent multiple linear regression after controlling for age, sex and body weight for e.g. waist and hip circumference, systolic blood pressure, body fat mass, lean body mass, body water, impedance, plasma concentrations of myristic acid (C14:0), linoleic acid (C18:2:6), BMR, total energy, total dietary fat (g/day), dietary MUFA (g/day), dietary starch (g/day), dietary protein (%Energy), dietary fibre and Vitamin E. In contrast, some variables became significant after controlling for age, gender and body weight that were not previously identified when entered univariatley e.g. centre, NEFA concentrations, plasma MUFA concentrations, dietary CHO (%energy), dietary protein (g/day), dietary alcohol intakes (g/day), work status and smoking status.

Table 5.16 shows six separate models for which multiple linear regression analysis was performed on each, using the dependent variable S_I pre-intervention. In the centre and IVGTT parameters model, DI, AIRg and P3 and were shown to be the strongest significant predictors of S_I; increasing DI and P3 were positively associated with S_I and increasing AIRg inversely associated with S_I ($p<0.05$). In the anthropometry model, increasing BMI was inversely associated with S_I and hip circumference positively associated with S_I ($p<0.05$). In the lipid and lipoproteins model, age, body weight and NEFA concentrations were significant predictors, negatively associated with S_I, ($p<0.05$). Total HDL cholesterol concentrations were positively associated with S_I. In the plasma fatty acid model, females compared to males and increasing body weight was positively and negatively associated with S_I, respectively, ($p<0.05$). From the dietary variables model, females compared to males were positively associated and increasing body weight and *trans* fatty acid intakes were negatively associated with S_I, ($p<0.05$). Finally, from the health and lifestyle model, females compared to males were positively associated whilst increasing body weight, being married or divorced compared to being single and taking no alcohol were significant predictors of increasing S_I, ($p<0.05$).

Table 5.17 illustrates the main predictors of S_I at baseline. The model had a moderate-to- high adjusted r^2 (68.8%), signifying a good fit for the population. AIRg, DI and P3 were the strongest predictors for S_I. Increasing DI and P3 were positive

predictors whilst increasing AIRg showed an inverse association with S_I. Increasing body weight was also negatively associated with S_I.

Table 5.18 demonstrates the independent relationship of each variable to HOMA-IR also measured pre-intervention, using univariate and multivariate analysis. Considerably more variables were significant predictors of HOMA-IR at the first step of the model development as compared to S_I e.g. age, S_G, G0, P2, TRL Cholesterol concentrations, plasma concentrations of palmitic (C16:0), palmitoleic (C16:1), stearic acid (C18:0), EPA (C20:5:3), saturated fat etc..

Table 5.19 shows the six separate models for which multiple linear regression analysis was performed on each, using the dependent variable HOMA-IR. In the IVGTT parameters model age, volunteers in JUMC, insulin sensitivity (S_I), glucose effectiveness (S_G), AIRg, DI, G0, P2, P3, fasting glucose and fasting insulin concentrations were significant predictors of HOMA-IR; increasing insulin sensitivity (SI), AIRg, G0, P2 and volunteers in JUMC were inversely associated with HOMA-IR and increasing age, S_G, DI, P3, fasting glucose and fasting insulin were positively associated with HOMA-IR. In the anthropometry model, increasing BMI, waist circumference and impedance were positively associated with HOMA-IR and hip circumference inversely associated with HOMA-IR. In the lipid and lipoprotein model, females compared to males, increasing body weight and increasing concentrations of apoE were positive predictors of HOMA-IR and increasing concentrations of TRL cholesterol and HDL cholesterol were associated with decreasing HOMA-IR levels. In the plasma fatty acid model, females compared to males, increasing body weight and increasing concentration of plasma myristic acid (C14:0) were positively associated with HOMA-IR whilst plasma palmitoleic acid (C16:1) was the only significant negative predictor of HOMA-IR. From the dietary variables, increasing body weight and dietary starch were positive predictors of increasing HOMA-IR and in the health and lifestyle model, females compared to males, increasing body weight and working in the home compared to working outside the home were positive predictors of an increasing HOMA-IR index, ($p<0.05$).

Table 5.20 illustrates the main predictors of HOMA-IR at baseline. Again there were a lot more significant predictors of HOMA-IR than S_I and the model had a better adjusted r^2 (99%) which signified an excellent fit for the population. Fasting insulin was the strongest predictor of HOMA-IR, increasing concentrations associated

with an increase in the HOMA-IR index. Other positive and significant predictors from the model were fasting glucose, age, S_G, DI, P3, UoR volunteers, BMI, and apoE concentrations. S_I, AIRg, G0, P2, JUMC volunteers, hip circumference and TRL cholesterol concentrations were significantly inversely associated with HOMA-IR index.

Table 5.15: The relationship of each of the individual variables to S_{I} measured at pre-intervention using univariate and multivariate (controlling for age, sex and post body weight) linear regression analysis.

	Univariate		Multivariate(controlling for age,sex and body weight)			Univariate		Multivariate(controlling for age,sex and body weight)	
	β	Sig.	β	Sig.		β	Sig.	β	Sig.
Age (y)	+	0.48	na	na	Plasma C18:0	+	0.253	+	0.362
Sex (male/female)	+	0.00	na	na	Plasma C18:1	-	0.118	-	0.080
Centre*					Plasma C18:2:6	+	0.055	+	0.152
TCD v Reading	-	0.40	-	0.38	Plamsa C20:4:6	-	0.620	-	0.747
TCD v Oslo	-	0.20	-	0.25	Plasma C20:5:3	+	0.189	+	0.207
TCD v INSERM	-	0.28	-	0.09	Plasma C22:6:3	+	0.901	+	0.868
TCD v Maastricht	-	0.01	-	0.05	Plasma SFA	-	0.443	-	0.855
TCD v Cordoba	-	0.04	-	0.02	Plasma MUFA	-	0.214	-	0.123
TCD v JUMC	-	0.35	-	0.36	Plasma PUFA	+	0.114	+	0.210
TCD v UU	-	0.84	-	0.27	Plasma n-6 PUFA	+	0.147	+	0.291
$S_{G\ min}^{-1}$	+	0.20	+	0.21	Plasma n-3 PUFA	+	0.351	+	0.290
AIRg $mUL^{-1}\ min^{-1}$	-	0.00	-	0.00	Basal Metabolic Rate (MJ/day)	-	0.000	+	0.225
DI	+	0.00	+	0.00	Total Energy (MJ)	-	0.134	+	0.216
G0 mg/dl^{-1}	-	0.33	+	0.99	Total fat (g/day)	-	0.092	+	0.638
P2 min^{-1}	+	0.20	+	0.64	Total fat (%Energy)	-	0.379	-	0.415
P3 $(mU/L)\ min^{-2}$	+	0.00	+	0.00	SAFA (g/day)	-	0.374	+	0.265
Fasting Glucose (mmol/L)	-	0.05	-	0.11	SAFA (%Energy)	+	0.764	+	0.754
Fasting Insulin(uIU/ml)	-	0.00	-	0.00	MUFA (g/day)	-	0.052	-	0.806
HOMA-IR	-	0.00	-	0.00	MUFA (%Energy)	-	0.259	-	0.269
QUICKI	+	0.07	+	0.16	PUFA (g/day)	-	0.401	+	0.265
Revised QUICKI	+	0.00	+	0.00	PUFA (%Energy)	-	0.968	+	0.460
C-Peptide ng/ml	-	0.00	-	0.00	n-3 (g/day)	-	0.424	+	0.601
Insulin to C-peptide Ratio	-	0.00	-	0.00	n-6 (g/day)	-	0.505	+	0.638
Height(m)	-	0.001	+	0.027	Trans fatty acids (g/day)	-	0.015	-	0.045
BodyWeight(kg)	-	0.000	na	na	CHO (g/day)	-	0.147	+	0.753
BMI (kg/m^2)	-	0.000	-	0.014	CHO (%Energy)	-	0.420	-	0.096
Waist circumference(cm)	-	0.000	-	0.181	Starch (g/day)	-	0.023	-	0.602
Hip circumference (cm)	-	0.123	+	0.318	Total Sugars (g/day)	-	0.809	+	0.781
Diastolic BP(mmHg)	-	0.003	-	0.099	Protein (g/day)	-	0.585	+	0.064
Systolic BP(mmHg)	-	0.055	-	0.162	Protein (%Energy)	+	0.133	+	0.187
Body Fat (%)	+	0.122	+	0.877	Alcohol (g/day)	+	0.269	+	0.002
Body Fat(kg)	-	0.064	+	0.522	Alcohol (%Energy)	+	0.134	+	0.001
Lean Body Mass(%)	-	0.034	+	0.726	Fibre (g)	-	0.059	-	0.386
Lean Mass Body(kg)	-	0.000	+	0.115	Vitamin E (mg/day)	-	0.054	-	0.340
Body Water(%)	-	0.007	-	0.589	Marital Status*				
Body Water (L)	-	0.000	+	0.307	Single v Married	-	0.015	-	0.033
Impedance(KHz)	+	0.001	+	0.913	Single v Widow/er	+	0.570	+	0.554
TAG (mmol/L)	-	0.008	-	0.007	Single v Divorced	-	0.048	-	0.052
Cholesterol (mmol/L)	+	0.001	+	0.080	Single v Separated	-	0.046	-	0.111
NEFA (µmol/L)	-	0.316	-	0.030	Single v Living with partner	-	0.161	-	0.249
TRL TAG (mmol/L)	-	0.005	-	0.015	Education*				
TRL Cholesterol (mmol/L)	-	0.172	-	0.189	No formal education v Primary	+	0.924	+	0.881
LDL (mmol/L)	+	0.030	+	0.137	No formal education v Secondary	+	0.761	+	0.375
Total HDL (mmol/L)	+	0.000	+	0.000	No formal education v Vocational	+	0.890	+	0.300
Apo A1(g/L)	+	0.001	+	0.088	No formal education v Higher Vocational	+	0.929	+	0.533
Apo B (g/L)	+	0.932	-	0.787	No formal education v Dipolma/Degree	-	0.984	+	0.347
Apo CII (mg/L)	-	0.390	-	0.145	Work Status*				
Apo CIII (mg/L)	-	0.636	-	0.157	Working v Working in the home	-	0.673	-	0.079
Apo E (mg/L)	-	0.124	-	0.028	Working v Unemployed	-	0.459	-	0.562
Apo B48 (mg/L)	-	0.929	+	0.987	Working v Retired	-	0.704	-	0.723
TRL ApoB (mg/L)	-	0.816	+	0.797	Working v Other	-	0.493	-	0.286
Plasma C14:0	-	0.038	-	0.147	Smoke(Yes v no)	-	0.148	-	0.106
Plasma C16:0	-	0.275	-	0.718	Drink alcohol (Yes v no)	-	0.013	-	0.001
Pasma C16:1	+	0.351	+	0.842	Play sport (Yes v No)	-	0.705	-	0.507

na, non applicable, Sig.=Significance value, β= Beta co-efficient. *Categorical variables with ≥3 categories for which dummy variables were created

$p=0.00 = p<0.001$

Table 5.16: Multiple linear regression predictors of S_I measured at pre-intervention in LIPGENE volunteers, analysis of six different regression models

		S_I			
				95% CI	
		β	Sig.	Lower	Upper
Model 1	**IVGTT Parameters and Centre** (Adjusted r^2=0.656, r^2=0.670)†				
	Age (y)	-0.01	0.78	-0.015	0.011
	Sex (male/female)	0.04	0.28	-0.108	0.372
	Body Weight(kg)	-0.09	0.01	-0.020	-0.002
	Centre*				
	TCD v Reading	-0.01	0.83	-0.442	0.355
	TCD v Oslo	-0.05	0.18	-0.687	0.131
	TCD v INSERM	-0.02	0.54	-0.578	0.304
	TCD v Maastricht	-0.02	0.61	-0.517	0.306
	TCD v Cordoba	-0.04	0.30	-0.600	0.183
	TCD v JUMC	-0.03	0.49	-0.517	0.250
	TCD v UU	-0.02	0.55	-0.561	0.298
	AIRg mUL^{-1} min^{-1}	-0.60	0.00	-0.004	-0.003
	DI	0.65	0.00	0.001	0.002
	P(3)	0.36	0.00	44431.321	66435.904
	Fasting Glucose (mmol/L)	0.03	0.40	-0.077	0.191
	Fasting Insulin(uIU/ml)	0.15	0.18	-0.022	0.117
	C-Peptide ng/ml	-0.12	0.16	-0.560	0.090
	Insulin to C-peptide Ratio	-0.10	0.23	-0.289	0.070
†variables: HOMA-IR, QUICKI, Revised QUICKI were removed from model due to their confounding effects					
Model 2	**Anthropometry** (Adjusted r2=0.120, r2=0.153)¥				
	Age (y)	-0.07	0.28	-0.038	0.011
	Sex (male/female)	0.04	0.73	-0.678	0.966
	Height(m)	-0.11	0.29	-5.289	1.571
	BMI (kg/m^2)	-0.46	0.00	-0.298	-0.082
	Waist circumference(cm)	-0.07	0.45	-0.040	0.018
	Hip circumference (cm)	0.23	0.02	0.005	0.073
	Diastolic BP(mmHg)	-0.05	0.50	-0.033	0.016
	Systolic BP(mmHg)	-0.04	0.61	-0.020	0.012
	Body Fat (%)	0.01	0.94	-0.040	0.043
	Body Water(%)	-0.13	0.15	-0.080	0.013
	Impedance(KHz)	-0.06	0.49	-0.004	0.002
¥variables: Body fat (kg), Lean Body Mass (% and Kg), Body Weight(kg) and Body Water (L) were removed from model due to their confounding effects					
Model 3	**Lipid and lipoprotein Markers** (Adjusted r2=0.155, r2=0.177)				
	Age (y)	-0.08	0.09	-0.034	0.003
	Sex (male/female)	0.06	0.26	-0.161	0.593
	Body Weight(kg)	-0.25	0.00	-0.043	-0.017
	TAG (mmol/L)	0.00	1.00	-0.464	0.467
	Cholesterol (mmol/L)	0.07	0.38	-0.169	0.440
	NEFA (µmol/L)	-0.12	0.01	-0.002	0.000
	TRL TAG (mmol/L)	-0.05	0.67	-0.748	0.484
	LDL (mmol/L)	0.04	0.60	-0.179	0.312
	Total HDL (mmol/L)	0.26	0.00	0.568	2.555
	Apo A1(g/L)	-0.11	0.16	-1.750	0.280
	Apo E (mg/L)	-0.06	0.32	-0.018	0.006
Model 4	**Plasma Fatty Acids** (Adjusted r2=0.116, r2=0.121)§				
	Age (y)	-0.08	0.12	-0.033	0.004
	Sex (male/female)	0.10	0.05	-0.003	0.717
	Body Weight(kg)	-0.28	0.00	-0.047	-0.021
	C14:0	-0.06	0.23	-0.296	0.071
	Plasma C18:2:6	0.02	0.84	-0.052	0.064
	Plasma PUFA	-0.02	0.86	-0.048	0.040
	Plasma C18:1	-0.07	0.17	-0.072	0.012
§variables: Plasma MUFA were removed from model due to their confounding effects					
Model 5	**Dietary Variables** (Adjusted r2=0.117, r2=0.170)¶				
	Age (y)	-0.07	0.39	-0.042	0.016
	Sex (male/female)	0.16	0.07	-0.050	1.194
	Body Weight(kg)	-0.29	0.00	-0.055	-0.014
	MUFA (g/day)	0.06	0.74	-0.035	0.050
	Trans fatty acids (g/day)	-0.16	0.04	-0.266	-0.004
	CHO (%Energy)	-0.01	0.95	-0.081	0.076
	Starch (g/day)	-0.09	0.54	-0.012	0.006
	Protein (g/day)	0.15	0.25	-0.006	0.024
	Protein (%Energy)	-0.04	0.76	-0.138	0.101
	Alcohol (g/day)	0.08	0.72	-0.043	0.062
	Alcohol (%Energy)	0.08	0.71	-0.149	0.218
	Fibre (g)	0.00	0.98	-0.035	0.037
	Vitamin E (mg/day)	-0.06	0.48	-0.075	0.035
¶variables: BMR, Total Energy(MJ/day), Total Fat (g/day) were removed from model due to their confounding effects					
Model 6	**Health and Lifestyle Markers** (Adjusted r2=0.134, r2=0.177)				
	Age (y)	-0.06	0.27	-0.034	0.010
	Sex (male/female)	0.14	0.01	0.100	0.867
	Body Weight(kg)	-0.27	0.00	-0.046	-0.020
	Marital Status*				
	Single v Married	-0.15	0.03	-0.989	-0.061
	Single v Widow/er	0.02	0.67	-0.766	1.195
	Single v Divorced	-0.09	0.06	-1.743	0.047
	Single v Separated	-0.08	0.11	-2.668	0.280
	Single v Living with partner	-0.08	0.18	-0.929	0.175
	Work Status*				
	Working v Working in the home	-0.03	0.63	-0.659	0.397
	Working v Unemployed	-0.02	0.74	-1.193	0.844
	Working v Retired	0.00	0.94	-0.469	0.509
	Working v Other	-0.05	0.24	-1.152	0.291
	Smoke(Yes v no)	-0.07	0.15	-0.707	0.105
	Drink alcohol (Yes v no)	-0.16	0.00	-1.003	-0.248

Sig. significance value, β= Beta co-efficient, CI = confidence intervals. Each model controlled for sex, age and body weight
*Categorical variables with ≥3 categories for which dummy variables were created. p=0.00 = p<0.001

Table 5.17: Multiple linear regression model of variables predicting increasing S_I pre-intervention in LIPGENE volunteers

			95% CI	
	β	Sig.	Lower	Upper
Age (y)	-0.01	0.77	-0.022	0.016
Sex (male/female)	-0.03	0.66	-0.622	0.393
Body Weight(kg)	-0.16	0.08	-0.042	0.003
AIRg mUL^{-1} min^{-1}	-0.58	0.00	-0.004	-0.002
DI	0.63	0.00	0.001	0.002
P(3)	0.36	0.00	38043.566	71225.622
BMI (kg/m^2)	-0.01	0.93	-0.076	0.069
Hip circumference (cm)	0.09	0.24	-0.011	0.043
NEFA (µmol/L)	0.02	0.75	-0.001	0.001
Total HDL (mmol/L)	0.03	0.56	-0.436	0.798
Trans fatty acids (g/day)	-0.05	0.30	-0.121	0.037
Marital Status*				
Single v Married	-0.10	0.10	-0.803	0.077
Single v Widow/er	-0.02	0.65	-1.148	0.717
Single v Divorced	-0.02	0.70	-1.041	0.703
Single v Separated	-0.04	0.42	-2.008	0.849
Single v Living with partner	-0.06	0.29	-0.826	0.248
Drink alcohol (Yes v no)	-0.04	0.37	-0.545	0.204

Adjusted r^2=0.645, r^2=0.688; p=0.00 = p<0.001
Model controlled for sex, age and body weight
* Categorical variables of 3 categories or more, for which dummy variables were created
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.

Table 5.18: The relationship of each of the individual variables to HOMA-IR measured at pre-intervention using univariate and multivariate (controlling for age, sex and post body weight) linear regression analysis.

	Univariate		Multivariate(controlling for age,sex and body weight)			Univariate		Multivariate(controlling for age,sex and body weight)	
	β	Sig.	β	Sig.		β	Sig.	β	Sig.
Age (y)	-	0.047	na	na	Plasma C18:0	+	0.029	+	0.020
Sex (male/female)	-	0.448	na	na	Plasma C18:1	-	0.964	-	0.917
Centre*					Plasma C18:2:6	-	0.004	-	0.015
TCD v Reading	+	0.121	+	0.059	Plamsa C20:4:6	-	0.225	-	0.147
TCD v Oslo	+	0.129	+	0.155	Plasma C20:5:3	-	0.084	-	0.075
TCD v INSERM	-	0.338	-	0.848	Plasma C22:6:3	+	0.888	-	0.985
TCD v Maastricht	+	0.431	+	0.336	Plasma SFA	+	0.001	+	0.001
TCD v Cordoba	+	0.037	+	0.024	Plasma MUFA	-	0.666	-	0.608
TCD v JUMC	+	0.308	+	0.605	Plasma PUFA	-	0.005	-	0.009
TCD v UU	-	0.026	-	0.130	Plasma n-6 PUFA	-	0.005	-	0.011
S_I $(mU/L)^{-1}min-1$	-	0.000	-	0.000	Plasma n-3 PUFA	-	0.414	-	0.308
$S_{G\ min}{}^{-1}$	-	0.026	-	0.007	Basal Metabolic Rate (MJ/day)	+	0.000	-	0.281
AIRg mUL^{-1} min^{-1}	+	0.000	+	0.000	Total Energy (MJ)	+	0.071	+	0.436
DI	-	0.006	-	0.003	Total fat (g/day)	+	0.001	+	0.015
G0 mg/dl^{-1}	+	0.008	+	0.261	Total fat (%Energy)	+	0.001	+	0.002
P2 min^{-1}	-	0.000	-	0.000	SAFA (g/day)	+	0.004	+	0.033
P3 (mU/L) min^{-2}	-	0.000	-	0.000	SAFA (%Energy)	+	0.015	+	0.015
Fasting Glucose (mmol/L)	+	0.000	+	0.000	MUFA (g/day)	+	0.000	+	0.009
Fasting Insulin(uIU/ml)	+	0.000	+	0.000	MUFA (%Energy)	+	0.004	+	0.010
QUICKI	-	0.000	-	0.000	PUFA (g/day)	+	0.327	+	0.996
Revised QUICKI	-	0.000	-	0.000	PUFA (%Energy)	+	0.953	-	0.629
C-Peptide ng/ml	+	0.000	+	0.000	n-3 (g/day)	+	0.126	+	0.501
Insulin to C-peptide Ratio	+	0.000	+	0.000	n-6 (g/day)	+	0.081	+	0.285
Height(m)	-	0.639	-	0.000	Trans fatty acids (g/day)	+	0.152	+	0.222
BodyWeight(kg)	+	0.000	na	na	CHO (g/day)	+	0.535	-	0.810
BMI (kg/m^2)	+	0.000	+	0.000	CHO (%Energy)	-	0.032	-	0.083
Waist circumference(cm)	+	0.000	+	0.000	Starch (g/day)	+	0.040	+	0.138
Hip circumference (cm)	+	0.000	+	0.908	Total Sugars (g/day)	+	0.839	+	0.991
Diastolic BP(mmHg)	+	0.222	+	0.845	Protein (g/day)	+	0.045	+	0.220
Systolic BP(mmHg)	+	0.623	+	0.862	Protein (%Energy)	+	0.893	+	0.782
Body Fat (%)	+	0.067	+	0.988	Alcohol (g/day)	-	0.031	-	0.010
Body Fat(kg)	+	0.000	+	0.551	Alcohol (%Energy)	-	0.025	-	0.008
Lean Body Mass(%)	-	0.166	-	0.173	Fibre (g)	+	0.634	+	0.999
Lean Mass Body(kg)	+	0.002	-	0.077	Vitamin E (mg/day)	+	0.818	-	0.765
Body Water(%)	-	0.619	-	0.558	Marital Status*				
Body Water (L)	+	0.002	-	0.239	Single v Married	-	0.893	-	0.895
Impedance(KHz)	-	0.066	+	0.712	Single v Widow/er	-	0.530	-	0.399
TAG (mmol/L)	+	0.000	+	0.000	Single v Divorced	+	0.511	+	0.596
Cholesterol (mmol/L)	-	0.197	-	0.566	Single v Separated	+	0.023	+	0.108
NEFA (µmol/L)	+	0.752	+	0.657	Single v Living with partner	-	0.662	-	0.401
TRL TAG (mmol/L)	+	0.001	+	0.002	Education*				
TRL Cholesterol (mmol/L)	+	0.061	+	0.034	No formal education v Primary	-	0.744	-	0.860
LDL (mmol/L)	-	0.294	-	0.402	No formal education v Secondary	-	0.732	-	0.356
Total HDL (mmol/L)	-	0.000	-	0.000	No formal education v Vocational	-	0.795	-	0.463
Apo A1(g/L)	-	0.442	-	0.889	No formal education v Higher Vocational	-	0.787	-	0.426
Apo B (g/L)	+	0.165	+	0.119	No formal education v Dipolma/Degree	-	0.875	-	0.344
Apo CII (mg/L)	+	0.370	+	0.132	Work Status*				
Apo CIII (mg/L)	+	0.265	+	0.093	Working v Working in the home	+	0.038	+	0.013
Apo E (mg/L)	+	0.003	+	0.001	Working v Unemployed	-	0.516	-	0.382
Apo B48 (mg/L)	+	0.007	+	0.021	Working v Retired	-	0.240	+	0.963
TRL ApoB (mg/L)	+	0.558	+	0.516	Working v Other	+	0.840	+	0.487
Plasma C14:0	+	0.022	+	0.072	Smoke(Yes v no)	+	0.698	+	0.724
Plasma C16:0	+	0.002	+	0.004	Drink alcohol (Yes v no)	+	0.088	+	0.206
Pasma C16:1	-	0.131	-	0.106	Play sport (Yes v No)	+	0.124	+	0.065

[na], non applicable, Sig.=Significance value, β= Beta co-efficient. *Categorical variables with ≥3 categories for which dummy variables were created

$p=0.00 = p<0.001$

Table 5.19: Multiple linear regression predictors of HOMA-IR measured at pre-intervention in LIPGENE volunteers, analysis of six different regression models

		HOMA-IR			
				95% CI	
		β	Sig.	Lower	Upper
Model 1	**IVGTT Parameters and Centre** (Adjusted r^2=0.998, r^2=0.999)				
	Age (y)	0.02	0.00	0.001	0.006
	Sex (male/female)	0.00	0.70	-0.053	0.035
	Body Weight(kg)	0.00	0.77	-0.001	0.002
	Centre*				
	TCD v Reading	0.00	0.81	-0.062	0.079
	TCD v Oslo	0.01	0.20	-0.027	0.124
	TCD v INSERM	-0.01	0.37	-0.114	0.043
	TCD v Maastricht	0.00	0.74	-0.060	0.085
	TCD v Cordoba	0.00	0.79	-0.079	0.060
	TCD v JUMC	-0.05	0.00	-0.302	-0.167
	TCD v UU	0.01	0.11	-0.013	0.139
	S_I $(mU/L)^{-1}min-1$	-0.06	0.00	-0.072	-0.030
	$S_{G\ min}^{-1}$	0.03	0.00	3.036	12.168
	AIRg mUL^{-1} min^{-1}	-0.08	0.00	0.000	0.000
	DI	0.06	0.00	0.000	0.000
	G0 mg/dl^{-1}	-0.03	0.00	-0.002	0.000
	P2 min^{-1}	-0.04	0.00	-3.359	-1.296
	P3 (mU/L) min^{-2}	0.06	0.00	4434.767	11827.118
	Fasting Glucose (mmol/L)	0.24	0.00	0.431	0.487
	Fasting Insulin(uIU/ml)	0.97	0.00	0.270	0.294
	QUICKI	0.00	0.73	-0.039	0.055
	Revised QUICKI	0.01	0.14	-0.327	2.367
	C-Peptide ng/ml	0.00	0.95	-0.063	0.059
	Insulin to C-peptide Ratio	0.02	0.21	-0.012	0.055
Model 2	**Anthropometry** (Adjusted r2=0.173, r2=0.196)¥				
	Age (y)	-0.01	0.81	-0.023	0.018
	Sex (male/female)	0.11	0.32	-0.335	1.028
	Height(m)	0.02	0.89	-3.933	4.549
	BMI (kg/m^2)	0.51	0.00	0.098	0.296
	Waist circumference(cm)	0.22	0.01	0.008	0.056
	Hip circumference (cm)	-0.23	0.01	-0.064	-0.008
	Body Fat (%)	-0.13	0.18	-0.060	0.011
	Lean Mass Body(kg)	0.02	0.88	-0.034	0.039
	Impedance(KHz)	0.16	0.04	0.000	0.005
¥variables: Body fat (kg), Body Weight(kg) and Body Water (L) were removed from model due to their confounding effects					
Model 3	**Lipid and lipoprotein Markers** (Adjusted r2=0.152, r2=0.180)				
	Age (y)	0.04	0.49	-0.012	0.025
	Sex (male/female)	0.17	0.00	0.184	0.935
	Body Weight(Kg)	0.32	0.00	0.023	0.049
	TAG (mmol/L)	0.13	0.35	-0.250	0.700
	TRL TAG (mmol/L)	0.19	0.20	-0.270	1.283
	TRL Cholesterol (mmol/L)	-0.32	0.01	-2.772	-0.482
	Total HDL (mmol/L)	-0.20	0.00	-1.818	-0.396
	Apo B (g/L)	0.02	0.71	-0.607	0.896
	Apo CII (mg/L)	-0.09	0.26	-0.022	0.006
	Apo CIII (mg/L)	0.01	0.87	-0.005	0.006
	Apo E (mg/L)	0.17	0.01	0.004	0.030
	Apo B48 (mg/L)	0.04	0.42	-0.125	0.298

		HOMA-IR			
				95% CI	
		β	Sig.	Lower	Upper
Model 4	**Plasma Fatty Acids** (Adjusted r2=0.124, r2=0.143)§				
	Age (y)	0.04	0.37	-0.009	0.024
	Sex (male/female)	0.18	0.00	0.252	0.903
	Body Weight(Kg)	0.36	0.00	0.029	0.052
	Plasma C14:0	0.12	0.03	0.019	0.381
	Plasma C16:0	0.05	0.46	-0.028	0.062
	Pasma C16:1	-0.14	0.02	-0.403	-0.037
	Plasma C18:0	0.05	0.37	-0.048	0.130
	Plasma C18:2:6	-0.16	0.13	-0.107	0.013
	Plasma C20:5:3	-0.09	0.12	-0.358	0.039
	Plasma PUFA	0.11	0.44	-0.039	0.090
§variables: Plasma n-6 PUFA and Plasma SFA were removed from model due to their confounding effects					
Model 5	**Dietary Variables** (Adjusted r2=0.090, r2=0.157)¶				
	Age (y)	-0.01	0.88	-0.030	0.026
	Sex (male/female)	0.14	0.13	-0.142	1.052
	Body Weight(Kg)	0.32	0.00	0.016	0.056
	SAFA (g/day)	-0.22	0.32	-0.086	0.028
	SAFA (%Energy)	0.22	0.25	-0.083	0.311
	MUFA (%Energy)	0.04	0.75	-0.073	0.101
	n-3 (g/day)	-0.03	0.76	-0.325	0.239
	n-6 (g/day)	0.07	0.51	-0.038	0.077
	CHO (%Energy)	-0.12	0.51	-0.097	0.048
	Starch (g/day)	0.25	0.05	0.000	0.015
	Protein (g/day)	0.04	0.78	-0.014	0.018
	Alcohol (g/day)	-0.14	0.54	-0.065	0.034
	Alcohol (%Energy)	0.03	0.90	-0.171	0.194
¶variables: BMR, Total Energy(MJ/day), Total Fat (g/day), MUFA (g/day) were removed from model due to their confounding effects					
Model 6	**Health and Lifestyle Markers** (Adjusted r2=0.112, r2=0.140)				
	Age (y)	0.01	0.83	-0.017	0.022
	Sex (male/female)	0.12	0.03	0.040	0.730
	Body Weight(Kg)	0.37	0.00	0.030	0.053
	Marital Status*				
	Single v Married	-0.04	0.58	-0.536	0.299
	Single v Widow/er	-0.06	0.24	-1.406	0.358
	Single v Divorced	0.02	0.64	-0.611	1.000
	Single v Separated	0.07	0.14	-0.332	2.324
	Single v Living with partner	-0.05	0.37	-0.722	0.272
	Work Status*				
	Working v Working in the home	0.12	0.03	0.059	1.011
	Working v Unemployed	-0.04	0.39	-1.318	0.519
	Working v Retired	0.00	1.00	-0.441	0.441
	Working v Other	0.03	0.50	-0.425	0.876
	Drink alcohol (Yes v no)	0.02	0.71	-0.276	0.407
	Play sport (Yes v No)	0.07	0.11	-0.052	0.512

Sig. significance value, β= Beta co-efficient, CI = confidence intervals. Each model controlled for sex, age and body weight

*Categorical variables with ≥3 categories for which dummy variables were created. p=0.00 = p<0.001

Table 5.20: Multiple linear regression model of variables predicting increasing HOMA-IR pre-intervention in LIPGENE volunteers

			95% CI	
	β	Sig.	Lower	Upper
Age (y)	0.02	0.01	0.001	0.006
Sex (male/female)	0.01	0.16	-0.017	0.109
Body Weight(kg)	0.02	0.13	-0.001	0.005
S_I $(mU/L)^{-1}min-1$	-0.05	0.00	-0.069	-0.029
$S_{G\ min}^{-1}$	0.03	0.00	2.399	11.023
AIRg mUL^{-1} min^{-1}	-0.07	0.00	0.000	0.000
DI	0.06	0.00	0.000	0.000
G0 mg/dl^{-1}	-0.02	0.03	-0.002	0.000
P2 min^{-1}	-0.05	0.00	-3.521	-1.533
P3 (mU/L) min^{-2}	0.06	0.00	5563.110	12678.732
Fasting Glucose (mmol/L)	0.24	0.00	0.423	0.476
Fasting Insulin(uIU/ml)	0.97	0.00	0.279	0.287
Centre*				
TCD v Reading	0.02	0.03	0.008	0.149
TCD v Oslo	0.00	0.96	-0.074	0.070
TCD v INSERM	-0.01	0.11	-0.142	0.014
TCD v Maastricht	0.00	0.95	-0.070	0.075
TCD v Cordoba	0.00	0.67	-0.089	0.057
TCD v JUMC	-0.04	0.00	-0.268	-0.133
TCD v UU	0.01	0.20	-0.027	0.127
BMI (kg/m^2)	0.03	0.00	0.004	0.021
Waist circumference(cm)	0.01	0.46	-0.002	0.004
Hip circumference (cm)	-0.07	0.00	-0.014	-0.008
TRL Cholesterol (mmol/L)	-0.02	0.01	-0.199	-0.029
Total HDL (mmol/L)	0.01	0.10	-0.013	0.140
Apo E (mg/L)	0.02	0.01	0.001	0.003
Plasma C14:0	-0.01	0.28	-0.036	0.010
Pasma C16:1	0.00	0.86	-0.023	0.020
Work Status*				
Working v Working in the home	0.00	0.64	-0.074	0.045
Working v Unemployed	0.01	0.11	-0.019	0.196
Working v Retired	-0.01	0.21	-0.083	0.019
Working v Other	0.01	0.19	-0.025	0.128

Adjusted r^2=0.990, r^2=0.990; p=0.00 = p<0.001
Model controlled for sex, age and body weight
* Categorical variables of 3 categories or more, for which dummy variables were created
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.

5.4.6 Number of Criteria of the MetS

The levels of insulin sensitivity, other IVGTT parameters, fasting insulin and glucose and other measures of insulin sensitivity and resistance were examined in relation to the number of MetS criteria (*Table 5.21*). Insulin sensitivity non-significantly decreased with the increasing number of criteria, however, QUICKI and Revised QUICKI significantly decreased with increasing numbers, ($p<0.05$). Similarly, fasting insulin levels, HOMA-IR and C-peptide concentrations significantly increased by the increasing number of MetS criteria, ($p<0.05$).

Table 5.21: Insulin sensitivity index and other IVGTT parameters, fasting insulin and glucose and measures of insulin sensitivity and resistance in relation to the number of metabolic syndrome criteria in LIPGENE subjects at baseline.

	Number of MetS Criteria													
	3				4				5					
	n	Mean		SD	SEM	n	Mean		SD	SEM	n	Mean		SD	SEM
S_I $(mU/L)^{-1}min^{-1}$	125	3.10		1.78	0.16	159	2.58		1.55	0.12	85	2.45		1.25	0.14
S_G min^{-1}	125	0.0158		0.0053	0.0005	160	0.0154		0.0069	0.0005	86	0.0156		0.0061	0.0007
AIRg mUL^{-1} min^{-1}	125	326.26		260.32	23.28	160	407.66		421.63	33.33	86	307.51		266.12	28.70
DI	125	897.54		731.17	65.40	160	904.43		731.58	57.84	86	699.82		646.87	69.75
G0 mg/dl^{-1}	125	296.17	a	36.52	3.27	160	299.39	ab	44.28	3.50	86	309.20	b	35.75	3.86
P2 min^{-1}	125	0.04604	a	0.03449	0.00309	160	0.04441	a	0.02887	0.00228	86	0.03570	b	0.02675	0.00288
P3 (mU/L) min^{-2}	125	0.0000146	a	0.0000130	0.0000012	160	0.0000120	a	0.0000106	0.0000008	86	0.0000086	b	0.0000074	0.0000008
Fasting Glucose (mmol/l)	139	5.87	a	0.90	0.08	173	6.01	a	0.75	0.06	93	6.34	b	0.75	0.08
Fasting Insulin(uIU/ml)	139	8.93	a	4.71	0.40	173	10.93	b	5.88	0.45	93	12.48	c	5.68	0.59
HOMA-IR	141	2.30	a	1.35	0.11	175	2.90	b	1.66	0.13	93	3.54	c	1.74	0.18
QUICKI	139	0.66	a	0.54	0.05	173	0.55	b	0.37	0.03	93	0.55	c	0.06	0.01
Revised QUICKI	139	0.23	a	0.02	0.00	173	0.22	b	0.02	0.00	93	0.22	c	0.01	0.00
C-Peptide ng/ml	138	2.49	a	0.83	0.07	173	2.75	b	0.91	0.07	92	3.04	c	0.88	0.09
Insulin to C-peptide Ratio	138	3.59	a	1.58	0.13	173	4.00	b	1.68	0.13	92	4.10	b	1.24	0.13

$^{abc...}$ denotes significance across age categories (when two superscripts contain the same letter there is no significant difference between two of the three age categories) $p<0.05$, One Way ANOVA

5.4.7 Binary logistic regression analysis

The influence of each of the individual variables on predicting "3 versus 4", "4 versus 5" and "3 versus 5" criteria of the MetS are presented in *Table 5.22, 5.23 and 5.24* respectively. Many variables were significant predictors independently. *Table 5.25, 5.26 and 5.27* show six separate models for which multiple binary logistic regression analysis was performed on each for the dependent dichotomous variable "3 versus 4", "4 versus 5" and "3 versus 5", respectively. In the IVGTT parameters and centre model, increasing fasting insulin and decreasing HOMA-IR index were significant predictors of having 3 versus 4 criteria of the MetS; there were no significant IVGTT parameters predictive of having 4 versus 5 criteria and males compared to females,

increasing body weight and increasing glucose concentrations were significant predictors of having 5 versus 3 criteria of the MetS criteria. In the anthropometry model, increasing body weight, BMI, diastolic blood pressure, body fat (%), and all BMI categories were significant predictors of having 4 versus 3 criteria of the MetS; increasing age only was a significant predictor of having 5 versus 4 criteria and there were no significant anthropometric predictors of having 5 versus 3 criteria. In model 3 (lipids and lipoproteins) females compared to males, body weight and apoB concentrations were significant negative predictors of having 3 versus 4 criteria of the MetS whilst apoA1 concentration was a positive predictor. For 4 versus 5 criteria, younger age, males compared to females, a decrease in body weight, decrease in TAG and TRL TAG, apoB and apoB48 concentrations and an increase in TRL apoB concentrations were significant predictors of having 4 versus 5 criteria. Similarly, younger ages, males compared to females, decrease in body weight, TAG, TRL TAG, apoB and an increase in apoCIII concentrations were significant predictors of having 3 versus 5 criteria of the MetS. For the plasma fatty acid model, males compared to females, decreasing concentrations of plasma myristic (C14:0), palmitic (C16:0) and n-6 PUFA and increasing concentrations of linoleic (C18:2:6), arachidonic (C20:4:6) and SFA are associated with 3 versus 4 criteria; decreas in age, males compared to females, decreasing body weights and an increase in plasma EPA (C20:5:3) are predictors of having 4 versus 5 criteria and males compared to females, decreasing body weights, increasing plasma concentrations of palmitic (C16:0), stearic (C18:0), EPA (C20:5:3) and MUFA and decreasing plasma concentrations of palmitoleic (C16:1) and oleic acids (C18:1) were predictive of having 3 versus 5 criteria of the MetS. No dietary variables had predictive properties in the models for each binary dichotomous variable. In the final health and lifestyle model, aside from gender and body weight which was controlled for in each model, living with your partner as compared to being single was a predictive of having 5 versus 4 criteria and working in the home and being retired were predictive of having 5 versus 3 criteria of the MetS.

The variables that were found to be significant ($p<0.05$) in the initial logistic regression models were entered simultaneously into a final multiple logistic regression model controlling for age, sex and body weight of the subject. *Table 5.28, 5.29 and 5.30* present the results of the final models along with the ORs and 95% CI values for each dependent variable respectively. Overall, seven, nine and nine variables significantly predicted "3 versus 4", "4 versus 5" and "3 versus 5" criteria of

the MetS respectively, (p<0.05). Males as compared to females, decreasing fasting insulin concentrations, increasing HOMA-IR index, increasing diastolic blood pressure, increasing TRL TAG and apoB concentrations and decreasing apoA1 concentrations were significantly associated with an increased likelihood of having "4 versus 3" criteria of the MetS. Whereas, increasing age, males compared to females, increasing body weight, TAG, TRL TAG, apoB and apoB48 concentrations, decreasing TRL apoB concentrations and living with your partner as compared to being single were significantly associated with an increased likelihood of having "5 versus 4" criteria of the MetS. Finally, males compared to females, increasing body weight, fasting glucose, TAG, TRL TAG, apoB, plasma palmitic acid (C16:0)and oleic acid (C18:1) concentrations, a decrease in apoCIII, plasma palmitoleic acid (C16:1) were associated with having "5 versus 3" criteria of the MetS.

Table 5.22: The influence of each of the individual variables on predicting 3 vs 4 criteria of the MetS as assessed by binary logistic regression.

	3vs4 criteria											
	Univariate		Controlling for sex, age and body weight)		Univariate		Controlling for sex, age and body weight)		Univariate		Controlling for sex, age and body weight)	
	β	Sig.	β	Sig.	β	Sig.	β	Sig.	β	Sig.	β	Sig.
Age (y)	+	0.19	na	na	-	0.00	-	0.00	-	0.29	-	0.23
Sex (male^, female)	-	0.15	na	na	-	0.01	-	0.01	+	0.41	+	0.55
S_I (mU/L)-1min-1	+	0.01	+	0.00	+	0.65	+	0.87	+	0.51	+	0.51
$S_{G\ min}^{-1}$	+	0.58	+	0.42	-	0.00	-	0.00	+	0.16	+	0.30
AIRg mUL^{-1} min^{-1}	-	0.06	-	0.12	-	0.00	-	0.00	+	0.20	+	0.34
DI	+	0.94	+	0.71	-	0.01	-	0.01	-	0.79	-	0.64
G0 mg/dl^{-1}	-	0.51	-	0.71	+	0.00	+	0.00	+	0.52	+	0.62
P2 min^{-1}	+	0.66	+	0.53	+	0.08	+	0.03		0.71		0.55
P3 (mU/L) min^{-2}	+	0.06	+	0.02	-	0.00	-	0.00	+	0.34	+	0.18
Fasting Glucose (mmol/L)	-	0.12	-	0.03	-	0.00	-	0.00	+	0.78	+	0.57
Fasting Insulin(uIU/ml)	-	0.00	-	0.01	-	0.00	-	0.00	+	0.34	+	0.26
HOMA-IR	-	0.00	-	0.00	-	0.01	-	0.01		0.97		0.94
QUICKI	+	0.00	+	0.00	-	0.04	-	0.06	+	0.85	+	0.74
Revised QUICKI	+	0.01	+	0.03	-	0.06	-	0.03	-	0.97	+	0.90
C-Peptide ng/ml	-	0.01	-	0.02	-	0.07	-	0.06		0.60		0.69
Insulin to C-peptide Ratio	-	0.03	-	0.10	-	0.06	-	0.03	-	0.41	-	0.47
TCD^		0.18		0.20	+	0.16	+	0.07	-	0.36	-	0.45
Reading	+	0.38	+	0.54	-	0.76	-	0.82		0.48		0.24
Oslo	+	0.95	+	0.96	-	0.01	-	0.01	-	0.79	-	0.56
INSERM	+	0.01	+	0.01	+	0.00	+	0.00	+	0.77	+	0.74
Maastricht	+	0.84	-	0.84	+	0.01	+	0.01	+	0.39	+	0.39
Cordoba	+	0.90	-	0.92	+	0.45	+	0.50	-	0.46	-	0.70
UMC	-	0.74	-	0.92	+	0.90	+	0.99	+	0.29	+	0.21
Uppsala	-	0.92	-	0.76	-	0.06	-	0.04		0.22		0.28
Low Tertile S_I^		0.02		0.01	-	0.03	-	0.04	+	0.67	+	0.79
Medium Tertile S_I	+	0.05	+	0.04	+	0.00	+	0.00	-	0.72	-	0.76
High Tertile S_I	+	0.00	+	0.00	+	0.00	+	0.00	+	1.00	+	1.00
Height(m)	+	0.48	+	0.76	+	0.87	+	0.97	+	0.16	+	0.17
BodyWeight(kg)	-	0.52			-	0.76	-	0.79	+	0.78	+	0.67
BMI (kg/m^2)	-	0.12	-	0.70	+	0.59	+	0.79		0.63		0.80
Waist circumference(cm)	-	0.73	-	0.91	+	0.44	+	0.47	-	0.34	-	0.35
Hip circumference (cm)	-	0.35	+	0.44	+	0.72	+	0.55	-	0.92	-	0.79
Diastolic BP(mmHg)	-	0.21	-	0.17	+	0.67	+	0.73	+	0.43	-	0.80
Systolic BP(mmHg)	-	0.79	-	0.50	+	0.88	+	0.76	+	0.53	+	0.63
Body Fat (%)	-	0.11	-	0.67	-	0.89	-	0.80	+	0.47	+	0.60
Body Fat(kg)	-	0.14	-	0.89	-	0.41	-	0.48	-	0.95	+	0.58
Lean Body Mass(%)	+	0.21	+	0.70	+	0.34	+	0.38	+	0.83	+	0.92
Lean Mass Body(kg)	+	0.78	-	0.93	+	0.46	+	0.43				
Body Water(%)	+	0.38	+	0.78	+	0.31	+	0.40				
Body Water (L)	+	0.67	+	0.85	+	0.28	+	0.30				
Impedance(KHz)	+	0.91	+	0.66	-	0.32	-	0.34				
Normal Weight^		0.26		0.13	+	0.79	-	0.61				
Overweight	+	0.11	+	0.06	-	0.18	-	0.16				
Obese	+	0.15	+	0.04	-	0.74	-	0.81				

^reference category, na non applicable, Sig.=Significance value, β = Beta co-efficient

$p=0.00 = p<0.001$

Table 5.23: The influence of each of the individual variables on predicting 4 vs 5 criteria of the MetS as assessed by binary logistic regression.

	4vs5 criteria											
	Univariate		Controlling for sex, age and body weight)			Univariate		Controlling for sex, age and body weight)			Univariate		Controlling for sex, age and body weight)	
	β	Sig.	β	Sig.		β	Sig.	β	Sig.		β	Sig.	β	Sig.
Age (y)	-	0.036			TAG (mmol/L)	-	0.001	-	0.000	Total Sugars (g/day)	+	0.320	+	0.442
Sex (male^, female)	-	0.782			Cholesterol (mmol/L)	-	0.304	-	0.159	Protein (g/day)	+	0.769	+	0.975
S_I (mU/L)-1min-1	+	0.506	+	0.671	NEFA (µmol/L)	+	0.504	+	0.775	Protein (%Energy)	+	0.966	+	0.998
$S_{G\ min}^{-1}$	-	0.786	-	0.775	TRL TAG (mmol/L)	-	0.002	-	0.000	Alcohol (g/day)	+	0.342	+	0.275
AIRg mUL^{-1} min^{-1}	+	0.044	+	0.037	TRL Cholesterol (mmol/L)	-	0.012	-	0.002	Alcohol (%Energy)	+	0.435	+	0.317
DI	+	0.033	+	0.082	LDL (mmol/L)	-	0.966	-	0.859	Fibre (g)	+	0.894	+	0.689
G0 mg/dl^{-1}	-	0.085	-	0.278	Total HDL (mmol/L)	+	0.000	+	0.000	Vitamin E (mg/day)	-	0.583	-	0.877
P2 min^{-1}	+	0.023	+	0.034	Apo A1(g/L)	+	0.048	+	0.022	Low Quartile Total Dietary Fat (%Energy)^		0.246		0.243
P3 (mU/L) min^{-2}	+	0.013	+	0.028	Apo B (g/L)	-	0.067	-	0.059	Low/Medium Quartile Total Dietary Fat (%Energy)	-	0.337	-	0.575
Fasting Glucose (mmol/L)	-	0.001	-	0.014	Apo CII (mg/L)	-	0.012	-	0.002	Medium/High Quartile Total Dietary Fat (%Energy)	-	0.043	-	0.052
Fasting Insulin(uIU/ml)	-	0.044	-	0.183	Apo CIII (mg/L)	-	0.033	-	0.006	High Quartile Total Dietary Fat (%Energy)	-	0.277	-	0.480
HOMA-IR	-	0.005	-	0.049	Apo E (mg/L)	-	0.016	-	0.009	Low Tertile SFA (%Energy)^		0.671		0.535
QUICKI	+	0.920	-	0.767	Apo B48 (mg/L)	-	0.000	-	0.000	Medium Tertile SfA (%Energy)	-	0.374	-	0.339
Revised QUICKI	+	0.005	+	0.038	TRL ApoB (mg/L)	-	0.066	-	0.025	High Tertile SFA (%Energy)	-	0.701	+	0.989
C-Peptide ng/ml	-	0.015	-	0.131	Plasma C14:0	-	0.459	-	0.424	Low Tertile MUFA (%Energy)^		0.614		0.796
Insulin to C-peptide Ratio	-	0.627	-	0.678	Plasma C16:0	-	0.141	-	0.140	Medium Tertile MUFA (%Energy)^	-	0.386	-	0.522
TCD^		0.791		0.800	Pasma C16:1	+	0.583	+	0.782	High Tertile MUFA (%Energy)^	-	0.377	-	0.587
Reading	-	0.801	-	0.971	Plasma C18:0	-	0.147	-	0.073	Single^		0.304		0.291
Oslo	-	0.939	+	0.862	Plasma C18:1	-	0.135	-	0.061	Married	-	0.642	-	0.652
INSERM	+	0.654	+	0.717	Plasma C18:2:6	+	0.012	+	0.006	Widow/er	-	0.202	-	0.387
Maastricht	-	0.869	+	0.598	Plamsa C20:4:6	+	0.091	+	0.062	Divorced	-	0.436	-	0.500
Cordoba	-	0.526	-	0.908	Plasma C20:5:3	+	0.146	+	0.046	Separated	+	0.913	+	0.857
JUMC	-	0.709	-	0.935	Plasma C22:6:3	-	0.226	-	0.433	Living with Partner	-	0.078	-	0.058
Uppsala	+	0.239	+	0.143	Plasma SFA	-	0.087	-	0.070	No formal education^		0.602		0.558
Low Tertile S_I^		0.837		0.775	Plasma MUFA	-	0.211	-	0.096	Primary	-	0.580	-	0.306
Medium Tertile S_I	-	0.745	-	0.555	Plasma PUFA	+	0.012	+	0.004	Secondary	+	0.649	+	0.970
High Tertile S_I	+	0.752	+	0.917	Plasma n-6 PUFA	+	0.004	+	0.002	Vocational	-	0.725	-	0.460
Height(m)	+	0.606	+	0.145	Plasma n-3 PUFA	-	0.782	+	0.764	Higher Vocational	+	0.753	-	0.808
BodyWeight(kg)	-	0.038			Basal Metabolic Rate (MJ/day)	+	0.928	+	0.172	Dipolma/Degree	+	0.826	-	0.902
BMI (kg/m^2)	-	0.003	-	0.120	Total Energy (MJ)	+	0.937	-	0.767	Working^		0.375		0.550
Waist circumference(cm)	-	0.059	-	0.955	Total fat (g/day)	-	0.515	-	0.473	Working in the home	-	0.067	-	0.146
Hip circumference (cm)	-	0.004	-	0.097	Total fat (%Energy)	-	0.472	-	0.737	Unemployed	-	0.436	-	0.257
Diastolic BP(mmHg)	+	0.731	+	0.471	SAFA (g/day)	-	0.833	-	0.645	Retired	-	0.076	-	0.179
Systolic BP(mmHg)	-	0.039	-	0.175	SAFA (%Energy)	+	0.918	+	0.937	Other work	-	0.359	-	0.293
Body Fat (%)	-	0.045	-	0.339	MUFA (g/day)	-	0.621	-	0.734	Smoke(Yes^ v no)	-	0.544	-	0.764
Body Fat(kg)	-	0.002	-	0.206	MUFA (%Energy)	-	0.827	+	0.827	Drink alcohol (Yes^ v no)	-	0.210	-	0.435
Lean Body Mass(%)	+	0.067	+	0.128	PUFA (g/day)	-	0.380	-	0.260	Play sport (Yes^ v no)	-	0.334	-	0.282
Lean Mass Body(kg)	-	0.837	+	0.100	PUFA (%Energy)	-	0.129	-	0.137					
Body Water(%)	+	0.069	+	0.170	n-3 (g/day)	-	0.268	-	0.173					
Body Water (L)	-	0.704	+	0.167	n-6 (g/day)	-	0.766	-	0.556					
Impedance(KHz)	+	0.776	-	0.577	Trans fatty acids (g/day)	+	0.958	-	0.719					
Normal Weight^		0.553		0.999	CHO (g/day)	+	0.789	+	0.785					
Overweight	-	0.999	-	0.999	CHO (%Energy)	+	0.755	-	0.938					
Obese	-	0.999	-	0.999	Starch (g/day)	+	0.620	+	0.868					

^reference category, na non applicable, Sig.=Significance value, β = Beta co-efficient
$p=0.00 = p<0.001$

Table 5.24: The influence of each of the individual variables on predicting 3 vs 5 criteria of the MetS, as assessed by binary logistic regression.

	3vs5 criteria											
	Univariate		Controlling for sex, age and body weight)			Univariate		Controlling for sex, age and body weight)			Univariate		Controlling for sex, age and body weight)	
	β	Sig.	β	Sig.		β	Sig.	β	Sig.		β	Sig.	β	Sig.
Age (y)	-	0.356	na	na	TAG (mmol/L)	-	0.000	-	0.000	Total Sugars (g/day)	+	0.845	+	0.729
Sex (male^, female)	-	0.139	na	na	Cholesterol (mmol/L)	-	0.001	-	0.000	Protein (g/day)	+	0.274	+	0.424
S_I (mU/L)-1min-1	+	0.006	+	0.015	NEFA (µmol/L)	-	0.318	+	0.677	Protein (%Energy)	+	0.529	+	0.430
$S_{G\ min}^{-1}$	+	0.830	+	0.767	TRL TAG (mmol/L)	-	0.000	-	0.000	Alcohol (g/day)	+	0.056	+	0.240
AIRg mUL^{-1} min^{-1}	+	0.609	+	0.678	TRL Cholesterol (mmol/L)	-	0.000	-	0.000	Alcohol (%Energy)	+	0.093	+	0.207
DI	+	0.048	+	0.134	LDL (mmol/L)	-	0.026	-	0.028	Fibre (g)	-	0.914	+	0.798
G0 mg/dl^{-1}	-	0.013	-	0.112	Total HDL (mmol/L)	+	0.000	+	0.000	Vitamin E (mg/day)	+	0.956	-	0.858
P2 min^{-1}	+	0.024	+	0.087	Apo A1(g/L)	+	0.001	+	0.000	Low Quartile Total Dietary Fat (%Energy)^		0.267		0.284
P3 (mU/L) min^{-2}	+	0.000	+	0.002	Apo B (g/L)	-	0.000	-	0.000	Low/Medium Quartile Total Dietary Fat (%Energy)	-	0.871	-	0.937
Fasting Glucose (mmol/l)	-	0.000	-	0.000	Apo CII (mg/L)	-	0.000	-	0.000	Medium/High Quartile Total Dietary Fat (%Energy)	-	0.094	-	0.105
Fasting Insulin(uIU/ml)	-	0.000	-	0.000	Apo CIII (mg/L)	-	0.000	-	0.000	High Quartile Total Dietary Fat (%Energy)	-	0.780	-	0.753
HOMA-IR	-	0.000	-	0.000	Apo E (mg/L)	-	0.000	-	0.000	Low Tertile SFA (%Energy)^		0.784		0.711
QUICKI	+	0.000	+	0.000	Apo B48 (mg/L)	-	0.000	-	0.000	Medium Tertile SfA (%Energy)	-	0.487	-	0.436
Revised QUICKI	+	0.000	+	0.000	TRL ApoB (mg/L)	-	0.005	-	0.003	High Tertile SFA (%Energy)	-	0.684	-	0.874
C-Peptide ng/ml	-	0.000	-	0.000	Plasma C14:0	-	0.035	-	0.055	Low Tertile MUFA (%Energy)^		0.200		0.190
Insulin to C-peptide Ratio	-	0.012	-	0.039	Plasma C16:0	-	0.003	-	0.001	Medium Tertile MUFA (%Energy)^	-	0.126	-	0.126
TCD^		0.180		0.196	Pasma C16:1	+	0.127	+	0.031	High Tertile MUFA (%Energy)^	-	0.106	-	0.097
Reading	+	0.623	+	0.551	Plasma C18:0	-	0.119	-	0.048	Single^		0.917		0.868
Oslo	-	0.984	+	0.867	Plasma C18:1	-	0.001	-	0.002	Married	-	0.510	-	0.497
INSERM	+	0.029	+	0.036	Plasma C18:2:6	+	0.000	+	0.000	Widow/er	-	0.324	-	0.622
Maastricht	+	0.983	-	0.862	Plamsa C20:4:6	+	0.000	+	0.000	Divorced	-	0.942	+	0.516
Cordoba	-	0.632	-	0.612	Plasma C20:5:3	+	0.062	+	0.019	Separated	-	0.610	-	0.952
JUMC	-	0.527	-	0.927	Plasma C22:6:3	-	0.299	-	0.328	Living with Partner	-	0.383	-	0.533
Uppsala	+	0.298	+	0.203	Plasma SFA	-	0.002	-	0.001	No formal education^		0.749		0.729
Low Tertile S_I^		0.028		0.059	Plasma MUFA	-	0.008	-	0.026	Primary	-	0.887	+	0.892
Medium Tertile S_I	+	0.166	+	0.292	Plasma PUFA	+	0.000	+	0.000	Secondary	+	0.918	+	0.593
High Tertile S_I	+	0.008	+	0.018	Plasma n-6 PUFA	+	0.000	+	0.000	Vocational	+	1.000	+	1.000
Height(m)	+	0.255	+	0.058	Plasma n-3 PUFA	-	0.909	+	0.831	Higher Vocational	+	0.372	+	0.307
BodyWeight(kg)	-	0.009	na	na	Basal Metabolic Rate (MJ/day)	-	0.851	+	0.393	Dipolma/Degree	+	0.592	+	0.274
BMI (kg/m^2)	-	0.000	-	0.034	Total Energy (MJ)	+	0.585	+	0.911	Working^		0.243		0.252
Waist circumference(cm)	-	0.022	-	0.893	Total fat (g/day)	+	0.989	-	0.739	Working in the home	-	0.012	-	0.017
Hip circumference (cm)	-	0.002	-	0.611	Total fat (%Energy)	-	0.688	-	0.646	Unemployed	-	0.409	-	0.612
Diastolic BP(mmHg)	-	0.442	-	0.403	SAFA (g/day)	+	0.891	-	0.852	Retired	-	0.286	-	0.079
Systolic BP(mmHg)	-	0.040	-	0.064	SAFA (%Energy)	+	0.811	+	0.830	Other work	-	0.711	-	0.690
Body Fat (%)	-	0.002	-	0.374	MUFA (g/day)	-	0.559	-	0.400	Smoke(Yes^ v no)	+	0.981	+	0.380
Body Fat(kg)	-	0.000	-	0.347	MUFA (%Energy)	-	0.333	-	0.326	Drink alcohol (Yes^ v no)	-	0.207	-	0.771
Lean Body Mass(%)	+	0.005	+	0.080	PUFA (g/day)	+	0.980	+	0.989	Play sport (Yes^ v no)	-	0.449	-	0.307
Lean Mass Body(kg)	+	0.968	+	0.126	PUFA (%Energy)	-	0.424	-	0.569					
Body Water(%)	+	0.011	+	0.075	n-3 (g/day)	-	0.880	+	0.951					
Body Water (L)	+	0.985	+	0.110	n-6 (g/day)	+	0.500	+	0.529					
Impedance(KHz)	+	0.850	+	0.781	Trans fatty acids (g/day)	-	0.468	-	0.110					
Normal Weight^		0.275		0.716	CHO (g/day)	+	0.976	+	0.929					
Overweight	-	1.000	-	1.000	CHO (%Energy)	-	0.335	-	0.493					
Obese	-	1.000	-	1.000	Starch (g/day)	+	0.869	-	0.877					

^reference category, na non applicable, Sig.=Significance value, β = Beta co-efficient

$p=0.00 = p<0.001$

Table 5.25: Multiple binary logistic regression predictors of 3vs4Criteria of the MetS, analysis of six different regression models

		3 vs 4				
		β	Sig.	OR	95% CI	
					Lower	Upper
Model 1	**IVGTT Parameters** (-2 Log likelihood = 345.769)†					
	Age (y)	0.026	0.14	1.03	0.99	1.06
	Sex (male^, female)	-0.700	0.04	0.50	0.25	0.98
	BodyWeight(kg)	0.003	0.80	1.00	0.98	1.03
	S_I (mU/L)-1min-1	0.137	0.32	1.15	0.88	1.50
	AIRg mUL^{-1} min^{-1}	-0.001	0.14	1.00	1.00	1.00
	Fasting Glucose (mmol/L)	0.218	0.59	1.24	0.56	2.74
	Fasting Insulin(uIU/ml)	0.513	0.05	1.67	1.01	2.77
	HOMA-IR	-1.594	0.08	0.20	0.03	1.18
	QUICKI	1.493	0.54	4.45	0.04	532.60
	C-Peptide ng/ml	-0.453	0.37	0.64	0.24	1.71
	Insulin to C-peptide Ratio	-0.237	0.43	0.79	0.44	1.42
	TCD^		0.60			
	Reading	0.276	0.61	1.32	0.45	3.83
	Oslo	0.215	0.71	1.24	0.41	3.78
	INSERM	1.008	0.15	2.74	0.69	10.94
	Maastricht	0.038	0.95	1.04	0.33	3.23
	Cordoba	0.204	0.71	1.23	0.43	3.52
	JUMC	-0.287	0.60	0.75	0.26	2.18
	Uppsala	-0.372	0.52	0.69	0.22	2.17
	Low Tertile S_I^		0.35			
	Medium Tertile S_I	0.530	0.16	1.70	0.81	3.55
	High Tertile S_I	0.400	0.48	1.49	0.49	4.51
†variables: P3, Revised QUICKI were removed from model due to their confounding effects						
Model 2	**Anthropometry** (-2 Log likelihood = 402.070)					
	Age (y)	0.018	0.22	1.02	0.99	1.05
	Sex (male^, female)	-0.288	0.53	0.75	0.30	1.85
	BodyWeight(Kg)	-0.080	0.06	0.92	0.85	1.00
	BMI (kg/m^2)	-0.117	0.07	0.89	0.79	1.01
	Diastolic BP(mmHg)	-0.020	0.10	0.98	0.96	1.00
	Body Fat (%)	-0.184	0.06	0.83	0.68	1.01
	Body Fat(kg)	0.210	0.06	1.23	0.99	1.54
	Lean Body Mass(%)	0.015	0.50	1.02	0.97	1.06
	Normal Weight^		0.01			
	Overweight	2.993	0.01	19.94	1.79	221.70
	Obese	3.849	0.00	46.95	3.46	637.21
Model 3	**Lipid Markers** (-2 Log likelihood = 252.973)¥					
	Age (y)	-0.019	0.32	0.98	0.95	1.02
	Sex (male^, female)	-1.780	0.00	0.17	0.08	0.37
	BodyWeight(kg)	-0.033	0.02	0.97	0.94	0.99
	TAG (mmol/L)	-0.804	0.16	0.45	0.15	1.37
	Cholesterol (mmol/L)	0.438	0.29	1.55	0.69	3.49
	TRL TAG (mmol/L)	-1.784	0.08	0.17	0.02	1.27
	TRL Cholesterol (mmol/L)	0.213	0.92	1.24	0.02	63.77
	LDL (mmol/L)	-0.131	0.61	0.88	0.53	1.45
	Apo A1(g/L)	2.400	0.01	11.02	1.80	67.27
	Apo B (g/L)	-2.617	0.06	0.07	0.00	1.17
	Apo CII (mg/L)	-0.025	0.12	0.97	0.94	1.01
	Apo CIII (mg/L)	0.005	0.38	1.00	0.99	1.02
	Apo E (mg/L)	-0.010	0.54	0.99	0.96	1.02
	Apo B48 (mg/L)	0.217	0.38	1.24	0.77	2.01
	TRL ApoB (mg/L)	0.007	0.33	1.01	0.99	1.02
¥variables: Total HDL was removed from model due to its confounding effects						
Model 4	**Plasma Fatty Acids** (-2 Log likelihood = 372.097)					
	Age (y)	0.025	0.12	1.03	0.99	1.06
	Sex (male^, female)	-1.079	0.00	0.34	0.18	0.65
	BodyWeight(kg)	-0.010	0.41	0.99	0.97	1.01
	Plasma C14:0	-0.860	0.00	0.42	0.28	0.63
	Plasma C16:0	-0.436	0.00	0.65	0.49	0.85
	Pasma C16:1	0.064	0.91	1.07	0.34	3.39
	Plasma C18:1	-0.887	0.12	0.41	0.13	1.26
	Plasma C18:2:6	0.952	0.02	2.59	1.20	5.61
	Plamsa C20:4:6	1.117	0.01	3.06	1.34	6.97
	Plasma SFA	0.354	0.00	1.42	1.12	1.81
	Plasma MUFA	0.803	0.16	2.23	0.73	6.79
	Plasma n-6 PUFA	-0.916	0.03	0.40	0.18	0.90
Model 5	**Dietary Variables** (-2 Log likelihood = 399.597)					
	Age (y)	0.015	0.30	1.02	0.99	1.04
	Sex (male^, female)	-0.450	0.13	0.64	0.35	1.15
	BodyWeight(kg)	-0.017	0.13	0.98	0.96	1.00
	CHO (%Energy)	-0.016	0.54	0.98	0.94	1.04
	Alcohol (g/day)	0.008	0.73	1.01	0.97	1.05
	Alcohol (%Energy)	0.000	1.00	1.00	0.85	1.17
	Low Quartile Total Dietary Fat (%Energy)^		0.69			
	Low/Medium Quartile Total Dietary Fat (%Energy)	0.372	0.32	1.45	0.70	3.02
	Medium/High Quartile Total Dietary Fat (%Energy)	0.030	0.95	1.03	0.44	2.43
	High Quartile Total Dietary Fat (%Energy)	0.156	0.78	1.17	0.40	3.46
Model 6	**Health and Lifestyle** (-2 Log likelihood = 394.243)					
	Age (y)	0.020	0.19	1.02	0.99	1.05
	Sex (male^, female)	-0.711	0.01	0.49	0.28	0.87
	BodyWeight(kg)	-0.019	0.08	0.98	0.96	1.00
	Single^		0.25			
	Married	-0.206	0.59	0.81	0.38	1.72
	Widow/er	0.375	0.61	1.45	0.34	6.20
	Divorced	0.617	0.42	1.85	0.41	8.31
	Separated	-0.390	0.75	0.68	0.06	7.60
	Living with Partner	0.589	0.20	1.80	0.73	4.42
	No formal education^		0.31			
	Primary	0.085	0.89	1.09	0.32	3.70
	Secondary	-0.214	0.73	0.81	0.24	2.67
	Higher Vocational	0.802	0.21	2.23	0.64	7.76
	Dipolma/Degree	0.115	0.85	1.12	0.33	3.80

Each model controlled for sex, age and body weight

^reference category, Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio

Table 5.26: Multiple binary logistic regression predictors of 4vs5 Criteria of the MetS, analysis of six different regression models

		4 vs 5				
		β	Sig.	OR	95% CI	
					Lower	Upper
Model 1	**IVGTT Parameters** (-2 Log likelihood = 286.073)†					
	Age (y)	-0.046	0.03	0.96	0.92	1.00
	Sex (male^, female)	-0.467	0.23	0.63	0.29	1.35
	BodyWeight(kg)	-0.036	0.02	0.96	0.94	0.99
	AIRg mUL^{-1} min^{-1}	0.001	0.24	1.00	1.00	1.00
	DI	0.000	0.71	1.00	1.00	1.00
	G0 mg/dl^{-1}	-0.003	0.52	1.00	0.99	1.01
	Fasting Glucose (mmol/l)	-0.197	0.66	0.82	0.34	1.98
	Fasting Insulin(uIU/ml)	0.025	0.92	1.02	0.62	1.70
	HOMA-IR	-0.188	0.83	0.83	0.15	4.68
	C-Peptide ng/ml	-0.138	0.58	0.87	0.54	1.42
	TCD^		0.89			
	Reading	-0.177	0.77	0.84	0.26	2.74
	Oslo	0.308	0.63	1.36	0.38	4.82
	INSERM	-0.167	0.87	0.85	0.12	5.90
	Maastricht	-0.219	0.73	0.80	0.23	2.78
	Cordoba	-0.361	0.52	0.70	0.23	2.09
	JUMC	-0.278	0.62	0.76	0.25	2.27
	Uppsala	0.492	0.50	1.64	0.39	6.83
†variables: P2, P3, Revised QUICKI were removed from model due to their confounding effects						
Model 2	**Anthropometry** (-2 Log likelihood = 248.642)					
	Age (y)	-0.068	0.00	0.93	0.89	0.98
	Sex (male^, female)	-0.126	0.85	0.88	0.24	3.18
	BodyWeight(kg)	-0.022	0.84	0.98	0.79	1.21
	BMI (kg/m^2)	0.026	0.73	1.03	0.88	1.19
	Waist circumference(cm)	0.011	0.67	1.01	0.96	1.06
	Hip circumference (cm)	-0.038	0.25	0.96	0.90	1.03
	Systolic BP(mmHg)	-0.001	0.96	1.00	0.98	1.02
	Body Fat (%)	0.067	0.59	1.07	0.84	1.36
	Body Fat(kg)	-0.076	0.59	0.93	0.70	1.22
	Lean Body Mass(%)	0.029	0.81	1.03	0.82	1.30
	Lean Mass Body(kg)	-0.176	0.40	0.84	0.56	1.27
	Body Water(%)	-0.021	0.91	0.98	0.66	1.44
	Body Water (L)	0.241	0.40	1.27	0.73	2.22
Model 3	**Lipid Markers** (-2 Log likelihood = 167.418)*					
	Age (y)	-0.093	0.00	0.91	0.86	0.96
	Sex (male^, female)	-1.191	0.03	0.30	0.10	0.88
	BodyWeight(kg)	-0.055	0.00	0.95	0.91	0.98
	TAG (mmol/L)	-1.146	0.03	0.32	0.11	0.92
	Cholesterol (mmol/L)	0.322	0.42	1.38	0.64	3.00
	TRL TAG (mmol/L)	-1.725	0.05	0.18	0.03	1.00
	Apo A1(g/L)	1.439	0.22	4.21	0.42	42.40
	Apo B (g/L)	-3.075	0.05	0.05	0.00	1.03
	Apo CII (mg/L)	0.013	0.49	1.01	0.98	1.05
	Apo CIII (mg/L)	0.004	0.56	1.00	0.99	1.02
	Apo E (mg/L)	-0.019	0.28	0.98	0.95	1.02
	Apo B48 (mg/L)	-0.466	0.03	0.63	0.42	0.95
	TRL ApoB (mg/L)	0.019	0.01	1.02	1.01	1.03
*variables: Total HDL, TRL Cholesterol were removed from model due to its confounding effects						
Model 4	**Plasma Fatty Acids** (-2 Log likelihood = 294.513)					
	Age (y)	-0.072	0.00	0.93	0.90	0.97
	Sex (male^, female)	-0.684	0.06	0.50	0.25	1.03
	BodyWeight(kg)	-0.053	0.00	0.95	0.92	0.97
	Plasma C16:0	0.077	0.71	1.08	0.72	1.62
	Plasma C18:0	-0.013	0.95	0.99	0.63	1.54
	Plasma C18:1	-0.092	0.70	0.91	0.57	1.45
	Plasma C18:2:6	0.067	0.90	1.07	0.38	2.98
	Plamsa C20:4:6	0.165	0.77	1.18	0.39	3.53
	Plasma C20:5:3	0.824	0.10	2.28	0.86	6.02
	Plasma C22:6:3	-0.229	0.60	0.80	0.34	1.88
	Plasma SFA	0.138	0.77	1.15	0.45	2.90
	Plasma MUFA	0.253	0.58	1.29	0.52	3.17
	Plasma n-6 PUFA	0.191	0.79	1.21	0.29	5.00
Model 5	**Dietary Variables** (-2 Log likelihood = 194.687)					
	Age (y)	-0.054	0.05	0.95	0.90	1.00
	Sex (male^, female)	0.451	0.71	1.57	0.14	17.58
	BodyWeight(kg)	-0.110	0.01	0.90	0.82	0.98
	Basal Metabolic Rate (MJ/day)	1.292	0.16	3.64	0.60	22.11
	PUFA (%Energy)	0.006	0.96	1.01	0.82	1.24
	n-3 (g/day)	-0.173	0.43	0.84	0.55	1.30
	Low Quartile Total Dietary Fat (%Energy)^		0.17			
	Low/Medium Quartile Total Dietary Fat (%Energy)	0.047	0.93	1.05	0.36	3.02
	Medium/High Quartile Total Dietary Fat (%Energy)	-0.710	0.17	0.49	0.18	1.35
	High Quartile Total Dietary Fat (%Energy)	0.314	0.56	1.37	0.48	3.89
Model 6	**Health and Lifestyle** (-2 Log likelihood = 302.906)					
	Age (y)	-0.040	0.06	0.96	0.92	1.00
	Sex (male^, female)	-0.638	0.08	0.53	0.26	1.09
	BodyWeight(kg)	-0.049	0.00	0.95	0.93	0.98
	Single^		0.35			
	Married	-0.143	0.77	0.87	0.33	2.25
	Widow/er	-0.534	0.51	0.59	0.12	2.88
	Divorced	-0.247	0.79	0.78	0.12	4.95
	Separated	-0.070	0.96	0.93	0.07	12.71
	Living with Partner	-0.990	0.07	0.37	0.13	1.08
	Working^		0.58			
	Working in the home	-0.602	0.16	0.55	0.23	1.28
	Unemployed	-0.871	0.30	0.42	0.08	2.20
	Retired	-0.574	0.19	0.56	0.24	1.33
	Other work	-0.711	0.29	0.49	0.13	1.84
	Drink alcohol (Yes^ v no)	-0.153	0.65	0.86	0.44	1.66

Each model controlled for sex, age and body weight

^reference category, Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio

Table 5.27: Multiple binary logistic regression predictors of 3vs5 Criteria of the MetS, analysis of six different regression models

		3 vs 5				
		β	Sig.	OR	95% CI	
					Lower	Upper
Model 1	**IVGTT Parameters** (-2 Log likelihood = 216.509)†					
	Age (y)	-0.015	0.50	0.99	0.94	1.03
	Sex (male^, female)	-1.591	0.00	0.20	0.08	0.50
	BodyWeight(kg)	-0.036	0.03	0.96	0.93	1.00
	S_I (mU/L)-1min-1	0.219	0.30	1.24	0.82	1.88
	DI	-0.001	0.10	1.00	1.00	1.00
	G0 mg/dl $^{-1}$	0.004	0.50	1.00	0.99	1.01
	Fasting Glucose (mmol/l)	-1.182	0.00	0.31	0.14	0.66
	HOMA-IR	-0.100	0.85	0.91	0.33	2.47
	C-Peptide ng/ml	-0.351	0.56	0.70	0.21	2.31
	Insulin to C-peptide Ratio	-0.105	0.77	0.90	0.45	1.82
	TCD^		0.48			
	Reading	0.443	0.51	1.56	0.41	5.90
	Oslo	0.442	0.55	1.56	0.37	6.57
	INSERM	1.285	0.19	3.61	0.52	25.00
	Maastricht	-0.194	0.79	0.82	0.20	3.39
	Cordoba	-0.033	0.96	0.97	0.26	3.64
	JUMC	-0.353	0.59	0.70	0.19	2.57
	Uppsala	0.907	0.28	2.48	0.48	12.73
	Low Tertile S_I^		0.82			
	Medium Tertile S_I	0.276	0.58	1.32	0.50	3.47
	High Tertile S_I	0.465	0.55	1.59	0.35	7.32
†variables: P2, P3, QUICKI, Revised QUICKI were removed from model due to their confounding effects						
Model 2	**Anthropometry** (-2 Log likelihood = 229.665)#					
	Age (y)	-0.024	0.32	0.98	0.93	1.02
	Sex (male^, female)	-0.106	0.88	0.90	0.23	3.55
	BodyWeight(kg)	-0.037	0.73	0.96	0.78	1.19
	BMI (kg/m^2)	-0.083	0.31	0.92	0.78	1.08
	Waist circumference(cm)	0.032	0.31	1.03	0.97	1.10
	Hip circumference (cm)	-0.002	0.93	1.00	0.94	1.06
	Systolic BP(mmHg)	-0.008	0.46	0.99	0.97	1.01
	Body Fat (%)	-0.090	0.55	0.91	0.68	1.22
	Body Fat(kg)	0.085	0.61	1.09	0.78	1.51
	Lean Body Mass(%)	0.241	0.36	1.27	0.76	2.12
	Lean Mass Body(kg)	-0.456	0.19	0.63	0.32	1.25
	Body Water(%)	-0.219	0.53	0.80	0.41	1.59
	Body Water (L)	0.538	0.23	1.71	0.71	4.14
#variables: Height was removed from the model due to its confounding effects						
Model 3	**Lipid Markers** (-2 Log likelihood = 108.465)¥					
	Age (y)	-0.079	0.02	0.92	0.86	0.99
	Sex (male^, female)	-2.048	0.00	0.13	0.04	0.46
	BodyWeight(kg)	-0.055	0.02	0.95	0.90	0.99
	TAG (mmol/L)	-2.500	0.02	0.08	0.01	0.66
	Cholesterol (mmol/L)	0.643	0.32	1.90	0.53	6.81
	TRL TAG (mmol/L)	-3.105	0.05	0.04	0.00	0.96
	LDL (mmol/L)	0.328	0.38	1.39	0.66	2.90
	Apo B (g/L)	-6.075	0.02	0.00	0.00	0.39
	Apo CII (mg/L)	-0.003	0.91	1.00	0.94	1.05
	Apo CIII (mg/L)	0.024	0.05	1.02	1.00	1.05
	Apo E (mg/L)	-0.016	0.48	0.98	0.94	1.03
	Apo B48 (mg/L)	-0.320	0.33	0.73	0.38	1.38
	TRL ApoB (mg/L)	0.009	0.34	1.01	0.99	1.03
¥variables: Total HDL, TRL Cholesterol and Apo A1 were removed from model due to its confounding effects						
Model 4	**Plasma Fatty Acids** (-2 Log likelihood = 205.292)					
	Age (y)	-0.036	0.13	0.96	0.92	1.01
	Sex (male^, female)	-1.614	0.00	0.20	0.09	0.46
	BodyWeight(kg)	-0.068	0.00	0.93	0.90	0.97
	Plasma C14:0	0.848	0.17	2.33	0.69	7.95
	Plasma C16:0	1.306	0.02	3.69	1.26	10.84
	Pasma C16:1	-2.756	0.05	0.06	0.00	1.02
	Plasma C18:0	1.473	0.01	4.36	1.50	12.69
	Plasma C18:1	-3.855	0.01	0.02	0.00	0.33
	Plasma C18:2:6	0.118	0.84	1.13	0.35	3.57
	Plamsa C20:4:6	0.423	0.49	1.53	0.46	5.10
	Plasma C20:5:3	2.268	0.00	9.66	2.49	37.51
	Plasma C22:6:3	0.960	0.12	2.61	0.78	8.71
	Plasma MUFA	5.063	0.00	158.11	7.34	3405.77
	Plasma n-6 PUFA	1.295	0.15	3.65	0.62	21.44
Model 5	**Dietary Variables** (-2 Log likelihood = 106.629)					
	Age (y)	-0.091	0.01	0.91	0.85	0.98
	Sex (male^, female)	-1.575	0.01	0.21	0.06	0.73
	BodyWeight(kg)	-0.075	0.00	0.93	0.89	0.97
	Trans fatty acids (g/day)	-0.156	0.30	0.86	0.64	1.15
	Alcohol (g/day)	-0.035	0.56	0.97	0.86	1.09
	Alcohol (%Energy)	0.092	0.65	1.10	0.73	1.64
	Low Quartile Total Dietary Fat (%Energy)^		0.69			
	Low/Medium Quartile Total Dietary Fat (%Energy)	0.023	0.97	1.02	0.26	4.10
	Medium/High Quartile Total Dietary Fat (%Energy)	0.724	0.37	2.06	0.42	10.08
	High Quartile Total Dietary Fat (%Energy)	-0.113	0.93	0.89	0.07	10.94
	Low Tertile MUFA (%Energy)^		0.71			
	Medium Tertile MUFA (%Energy)^	-0.569	0.41	0.57	0.15	2.16
	High Tertile MUFA (%Energy)^	-0.453	0.72	0.64	0.05	7.71
Model 6	**Health and Lifestyle** (-2 Log likelihood = 271.040)					
	Age (y)	-0.001	0.96	1.00	0.96	1.04
	Sex (male^, female)	-1.097	0.00	0.33	0.16	0.69
	BodyWeight(kg)	-0.061	0.00	0.94	0.92	0.97
	Working^		0.25			
	Working in the home	-1.078	0.02	0.34	0.14	0.82
	Unemployed	-0.487	0.60	0.61	0.10	3.82
	Retired	-0.806	0.08	0.45	0.18	1.09
	Other work	-0.234	0.72	0.79	0.22	2.85
	Drink alcohol (Yes^ v no)	0.089	0.79	1.09	0.57	2.11

Each model controlled for sex, age and body weight

^reference category, Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio

Table 5.28: Multiple binary logistic regression model of variables predicting 3 vs 4 Criteria of the MetS

	3 vs 4				
	β	Sig.	OR	95% CI	
				Lower	Upper
Age (y)	0.019	0.29	1.02	0.98	1.06
Sex (male^, female)	-1.080	0.05	0.34	0.11	1.01
BodyWeight(kg)	-0.009	0.85	0.99	0.91	1.08
Fasting Insulin(uIU/ml)	0.317	0.01	1.37	1.09	1.72
HOMA-IR	-1.358	0.00	0.26	0.11	0.59
BMI (kg/m^2)	-0.092	0.13	0.91	0.81	1.03
Diastolic BP(mmHg)	-0.038	0.01	0.96	0.93	0.99
Body Fat (%)	-0.049	0.66	0.95	0.77	1.18
Body Fat(kg)	0.046	0.71	1.05	0.82	1.33
TRL TAG (mmol/L)	-1.545	0.00	0.21	0.09	0.51
Apo A1(g/L)	2.270	0.00	9.68	2.46	38.14
Apo B (g/L)	-2.563	0.00	0.08	0.02	0.34
Plasma C14:0	0.164	0.41	1.18	0.80	1.74
Plasma C16:0	-0.174	0.25	0.84	0.62	1.13
Plasma C18:2:6	0.419	0.35	1.52	0.63	3.67
Plamsa C20:4:6	0.566	0.24	1.76	0.68	4.53
Plasma SFA	0.189	0.11	1.21	0.96	1.53
Plasma n-6 PUFA	-0.368	0.43	0.69	0.28	1.71

Each model controlled for sex, age and body weight (-2LL=304.789)
Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio

Table 5.29: Multiple binary logistic regression model of variables predicting 4 vs 5 Criteria of the MetS

	4 vs 5				
	β	Sig.	OR	95% CI	
				Lower	Upper
Age (y)	-0.097	0.001	0.91	0.86	0.96
Sex (male^, female)	-1.262	0.016	0.28	0.10	0.79
BodyWeight(kg)	-0.071	0.001	0.93	0.89	0.97
TAG (mmol/L)	-0.893	0.047	0.41	0.17	0.99
TRL TAG (mmol/L)	-1.626	0.064	0.20	0.04	1.10
Apo B (g/L)	-1.667	0.087	0.19	0.03	1.28
Apo B48 (mg/L)	-0.383	0.063	0.68	0.46	1.02
TRL ApoB (mg/L)	0.016	0.018	1.02	1.00	1.03
Plasma C20:5:3	0.421	0.152	1.52	0.86	2.71
Single^		0.633			
Married	-0.920	0.196	0.40	0.10	1.61
Widow/er	-1.200	0.263	0.30	0.04	2.46
Divorced	-0.685	0.576	0.50	0.05	5.56
Separated	0.039	0.978	1.04	0.07	16.55
Living with Partner	-1.473	0.096	0.23	0.04	1.30

Each model controlled for sex, age and body weight (-2LL=107.874)
Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio

Table 5.30: Multiple binary logistic regression model of variables predicting 3 vs 5 Criteria of the MetS

	3 vs 5				
	β	Sig.	OR	95% CI	
				Lower	Upper
Age (y)	-0.029	0.44	0.97	0.90	1.05
Sex (male^, female)	-3.962	0.00	0.02	0.00	0.10
BodyWeight(kg)	-0.115	0.00	0.89	0.85	0.94
Fasting Glucose (mmol/L)	-1.634	0.00	0.20	0.10	0.39
TAG (mmol/L)	-2.757	0.00	0.06	0.01	0.38
TRL TAG (mmol/L)	-1.767	0.09	0.17	0.02	1.34
Apo B (g/L)	-3.146	0.03	0.04	0.00	0.79
Apo CIII (mg/L)	0.010	0.28	1.01	0.99	1.03
Plasma C16:0	-0.275	0.00	0.76	0.65	0.89
Pasma C16:1	2.391	0.00	10.92	4.33	27.54
Plasma C18:0	0.121	0.46	1.13	0.82	1.56
Plasma C18:1	-0.182	0.01	0.83	0.73	0.95
Plasma C20:5:3	-0.286	0.49	0.75	0.33	1.71
Working^		0.60			
Working in the home	1.028	0.23	2.80	0.51	15.20
Unemployed	1.183	0.33	3.27	0.30	35.67
Retired	0.008	0.99	1.01	0.19	5.36
Other work	2.273	0.15	9.71	0.45	210.24

Each model controlled for sex, age and body weight (-2LL=107.874)
Sig.=Significance value, β = Beta co-efficient, OR = Odds ratio, CI = confidence ratio
Plasma MUFA removed from the model due to its confounding effects

5.5 DISCUSSION

The MetS is now a recognised precursor of T2DM and CVD risk (Haffner *et al.*, 1992; Ford, 2005a). Much time and effort is now given to screen for and subsequently treat the MetS as a primary preventitative measure for these complex diseases. Obesity is considered the most important environmental factor in the increase of the incidence of T2DM (Wannamethee & Shaper, 1999). We identified body weight as a re-occurring risk factor to emerge from the regression models. Body weight significantly decreased from low to high insulin sensitivity tertiles as did BMI, confirming its underlying causality in the MetS. It has been put forward that obesity is a pro-inflammatory state with increased cytokine levels (TNFα, IL6, leptin, resistin, CRP, PAI-1) and NEFA concentrations that interfere with insulin signalling and insulin secretion (Tierney & Roche, 2007). In BMI stratified analysis HDL-cholesterol decreased, although not significantly, whilst systolic blood pressure and diastolic blood pressure increased significantly with increasing BMI index. Other variables influenced by obesity included AIRg, G0, fasting insulin, HOMA-IR, QUICKI, C-peptide and total cholesterol concentrations. From the dietary perspective, BMR, total fat (%energy), MUFA (%energy), dietary palmitoleic acid and stearic acid increased with increasing BMI.

From the present analysis, body weight, BMI, waist circumference, hip circumference, AIRg, fasting insulin, HOMA-IR, BMR, total fat (g/day), SFA (g/day) and MUFA (g/day) decreased with increasing age, whilst QUICKI, plasma EPA and n-3 PUFA concentrations increased. Fasting glucose, total and HDL-cholesterol, apoA1 and apoB concentrations were highest in the middle-aged group. Given the differences in metabolic variables with increasing age and BMI, the prevalence of obesity and impact of age could be main determinants of the MetS. The finding that the MetS increases with age was not apparent in this study (examining the five components) but this could be as a result of recruitment strategies or medication usage.

In young and middle-aged women, blood pressure, serum TAG and waist circumference are lower than their male counterparts (Carr, 2003). We examined gender differences in the characteristics of metabolic components and found differences in waist and hip circumference, diastolic blood pressure, insulin sensitivity, AIRg, fasting glucose, total cholesterol and NEFA concentrations, among others. Using the NCEP ATPIII criteria, the prevalence of the MetS is higher in men

(Hwang *et al.*, 2007), with dyslipidaemia occurring earlier in both genders and blood pressure seen in older people. It has previously been demonstrated that men are more insulin resistant than women (Nuutila *et al.*, 1995). Abnormal insulin sensitivity is a central feature of the MetS. Metabolic control varies among the genders (Hwang *et al.*, 2007) with molecular and cellular hormonal, sexual and body fat composition differences. The protective effect of female sex hormones and the unfavourable effects of testosterone on substrate metabolism has been suggested (Hwang *et al.*, 2007).

Dietary fat quality affects insulin sensitivity (Vessby *et al.*, 2001) and a diet rich in SFAs induces a similar fatty acid pattern seen in subjects at risk of the MetS (Vessby *et al.*, 2002). In our cohort of MetS subjects, fatty acid composition was characterised by high levels of linoleic acid and palmitic acid. Total PUFA was the predominant plasma fatty acid compared to SFA and MUFA. It is difficult to compare these results to results of plasma fat composition measured in serum lipid esters. Fatty acid composition of serum lipid esters (Ma *et al.*, 1995a) or adipose tissue triglycerides (van Staveren *et al.*, 1986; Wolk *et al.*, 1998) reflects fatty acid intakes over several weeks and months. Due to cost and labour limitations plasma fatty acids were analysed for whole serum without standardisation for lipids and may only weakly represent dietary fat composition. However, we showed that plasma fatty acid composition was significantly different at baseline in men and women. Myristic, palmitic and total SFA was significantly higher in males and palmitoleic and EPA significantly higher in females. This corresponded to similar significant differences in dietary myristic, palmitic and total SFA but not for palmitoleic or EPA. Whether plasma fatty acid differences are an effect of dietary intakes or genetic differences in the regulation of fatty acid composition cannot be determined from this study. Perhaps males are at greater risk of this plasma fatty acid phenomenon and are, therefore, at greater risk of T2DM and CVD. Polyunsaturated fatty acids downregulate the expression of stearyl CoA desaturase (SCD) and delta 6 desaturase (D6D) (Jones *et al.*, 1996) which are involved in endogenous fatty acid synthesis and introduce a double bond in specific positions of long chain fatty acids (Warensjo *et al.*, 2005). In contrast SFAs coupled with a high fat diet have been shown to upregulate expression of SCD and D6D (Warensjo *et al.*, 2005). In another study, Warensjo *et al.*, (2006) found that SCD-1 activity was higher for women.

An association between saturated fat content in the diet and increased fasting insulin levels has been previously reported (Parker *et al.*, 1993). In this study, when stratifying the group according to low, medium and high intakes of dietary SFA, an increase in fasting insulin, HOMA-IR and C-peptide concentrations was observed. In animal studies, diets high in saturated fat caused distinct insulin resistance (Storlien *et al.*, 1991). Long term basal hyperinsulinaemia may eventually cause β-cell toxicity and damage (Stein *et al.*, 1997). Dietary intervention studies, where modification of fat intakes are undertaken, have produced conflicting results in terms of ideal amounts and types of fat to ameliorate insulin sensitivity. This may be due to study design differences or type of patient group studied. Some epidemiological studies have shown beneficial outcomes for risk of T2DM with dietary fat quality, however results are not conclusive (Salmeron *et al.*, 1997). Single plasma phospholipids fatty acid measurement to reflect habitual status is a potential flaw.

Although subjects in the UO cohort had the highest plasma and dietary amounts of EPA and DHA this did not manifest to a superior insulin sensitivity status or more desirable lipid profiles at baseline. A positive relationship between insulin resistance and plasma cholesterol ester levels of EPA and DHA in subjects with the metabolic syndrome has not been found (Klein-Platat *et al.*, 2005). A potential oversight could be that plasma does not represent the true levels or effects of these essential fatty acids as compared to insulin sensitive tissues like muscle.

No clinical procedures are in place to definitively predict insulin resistance in an individual. Through multiple linear regression analysis, we assessed how effectively anthropometric measurements, biochemical markers and health and lifestyle variables can predict insulin sensitivity. Waist circumference has already been established as an independent risk factor for CVD. Entered univariately, waist circumference was negatively associated with S_I and positively associated with HOMA-IR, highlighting the strong independent risk of abdominal obesity. However, unlike other studies (Wahrenberg *et al.*, 2005) it was not the most powerful regressor in our model. Larger hip circumference (a measure of peripheral obesity) was associated with lower levels of insulin resistance. Body weight was a strong significant and negative indicator of insulin sensitivity both univariatley and when entered into each model respectively. BMI was positively associated with an increasing HOMA-IR index in the final model of predictors. The large number of overweight and obese persons in Westernised populations is increasing the incidence

of T2DM and CVD. Using BMI as a quick diagnostic tool to indicate whether a person is insulin resistant may be advocated in clinical settings. The presence of dyslipidaemia characteristic of insulin resistance might also be a useful predictive tool. Plasma TAG and HDL-cholesterol concentrations are independently associated with insulin resistance. As the underlying component, increasing insulin resistance as measured by HOMA-IR is associated with increasing age, fasting glucose and insulin concentrations, BMI and apoE concentrations and a decrease in insulin sensitivity, AIRg, G0, P2, hip circumference and TRL-cholesterol concentrations. Being a volunteer in UoR and JUMC was positively and negatively associated with having an increased HOMA-IR index, respectively. One could hypothesise that habitual dietary patterns or genetic disposition could account for this centre difference. Interestingly, of the examined lifestyle factors, not playing sport was a significant univariate predictor of increasing HOMA-IR but did not feature in the logistic regression analysis. This is in contrast to other studies which demonstrated that the most strongly associated lifestyle factor with a lower risk of having the MetS was physical activity (Carroll *et al.*, 2000; Irwin *et al.*, 2002; Zhu *et al.*, 2004). In our health and lifestyle questionnaire subjects were asked do they play sport or not and if so, what sport and how many times a week? This is a crude estimate of physical activity levels and as compared to dietary intakes, possible overestimation of activity units could have occurred. Perhaps this is the reasoning to the surprising lack of significant effect or predictive power of this lifestyle variable. In addition to a lower level of physical activity, a low socioeconomic status (Rosengren & Wilhelmsen, 1997) and a low education level may increase CVD risk (Jacobsen & Thelle, 1988). Interestingly, being married, divorced or separated and working in the home were negatively associated with insulin sensitivity when entered univariately into the model. In common, being separated and working in the home were positively associated with an increase in HOMA-IR. Perhaps underlying stresses associated with living and working experiences should be examined in further detail. DI which represents β-cell function, decreased significantly from high to low insulin sensitivity as waist circumference increased. In the Insulin Resistance Atherosclerosis Study, it was reported that elevated waist circumference was associated with a reduced DI (Mayer-Davis *et al.*, 2001). The significant reduction in DI was not however seen with increasing BMI.

The purpose of developing the logistic models was to investigate if certain factors were causative in the increase of the number of MetS criteria a subject possesses from 3 to 4 to 5 whilst controlling for various potential confounding factors such as age, gender and body weight. An ideal way to explore this would be in a cohort who was followed longitudinally. Several aetiological factors are involved in the development and progression of the MetS. Body weight was a significant negative predictor in the final model of "4 versus 5" and "3 versus 5" criteria of the MetS. Diet composition and physical inactivity did not feature in any of the final models. However, a volunteer "living with a partner" versus being "single" was more likely to have "5 versus 4" criteria, in the univariate and in the final prediction model. "Working in the home" and being "retired" were associated with "5 versus 3" criteria in the univariate model. When predicting increasing components of the MetS, interesting to note that HOMA-IR or S_I did not feature in the final model, which raises the question: is insulin resistance an underlying component of the MetS or at least in this cohort? However, subjects were recruited on various criteria e.g. BP clinics, lipid clinics and with the modified version of the NCEP ATPIII criteria being used which does not stipulate insulin resistance as one of the defining criteria, this may in part explain this.

A high proportion of palmitic acid at baseline is associated with unfavourable changes in fasting serum insulin concentrations (Laaksonen *et al.*, 2002). In the correlation analysis S_I and QUICKI were negatively whilst fasting insulin, HOMA-IR and C-peptide concentrations were positively associated with myristic, palmitic and SFAs. As the main SFA, palmitic acid has the greatest concentration in plasma and is suggested in the development of insulin resistance (Storlien *et al.*, 1996). In this study palmitic acid was a negative and a positive univariate predictor of S_I and HOMA-IR, respectively, and a strong final predictor of having "5 versus 3" criteria of the MetS, further instigating it in the development and progression of the MetS.

Much debate surrounds the role of n-6 and n-3 PUFAs in insulin resistance (Berry, 1997). From this analysis, plasma n-3 PUFA was significantly inversely associated with increasing AIRg and insulin to C-peptide ratio, with plasma n-6 PUFA significantly and positively associated with QUICKI, RQUICKI and negatively with fasting insulin, HOMA-IR and C-peptide concentrations. Plasma n-6 PUFA was significantly associated with having a lower number of criteria when entered univariatley in the regression models. This significance was lost however when

entered multivariately. Laaksonen *et al.*, (2002) demonstrated that the beneficial role of PUFA in preventing IFG and T2DM were mediated mainly by the n-6 fatty acid linoleate. Plasma C18:2:6 was positively associated with increasing insulin sensitivity index and QUICKI and negatively with insulin resistance measured by HOMA-IR and fasting insulin values. It should be noted that while plasma fatty acids may reflect proportions of dietary SFAs and PUFAs it does not represent total fat consumption. Saturated fatty acids are also somewhat metabolised to MUFAs, therefore it is difficult to draw conclusions or relate dietary MUFA to corresponding plasma intakes.

Recently, it was proposed that the dyslipidemia central to the diagnosis of the metabolic syndrome should be redefined to include apolipoproteins B and A1 (Sniderman & Faraj, 2007). In our cohort, it was found that, apoA1 increased significantly with increasing insulin sensitivity, increasing MUFA intakes and was a significant independent predictor of insulin sensitivity. ApoB was a positive univariate predictor of increasing insulin resistance. ApoA1 and apoB were positively and negatively associated with having a lower number of MetS criteria, respectively, and multivariatley with having "3 versus 4" criteria. ApoB was a strong significant predictor of having "5 versus 4" and "5 versus 3" criteria.

Poor dietary quality, characterised by high percentage of energy from total and saturated fat, low intakes of total CHO and fibre illustrated in this cohort of MetS subjects, is associated with the development of abdominal obesity (Millen *et al.*, 2006). In this study, however, poor dietary attributes did not predict progression of the MetS or indeed predict worsening insulin sensitivity or contribute to an increase in HOMA-IR. It would seem paramount that weight management be the focus of preventative target measures in alleviating insulin resistance and MetS risk as obesity is a recognised risk factor for the development of IHD, stroke and CVD (Keys, 1980). Hyperinsulinaemia which accompanies obesity is also associated with CVD (Parker *et al.*, 1993). Animal studies have indicated that diets high in fat lead to the inducement of insulin resistance and hyperinsulinaemia (Storlien *et al.*, 1986). From our analysis increased intakes of total and SFA but not MUFA and overall adiposity (BMI) was associated with increasing insulin levels. In the Normative Aging study, BMI was found to be positively associated with both fasting and postprandial serum insulin concentrations (Parker *et al.*, 1993). With respect to diet, increasing SFA (% Energy) was also positively related to serum insulin in the fasted and the fed state. These findings are consistent with our results of fasting insulin. This has also been

demonstrated in adults (Olefsky *et al.*, 1982) and children (Folsom *et al.*, 1989). The underlying mechanisms behind this phenomenon are not completely understood and elucidate to the increase of free fatty acid release into the portal circulation. A reduction in dietary SFA intake from <13% of total energy to <10% of total energy in our study population was associated with a reduction in fasting insulin of 21%. This finding is similar to the Normative Aging study where they experienced a reduction in fasting insulin of 26% and 32% in postprandial insulin with a reduction of SFA (Parker *et al.*, 1993). Similar to their study, family history was not accounted for in this analysis which places volunteers at further risk. However, the results suggest that a reduction in body weight and SFA intake may ameliorate fasting insulin concentrations and thus CVD risk.

In the present study, we described the determinants of insulin sensitivity in MetS subjects. For a more detailed approach, we created a model in which the influence of health and lifestyle factors and biochemical factors were incorporated concomitantly with gender, age and body weight. Insulin sensitivity correlated negatively with increasing body weight and AIRg and positively with DI and P3. The remaining insulin sensitivity variance that remains unaccounted for (35.5%) after modelling the study variables, suggests that other factors are involved. These may include inflammatory cytokines such as TNFα which is known to impair insulin signalling (Trischitta *et al.*, 1997). Univariatley, low insulin sensitivity was associated with increased waist circumference, glucose, TAG, blood pressure and reduced HDL cholesterol concentrations. This was independent of body weight, age and gender. Fasting glucose and fasting insulin levels increased with increasing number of metabolic disorders which has been demonstrated previously (Haffner *et al.*, 1992; Mykkanen *et al.*, 1993; Mykkanen *et al.*, 1997). Subjects with 5 disorders had 28.4% higher fasting insulin concentrations than subjects with 3. Fasting insulin and HOMA-IR was significantly associated with a clustering of metabolic disorders compared to insulin sensitivity. Reasons for this could be the close association of hyperinsulinaemia to the components that cluster in the MetS.

From our analysis it would seem that males are more at risk (higher fasting glucose and insulin concentrations, blood pressure, waist circumference, TAG, lower insulin sensitivity and HDL-cholesterol concentrations). This might be explained by the more favourable gluteal fat distribution in women. Abdominal obesity is more attributable to men which confers a greater risk of developing insulin resistance.

Majority of subjects (n=337) fell into the obese category. We know that underestimation of energy intake is more pronounced in obese people (Blundell & Cooling, 2000). The high intake of fat may be a major contributor to the development of obesity (Astrup *et al.*, 2000) and impaired insulin sensitivity (Lovejoy & DiGirolamo, 1992; Mayer *et al.*, 1993; Marshall *et al.*, 1997). However, our understanding of the associations between dietary total fat, SFA and MUFA and MetS components is limited by the accuracy of the measurement of habitual fat intake. The worsening of the MetS was associated with modifiable risk factors including increasing body weight and lipid factors. One simple public health message to convey is that increasing body weight predicts a decline in S_I, increasing risk of T2DM and CVD. The significant age and gender differences in the MetS and its components are required for health promotion programs and in the screening of high risk individuals to aid recognition and treatment of the MetS. As well as focusing on dietary fat intake to truly understand mechanisms and results, there is a need to also focus on energy expenditure which was not considered in detail in this study.

A clear strength of the study is that it was carried out in 8 European countries which enabled a comparison of baseline anthropometry and biochemical measures. However subjects for the study were in a way preselected from certain clinics based on blood pressure, lipids, weight management etc. which increases the possibility of bias.

A few limitations to this study must be addressed. Although there still exists debate about the most accurate way to assess insulin sensitivity, our study demonstrated a strong correlation with fasting insulin and QUICKI and the S_I index exported from the minimal model analysis in individuals with varying insulin concentrations. However, S_I is an imperfectly model based measured variable as insulin sensitivity was measured by the minimal model and not the gold standard euglycaemic clamp method. Another limitation of the study is the failure of follow up assessment of the subjects and their risk factors over time. It should also be considered that with all the statistical tests performed on the data, statistically significant findings may have occurred by chance. A p value of 0.05 means that the result obtained would have occurred by chance 5% of the time. The more independent tests that are carried out, the more likely that false positives will be obtained.

This study examined the relationship of anthropometric, biochemical and health and lifestyle patterns and the MetS between males and females, age groups,

BMI categories and centres. We developed logistic regression models examining the independent influence of these factors on the odds of having 3, 4 or 5 criteria of the MetS. In summary, body weight emerged several times as a risk factor for decreasing S_I and increasing insulin resistance and decreased significantly from low to high insulin sensitivity tertiles as did BMI. This suggests its role as an underlying cause in the MetS. With the significant differences in metabolic variables also observed with increasing age and BMI, the impact of age and obesity are factors to consider in this pre-diabetic syndrome. Dietary fat quality affects insulin sensitivity and in this analysis a significant increase in fasting insulin, HOMA-IR and c-peptide concentrations were observed with increasing intakes of dietary SFA. A reduction in dietary SFA with as little as 3% reduced fasting insulin concentrations by 20%. Subjects with 5 criteria had 28% higher fasting insulin concentrations than subjects with 3 criteria which corroborates the close association of hyperinsulinaemia to the components that cluster in the MetS. With plasma fat, palmitic acid is suggested in the development of insulin resistance and positively associated with HOMA-IR and was a strong predictor of having 5 criteria of the MetS.

ACKNOWLEDGEMENTS

The LIPGENE Dietary Intervention Study was carried out in 8 centres where IVGTTs, anthropometric measurements, blood sampling and dietary assessments were done locally. Anthropometry results and dietary analysis data was sent by each centre to TCD and all data was collated by TCD. Insulin and C-peptide analysis were completed in the laboratory of St. James's Hospital, Dublin. Data preparation and statistical analysis was completed by the author. Lipid (TAG, HDL-cholesterol, LDL-cholesterol, Total Cholesterol, TRL TAG, TRL Cholesterol, NEFA) and glucose analysis was conducted in the University of Reading. Apolipoprotein (apoAI, CII, CIII, B, B48, E, TRL apoB) analysis was conducted in INSERM, Marseille, France. Extractions and transmethylations for the plasma fatty acid analysis was conducted in TCD by the author and Jolene McMonagle, Nutrigenomics research Group, TCD. Analysis of the data on the GC was carried out by Jane Ferguson, Nutrigenomics Research Group, TCD. IVGTT modelling was carried out by the author and Danielle Shaw (UoR) in the University of Reading. All analysis for this chapter was carried out by the author.

Chapter Six

The effects of different amounts and types of dietary fat on metabolic markers of the metabolic syndrome - Findings from the LIPGENE Dietary Intervention Study

---

6.1 ABSTRACT

Background: Evidence suggests that dietary fatty acid composition affects insulin sensitivity.

Objective: The LIPGENE Human Dietary Intervention Study is a multi-centre, Trans–European, randomised, controlled trial with the aim of determining the relative efficacy of reducing dietary SFA consumption, by altering quality and reducing the quantity of dietary fat, on metabolic risk factors of the MetS.

Design: 417 free-living subjects with the MetS received one of four dietary treatments for 12 weeks: (1) High-fat (38% energy) SFA-rich diet (HFSFA); (2) High-fat (38% energy), monounsaturated fatty acid (MUFA)-rich diet (HFMUFA); (3) Low-fat (28% energy), high-complex carbohydrate diet (LF) and (4) Low-fat (28% energy), high-complex carbohydrate diet, with 1.24 g/d LC n-3 PUFA (LFn-3PUFA). An IVGTT, lipoprotein analysis, cytokines, adhesion molecules and coagulation factors were determined pre- and post-intervention.

Results: Dietary fat modification had no significant effect on insulin sensitivity (S_I). The effect of the four dietary treatments were determined in volunteers with a habitual high- or low-fat intake pre-intervention, defined as being above or below the median (36% of Total Energy). S_I was significantly lower following the high-fat, SFA-rich diet (P=0.021) in subjects with a habitual low-fat intake pre-intervention. The sensitivity to SFA was more applicable to females wherein S_I was reduced following the high-fat, SFA rich diet. HOMA-IR was also reduced in females following the high-fat MUFA diet (P<0.05).

Conclusion: The effectiveness of dietary fat modification was dependent upon pre-intervention dietary fat intake. Altering dietary fat seems to be more effective in females. Further investigation is necessary to elucidate these diet*gender effects.

6.2 INTRODUCTION

The metabolic syndrome (MetS) is a very common, multi-component, condition characterised by insulin resistance or impaired insulin sensitivity, dyslipidaemia, abdominal obesity and hypertension that is associated with an increased risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and atherosclerosis (Moller & Kaufman, 2005). It has been hypothesised that insulin resistance, glucose intolerance and hyperinsulinaemia are the underlying components of the MetS or 'Syndrome X', and increase the likelihood of other abnormalities occurring (dyslipidaemia, endothelial dysfunction, coagulation and inflammatory disorders and abnormal uric acid metabolism)(Reaven, 1988a). The relationship between inflammatory status and metabolic disease is now well recognised. Inflammatory markers such as TNFα, IL6, Leptin and PAI-1 are increased in obese (Festa *et al.*, 2001) and insulin resistant (Yudkin *et al.*, 1999) states. Low plasma levels of adiponectin, a cytokine originating from adipose tissue with potent anti-inflammatory effects, is also an independent risk factor for the development of T2DM (Lindsay *et al.*, 2002). Several definitions for what constitutes the MetS have been proposed. Whilst the clustering of the components that define the different versions of the MetS are similar each place slightly different emphasis on the metabolic phenotype. In a recent perspective review, Gerald Reaven (2006) summarised the similarities and differences of three widely used definitions of the MetS (World Health Organisation (WHO), International Diabetes Federation (IDF) and National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATPIII)) but categorically stated that the components clustered in the syndrome occur only in insulin resistant people (Reaven, 2006). This is supported by results from animal studies (Shafrir, 1996; Zimmet *et al.*, 1999), and from human metabolic studies including the insulin resistance atherosclerosis study (IRAS) (Karter *et al.*, 1996; Howard *et al.*, 1998; Hanley *et al.*, 2003). As there is no universal definition of the MetS, this hinders the determination of the true global prevalence both locally and internationally. In a recent publication (Hossain *et al.*, 2007), it was estimated that 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the MetS. This is expected to increase to 420 million by the year 2025 (Hossain *et al.*, 2007). Using the NCEP ATPIII definition, the prevalence of the MetS is estimated to be 25% of the general population, varying with genetic background (Ford *et al.*, 2002). The IDF definition tends towards higher prevalence rates (Athyros *et al.*, 2005;

Lawlor *et al.*, 2006), as it includes lower cut-off points for waist circumference, as a prerequisite for the diagnosis of the MetS. However, it remains to be seen if one definition over another can predict the development of T2DM. More in-depth prospective data analysis is required.

There are many facets to the MetS, of which diet plays an important role and one that can be possibly targeted by public health initiatives. The typical Western dietary pattern is associated with higher risks of the MetS (Esmaillzadeh *et al.*, 2007). The increased intakes of saturated fat in the "Westernised diet" which is thought to have a detrimental effect on lipoprotein metabolism and insulin sensitivity, could in part explain the association. Saturated fat is a significant predictor of fasting and postprandial insulin concentration, even when controlling for total body adiposity and body fat distribution (Parker *et al.*, 1993). In contrast, dietary patterns attributable to the Mediterranean diet, high in monounsaturated fat, have been negatively associated with features of the MetS (Williams *et al.*, 2000; Esposito *et al.*, 2004; Panagiotakos & Polychronopoulos, 2005; Vincent-Baudry *et al.*, 2005). There is consistent information in the literature of the beneficial effects of n-3 PUFA on insulin action in rats (Storlien *et al.*, 1991). Its effects on insulin sensitivity in humans, however, are not as clearly defined, with some studies showing favourable benefits (Popp-Snijders *et al.*, 1987; Fasching *et al.*, 1991; Delarue *et al.*, 1996) and others showing little or no influence (Vessby *et al.*, 2001). Epidemiological evidence suggests that long chain (LC) n-3 PUFAs have protective properties against the development of T2DM (Friedberg *et al.*, 1998), modifying plasma lipid and lipoprotein concentrations, lowering triglyceride (TAG) levels effectively (Popp-Snijders *et al.*, 1987; Friedberg *et al.*, 1998) and increasing high density lipoprotein (HDL) cholesterol concentrations (Mori *et al.*, 1999). In individuals with an inflammatory phenotype at risk of developing insulin resistance, supplementation with LC n-3 PUFAs may attenuate the inflammation process and the impact on the MetS risk factors. Browning *et al.*, (2007) demonstrated an improvement in insulin sensitivity (insulin area under the curve) in overweight individuals with a susceptible inflammatory status, which was not observed in a similar group with low levels of background inflammation after a 12 week supplementation with LC n-3 PUFA (Browning *et al.*, 2007).

Whilst diet and/or poor nutritional status is not a risk factor for the MetS, reducing body weight, through manipulation of diet, is a well accepted therapy to reduce the incidence of T2DM (NIH, 1998; Klein *et al.*, 2004) and ameliorate risk

factors such as insulin resistance, hyperlipidaemia and hypertension. However poor compliance means that this therapeutic approach is largely ineffective and the prevalence of obesity continues to rise. Therefore other strategies to attenuate the impact of insulin resistance in the presence of obesity are required. The best diet for the treatment of the MetS is still under debate and scrutiny, with attention focussed on the composition of the diet, particularly quality and quantity of dietary fat. Human intervention studies determining the relationship between dietary fat composition and insulin sensitivity, evaluated by sound methodologies, whilst maintaining isoenergetic conditions (to outrule the effects of weight loss) are relatively few. The well-cited KANWU study, a controlled multi-centre study with 162 healthy volunteers, randomised to a high saturated or monounsaturated fat, isoenergetic diet showed that decreasing SFA and increasing MUFA improved insulin sensitivity but had no effect on insulin secretion. The positive effects, however, were observed when total fat intake was below 37% energy (Vessby *et al.*, 2001). In a cohort of 59 young healthy subjects, with a crossover study design, an improvement in insulin sensitivity was demonstrated with an isoenergetic substitution of carbohydrates or monounsaturated fat for saturated fat (Perez-Jimenez *et al.*, 2001). This group have recently shown an improvement in HOMA-IR and fasting pro-insulin levels in insulin-resistant subjects assigned a MUFA-rich isoenergetic diet for 28 days, compared to a diet high in saturated fat and a carbohydrate-rich diet (Paniagua *et al.*, 2007). Taken together, these studies suggest that manipulating the quality of dietary fat can improve insulin sensitivity when SFA is replaced with unsaturated fat by mechanisms still to be elucidated e.g. insulin secretion, insulin signalling, gene-nutrient interactions and membrane lipid composition. Decreasing the amount of total dietary fat in individuals who are at an increased risk of developing T2DM, due to their obese status, may also improve their insulin sensitivity (Mayer-Davis *et al.*, 1997). However, the need for controlled randomised clinical trials with sufficient study power and duration are required to further investigate the effects of dietary fats on insulin sensitivity and the factors of the MetS, and to examine whether insulin sensitivity can affect individual responses to the type and amount of dietary fat in an isoenergetic diet. Therefore, LIPGENE (contract no. FOOD-CT-2003-505944) is an integrated project of the EU Sixth Framework Programme for Research and Technology Development that is being conducted by a consortium of twenty-five research centres across Europe. The project is entitled: Diet, Genomics and the MetS: an integrated nutrition, agri-food,

social and economic analysis. It began in February 2004 and comprises six workpackages: Human Nutrition, Consumer Science, Animal Nutrition, Plant Biotechnology, Dissemination and Economic science. The objective of the human nutrition component and this present study was to determine the relative efficacy of reducing dietary SFA consumption, by altering the quality of dietary fat and reducing the quantity of dietary fat on multiple metabolic and molecular risk factors of the MetS; to determine insulin sensitivity and β-cell dysfunction using the frequently sampled intravenous glucose tolerance test (IVGTT) with minimal modelling and multivariate statistical approaches to identify determinants of increasing insulin sensitivity (S_I) after a 12-week intervention in four dietary treatment groups. A secondary aim of this present chapter was to ascertain whether background total fat intake as a percentage of energy determined the effects of the four intervention diets on insulin sensitivity and other IVGTT parameters and measures of insulin resistance.

6.3 SUBJECTS AND METHODS

The LIPGENE human dietary intervention study was a randomised, controlled trial approved by the local ethics committees at each of the 8 clinical intervention centres, all of which conformed to the Helsinki Declaration of 1975 as revised in 1983. The study was registered with The US National Library of Medicine Clinical Trials registry (NCT00429195). The nature of the LIPGENE study was explained to each subject and written informed consent was attained before assessment of anthropometric indices, blood pressure, and collection of a fasting blood sample at the screening visit. The statistical power of the LIPGENE dietary intervention study was based on insulin sensitivity as the primary biomarker of the MetS and was calculated with the accuracy to detect at least 1 unit difference at a SD of 2, between diets. The power calculation was based on the assumption that 15% difference in insulin sensitivity was physiologically important and that this difference can be detected at a significance level of 0.05 and a power of 0.8. Thus, a minimum of 98 subjects per dietary group would be required to detect significance differences. The group size was increased to 120 per group, to account for an anticipated 20% drop-out rate.

417 free-living subjects with the MetS, defined by a modified version of the NCEP criteria (2001), characterised by three or more of the following criteria, completed the LIPGENE dietary intervention programme:

- Fasting glucose concentration — 5.5 - 7.0 mmol/L
- Serum TAG concentrations — ≥ 1.5 mmol/L
- Serum HDL cholesterol concentration — < 1.0 mmol/L (men)
  < 1.3 mmol/L (women)
- Blood pressure — systolic BP≥130
  diastolic BP≥85mmHg
  or treatment of previously diagnosed hypertension
- Waist girth — > 102 cm (men)
  > 88 cm (women).

Each volunteer conformed to the LIPGENE inclusion and exclusion criteria. None of the subjects were receiving pharmacological treatment for glycaemic control, hyperlipidaemic medication, anti-inflammatory treatment or weight loss drugs. Subjects who were taking hypertensive medication had to have been prescribed them

for 3months prior to commencing the study. (Chapter 3 provides a more detailed description of the inclusion and exclusion criteria, aswell as information on the recruitment procedures and screening process). There was no change in allowed prescribed medication throughout the study.

The intervention study was completed in 8 European centres (Trinity College Dublin, Ireland (TCD), University of Reading, URREADNSI, UK (UoR), University of Oslo, Norway (UO), INSERM U476 Marseille, France (INSERM), Maastricht University, The Netherlands (UM), Hospital Universitario Reina Sofía-University of Córdoba, Spain (HURS-UCO), University of Krakow, Poland (JUMC) and Uppsala University, Sweden (UU).

6.3.1 Study Design

This randomised, controlled trial was conducted on a free-living population of MetS individuals. Subjects were recruited and randomly assigned to one of four dietary interventions per centre. Randomisation was completed by the coordinating centre, according to age, gender and fasting plasma glucose concentration. An equal number of subjects were allocated to each dietary group at each centre using the MINIM (Minimisation Programme for Allocating patients to Clinical Trials, Dept of Clinical Epidemiology, The London Hospital Medical College, UK) randomisation programme. The objective of the LIPGENE dietary intervention study was to determine the relative efficacy of replacing or reducing dietary SFA on insulin sensitivity, by comparing the effect of four different dietary treatments:

- Diet A: High-fat (38% energy), SFA-rich diet (16% SFA, 12 % MUFA 6% PUFA) (HFSFA).
- Diet B: High-fat (38% energy), MUFA-rich diet (8% SFA, 20% MUFA, 6% PUFA) (HFMUFA).
- Diet C: Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11%; MUFA; 6% PUFA) (LF).
- Diet D: Isoenergetic low-fat (28% energy), high-complex carbohydrate diet (8% SFA, 11% MUFA; 6% PUFA), with 1.24 g/d LC n-3 PUFA supplement (LFn3PUFA).

A range of fat-modified food products (margarines, cooking fats, baking fats, mayonnaise, dressings, biscuits, etc) were produced by Unilever Best Foods (Vlaardingen, The Netherlands). The LC n-3 PUFA supplement (Marinol ^{TM} C-38) and the comparator high-oleic acid sunflower seed oil (HOSO) supplement were provided by Lipid Nutrition, Loders Croklaan (Wormerveer, The Netherlands), the composition of which is presented in *Table 6.1*. Each volunteer received his or her capsules on day 0 (pre-intervention clinical investigation day) of the study. A capsule count was taken at the end of the study period (week 12) and volunteers were asked to note down any capsules missed or lost during the 12 weeks.

Table 6.1: Fatty Acid Composition and tocopherol content of the LC n-3 PUFA and the comparator HOSO supplement

	LC n-3 PUFA	**HOSO Supplement**
Description:	Marinol^{TM} C-38 is a concentrated natural fish oil with a high content of EPA and DHA	High Oleic Acid Sunflower Seed Oil supplement
Fatty Acid Composition	w/w %	w/w %
C14:0	5	0
C16:0	10.5	3.5
C16:1	4.5	0
C17:0	0.2	0
C18:0	2.5	3.4
C18:1	8.0	79.2
C18:2	1.0	11.8
C18:3	0.5	0.2
C20:0	0.2	0.3
C20:1	1.1	0.3
C22:0	0.2	0.9
C24:0	0.2	0.3
C20:5	24	-
C22:6	17.5	-
Total EPA and DHA	38	-
Mixed Natural Tocopherol	3.0mg	3.0mg

6.3.2 Dietary Assessment

Subjects completed a 3-day weighed food dietary record (two weekdays and one weekend day, not necessarily consecutive) to assess pre-intervention habitual dietary intake. This assessment formed the basis for dietary advice to achieve the dietary fat modification intervention targets using the intervention foods, without a significant alteration in body weight (<2% body weight change). Each volunteer had individualised dietetic counselling to attain their dietary targets.

Two weighed 3-day food dietary records were completed at week 6 and 12 to assess dietary intake mid- and post-intervention. The nutrient composition of the subject's diets was assessed using centre specific dietary analysis programmes to ensure a comprehensive assessment of culturally specific foods (Ireland: WISP version 3.0; UK: Food Base version 3.1; Norway: Kostberegningssystem (nutrient calculation software developed at the Department of Nutrition, University of Oslo); INSERM, France: Nutrilog; Maastricht, The Netherlands: Komeet to enter the dietary data and Orion to analyse the data; Poland: "Dietitian software"; Cordoba, Spain: Dietsource version 2.0; Uppsala, Sweden: MATs connected to the Swedish Board of Food Administration database for foods). These packages were modified to include the nutrient composition of the LIPGENE dietary intervention foods. Compliance was promoted and assessed by regular contact with each volunteer by 24-hour recalls and food use questionnaires, every two weeks of the trial. The food use questionnaire ensured that the subjects used the study foods as intended. Body weight was measured at week 0, 6 and 12 and subjects' energy intake was adjusted if greater than 2kg weight change occurred to ensure an isoenergetic state. Study foods were delivered by the investigators or subjects collected them from the study centres throughout the 12-week study period. The level of physical activity and alcohol consumption did not deviate throughout the intervention period. A more in-depth assessment on the methodological approach used in the dietary advice/strategy to achieve the intervention targets and the composition of the study foods are given in Chapter 4.

6.3.3 Clinical Investigations

Pre- and post-intervention, subjects reported to one of the eight intervention centres following a 12-hour overnight fast. Subjects abstained from strenuous exercise and

alcohol intake for 24 hours before the examination. Subjects were asked to provide details of their last meal eaten i.e. time and amounts in household quantities.

6.3.4 Collection of urine and blood samples

At each clinical investigation day subjects brought their fasting urine sample (first void) and 2ml was transferred into 2 ependorf tubes without any addition of preservative. Samples were kept frozen at -70°C until they were transported under dry ice to Uppsala, Sweden for analysis of urinary isoprostanes and prostaglandin metabolites (results of which are not presented in this thesis). Fasting blood samples were taken at the first sampling (-5 minutes) of the intravenous glucose tolerance test (IVGTT). Blood for the future measurement of leptin, resistin, adiponectin, C-reactive protein (CRP), interleukin (IL)-6, Tumour necrosis factor alpha (TNFα), sICAM and sVCAM was collected in serum separator tubes (Becton Dickinson, Oxford, UK). Blood for insulin, C-peptide and low density lipoprotein (LDL) isolation was collected in serum tubes (Becton Dickinson, Oxford, UK). Samples for plasma cholesterol, triacylgycerol (TAG), non-esterified fatty acids (NEFA), total high density lipoprotein (HDL), triacylglyceride rich lipoproteins (TRL), plasma apolipoproteins (apo) A1, B, B48, CII, CIII, E, TRL apoB and for plasma fatty acid analysis were collected in K3-EDTA tubes (Becton Dickinson, Oxford, UK). Samples for glucose analysis were collected in tubes containing sodium fluoride, and blood for fibrinogen, plasminogen activator inhibitor (PAI)-1 and tissue plasminogen activator (tPA) were collected in tubes containing citrate (Becton Dickinson, Oxford, UK). All tubes were inverted 8-10 times to ensure adequate mixing of anti-coagulant/preservative. The sodium citrate tube was pre-chilled on ice before blood collection. All samples, except for the serum samples were put on ice after collection and processed within 1 hour. The serum separator tubes were allowed to clot for 30 minutes before centrifugation. Blood samples were centrifuged and the plasma was harvested following centrifugation and was stored at the appropriate temperature. TRL, LDL and HDL fractions were isolated within 24 hours, with the buffy coat isolated within 2 hours. In Chapter 2, *Table 2.4* details the organisation of blood samples for analysis (vaccutainer type, plasma or serum harvested, volume, preparation, analysing centre and temperature storage). *Table 2.5* demonstrates the centrifugation details.

6.3.5 Intravenous Glucose Tolerance Test (IVGTT)

On the morning of the IVGTT two cannulae were inserted into the antecubital veins of both forearms. Following donation of the fasting blood samples from cannula 1, a bolus of 50% glucose solution (Phoenix Pharma Ltd, Gloucester, UK) (0.3g/kg body weight) was infused into cannula 2 over a 1 minute period followed by 20ml of saline. Twenty minutes later a dose of insulin (Novo Nordisk Pharmaceuticals Ltd, West Sussex, UK) (0.03U/kg body weight) was infused into the same cannula (cannula 2). Meanwhile, blood was sampled through cannula 1 at frequent intervals over a 3 hour period (-5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 minutes) after the start of the glucose injection. A total of 8ml of blood was collected at each time point (2ml into paediatric fluoride oxalate tubes for subsequent glucose analysis and 6ml into serum tube for subsequent analysis of insulin). At each time point real-time blood glucose monitoring was performed using a gluco-trend machine to confirm that the volunteer's blood glucose was not excessively low (<2.0mmol/l). If blood glucose dropped below this level, the IVGTT was discontinued and an isotonic high glucose drink was administered immediately. Blood samples were spun at 3000rpm for 10 minutes after which the plasma/serum was aliquoted and stored at -20°C for future analysis. After analysis, glucose and insulin values at each timepoint were entered into the MINMOD computer programme (version 6.02, Richard N. Bergman) to determine insulin sensitivity (S_I), glucose effectiveness (S_G), acute insulin response to glucose (AIRg), disposition index (DI), removal rate of insulin from the interstitial space (P2), movement of circulating insulin to the interstitial space (P3) and distributed glucose concentration at time 0 (G0) (Bergman *et al.*, 1985; Beard *et al.*, 1986; Pacini & Bergman, 1986), described in detail in sections *2.5.3* and *2.7* Homeostasis model assessment-insulin resistance (HOMA-IR) (fasting glucose x fasting insulin/22.5)(Matthews *et al.*, 1985) and quantitative insulin sensitivity check index (QUICKI) (1/(log fasting insulin concentration + log fasting glucose concentration) (Katz *et al.*, 2000) were also calculated from fasting data. First phase insulin response was calculated from insulin values at the 2, 4 and 8 minute IVGTT timepoints.

6.3.6 Biochemical Analysis

Serum insulin and C-peptide concentrations were measured by solid-phase, two-site fluoroimmunometric assays (AutoDELFIA Insulin kit and AutoDELFIA C-peptide

kit, respectively; Wallac Oy, Turky, Finland) on a 1235 AutoDELFIA automatic immunoassay system (Wallac Oy, Turku, Finland) at the Central Pathology laboratory, St. James's Hospital, Dublin 8, Ireland (Section *2.6.7*). The IL Test™ Glucose Hexokinase Clinical Chemistry kit (Instrumentation Laboratories, Warrington, UK) was used to determine plasma glucose concentration (Sections *2.2.6* and *2.6.5*).

The TRL fraction was isolated from each patient's fasting blood sample. The preparation of the TRL fraction, and the preservative used for the lipoprotein isolation are described in Section *2.6.1.* Total HDL concentrations were determined after precipitation with Quantolip total HDL precipitation Reagent (Immuno Ag, Vienna, Austria) as described in sections *2.2.7* and *2.6.3*. LDL-cholesterol concentrations were measured after precipitation of LDL using Randox Precipitation Reagent (Randox CH1350, Antrim, Ireland) as outlined in section *2.6.2*. The IL Test™ HDL-C Kit (Instrumentation Laboratories, Warrington, UK) was used for direct quantification of HDL-C. Quantification of total cholesterol, LDL cholesterol and TRL cholesterol from defrosted plasma and supernatant samples was performed using the IL Test™ Cholesterol Kit (Instrumentation Laboratories, Warrington, UK) on the ILAB 600 clinical chemistry analyzer (Instrumentation Laboratory, Warrington, UK). Quantification of plasma and TRL TAG was performed using the IL Test™ Triglycerides Kit (Instrumentation Laboratories, Warrington, UK). The WAKO NEFA C enzymatic colour kit (Alpha Laboratories, Hampshire, UK) was used to quantitate plasma NEFA concentration. The intensity of coloured product (quinoneimine) is proportional to the concentration of free fatty acids and measured by absorbance using the ILAB. Plasma apoA1, B and E concentrations were measured using an immunonephelometric methods on a BN Prospec® System with commercial kits (Dade, Behring, Deerfield, USA) at the Analytic Biochemistry Laboratory, Ste Marguerite Hospital, Marseille, France. Plasma apoCII and CIII concentrations were measured by immuno-turbidimetry on a Hitachi 911 system with commercial kits (Diasys, Bouffemont, France) at the Analytic Biochemistry Laboratory, Ste Marguerite Hospital, Marseille, France. Assessment of apoB-48 was by a competitive ELISA with a specific apoB-48 antibody obtained in rabbits. Human CRP, IL-6, TNFα, total Adiponectin, Resistin, sICAM, sVCAM and Leptin concentrations were measured on the basis of a solid phase sandwich enzyme linked immunosorbent assay

(ELISA). CRP was determined by high sensitivity (hs) CRP kits (BioCheck Inc, CA, USA). Human IL-6 ultrasensitive kits were purchased from Biosource International Inc, CA, USA. TNFα was determined using hs TNFα kits (R&D Systems, Abingdon, UK). Adiponectin, Resistin and Leptin were analysed using a DuoSet® ELISA Development System (R&D Systems, MN, USA) and Human sICAM and sVCAM with kits from R&D Systems, Abington, UK. Fibrinogen clotting activity was measured by using an automated clotting assay as described in Section *2.6.14*. Plasma tPA and PAI-1 concentrations were determined using the commercially available kits (ASSERACHROM® tPA, ASSERACHROM® PAI-1, respectively) by enzyme immunoassay (EIA) procedure by ELISA (Diagnostica Stago, France) as described in section *2.6.13*.

6.3.7 Fatty acid compositional analysis

Dietary compliance was verified by conducting a capsule count. Measurement of plasma fatty acid composition was determined with the use of gas liquid chromatography. Lipid extraction (Bligh and Dyer Method) and transesterfication (OHTA Method) of samples were performed (Section *2.6.8*) before analysis of the fatty acids methyl esters (FAME) of plasma fatty acids by gas chromatography (GC) on a Shimadzu GC 2010 (Shimadzu, Japan) using a fused silica capillary column Omegawax 250, (30 m x 0.25 mm inner diameter, 0.25μm film) (Supelco). GC was equipped with a flame ionisation detector and GC solutions software (Shimadzu, Japan). Fatty acids were identified by comparing the relative retention times of fatty acids with a FAME standard (PUFA, n-3, PUFA n-2 and C17 (standard). The fatty acid composition was represented as a relative percentage of the total of the quantified fatty acids (Section *2.6.8*).

6.3.8 Anthropometric measurements

Body weight was measured in duplicate on a centre specific, calibrated scale, to the nearest 0.1kg. Subjects were weighed in light clothing, without shoes and after voiding. Height was measured in duplicate to the nearest 0.1cm, in the upright position using a stadiometer. Waist circumference was measured directly on the skin, at the mid-point between the supra iliac crest and lower ribs margin at the end of a normal expiration, in duplicate to the nearest 0.1cm using a non-stretch tape measure.

Hip circumference was measured in duplicate to the nearest 0.1cm, using a non-restrictive tape measure, at the level of the greater trochanter without compressing the skin. Blood pressure measurements were recorded in duplicate in the seated position after a 5 minute rest, using an appropriately sized cuff before blood samples were taken. Body weight, height, waist and hip circumference measurements and blood pressure readings are described in section *2.2.1*. Bioelectric impedance analysis was measured in subjects in the supine position, using a body fat analyser (TBF-300; Tanita Corporation, Arlington Heights, IL, USA) (section *2.2.2)*.

6.3.9 Data preparation and statistical analysis

All data is presented as group means ± standard error of the mean unless stated otherwise. Data that was not normally distributed, as assessed by skewness and kurtosis, was normalised by log, square root, inverse or sin transformation.

A repeated measure ANOVA (RM ANOVA) was used to compare differences in outcome variables among the four diet groups. The analysis was adjusted for between-subject factors: gender, centre and diet group with age and change of body weight as covariates. All significant interactions (time*diet group, time*centre, time*change in body weight, time*age, time*gender, gender*centre, time*gender*centre*diet group) and all significant main effects were investigated using post-hoc analysis, paired and independent t-tests (non parametric alternatives: Wilcoxan and Mann Whitney, respectively) and one-way ANOVA (non parametric alternative: Kruskal Wallis). The Bonferroni and Tamhane post-hoc tests were used to identify significant differences between the groups as appropriate (Coakes & Steed, 1999). A Bonferroni correction was applied if multiple testing occurred in order to account for the increase in type 1 error. As age and change of body weight are continuous variables, these were categorised for post-hoc analysis. Age categories were defined into three groups: 35-50y, 50-65y and 65-70y. Change in body weight was categorised into 5 categories: Lost 4-8kg, Lost 2-4kg, Lost 0-2kg, Gained 0-2kg and Gained 2-5kg.

Correlation analysis was used to describe the strength and direction of the linear relationship between two variables. Pearson product-moment coefficient was used for continuous variables. Spearman rank order correlation was used for variables not normalised by transformation. Interrelationships among the post-intervention variables were explored irrespective of dietary treatment. When significant or

moderate-to-strong (r>0.25) correlations were observed, the file was split by diet to see where the relationship was strongest as part of a post-hoc analysis. All correlations reported are significant at $p<0.05$.

Linear regression analysis was used to determine the best fitting model to describe the relationship between the dependent variable S_I measured at post-intervention and a set of explanatory variables. When constructing the complex model with several predictors, care was taken in selecting the type of regression analysis to perform, as the predictors included, and the way in which they are entered into the model, can have a great impact. The forced entry linear regression analysis was chosen as all known and suspected predictors are forced into the model simultaneously based on theory, logic and past research. The stepwise method was not used for this analysis as predictors may be entered (and others removed) based on slight differences in their semi-partial correlations rather than on their theoretical importance (Field, 2005). Three separate regression analysis steps were carried out in the final model development. Firstly, each of the biochemical parameters and dietary variables were entered individually in a univariate regression analysis against the dependent variable S_I measured at post-intervention (Enter method). Secondly, multivariate regression analysis was performed for each of the individual variables, controlling for the volunteer's age, sex and body weight at post-intervention. These models were then examined to identify the variables that would be used in further multiple linear regression modelling. Two approaches were taken to decide which variables to include; variables that were significant ($p<0.1$) in both models were included and variables that were significant ($p<0.1$) in only one of the models for which there was strong literature support for their inclusion. At this early stage the cut-off used to denote significance was $p<0.1$, as the customary cut-off of $p<0.05$ may fail to identify variables that are influential in the final model (Hosmer & Lemeshow, 2000). The explanatory variables to be included were then grouped into seven further models: 1. Centre, IVGTT parameters, Smoking and playing sport, 2. Anthropometry, 3. Coagulation factors, 4. Inflammatory markers, 5. Lipid and lipoprotein markers, 6. Plasma Fatty Acids and 7. Dietary variables. Multiple linear regression analysis, controlling for the volunteer's age, sex and post-intervention body weight, was performed for each of the seven different models. Finally, the variables that were significant ($p<0.05$) in each of the seven different models were included in a final model controlling for age, sex and post-intervention body weight. The strength

of each explanatory variable was determined using the beta co-efficient value. In the final multiple linear regression model significance was taken at $p<0.05$. This analysis was carried out for S_I at post-intervention, irrespective of dietary treatment; however diet was entered as a predictor variable. The exact same procedure was carried out in each of the four diet groups, to explore how well a set of variables is able to predict increasing S_I at post-intervention.

Multicollinearity and singularity were checked at the end of each model. Tolerance and Variance Inflation Factor (VIF) values were noted to identify highly intercorrelated independent variables that would confound the model. If multicollinearity was present, one predictor was removed as it restricted the accuracy and predictive power of the model.

All statistical analysis was performed using SPSS® for Windows™ version 14.0 (SPSS Inc, Chicago, Ill).

6.4 RESULTS

The effect of dietary fat modification was determined in 417 subjects (n=185 males and 232 females) who completed the 12-week intervention study. This cohort was generated from the 486 volunteers that went forward to the pre-intervention clinical investigation day. Completion was defined as having completed the 12-week dietary intervention programme with donation of a fasting blood sample, completed an intravenous glucose tolerance test or both at post-intervention clinical investigation day. Of the completers, 100 subjects had been randomised to the High-fat, SFA-rich diet (HFSFA, Diet A), 111 subjects to the High-fat, MUFA-rich diet (HFMUFA, Diet B), 106 subjects to the Low-fat diet (LF, Diet C) and 100 subjects to the Low-fat diet with 1.24g /day LC n-3 PUFA (LF n3 PUFA, Diet D).

6.4.1 Repeated Measures ANOVA

Anthropometry

Table 6.2a presents the adjusted means and SEMs of the effect of dietary fat modification on anthropometric measurements for the 417 volunteers that completed the LIPGENE dietary intervention study. There were no significant differences in the anthropometric profile of the subjects between diet groups at baseline (p<0.05).

The mean age in each of the four diet groups were 54.91y, 54.62y, 54.70y and 55.39y in Diets HFSFA, HFMUFA, LF and LFn3PUFA respectively. Initial weight, height and BMI (kg/m^2) were not significantly different between diet groups. A repeated measures ANOVA (RM ANOVA) demonstrated a significant within-subject time*diet group effect (p=0.001) wherein body weight was significantly reduced post-intervention in the LF and LFn3PUFA diets (p<0.0005). Change of body weight was computed for each of the four diet groups (post-intervention body weight minus pre-intervention body weight). One-way ANOVA demonstrated that subjects in the LF (Diet C) lost significantly more weight compared to the HFSFA (Diet A) and HFMUFA (Diet B) diets (p<0.05). Volunteers in the LFn3PUFA (Diet D) diet lost significantly more weight compared to the HFSFA diet (Diet A), (p<0.05) (*Figure 6.1*).

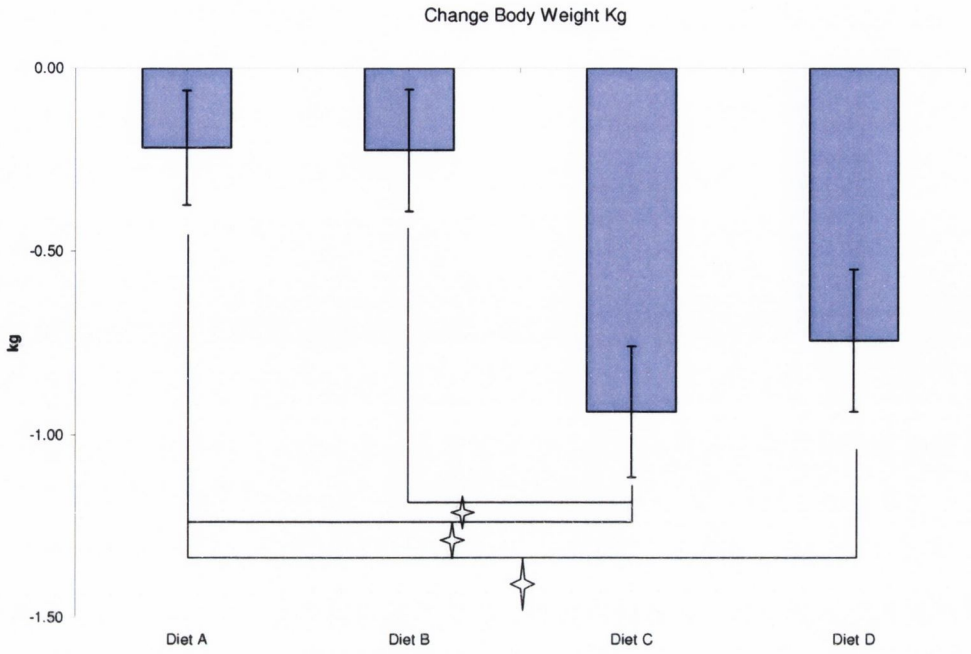


Figure 6.1: Change of body weight (kg) in each diet group over the 12 week dietary intervention period. ✦ $p<0.05$

Table 6.2b presents more detailed changes in anthropometric variables between the study centres, pre- and post-intervention, irrespective of dietary treatment. Interestingly a time*centre interaction showed a significant (p=0.015) decrease in body weight after the 12-week dietary intervention in the following centres: INSERM (87.42kg to 85.36kg p=0.001), UM (92.68kg to 91.99kg, p=0.002), HURS-UCO (90.95kg to 90.31kg, p=0.0005) and UU (83.38kg to 83.04kg, p=0.044). Body Mass Index (kg/m^2) was calculated and the effect of dietary fat modification determined (non-significant) using the RM ANOVA model. However, between-centre differences were achieved for BMI pre- and post-intervention. Volunteers in HURS-UCO and JUMC had significantly greater BMI's than volunteers in UoR, UO, INSERM and UM (p<0.05). Waist circumference differed between the centres with volunteers in TCD, UoR, UO, UM, HURS-UCO and JUMC having a higher waist circumference in comparison with UU pre- and post-intervention (p<0.002). As presented in *Table 6.2b* a time*centre interaction showed that volunteers in TCD (p=0.0005), UoR (p=0.029), UO (p=0.0005) and UM (p=0.001) had a lower hip circumference post-intervention. Significant differences between the centres existed for hip measurements and were the same pre- and post-intervention. Subjects in TCD, UoR, HURS-UCO and JUMC had

greater hip circumference than their study counterparts in UO, INSERM, UM and UU ($p<0.002$). There were no between-centre differences for systolic blood pressure post-intervention. However, there were significant differences pre-intervention with volunteers in INSERM having lower readings as compared to volunteers in UoR, HURS-UCO and JUMC ($p<0.05$). Differences between centres pre- and post-intervention existed for body fat (%). Volunteers in the UM cohort had the lowest percentage body fat (31.26%), and significantly lower than TCD, UO, JUMC and UU (38.43%, 36.72%, 38.63%, 43.33%, respectively, $p<0.05$) with volunteers in the UU cohort having the greatest percentage of body fat, pre-intervention. UM, again, had the lowest percentage body fat (31.86%), significantly lower than TCD (38.07%), JUMC (38.11%) and UU (43.25%) post-intervention ($p<0.05$). RM ANOVA for pre- and post-percentage lean body mass showed a significant main effect for centre ($p=0.0005$). With the lowest percentage body fat, volunteers in the UM cohort also had a significantly higher percentage lean body mass pre- and post-intervention (68.74%, 68.14%, respectively) compared to TCD, HURS-UCO, JUMC and UU ($p<0.05$). Differences between centres for lean body mass (kg) at pre- and post-intervention were varied, with each centre different to at least one other centre. Similar to the percentage, UM had greater amounts of lean body mass (kg) pre- and post-intervention compared to HURS-UCO and UU ($p<0.05$).

For body water (% and Litres), a significant between-subject effect ($p=0.0005$) for centre was noted wherein HURS-UCO volunteers had the lowest % body water and UU had the lowest body water measured in Litres, pre- and post-intervention ($p<0.05$).

A main effect of centre ($p=0.0005$), showed many differences pre- and post-intervention for impedance values. UO had the highest impedance values pre- (592.94 KHz) and post- (576 KHz) intervention compared to TCD, INSERM, UM and HURS-UCO ($p<0.002$). A time*gender*centre*diet group interaction showed significant differences for males randomised to the LF diet and females to the HFMUFA diet from the UoR cohort over the 12-week intervention period (LF diet: Males: 548 KHz pre, 527.63 KHz post; HFMUFA diet: Females: 622.83 KHz pre, 594.83 KHz, post, data not shown).

Table's 6.2c and *6.2d* present anthropometric data significantly affected by age and gender, respectively. Statistical analysis showed that subjects aged between 35-49y had greater body weights pre- and post-intervention than subjects aged 50-64y

and 65-70y ($p<0.05$). Males had greater body weights than females ($p=0.0005$). For 35-49 and 50-64 age groups, BMI was significantly higher than the 65-70 age group pre- ($p<0.05$) and post-intervention ($p<0.05$). For males and females, a time*gender interaction showed small but significant decreases in BMI from pre- to post-intervention ($p=0.0005$), irrespective of dietary treatment. There was a significant difference in waist circumference according to age ($p=0.009$) similar to body weight, waist circumference was greatest in the 35-49 age group as compared to the 50-64 and 65-70 age groups ($p<0.02$). A gender effect ($p=0.0005$) showed that males had a greater waist circumference than females pre- and post-intervention ($p=0.0005$). Age and gender were significant between-subject effects ($p=0.0005$) for hip circumference wherein volunteers in the 35-49 and 50-64 age categories and females had significantly greater hip circumferences than the 65-70 age group ($p<0.017$) and males ($p=0.0005$) pre- and post-intervention. RM ANOVA demonstrated a time effect wherein a significant decrease in diastolic blood pressure was observed from pre- to post-intervention (85.66mmHg, 83.95mmHg respectively, $p=0.005$). A decrease in the 50-64 ($p=0.0005$) and 65-70 ($p=0.006$) age groups was also observed (*Table 6.2c*). Systolic blood pressure was also significantly affected by age, where this parameter increased with increasing age pre- ($p<0.05$) and post-intervention ($p<0.05$). Systolic blood pressure decreased in all age groups over the intervention period, but this did not reach significance. A time*gender interaction showed systolic blood pressure decreased from pre- to post-intervention for both males ($p=0.007$) and females ($p=0.0005$).

For percentage body fat, a significant main effect for gender ($p=0.0005$) was observed where females had greater percentage body fat pre- and post-intervention compared to males ($p=0.0005$). Comparably males had significantly lower body fat mass pre- and post-intervention compared to females ($p=0.0005$) also.

RM ANOVA of percentage lean body mass showed significant main effects for age ($p=0.004$) and gender ($p=0.0005$). Volunteers in the 35-49 age group had a higher percentage of lean body mass pre-intervention (65.28%) and post-intervention (64.78%) compared to those in the 50-64 ($p<0.05$) and 65-70 ($p<0.05$) age groups. Males had a higher percentage and weight (kg) of lean body mass than females pre- and post-intervention ($p=0.0005$). As with the percentage, volunteers in the 35-49 age group had greater lean body mass pre- (64.05kg) and post- (63.85kg) intervention compared to those in the 50-64 and 65-70 age groups ($p<0.05$). For body water (%

and Litres), between-subject effects of age and gender were significant (p=0.0005). Body Water (% and L) decreased with increasing age pre- and post-intervention (p<0.05). Males had higher body water (% and L) than females pre- and post-intervention (p=0.0005).

For impedance values, females had higher impedance than males pre- (p=0.0005) and post- (p=0.0005) intervention.

Table 6.2e illustrates significant gender*centre interactions from the RM ANOVA model. A significant gender*centre interaction effect was observed for percentage body fat. Post-hoc analysis showed significant differences between males in UM and JUMC both pre- and post-intervention (p<0.05). However, there were more centre differences for females. Females in TCD had higher percentage body fat pre-intervention (45.89%) and post-intervention (45.47%) compared to females in INSERM, HURS-UCO and JUMC (p<0.5). Females in UU had greater body fat (%) than females in INSERM, HURS-UCO and JUMC at pre- and post-intervention (p<0.05). Females in UoR had greater body fat (%) than their counterparts in HURS-UCO post-intervention (p<0.05).

RM ANOVA of percentage lean body mass also showed a significant gender*centre interaction effect (p=0.012). Males in UM had greater lean body mass (%) pre- (73.41%) and post- (73.83%) intervention compared to HURS-UCO and UoR (p<0.05). There were more differences for females with HURS-UCO having significantly less lean body mass (%) pre- (48.59%) and post- (48.59%) intervention than all other centres (p<0.05).

Table 6.2f illustrates significant time*change in body weight interactions from the RM measures ANOVA model. A significant time*change in body weight interaction effect was demonstrated for waist circumference (p=0.0005) A significant decrease in those who lost weight (Lost 4-8kg p=0.048; lost 2-4kg p=0.0005 and lost 0-2kg, p=0.0005) and conversely an increase in waist circumference in those who gained more than 2kg over the duration of the study (p=0.046). RM ANOVA established a time*change in body weight (p=0.0005) interaction for hip circumference (cm), with the same effects as for waist circumference for those who lost weight. *Table 6.2f* illustrates that hip circumference was significantly lower in those who lost 4-8kg (p=0.023); lost 2-4kg (p=0.001) and lost 0-2kg (p=0.0005). For body fat measured in kg, a time*change in body weight effect (p=0.0005) was further investigated with post-hoc analysis. Body fat (kg) decreased over the intervention

period in subjects who lost between 4-8kg (p=0.025) and 0-2kg (p=0.0005) and increased in those who gained between 2-5kg (p=0.018). For lean body mass in kg, a time*change in body weight interaction effect (p=0.0005) showed a decrease in lean body mass (kg) in those who lost between 2-4kg (p=0.035), and an increase in those who gained 0-2kg (p=0.001) and 2-5kg (p=0.035).

A time*change in body weight interaction effect was also observed for body water in litres (p=0.0005). Those who lost 2-4kg had less body water (L) post- compared to pre- intervention, (44.50L versus 45,46L, respectively, p=0.004). In contrast, volunteers who lost 0-2 kg and those who gained 2-5kg had greater body water (L) post-intervention (p<0.010).

A time*change in body weight interaction effect (p=0.0005) showed an increase in impedance values in volunteers who lost between 4-8kg (p<0.05) and 2-4kg (p<0.05), and a decrease in those who gained 0-2kg (p=0.001).

Table 6.2a: The effect of dietary fat modification on the anthropometric variables of subjects who completed the LIPGENE Human Dietary Intervention Study (n=417).

	High-Fat SFA-rich Diet Diet A n=100				High-Fat MUFA-rich Diet Diet B n=111				Low-Fat Diet Diet C n=106				Low-Fat Diet with 1.24g LC n3 PUFA Diet D n=100			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age(y)	54.91	0.86			54.62	0.83			54.70	0.91			55.39	0.96		
Height(m)	1.68	0.01			1.68	0.01			1.68	0.01			1.68	0.01		
BodyWeight(kg)	89.91	1.44	89.70	1.43	91.17	1.35	90.94	1.35	91.96	1.38	91.03†	1.38	91.20	1.43	90.45†	1.43
BMI (kg/m^2)	31.81	0.43	31.74	0.43	32.44	0.41	32.37	0.41	32.51	0.42	32.18	0.42	32.42	0.43	32.16	0.43
Waist circumference(cm)	105.21	1.11	104.52	1.09	105.92	1.05	106.13	1.03	106.95	1.07	105.24	1.05	106.42	1.11	105.51	1.09
Hip circumference (cm)	112.57	1.00	111.52	1.02	112.03	0.94	111.84	0.95	113.50	0.96	112.31	0.95	111.94	1.00	111.08	1.01
Diastolic BP(mmHg)	85.88	0.91	84.07	0.87	86.71	0.85	85.31	0.82	85.50	0.87	83.64	0.84	85.52	0.91	82.94	0.87
Systolic BP(mmHg)	137.90	1.52	135.24	1.50	139.40	1.43	136.19	1.41	139.53	1.46	135.18	1.44	137.73	1.52	133.52	1.50
Body Fat (%)	37.24	0.90	36.88	0.90	37.13	0.83	36.41	0.83	37.17	0.86	36.58	0.86	36.70	0.90	36.58	0.90
Body Fat(kg)	33.46	0.96	33.22	0.95	33.61	0.89	32.85	0.88	33.54	0.92	32.76	0.91	33.46	0.96	33.13	0.95
Lean Body Mass(%)	61.16	1.01	61.28	1.00	60.70	0.94	61.34	0.93	60.75	0.96	60.67	0.96	61.71	1.01	61.33	1.00
Lean Mass Body(kg)	55.18	1.42	55.15	1.42	55.44	1.32	55.96	1.32	54.98	1.36	55.05	1.36	56.72	1.42	56.02	1.42
Body Water(%)	44.32	0.78	44.70	0.76	44.44	0.73	44.92	0.71	43.93	0.74	44.24	0.72	44.51	0.77	44.58	0.75
Body Water (L)	39.78	1.15	39.69	1.12	39.77	1.05	40.40	1.03	40.50	1.08	40.15	1.06	40.89	1.13	40.71	1.10
Impedance(KHz)	529.20	10.44	531.51	9.76	524.82	10.01	520.15	9.37	536.37	9.94	527.19	9.30	509.89	10.36	513.92	9.69

Values represent adjusted means and standard error of the means (SEMs) for diet*time interactions

† significant time*diet interaction $p<0.0005$, post- compared to pre-intervention

BMI (kg/m2), Body Mass Index; BP, blood pressure, SEM standard error of the mean

Table 6.2b: Between- and within-centre differences for Anthropometric variables (mean ±SEM) pre- and post-intervention, irrespective of dietary treatment in subjects who completed the LIPGENE Dietary Intervention Study (n=417)

	TCD n=55		UoR n=58		UO n=54		INSERM n=38		UM n=49		HURS-UCO n=74		JUMC n=43		UU n=46	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre Body Weight(kg)	93.21	2.12	91.99	1.84	92.89	1.98	87.42	2.62	92.68	1.96	90.95	1.42	96.03	2.07	83.38	1.85
Post BodyWeight(kg)	92.76	2.12	92.02	1.87	92.31	1.99	85.36 †	2.59	91.99 †	2.00	90.31 †	1.40	94.97	1.88	83.04 †	1.83
Pre BMI (kg/m^2)	33.18 ^{ac}	0.60	31.51 ^{ab}	0.52	30.94 ^{ab}	0.43	30.95 ^{ab}	0.79	31.09 ^{ab}	0.63	35.04 ^{c}	0.41	34.55 ^{c}	0.50	30.00 ^{b}	0.55
Post BMI (kg/m2)	32.93 ^{ac}	0.60	31.53 ^{ab}	0.54	30.74 ^{ab}	0.44	30.45 ^{ab}	0.78	30.86 ^{ab}	0.64	34.79 ^{c}	0.40	34.39 ^{c}	0.50	29.88 ^{b}	0.54
Pre Waist circumference (cm)	106.91 ^{a}	1.62	107.12 ^{a}	1.85	105.20 ^{a}	1.23	103.70 ^{ab}	1.86	108.16 ^{a}	1.25	108.47 ^{a}	1.04	109.23 ^{a}	1.61	98.78 ^{b}	1.46
Post Waist circumference (cm)	105.57 ^{a}	1.65	105.87 ^{a}	1.39	104.74 ^{a}	1.25	103.09 ^{ab}	2.05	107.00 ^{a}	1.35	108.43 ^{a}	1.06	109.26 ^{a}	1.56	96.80 ^{b}	1.46
Pre Hip circumference (cm)	114.02 ^{a}	1.67	114.65 ^{a}	0.92	108.25 ^{b}	0.83	107.09 ^{b}	1.62	107.45 ^{b}	1.27	117.20 ^{a}	0.95	120.63 ^{a}	1.46	108.30 ^{b}	1.14
Post Hip circumference (cm)	112.57 ^{a†}	1.67	113.30 ^{a†}	0.99	107.17 ^{a†}	0.74	106.12 ^{b}	1.67	106.22 ^{b†}	1.31	117.74 ^{a}	0.95	119.64 ^{a}	1.49	107.33 ^{b}	1.01
Pre Systolic BP (mmHg)	138.49 ^{ab}	1.84	139.86 ^{b}	1.88	136.46 ^{ab}	2.03	129.21 ^{a}	2.48	137.35 ^{ab}	2.30	140.72 ^{b}	1.77	143.77 ^{b}	2.29	137.02 ^{ab}	2.33
Post Systolic BP (mmHg)	135.98	1.89	135.16	1.81	132.56	1.97	127.32	2.47	134.47	2.13	133.92	1.87	138.21	1.66	133.90	2.54
Pre Body Fat (%)	38.43 ^{ac}	1.27	35.73 ^{ab}	1.05	36.72 ^{a}	1.04	34.56 ^{ab}	1.47	31.26 ^{b}	1.34	35.68 ^{ab}	0.86	38.63 ^{ac}	1.25	43.33 ^{c}	1.08
Post Body Fat (%)	38.07 ^{ac}	1.32	34.80 ^{ab}	1.12	35.92 ^{ab}	1.02	35.41 ^{ab}	1.24	31.86 ^{b}	1.51	35.27 ^{ab}	0.88	38.11 ^{a}	1.22	43.25 ^{c}	1.12
Pre Lean Body Mass(%)	61.66 ^{ad}	1.28	64.33 ^{ab}	1.06	62.68 ^{a}	1.04	65.24 ^{ab}	1.44	68.74 ^{b}	1.34	54.78 ^{cd}	1.23	61.37 ^{ad}	1.25	56.71 ^{d}	1.08
Post Lean Body Mass(%)	61.29 ^{ad}	1.40	65.06 ^{ab}	1.12	62.86 ^{ab}	1.00	64.55 ^{ab}	1.24	68.14 ^{b}	1.51	54.58 ^{cd}	1.12	61.36 ^{ad}	1.31	56.80 ^{d}	1.11
Pre Lean Mass Body (kg)	57.54 ^{ac}	1.82	58.70 ^{a}	1.59	58.11 ^{a}	1.80	55.91 ^{abc}	1.82	63.86 ^{a}	1.86	50.72 ^{c}	1.90	58.33 ^{ac}	1.66	47.48 ^{b}	1.62
Post Lean Mass Body (kg)	57.07 ^{acd}	1.85	59.34 ^{ad}	1.64	59.70 ^{ad}	1.90	53.78 ^{abc}	1.89	63.15 ^{d}	2.11	49.99 ^{c}	1.69	58.22 ^{ad}	1.66	46.94 ^{b}	1.55
Pre Body Water (%)	45.37 ^{ac}	0.81	47.10 ^{a}	0.77	46.30 ^{ac}	0.76	46.94 ^{ac}	1.25	not measured		39.52 ^{b}	0.93	45.05 ^{ac}	0.97	42.52 ^{c}	0.77
Post Body Water (%)	45.92 ^{ac}	0.84	47.68 ^{abd}	0.81	46.94 ^{a}	0.74	47.42 ^{a}	0.98	52.99 ^{b}	1.84	39.63 ^{d}	0.87	45.58 ^{a}	0.90	42.47 ^{cd}	0.79
Pre Body Water (L)	41.99 ^{ab}	1.85	42.98 ^{a}	1.16	42.81 ^{a}	1.35	41.81 ^{ab}	1.34	not measured		36.62 ^{b}	1.41	42.50 ^{a}	1.25	35.77 ^{b}	1.19
Post Body Water (L)	42.28 ^{a}	1.22	43.49 ^{a}	1.20	43.41 ^{a}	1.36	40.38 ^{ab}	1.22	49.65 ^{a}	1.82	36.33 ^{b}	1.27	42.95 ^{a}	1.25	35.33 ^{b}	1.14
Pre Impedance (KHz)	505.09 ^{a}	10.84	567.03 ^{b}	9.09	592.54 ^{b}	16.36	501.00 ^{ac}	12.01	447.03 ^{c}	13.74	500.60 ^{a}	7.54				
Post Impedance (KHz)	501.66 ^{ad}	11.54	558.45 ^{b}	9.81	576.00 ^{c}	9.58	524.04 ^{ab}	11.87	450.66 ^{d}	14.24	497.36 ^{a}	6.93	not measured			

Values represent time*centre adjusted means (±SEM)

†Significant time*centre interaction, $p<0.05$, post- compared to pre-intervention in that centre

^{abc} denotes significance ($p<0.05$, $p<0.0018$ where bonferroni correction applied) across centres (when two superscripts contain the same letter there is no significant difference between two of the eight centres)

SEM: standard error of the mean

BMI (kg/m2), Body Mass Index; BP, blood pressure

Table 6.2c: Between- and within-age category differences for anthropometric variables pre-and post-intervention, irrespective of dietary treatment in subjects who completed the LIPGENE Dietary Intervention Study (n=417)

	35-49y n=107		50-64y n=249		65-70y n=61	
	Mean	SEM	Mean	SEM	Mean	SEM
Pre Body Weight(kg)	98.73 [a]	1.46	89.59 [b]	0.82	84.64 [c]	1.63
Post BodyWeight(kg)	97.87 [a]	1.45	89.02 [b]	0.82	84.19 [c]	1.62
Pre BMI (kg/m^2)	33.41 [a]	0.40	32.33 [a]	0.27	30.43 [b]	0.51
Post BMI (kg/m2)	33.24 [a]	0.40	32.11 [a]	0.27	30.27 [b]	0.51
Pre Waist circumference (cm)	108.57 [a]	1.06	105.81 [b]	0.71	103.57 [b]	1.27
Post Waist circumference (cm)	108.30 [a]	1.09	104.85 [b]	0.67	102.42 [b]	1.32
Pre Hip circumference (cm)	114.40 [a]	0.97	112.63 [a]	0.64	109.14 [b]	1.04
Post Hip circumference (cm)	113.32 [a]	1.01	111.82 [a]	0.65	108.44 [b]	0.98
Pre Diastolic BP (mmHg)	85.08	0.90	85.83	0.58	86.02	1.05
Post Diastolic BP (mmHg)	84.54	1.09	83.84 †	0.55	83.38 †	1.03
Pre Systolic BP (mmHg)	133.33 [a]	1.30	138.45 [b]	0.97	145.83 [c]	1.96
Post Systolic BP (mmHg)	129.48 [a]	1.35	134.13 [b]	0.91	141.89 [c]	1.91
Pre Lean Body Mass(%)	65.28 [a]	0.79	59.96 [b]	0.63	60.27 [b]	1.28
Post Lean Body Mass(%)	64.78 [a]	0.90	60.09 [b]	0.62	59.94 [b]	1.19
Pre Lean Mass Body (kg)	64.05 [a]	1.29	53.70 [b]	0.81	50.92 [b]	1.62
Post Lean Mass Body (kg)	63.85 [a]	1.34	53.66 [b]	0.82	50.03 [b]	1.51
Pre Body Water (%)	47.34 [a]	0.59	43.15 [b]	0.49	43.51 [b]	0.95
Post Body Water (%)	47.81 [a]	0.64	43.97 [b]	0.48	43.99 [b]	0.89
Pre Body Water (L)	47.14 [a]	1.08	38.48 [b]	0.63	36.31 [b]	1.18
Post Body Water (L)	46.40 [a]	1.01	39.21 [b]	0.61	36.93 [b]	1.09

Values represent group means and SEMs

[abc] denotes significance across age categories (when two superscripts contain the same letter there is no significant difference between two of the three age categories) p<0.017 where bonferroni correction applied

†Significant time*age interaction, p<0.05, post- compared to pre-intervention in that age-category

SEM: standard error of the mean

Table 6.2d: Between- and within-gender differences for anthropometric variables pre-and post-intervention, irrespective of dietary treatment in subjects who completed the LIPGENE Dietary Intervention Study (n=417)

	Males n=185		Females n=232	
	Mean	SEM	Mean	SEM
Pre Body Weight(kg)	98.91 [a]	1.01	85.08 [b]	0.75
Post BodyWeight(kg)	97.98 [a]	1.02	84.66 [b]	0.75
Pre BMI (kg/m^2)	31.98 [a]	0.29	32.61 [a]	0.29
Post BMI (kg/m2)	31.73 [b]	0.30	32.44 [b]	0.29
Pre Waist circumference (cm)	110.69 [a]	0.76	102.60 [b]	0.67
Post Waist circumference (cm)	109.70 [a]	0.79	101.93 [b]	0.63
Pre Hip circumference (cm)	110.06 [a]	0.69	114.58 [b]	0.65
Post Hip circumference (cm)	108.92 [a]	0.70	113.91 [b]	0.65
Pre Systolic BP (mmHg)	138.40	1.07	138.07	1.05
Post Systolic BP (mmHg)	135.80 †	1.04	132.74	1.00
Pre Body Fat (%)	29.92 [a]	0.43	42.13 [b]	0.42
Post Body Fat (%)	29.43 [a]	0.46	41.66 [b]	0.40
Pre Body Fat (kg)	29.88 [a]	0.66	36.10 [b]	0.57
Post Body Fat (kg)	29.21 [a]	0.66	35.61 [b]	0.55
Pre Lean Body Mass(%)	69.38 [a]	0.48	55.28 [b]	0.45
Post Lean Body Mass(%)	69.45 [a]	0.50	55.32 [b]	0.43
Pre Lean Mass Body (kg)	68.05 [a]	0.76	46.76 [b]	0.48
Post Lean Mass Body (kg)	67.87 [a]	0.79	46.88 [b]	0.49
Pre Body Water (%)	50.10 [a]	0.42	40.56 [b]	0.35
Post Body Water (%)	50.97 [a]	0.36	40.84 [b]	0.34
Pre Body Water (L)	49.66 [a]	0.68	34.15 [b]	0.38
Post Body Water (L)	49.62 [a]	0.56	34.55 [b]	0.38
Pre Impedance (KHz)	474.02 [a]	5.63	568.24 [b]	7.35
Post Impedance (KHz)	474.53 [a]	5.51	562.55 [b]	5.65

Values represent group means and SEMs

[a, b] denotes significance across gender, p<0.05

†Significant time*gender interaction, p<0.05, post- compared to pre-intervention in males or females

SEM: standard error of the mean

Table 6.2e: Body Fat (%) and Lean Body Mass (%) for males and females in each of the study centres, who completed the LIPGENE Dietary Intervention Study (gender*centre interactions)

	TCD				UoR				UO				INSERM				UM				HURS-UCO				JUMC				UU			
	Males		Females		Males		Females		Males		Females		Males		Females		Males		Females		Males		Females		Males		Females		Males		Females	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Body Fat (%)	30.39 ^{ab}	1.28	45.89 ^{a}	0.66	29.53 ^{ab}	0.85	42.86 ^{ab}	0.74	29.68 ^{ab}	0.86	41.95 ^{ab}	0.88	28.99 ^{ab}	1.51	39.29 ^{b}	1.84	26.59 ^{a}	0.83	42.18 ^{ab}	1.38	31.14 ^{ab}	1.18	38.41 ^{b}	0.98	34.14 ^{b}	1.86	40.79 ^{b}	1.49	32.33 ^{ab}	1.57	46.00 ^{a}	0.81
Post Body Fat (%)	29.67 ^{ab}	1.16	45.47 ^{a}	0.58	28.26 ^{ab}	0.84	42.30 ^{ab}	0.94	28.75 ^{ab}	0.73	41.41 ^{abc}	0.75	30.78 ^{ab}	1.24	39.55 ^{bc}	1.57	26.17 ^{a}	1.10	41.93 ^{abc}	1.15	30.92 ^{ab}	1.49	37.82 ^{c}	0.91	32.66 ^{b}	1.96	40.56 ^{bc}	1.31	31.67 ^{ab}	2.16	45.45 ^{a}	0.88
Pre Lean Body Mass(%)	69.78 ^{abc}	1.25	54.11 ^{a}	0.66	70.59 ^{ac}	0.86	57.14 ^{ab}	0.74	69.39 ^{abc}	0.85	57.70 ^{ab}	0.99	70.51 ^{abc}	1.49	60.77 ^{b}	1.84	73.41 ^{a}	0.83	57.83 ^{ab}	1.38	65.10 ^{b}	1.63	48.59 ^{c}	0.81	65.86 ^{c}	1.86	59.21 ^{b}	1.49	67.72 ^{abc}	1.57	54.03 ^{a}	0.81
Post Lean Body Mass(%)	68.96 ^{ab}	1.87	54.53 ^{a}	0.58	71.75 ^{a}	0.84	57.37 ^{ab}	0.83	69.30 ^{ab}	1.06	57.92 ^{ab}	0.75	69.14 ^{ab}	1.24	60.45 ^{b}	1.57	73.83 ^{a}	1.10	58.07 ^{ab}	1.15	64.77 ^{b}	1.12	48.59 ^{c}	0.76	68.03 ^{ab}	1.60	58.37 ^{ab}	1.44	68.39 ^{ab}	2.18	54.60 ^{a}	0.88

Values represent group means and SEMs

^{abc} denotes significance across a gender in centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories for each gender), $p<0.05$, $p<0.0018$ where bonferroni correction applied

SEM: standard error of the mean

Table 6.2f: Anthropometric variables across body weight change categories, pre- and post-intervention (time*change in body weight) in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417)

	Lost 4-8Kg n=14		Lost 2-4Kg n=57		Lost 0-2Kg n=191		Gained 0-2Kg n=129		Gained 2-5Kg n=23	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Waist circumference (cm)	107.40	2.60	111.26	1.77	105.53	0.76	104.85	0.89	104.89	2.26
Post Waist circumference (cm)	104.33 †	3.03	107.97 †	1.46	104.96 †	0.78	104.67	0.96	106.85 †	1.94
Pre Hip circumference (cm)	112.45	2.81	112.44	1.37	112.91	0.70	112.21	0.81	111.32	2.84
Post Hip circumference (cm)	109.80 †	2.94	111.06 †	1.40	111.75 †	0.72	112.10	0.81	111.82	2.78
Pre Body Fat (kg)	35.88	2.77	33.07	1.30	33.51	0.71	32.79	0.73	33.66	1.78
Post Body Fat (kg)	33.26 †	2.45	32.12	1.28	32.63 †	0.70	33.28	0.74	35.10 †	1.85
Pre Lean Mass Body (kg)	58.64	2.56	61.70	1.91	54.93	1.00	54.12	1.19	58.40	2.40
Post Lean Mass Body (kg)	56.30	2.73	59.48 †	1.94	54.87	1.01	54.39 †	1.21	60.03 †	2.83
Pre Body Water (L)	42.56	2.00	45.46	1.70	39.55	0.79	38.47	0.91	42.50	2.00
Post Body Water (L)	41.16	1.78	44.50 †	1.38	39.99 †	0.75	39.55	0.89	44.01 †	2.03
Pre Impedance (KHz)	496.96	19.68	506.89	13.08	516.51	8.92	542.52	9.24	526.48	14.96
Post Impedance (KHz)	534.88 †	11.37	516.96 †	12.00	510.27	7.34	536.87 †	8.64	525.84	15.87

Values represent time*change in body weight adjusted means

†significant time*change in body weight interaction p<0.0005, post- compared to pre-intervention in that weight-change category

SEM: standard error of the mean

BMI, Body mass index

IVGTT Parameters

Preliminary analysis was performed to assess outlier status and subsequent skewness of the data. Four outliers for S_I were determined from this. These were previously highlighted as outliers at the MINMOD programming stage but because there was no concrete reason to eliminate them at that stage they were left in the dataset, however they were now removed from the dataset for the purpose of this analysis due to their extreme outlier status.

Table 6.3a presents the effects of dietary fat modification on the parameters from the IVGTT modelling process. HOMA-IR, QUICKI and first phase insulin response were calculated as common surrogate measures of insulin action. Insulin sensitivity (S_I) is the main outcome variable of the IVGTT. There were no significant diet*time interactions for any of the parameters exported from the MINMOD millennium programme. Thus dietary fat modification per se, had no significant effect on insulin sensitivity. One-way ANOVA revealed no significant differences in any of the IVGTT parameters between the four diet groups pre-intervention. Insulin to C-peptide ratio was calculated and inputted into the RM ANOVA model. In all diet groups a decrease was seen (HFSFA p=0.030; HFMUFA p=0.005; LF p=0.003; LFn3PUFA p=0.002), the greatest changes seen for the HFMUFA diet and the LFn3PUFA diet.

RM ANOVA demonstrated a significant effect of gender on several components of the IVGTT (*Table 6.3b*). Insulin sensitivity index (S_I $(mU/L)^{-1}min^{-1}$) was significantly (p=0.004) greater in females compared to males (p=0.007) pre-intervention. The greater insulin sensitivity observed in females was also present post-intervention, albeit to a lesser extent (p=0.056). The movement of circulating insulin to the interstitial space (P3 (mU/L) min^{-2}) showed an effect for gender (p=0.004) which was consistent at both pre- and post-intervention (p<0.005). Insulin to C-peptide ratio decreased in both males (p=0.007) and females (p=0.0005) over the study period.

As expected, age also affected a number of IVGTT parameters (*Table 6.3c*). The Disposition Index which is a product of the acute insulin response to glucose (AIRg) and S_I, demonstrated significant differences according to age (p=0.0005). This effect was observed pre- and post-intervention, with the 35-49 age group having a significantly higher disposition index as compared to the 50-64 and 65-70 age categories (p<0.05). As anticipated, fasting glucose concentrations were also

significantly affected by age (p=0.005). Volunteers in the 35-49 age group had lower fasting glucose concentrations pre- and post-intervention compared to the 50-64 and 65-70 age groups (p<0.05). A significant age effect (p=0.0004) demonstrated that volunteers in the 35-49 and 50-64 age categories had significantly higher concentrations of fasting insulin pre-intervention compared to the 65-70 age group (p<0.05). This difference was only apparent between the 35-49 and 65-70 age groups post-intervention.

A number of the IVGTT parameters were significantly different between centres, the significant variables being presented in *Table 6.3d.* A centre effect showed that INSERM subjects had significantly lower fasting glucose concentrations pre- and post-intervention compared to subjects in UO at pre-intervention and HURS-UCO pre- and post-intervention (p<0.05). Centre differences (p=0.001) for fasting insulin concentrations were significant between UU and UoR, UO, UM, HURS-UCO and JUMC. These differences were maintained at post-intervention (p=0.0005). Centre differences (p=0.005) for QUICKI values were also similar to centre differences for fasting insulin. UU had significantly higher QUICKI levels at pre- (0.79) and post-intervention (0.70) compared to pre- and post-levels in UoR, UO, UM, HURS-UCO and JUMC.

A significant time*centre interaction effect (p=0.015) for C-peptide concentrations is explained by a decrease from 3.23ng/ml to 3.11ng/ml (p=0.043), in the UO cohort, and an increase in HURS-UCO (p=0.003), JUMC (p=0.004) and UU (p=0.048). UO had the highest C-peptide concentrations pre- and post-intervention compared to TCD pre-intervention, INSERM pre- and post-intervention, HURS-UCO pre- and UU pre- and post-intervention. Centre differences for insulin to C-peptide ratio at pre-intervention were apparent between subjects in UO and TCD, UoR, HURS-UCO and JUMC. Subjects in UU also had lower insulin to C-peptide ratios than HURS-UCO and JUMC subjects at pre-intervention. Post-intervention, subjects in UU had a lower ratio than TCD, UoR, UO, INSERM, HURS-UCO and JUMC subjects (p<0.05).

Importantly the RM ANOVA model demonstrated a significant time*change in body weight (p=0.017) interaction effect for fasting insulin concentrations, QUICKI, C-peptide concentrations and the insulin to C-peptide ratio. Post-hoc tests were performed to further investigate these interactions. Fasting insulin concentrations were significantly reduced in subjects who lost between 4 and 8kg

(p=0.003). Fasting insulin concentrations were also significantly reduced in those who lost 0-2kg (p=0.006), albeit to a lesser extent (*Table 6.3e*).

From the RM ANOVA model, a significant time*change in body weight (p=0.001) interaction effect was observed for QUICKI. QUICKI values increased significantly in subjects who lost 4-8 kg (p=0.005) and 2-4 Kg (p=0.013). However, in subjects who lost only 0-2 kg, QUICKI values decreased from 0.62 to 0.60 (p=0.005).

A significant time*change in body weight (p=0.001) interaction effect for C-peptide concentrations demonstrated a decrease in those who lost 4-8kg (p=0.011), and who lost 0-2kg (p=0.001) and an increase in those who gained 2-5kg (p=0.022). Subjects who lost 4-8kg, 0-2kg and gained 0-2kg had significant decreases in insulin to C-peptide ratios, the greatest being in those who lost the most weight (p=0.011).

Although there were no significant diet*time interactions, importantly, there was a significant time*centre*diet group interaction (p=0.004) for fasting insulin, QUICKI and the First phase insulin response (*Table 6.3f*). For fasting insulin concentrations, the interesting centre specific effects are as follows: Fasting insulin concentrations in subjects from the UU cohort (p=0.0023) significantly reduced in the HFMUFA diet (Diet B) group. Fasting insulin concentrations in the HURS-UCO cohort (p=0.003) significantly decreased in the LFn3PUFA diet (Diet D) group but in contrast a significant increase in fasting insulin concentrations in the JUMC cohort (p=0.017) was observed. As with fasting insulin, QUICKI values for subjects in the UU cohort on the HFMUFA diet (Diet B) increased significantly post-intervention (p=0.046). Subjects in this centre on the LF diet (Diet C) also had an increase in QUICKI values from 0.64 to 0.70 (p=0.033). With the LF n3 PUFA diet (Diet D), QUICKI values in volunteers in the HURS-UCO cohort increased (p=0.008) whilst subjects in JUMC decreased (p=0.036) over the 12 week intervention. RM ANOVA demonstrated one significant centre*diet group (p=0.001) interaction effect for the first phase insulin response. The first phase insulin response was significantly greater in UoR (56.18) compared to UO (24.04) and HURS-UCO (24.52), post-intervention in Diet D (LFn3PUFA diet).

A time*gender*centre*diet group (p=0.037) interaction for the Insulin to C-peptide ratio highlighted that this was significantly reduced by the LFn3PUFA diet in males in the HURS-UCO cohort (p=0.038). Furthermore, females in the HURS-UCO

cohort assigned to the LF diet (Diet C) had significantly lower Insulin to C-peptide ratios following intervention (p=0.019). Also, in the UU cohort, the Insulin to C-peptide ratio was significantly reduced by the HFMUFA diet in females only (p=0.007).

Table 6.3a: The effects of dietary fat modification on the parameters from the IVGTT modelling process in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417).

	High-Fat SFA-rich Diet						High-Fat MUFA-rich Diet						Low-Fat Diet						Low-Fat Diet with 1.24g LC n3 PUFA					
	Diet A						Diet B						Diet C						Diet D					
	Pre			Post			Pre			Post			Pre			Post			Pre			Post		
	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM
S_I $(mU/L)^{-1}min-1$	77	3.09	0.19	73	2.89	0.18	94	2.89	0.17	94	2.89	0.16	83	2.75	0.18	84	2.79	0.17	77	2.58	0.19	77	2.53	0.18
S_G min-1	77	0.016	0.001	74	0.015	0.001	94	0.016	0.001	94	0.016	0.001	84	0.016	0.001	84	0.017	0.001	78	0.015	0.001	78	0.017	0.001
AIRg mUL^{-1} min^{-1}	77	388.62	42.08	74	398.31	44.04	94	359.35	37.33	94	383.80	39.08	84	365.53	39.49	84	388.66	41.34	78	385.75	40.99	78	378.75	42.90
DI	77	955.96	84.00	74	994.03	94.36	94	955.33	74.53	94	957.50	83.72	84	814.73	78.84	84	913.38	88.57	78	879.87	81.82	78	921.61	91.91
G0 mg/dl^{-1}	77	297.46	4.74	74	299.63	10.49	94	297.83	4.21	94	300.71	9.31	84	296.09	4.45	84	295.76	9.85	78	297.67	4.62	78	313.00	10.22
P2 min^{-1}	77	0.037	0.004	74	0.043	0.015	94	0.045	0.003	94	0.047	0.013	84	0.043	0.003	84	0.053	0.014	78	0.044	0.003	78	0.070	0.014
P3 (mU/L) min^{-2}	77	0.000011	0.000001	74	0.000013	0.000002	94	0.000013	0.000001	94	0.000012	0.000001	84	0.000012	0.000001	84	0.000011	0.000001	78	0.000012	0.000001	78	0.000012	0.000001
Fasting Glucose (mmol/l)	100	5.96	0.09	97	6.00	0.11	110	5.90	0.08	110	5.97	0.10	105	5.94	0.08	105	5.81	0.10	96	6.02	0.09	96	6.16	0.11
Fasting Insulin (uIU/ml)	100	9.83	0.54	97	10.05	0.59	110	9.86	0.51	110	9.20	0.56	105	10.31	0.52	105	10.22	0.57	96	10.22	0.55	96	9.60	0.60
HOMA-IR	100	2.65	0.16	97	2.82	0.19	110	2.59	0.15	110	2.45	0.18	105	2.75	0.15	105	2.66	0.18	96	2.77	0.16	96	2.63	0.19
QUICKI	100	0.67	0.04	97	0.61	0.02	110	0.60	0.04	110	0.60	0.01	105	0.54	0.04	105	0.61	0.02	96	0.59	0.04	96	0.61	0.02
First Phase Insulin Response (uIU/ml)	98	40.77	4.10	96	42.05	4.32	106	40.25	3.87	109	41.77	4.09	104	38.69	3.99	100	42.23	4.21	96	39.31	4.05	96	38.82	4.28
C-Peptide ng/ml	99	2.67	0.09	96	2.82	0.10	109	2.54	0.09	108	2.68	0.10	105	2.68	0.09	105	2.78	0.10	95	2.64	0.09	95	2.81	0.11
Insulin to C-peptide Ratio	99	3.67	0.16	96	3.38†	0.15	109	3.86	0.15	108	3.33†	0.14	105	3.81	0.15	105	3.61†	0.14	95	3.89	0.16	95	3.32†	0.15

Values represent diet*time adjusted means(±SEM)

† significant time*diet interaction $p<0.0005$, post compared to pre-intervention in that diet

not all IVGTT data modelled sucessfully, therefore different n numbers

SEM standard error of the mean

S_I, insulin sensitivity; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; G0, distributed glucose at time 0min

Table 6.3b: S_I, P3 and Insulin to C-peptide ratio pre-and post-intervention for males and females, who completed the LIPGENE Human Dietary Intervention Study (n=417),irrespective of diet

	Males			Females		
	Mean	**n**	**SEM**	**Mean**	**n**	**SEM**
Pre S_I (mU/L)-1min-1	2.48 [a]	147	0.13	3.19 [b]	186	0.15
Post S_I (mU/L)-1min-1	2.64	145	0.20	3.06	185	0.13
Pre P3 (mU/L) min^{-2}	1.00568E-05 [a]	147	6.91249E-07	1.42424E-05 [b]	186	9.01236E-07
Post P3 (mU/L) min^{-2}	1.01815E-05 [a]	145	8.28321E-07	1.31889E-05 [b]	185	9.18495E-07
Pre Insulin to C-peptide Ratio	3.75	184	0.12	3.86	224	0.10
Post Insulinto C-peptide Ratio	3.58 [†]	181	0.13	3.27 [†]	223	0.08

[a,b] denotes significance across gender, p<0.05

[†]Significant time*gender interaction, p<0.05

SEM standard error of the mean

S_I, insulin sensitivity

Table 6.3c: DI, fasting glucose and fasting insulin concentrations pre-and post-intervention, in volunteers who completed the LIPGENE Dietary Intervention Study, across three age categories, irrespective of diet

	35-49			50-64			65-70		
	Mean	**n**	**SEM**	**Mean**	**n**	**SEM**	**Mean**	**n**	**SEM**
Pre DI	1184.04 [a]	91	86.35	781.76 [b]	197	46.42	824.74 [b]	45	94.67
Post DI	1257.36 [a]	88	85.38	805.65 [b]	197	52.44	951.37 [b]	45	143.37
Pre Fasting Glucose (mmol/l)	5.64 [a]	106	0.07	6.08 [b]	245	0.06	6.02 [b]	60	0.10
Post Fasting Glucose (mmol/l)	5.66 [a]	103	0.07	6.07 [b]	245	0.06	6.18 [b]	60	0.21
Pre Fasting Insulin(uIU/ml)	11.46 [a]	106	0.57	9.92 [a]	245	0.33	8.08 [b]	60	0.56
Post Fasting Insulin(uIU/ml)	10.66 [a]	103	0.54	9.59 [ab]	245	0.36	8.90 [b]	60	0.88

[abc...] denotes significance across age categories (when two superscripts contain the same letter there is no significant difference between two of the three categories)$p<0.05$, p<0.017 where bonferroni correction applied

SEM standard error of the mean

DI, disposition index

Table 6.3d: Significant between- and within-centre differences in the IVGTT parameters (mean ±SEM) of subjects who completed the LIPGENE Dietary Intervention Study (n=417).

	TCDn=55		UoR n=58		Oslo n=54		INSERM n=38		UM n=49		HURS-UCO n=74		JUMC n=43		UU n=46	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre Fasting Glucose (mmol/l)	5.87 ^{abc}	0.13	5.76 ^{ac}	0.09	6.13 ^{ab}	0.13	5.53 ^{c}	0.11	5.84 ^{ac}	0.08	6.35 ^{b}	0.12	5.86 ^{ac}	0.09	5.97 ^{abc}	0.11
Post Fasting Glucose (mmol/l)	5.93 ^{a}	0.15	5.78 ^{a}	0.10	6.03 ^{ab}	0.14	5.64 ^{a}	0.14	5.86 ^{ab}	0.09	6.40 ^{b}	0.14	5.82 ^{ab}	0.10	6.05 ^{ab}	0.26
Pre Fasting Insulin(uIU/ml)	9.88 ^{ab}	0.87	11.27 ^{a}	0.81	10.42 ^{a}	0.74	8.74 ^{ab}	0.73	9.92 ^{a}	0.62	10.96 ^{a}	0.62	11.13 ^{a}	0.69	6.82 ^{b}	0.52
Post Fasting Insulin(uIU/ml)	9.38 ^{ab}	0.78	11.40 ^{a}	0.95	10.28 ^{a}	0.79	8.36 ^{ab}	0.80	9.66 ^{a}	0.86	10.35 ^{a}	0.70	11.44 ^{a}	0.74	6.11 ^{b}	0.43
Pre QUICKI	0.53 ^{ab}	0.09	0.59 ^{a}	0.01	0.58 ^{a}	0.01	0.63 ^{ab}	0.02	0.59 ^{a}	0.01	0.57 ^{a}	0.01	0.57 ^{a}	0.01	0.79 ^{b}	0.14
Post QUICKI	0.62 ^{ab}	0.02	0.60 ^{a}	0.02	0.59 ^{a}	0.01	0.58 ^{ab}	0.06	0.61 ^{ab}	0.01	0.58 ^{a}	0.01	0.57 ^{a}	0.01	0.70 ^{b}	0.03
Pre C-Peptide ng/ml	2.42 ^{ac}	0.12	2.80 ^{ab}	0.10	3.23 ^{b}	0.13	2.19 ^{c}	0.14	2.77 ^{ab}	0.11	2.53 ^{ac}	0.11	2.75 ^{ab}	0.12	2.16 ^{c}	0.10
Post C-Peptide ng/ml	2.62 ^{ab}	0.11	3.06 ^{a}	0.19	3.11 $^{\dagger a}$	0.15	2.28 ^{b}	0.16	2.77 ^{ab}	0.14	2.80 $^{\dagger a}$	0.09	3.01 $^{\dagger a}$	0.14	2.28 $^{\dagger b}$	0.10
Pre Insulin to C-peptide Ratio	3.91 ^{ac}	0.26	3.89 ^{ac}	0.18	3.12 ^{bc}	0.12	3.92 ^{abc}	0.22	3.51 ^{abc}	0.15	4.58 ^{a}	0.25	4.05 ^{a}	0.19	3.17 ^{c}	0.18
Post Insulinto C-peptide Ratio	3.40 ^{ac}	0.17	3.63 ^{ac}	0.19	3.18 ^{a}	0.13	3.51 ^{ac}	0.21	3.55 ^{abc}	0.35	3.61 ^{ac}	0.18	3.74 ^{c}	0.14	2.60 ^{b}	0.11

Values represent time*centre adjusted means (±SEM)

†Significant time*centre interaction, p<0.05, post- compared to pre-intervention

$^{abc...}$ denotes significance across centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories)

SEM standard error of the mean

Table 6.3e: IVGTT paramters within body weight change categories, in volunteers who completed the LIPGENE Dietary Intervention Study (time*change in body weight)

	Lost 4-8Kg n=14		Lost 2-4Kg n=57		Lost 0-2Kg n=191		Gained 0-2Kg n=129		Gained 2-5Kg n=23	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre Fasting Insulin(uIU/ml)	10.23	1.76	9.25	0.65	10.17	0.38	10.09	0.48	10.56	1.34
Post Fasting Insulin(uIU/ml)	7.06 †	1.21	8.65	0.69	9.93 †	0.48	9.95	0.42	11.38	1.53
Pre QUICKI	0.60	0.02	0.52	0.09	0.62	0.03	0.60	0.01	0.59	0.02
Post QUICKI	0.69 †	0.04	0.63 †	0.01	0.60 †	0.01	0.59	0.01	0.59	0.02
Pre C-Peptide ng/ml	2.70	0.22	2.62	0.11	2.56	0.06	2.69	0.08	2.84	0.20
Post C-Peptide ng/ml	2.31 †	0.28	2.55	0.12	2.75 †	0.08	2.89 †	0.08	3.04 †	0.20
Pre Insulin to C-peptide Ratio	3.63	0.35	3.48	0.17	3.96	0.11	3.77	0.15	3.57	0.25
Post Insulinto C-peptide Ratio	2.91 †	0.22	3.25	0.14	3.48 †	0.13	3.39 †	0.09	3.53	0.28

Values represent time*change in body weight adjusted means (±SEM)

†significant time*change in body weight interaction p<0.0005, post- compared to pre-intervention

SEM standard error of the mean

Table 6.3f: Time*centre*diet group interaction effects for fasting insulin, QUICKI and First phase insulin response in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417)

	Diet A, High SFA Diet															
	TCD		UoR		UO		INSERM		UM		HURS-UCO		JUMC		UU	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Fasting Insulin(uIU/ml)	10.31	1.41	10.76	1.41	12.24	1.59	8.52	1.99	10.30	1.41	9.73	1.32	11.13	1.75	5.31	1.52
Post Fasting Insulin(uIU/ml)	9.37	1.51	9.12	1.51	12.68	1.70	7.63	2.14	11.47	1.51	11.89	1.41	10.83	1.88	6.25	1.63
Pre QUICKI	0.64	0.11	0.60	0.11	0.54	0.12	0.61	0.15	0.60	0.11	0.60	0.10	0.57	0.13	1.20	0.12
Post QUICKI	0.63	0.04	0.61	0.04	0.56	0.04	0.68	0.06	0.60	0.04	0.56	0.04	0.58	0.05	0.69	0.04
Pre First Phase Insulin Response	65.79	11.50	37.47	11.05	34.20	12.02	26.92	15.06	46.26	10.65	35.16	9.96	46.09	13.28	30.49	11.50
Post First Phase Insulin Response	73.48	12.07	33.63	11.60	34.15	12.61	24.57	15.81	45.50	11.18	34.27	10.46	53.11	13.94	35.23	12.07

	Diet B, High MUFA diet															
	TCD		UoR		UO		INSERM		UM		HURS-UCO		JUMC		UU	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Fasting Insulin(uIU/ml)	9.32	1.41	11.03	1.36	8.97	1.36	8.84	1.66	10.44	1.52	11.36	1.28	10.80	1.41	7.37	1.46
Post Fasting Insulin(uIU/ml)	9.53	1.51	10.28	1.46	8.69	1.46	7.42	1.79	9.82	1.63	11.32	1.37	9.74	1.51	5.65 †	1.57
Pre QUICKI	0.62	0.11	0.58	0.10	0.60	0.10	0.65	0.13	0.58	0.12	0.57	0.10	0.58	0.11	0.62	0.11
Post QUICKI	0.62	0.04	0.60	0.04	0.60	0.04	0.51	0.05	0.59	0.04	0.57	0.04	0.58	0.04	0.75 †	0.04
Pre First Phase Insulin Response (uIU/ml)	37.18	10.65	47.36	10.29	40.42	10.65	42.20	13.28	59.85	11.50	32.84	10.29	35.69	11.05	28.84	11.05
Post First Phase Insulin Response (uIU/ml)	41.78	11.18	53.01	10.80	39.36	11.18	31.22	13.94	54.64	12.07	39.47	10.80	39.47	11.60	31.74	11.60

	Diet C, Low fat diet															
	TCD		UR		UO		INSERM		UM		HURS-UCO		JUMC		UU	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Fasting Insulin(uIU/ml)	9.97	1.41	12.47	1.36	11.43	1.36	7.96	1.86	10.70	1.59	9.74	1.18	11.92	1.59	7.00	1.59
Post Fasting Insulin(uIU/ml)	10.97	1.51	13.47	1.46	11.47	1.46	9.36	2.00	9.84	1.70	8.69	1.26	11.13	1.70	6.01	1.70
Pre QUICKI	0.25	0.11	0.57	0.10	0.58	0.10	0.63	0.14	0.56	0.12	0.59	0.09	0.56	0.12	0.64	0.12
Post QUICKI	0.59	0.04	0.58	0.04	0.60	0.04	0.60	0.05	0.60	0.04	0.62	0.03	0.57	0.04	0.70 †	0.04
Pre First Phase Insulin Response (uIU/ml)	56.90	10.65	41.14	11.05	33.07	10.29	34.35	15.06	46.53	12.02	34.99	9.14	32.48	13.28	26.72	12.02
Post First Phase Insulin Response (uIU/ml)	62.53	11.18	46.12	11.60	27.82	10.80	50.02	15.81	39.62	12.61	37.93	9.59	51.43	13.94	29.01	12.61

	Diet D, Low fat diet with 1.24g LC n-3 PUFA															
	TCD		UoR		UO		INSERM		UM		HURS-UCO		JUMC		UU	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre Fasting Insulin(uIU/ml)	9.92	1.46	10.74	1.41	9.38	1.46	9.40	2.15	8.24	1.52	13.18	1.18	10.38	1.86	7.75	1.66
Post Fasting Insulin(uIU/ml)	7.54	1.57	12.67	1.51	8.72	1.57	9.44	2.31	7.23	1.63	9.95 †	1.26	15.52 †	2.00	6.64	1.79
Pre QUICKI	0.61	0.11	0.59	0.11	0.59	0.11	0.60	0.16	0.61	0.12	0.53	0.09	0.59	0.14	0.67	0.13
Post QUICKI	0.66	0.04	0.59	0.04	0.61	0.04	0.59	0.06	0.65	0.04	0.57 †	0.03	0.53 †	0.05	0.66	0.05
Pre First Phase Insulin Response (uIU/ml)	41.07	11.05	51.54	10.65	21.07	11.05	46.82	16.27	52.51	11.50	30.64	8.91	31.90	14.09	46.50	12.60
Post First Phase Insulin Response (uIU/ml)	47.54 ab	11.60	56.18 a	11.18	24.04 b	11.60	39.18 ab	17.07	49.77 ab	12.07	24.52 b	9.35	33.73 ab	14.79	41.66 ab	13.23

Values represent adjusted means (±SEM) for time*centre*diet group

abc denotes significance across centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories in that diet group)$p<0.05$, $p<0.0018$ where bonferroni correction applied

†significant time*centre*diet group interaction $p<0.0005$, post- compared to pre-intervention

SEM standard error of the mean

Inflammatory Cytokines

The analysis for the inflammatory cytokines was carried out by Jolene McMonagle. Only significant time*diet interactions are presented here for unity of the effects of diet on all parameters measured. There were no significant diet*time interactions for any of the inflammatory markers pre- and post-intervention, as shown in *Table 6.4.* However, RM ANOVA revealed a significant time*gender*centre*diet interaction ($p<0.05$) with respect to resistin concentrations. This observation related to the HFMUFA diet (Diet B) for HURS-UCO and UU cohorts whereby resistin concentrations were significantly reduced following the 12-week dietary intervention ($p<0.05$). For the HURS-UCO cohort this reduction was identified in males only (9805.4 mg/L v's 8143.80 mg/L). Within the UU cohort the significant decrease was noted in females only (10350.7 mg/L v's 6838.50 mg/L) (Data not shown).

Coagulation Markers

The effect of dietary fat modification on markers of coagulation is presented in *Table 6.5a.* RM ANOVA analysis demonstrated no significant diet*time interactions for PAI-1, tPA or fibrinogen concentrations. It was evident that change in body weight affected PAI-1 and tPA as shown in *Table 6.5b.* For PAI-1 (ng/ml) a significant time*change in body weight interaction ($p=0.006$) demonstrated a significant increase in subjects who gained more than 2kg, ($p=0.017$). tPA concentrations significantly decreased in those who lost between 0 and 2kg body weight ($p=0.051$).

Between-centre differences also highlighted varying concentrations of coagulation markers between the partners (*Table 6.5c*). A main effect for centre ($p=0.0005$) from a RM ANOVA was observed for PAI-1 concentrations. INSERM had significantly lower concentrations of PAI-1 pre- and post-intervention compared to TCD, UoR, UO, UM, HURS-UCO and JUMC ($p<0.05$). UoR had higher tPA concentrations than TCD, INSERM, UM and UU pre-intervention, ($p<0.05$). UoR also had higher tPA concentrations post-intervention as compared to INSERM and UM, ($p<0.05$). JUMC had higher tPA concentrations post-intervention compared to INSERM and UM also, ($p<0.05$). UO had significantly lower fibrinogen concentrations compared to TCD, UoR, INSERM and JUMC and UM also had lower concentrations than JUMC pre-intervention, ($p<0.05$). At post-intervention UO had lower fibrinogen concentrations than UoR and JUMC, ($p<0.05$).

Significant age and gender differences for the markers of coagulation are presented in *Table 6.5d and 6.5e*, respectively. tPA concentrations increased with increasing age and was significantly higher in the 65-70 age group compared to the 35-49 age group pre- and post-intervention ($p<0.05$). Males had higher tPA concentrations pre- ($p=0.0005$) and post-intervention ($p=0.004$) compared to females. Males had lower fibrinogen concentrations than females pre- ($p=0.0005$) and post-intervention ($p=0.0005$).

Table 6.4: The effects of dietary fat modification on inflammatory markers in subjects who completed the LIPGENE Dietary Intervention Study (n=417)

	High-Fat, SFA-rich Diet Diet A, n=100				High-Fat, MUFA-rich Diet Diet B, n=111				Low-Fat Diet Diet C, n=106				Low-Fat Diet with 1.24g LC n3 PUFA Diet D, n=100			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mesn	SEM	Mean	SEM	Mean	SEM
CRP (mg/L)	5.00	0.42	4.85	0.39	4.82	0.40	4.60	0.37	5.54	0.41	5.27	0.38	5.10	0.42	4.80	0.39
IL-6 (pg/mL)	4.57	0.42	4.56	0.41	5.69	0.40	4.81	0.39	4.76	0.40	4.99	0.41	4.99	0.42	4.53	0.41
TNFα (pg/mL)	5.68	0.49	5.13	0.41	5.44	0.47	4.86	0.39	4.77	0.47	4.23	0.40	4.90	0.49	4.71	0.41
sICAM (ng/mL)	286.27	7.45	284.90	7.53	287.32	7.07	289.20	7.14	287.35	7.20	286.63	7.28	279.34	7.41	280.75	7.49
sVCAM (ng/mL)	601.12	17.06	609.69	18.15	591.18	16.18	608.86	17.22	632.70	16.48	625.60	17.54	583.29	16.97	597.70	18.06
Resistin (mg/L)	10452.40	503.13	10114.70	551.70	10082.77	477.31	9709.70	523.40	10088.80	486.24	10582.88	533.20	9985.42	503.13	9965.88	551.73
Adiponectin (mg/L)	3.76	0.25	3.89	0.25	3.70	0.23	3.66	0.24	3.82	0.24	4.20	0.24	3.45	0.24	3.79	0.25
Leptin (ng/mL)	22.43	2.11	21.87	2.14	21.95	1.98	22.45	2.01	20.04	2.02	18.99	2.05	23.80	2.07	21.96	2.10

Values represent diet*time adjusted means (±SEM)

SEM standard error of the mean

Table 6.5a: The effect of dietary fat modification on markers of coagulation in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417).

	High-Fat SFA-rich Diet				High-Fat MUFA-rich Diet				Low-Fat Diet				Low-Fat Diet with 1.24g LC n3 PUFA			
	Diet A n=100				Diet B n=110				Diet C n=106				Diet D n=100			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
PAI-1 ng/ml	53.47	3.68	54.17	3.58	51.75	3.49	51.38	3.40	58.68	3.55	55.34	3.46	51.07	3.68	58.17	3.58
tPA ng/ml	8.86	0.41	8.63	0.42	8.30	0.39	8.44	0.40	8.64	0.40	8.02	0.41	8.77	0.41	8.51	0.42
Fibrinogen mg/dl	335.13	8.54	322.60	7.43	327.25	8.14	312.95	7.08	327.81	8.26	328.02	7.18	325.85	8.54	317.94	7.43

Values represent adjusted means (±SEM) for diet*time interactions

SEM standard error of the mean

Table 6.5b: PAI-1 and tPA concentrations across body weight change categories, in sujects who completed the LIPGENE Dietary Intervention Study, irrespective of dietary treatment

	Lost 4-8Kg n=14		Lost 2-4Kg n=57		Lost 0-2Kg n=191		Gained 0-2Kg n=127		Gained 2-5Kg n=23	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre PAI-1 ng/ml	35.57	7.70	60.28	5.46	55.35	2.71	50.26	2.85	55.55	8.30
Post PAI-1ng/ml	34.16	8.80	54.09	5.18	54.33	2.52	56.97	3.05	57.76 †	7.82
Pre tPA ng/ml	7.42	0.48	8.81	0.47	8.46	0.32	8.93	0.36	8.64	0.88
Post tPA ng/ml	6.34	0.82	8.23	0.45	8.11 †	0.32	9.06	0.36	8.76	0.96

Values represent adjusted means (±SEM) for time*change in body weight interactions

†significant time*change in body weight interaction p<0.0005, post- compared to pre-intervention in that weight change category

SEM: standard error of the mean

Table 6.5c: Between-centre differences for markers of coagulation (mean ±SEM), in subjects who completed the LIPGENE Dietary Intervention Study, irrespective of dietary treatment

	TCD n=55		UoR n=58		UO n=54		INSERM n=38		UM n=49		HURS-UCO n=74		JUMC n=43		UU n=46	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre PAI-1ng/ml	60.89 ^{ac}	5.06	53.76 ^{ac}	4.96	53.00 ^{ac}	5.52	25.00 ^{b}	2.82	73.80 ^{a}	5.17	49.19 ^{c}	3.77	67.06 ^{ac}	4.48	44.66 ^{bc}	5.46
Post PAI-1ng/ml	60.23 ^{acd}	4.44	51.03 ^{ad}	4.71	53.23 ^{b}	6.74	27.95 ^{ac}	3.80	68.93 ^{ad}	4.46	51.14 ^{ad}	3.41	77.84 ^{c}	4.02	45.81 ^{bd}	4.28
Pre tPA ng/ml	7.95 ^{a}	0.79	10.65 ^{b}	0.50	8.89 ^{ab}	0.50	7.08 ^{a}	0.48	8.06 ^{a}	0.52	8.92 ^{ab}	0.45	9.38 ^{ab}	0.48	7.46 ^{a}	0.54
Post tPA ng/ml	8.49 ^{ab}	0.75	9.35 ^{a}	0.48	8.59 ^{ab}	0.53	6.73 ^{b}	0.56	7.30 ^{b}	0.55	8.64 ^{ab}	0.46	9.85 ^{a}	0.61	7.74 ^{ab}	0.58
Pre Fibrinogen mg/dl	346.37 ^{ac}	13.59	355.01 ^{ac}	12.16	288.74 ^{b}	7.80	337.14 ^{ac}	10.42	314.26 ^{ab}	11.12	310.30 ^{abc}	10.97	360.62 ^{c}	10.73	332.83 ^{abc}	11.94
Post Fibrinogen mg/dl	318.74 ^{ab}	11.53	344.44 ^{a}	9.46	290.49 ^{b}	7.40	323.07 ^{ab}	11.17	313.23 ^{ab}	10.36	311.94 ^{ab}	9.13	354.74 ^{a}	11.13	317.18 ^{ab}	9.24

$^{abc...}$ denotes significance across centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories) $p<0.05$ or $p<0.0017$ where bonferroni correction applied

SEM: standard error of the mean

Table 6.5d: Between-age category differences for tPA concentrations pre-and post-intervention across age categories, irrespective of dietary treatment

	35-49 n=107		50-64 n=249		65-70 n=61	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre tPA ng/ml	7.87 [a]	0.34	8.66 [a]	0.28	9.85 [b]	0.46
Post tPA ng/ml	7.55 [a]	0.32	8.61 [ab]	0.29	9.08 [b]	0.48

[abc...] denotes significance across the age categories (when two superscripts contain the same letter there is no significant difference between two of the three categories) $p<0.05$, $p<0.017$ where bonferroni correction applied

Table 6.5e: Between-gender differences for tPA and fibrinogen concentrations pre-and post-intervention, irrespective of dietary treatment

	Males n=185		Females n=232	
	Mean	**SEM**	**Mean**	**SEM**
Pre tPA ng/ml	9.70 [a]	0.35	7.79 [b]	0.21
Post tPA ng/ml	9.15 [a]	0.34	7.81 [b]	0.24
Pre Fibrinogen mg/dl	310.72 [a]	5.89	343.59 [b]	5.68
Post Fibrinogen mg/dl	303.51 [a]	5.02	334.47 [b]	4.94

[a,b] denotes significance across genders, $p<0.05$

Lipids and Apolipoproteins

The effects of dietary fat modification on lipid and apolipoprotein metabolism pre- to post-intervention are presented in *Table 6.6a*. RM ANOVA demonstrated several significant diet*time interactions. Plasma TAG concentrations showed a significant diet*time interaction (p=0.006), whereby plasma TAG concentrations were significantly reduced following the HFSFA diet and the LFn3PUFA diet (p=0.018 and p=0.005, respectively). Interestingly a significant time*gender*diet interaction was observed (p=0.017), wherein plasma TAG concentrations are significantly reduced in men only (p=0.001) with TAG concentrations significantly reduced following the HFSFA diet (Diet A) and the LFn3PUFA diet (Diet D) (p=0.039 and p=0.002, respectively). The time*gender*diet interactions are demonstrated in *Table 6.6b*. It is also noteworthy that plasma TAG concentrations showed a significant time*change in body weight interaction (p=0.011) whereby plasma TAG was significantly reduced over time in subjects who lost 0-2kg (1.76mmol/L pre- to 1.70mmol/L post-intervention, p=0.031). Also, plasma NEFA concentrations showed a diet*time interaction which approached significance (p=0.061) and post-hoc analysis showed that there was a significant reduction in plasma NEFA concentrations following the LC n3 PUFA diet (Diet D) post intervention (p=0.006). Plasma total HDL-cholesterol concentrations showed a significant diet*time interaction (p=0.005), whereby HDL-cholesterol levels were significantly increased by both high-fat diets, HFSFA and HFMUFA post-intervention (p<0.0005 and p=0.006, respectively). TRL TAG concentrations also showed a significant diet*time interaction (p=0.049), this was explained by a significant reduction in TRL TAG levels following the LF n3 PUFA diet (Diet D) only (p=0.032). Interestingly TRL TAG concentrations also showed a significant time*gender*diet interaction (p=0.033), whereby TRL TAG levels showed a significant time*diet interaction (p=0.002) in men only, wherein TRL TAG concentrations were significantly reduced by the HFSFA diet (Diet A) and the LFn3PUFA diet (Diet D) in men only (p=0.013 and p=0.008). TRL cholesterol concentrations showed a significant time*gender*diet interaction (p=0.026). Again it was evident that TRL cholesterol concentrations showed a significant time*diet interaction (p=0.002) for men only, wherein TRL cholesterol concentrations were significantly reduced by HFSFA diet (Diet A) and the LFn3PUFA diet (Diet D) in men only (p=0.024 and p=0.036). Plasma cholesterol concentrations were not altered specifically by one diet but showed a significant time*change in body weight

interaction, with reduced levels in subjects who lost 4-8kg (5.72mmol/L pre- to 5.58mmol/L post-intervention, p=0.024) and in those who lost 0-2kg, (5.34mmol/L pre- to 5.23mmol/L post-intervention, p=0.005, respectively).

Neither plasma apoAI nor apoB were significantly altered by dietary fat modification, as demonstrated by a non significant time*diet interaction. Nevertheless plasma apoAI levels showed a significant time*change in body weight interaction (p=0.039), wherein levels were significantly reduced in subjects who lost 0-2kg (1.39g/L pre- to 1.38g/L post-intervention, p=0.009). Plasma apoCIII demonstrated a significant time*diet interaction (p=0.034), whereby levels were significantly reduced in the HFSFA diet group (Diet A) and the LFn3PUFA diet group (Diet D) (p=0.014 and p==0.008, respectively). Interestingly apoCIII concentrations also showed a significant time*change in body weight interaction (p=0.001), with a significant reduction in apoCIII post-intervention in subjects who lost 4-8kg (48.21mg/L pre- to 46.35mg/L post-intervention, p=0.029), whereas apoCIII was significantly increased post-intervention in subjects who lost 0-2kg (45.44mg/L pre- to 46.45mg/L post-intervention, p=0.010).

Table 6.6a: The effect of dietary fat modification on plasma lipid and apolipoprotein concentrations in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417).

	High-Fat SFA-rich Diet Diet A n=100				High-Fat MUFA-rich Diet Diet B n=111				Low-Fat Diet Diet C n=106				Low-Fat Diet with 1.24g LC n3 PUFA Diet D n=100			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cholesterol (mmol/L)	5.32	0.10	5.26	0.10	5.15	0.09	5.04	0.10	5.22	0.10	5.12	0.10	5.38	0.11	5.16	0.11
NEFA (µmol/L)	595.21	24.99	616.83	25.18	604.67	23.95	627.16	24.14	622.32	25.27	617.74	25.46	661.51	27.88	597.46†	28.09
LDL (mmol/L)	3.17	0.12	3.09	0.12	3.04	0.10	2.97	0.11	3.17	0.11	3.09	0.11	3.31	0.12	3.09	0.12
Apo A1 (g/L)	1.38	0.02	1.38	0.02	1.36	0.02	1.38	0.02	1.38	0.02	1.36	0.02	1.39	0.02	1.35	0.02
Apo B (g/L)	1.04	0.02	1.03	0.02	0.99	0.02	0.99	0.02	0.99	0.02	0.99	0.02	1.04	0.03	1.01	0.03
Apo CIII (mg/L)	162.53	5.59	151.17†	5.29	156.39	5.37	150.93	5.08	148.51	5.69	151.83	5.39	156.12	6.19	151.17†	5.86
TAG (mmol/L)	1.99	0.10	1.70†	0.09	1.78	0.10	1.68	0.08	1.67	0.10	1.78	0.09	1.68	0.11	1.57†	0.10
TRL TAG (mmol/L)	0.96	0.06	0.84	0.06	0.85	0.06	0.85	0.05	0.80	0.06	0.92	0.06	0.86	0.07	0.78†	0.06
TRL Cholesterol (mmol/L)	0.43	0.03	0.40	0.03	0.38	0.03	0.38	0.03	0.37	0.03	0.44	0.04	0.38	0.04	0.36	0.04
Apo B48 (mg/L)	1.00	0.09	0.76	0.09	0.62	0.09	0.73	0.09	0.81	0.09	0.66	0.09	0.66	0.11	0.65	0.11
TRL Apo B (mg/L)	52.22	4.35	56.85	4.73	52.57	4.12	53.66	4.47	49.43	4.38	52.63	4.75	50.53	4.70	54.72	5.10
Apo CII (mg/L)	46.64	1.95	46.16	2.00	44.40	1.87	43.47	1.92	43.51	1.99	47.18	2.05	43.73	2.16	43.71	2.22
HDL (mmol/L)	1.05	0.03	1.11†	0.03	1.05	0.03	1.10†	0.03	1.09	0.03	1.08	0.03	1.11	0.03	1.12	0.03
Apo E (mg/L)	43.83	1.84	40.56	1.87	39.61	1.76	39.39	1.79	41.40	1.86	42.13	1.89	39.57	2.03	38.56	2.06

Values represent adjusted means and standard error of the means (SEMs) for diet*time interactions

† significant time*diet interaction $p<0.0005$, post- compared to pre-intervention

NEFA, non esterified fatty acids; LDL, low density lipoproteins; Apo, Apolipoprotein; TAG, triglyceride; HDL, high density lipoprotein

Table 6.6b: The effect of dietary fat modification on plasma TAG, TRL TAG and TRL cholesterol concentrations in males and females who completed the LIPGENE Human Dietary Intervention Study (diet*gender*time interactions)

	High-Fat SFA-rich Diet Diet A n=45				High-Fat MUFA-rich Diet Diet B n=46				Low-Fat Diet Diet C n=47				Low-Fat Diet with 1.24g LC n3 PUFA Diet D n=45			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
MALE	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAG (mmol/L)	2.094	0.164	1.743†	0.13	1.752	0.171	1.596	0.135	1.723	0.175	1.986	0.138	1.678	0.199	1.486†	0.157
TRL TAG (mmol/L)	1.132	0.103	0.893†	0.083	0.854	0.107	0.828	0.086	0.858	0.108	1.067	0.088	0.897	0.12	0.78†	0.097
TRL Cholesterol (mmol/L)	0.491	0.05	0.42†	0.053	0.376	0.052	0.362	0.055	0.383	0.053	0.526	0.056	0.375	0.058	0.342†	0.062
	Diet A n=53				Diet B n=65				Diet C n=59				Diet D n=54			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
FEMALE	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAG (mmol/L)	1.89	0.13	1.68	0.12	1.82	0.11	1.76	0.10	1.63	0.12	1.57	0.11	1.68	0.12	1.65	0.12
TRL TAG (mmol/L)	0.81	0.08	0.81	0.08	0.86	0.07	0.87	0.07	0.74	0.08	0.78	0.07	0.81	0.08	0.78	0.08
TRL Cholesterol (mmol/L)	0.38	0.05	0.40	0.05	0.39	0.04	0.41	0.04	0.36	0.04	0.36	0.04	0.39	0.05	0.37	0.05

Values represent adjusted means and standard error of the means (SEMs) for diet*time*gender interactions

† significant time*diet interaction $p<0.0005$, post- compared to pre-intervention

NEFA, non esterified fatty acids; LDL, low density lipoproteins; Apo, Apolipoprotein; TAG, triglyceride; HDL, high density lipoprotein

Plasma Fatty Acids

Mean (±SEM) plasma fatty acid composition (w/w %) in the four diet groups, pre- and post-intervention are presented in *Table 6.7a*. There were no significant differences in the composition of the plasma fatty acids between subjects assigned to the four dietary treatments pre-intervention.

Importantly, a significant diet*time interaction demonstrated that plasma oleic acid levels (C18:1) were significantly reduced (p=0.0005) by the HFSFA diet (Diet A) and the LF n-3 PUFA diet (Diet D) (p=0.004). In contrast, plasma oleic acid levels (C18:1) increased significantly (p=0.033) in subjects adhering to the HFMUFA diet (Diet B). The effect of the four dietary treatments on the change in plasma oleic acid levels demonstrated that the HFMUFA diet (Diet B) significantly increased in plasma oleic acid (C18:1) levels compared to the HFSFA (Diet A) and the LFn3PUFA (Diet D) diets (*Figure 6.2*).

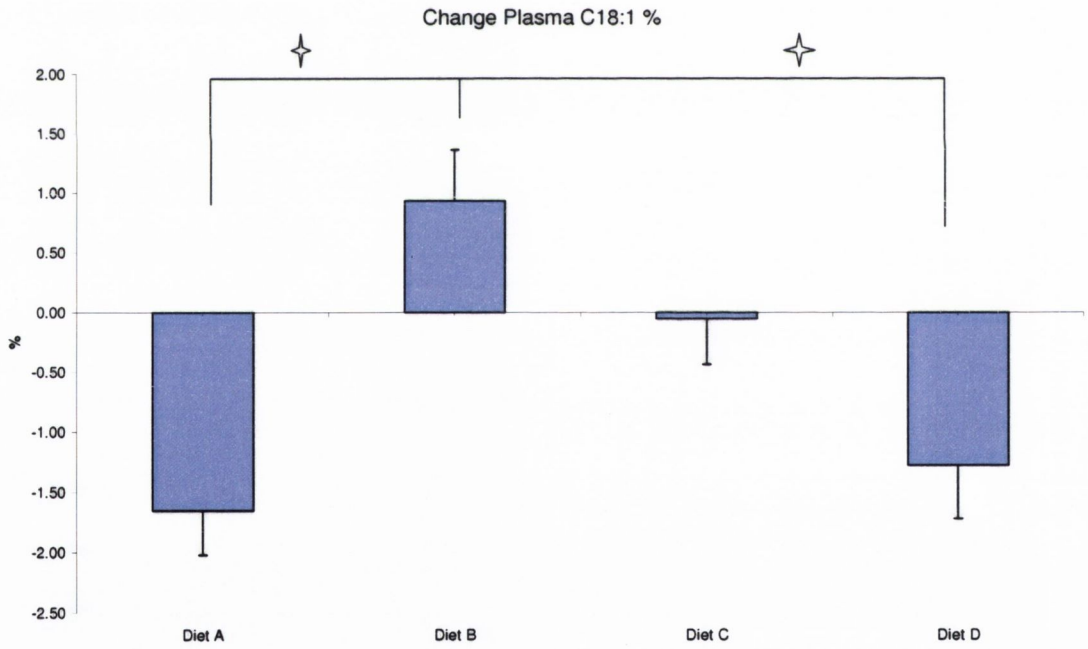


Figure 6.2: Change of Plasma C18:1 from pre- to post-intervention in each of the four diet groups. ✧p<0.05

Total plasma MUFA levels (C16:1+C18:1+C20:1) were significantly reduced following the HFSFA diet (Diet A) and the LF n3 PUFA diet (Diet D), (p=0.0005). It is important to note that total MUFA levels were not significantly different pre-intervention. However, post-intervention the HFMUFA diet (Diet B) was associated with significantly higher MUFA levels as compared to the HFSFA and LFn3PUFA diets. Also when total plasma MUFA concentrations were expressed as the change between pre- and post-intervention, HFMUFA diet had significantly greater MUFA levels compared to the HFSFA and LFn3PUFA diets (Diets A and D) (*Figure 6.3*).

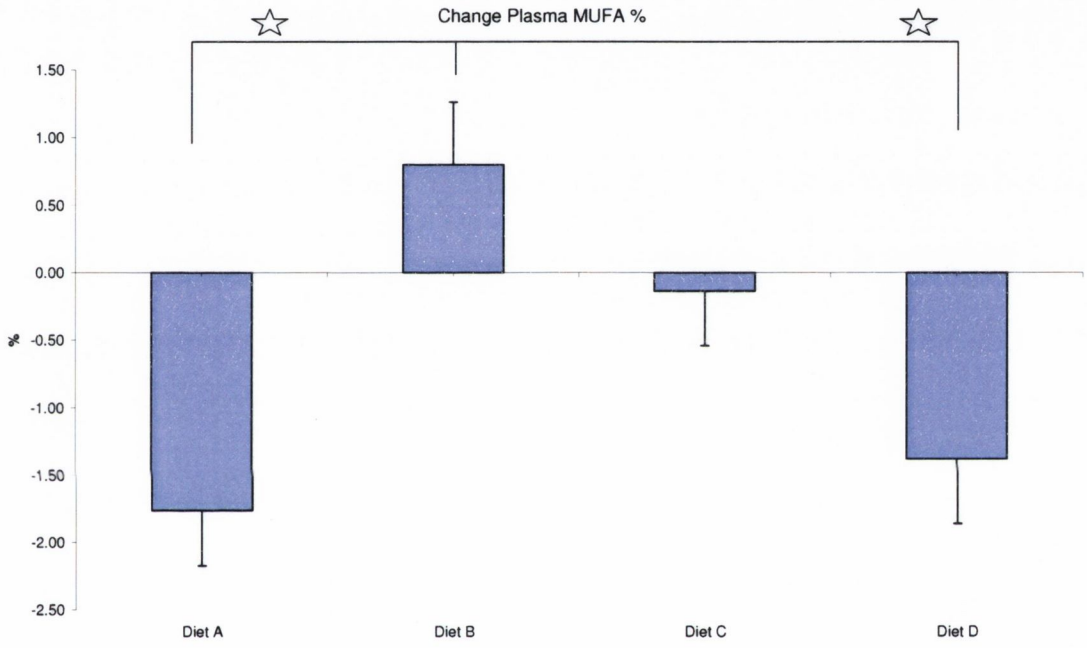


Figure 6.3: Change of Plasma MUFA from pre- to post-intervention in each of the four diet groups.✧ p<0.05

Total plasma arachidonic acid (C20:4:6) levels were significantly increased following the HFSFA and LF diets compared to the LF n3 PUFA diet (Diet D). Total plasma EPA and DHA levels were used as a biomarker of compliance to the n-3 PUFA supplement. Importantly the LF n3 PUFA diet showed a quantitatively important and significant increase in EPA (C20:5:3) (p=0.0005). Furthermore, EPA levels were significantly higher following the LFn3PUFA compared to the three other diet groups, (p<0.05) post-intervention. Plasma EPA (C20:5:3) levels increased in the HFSFA diet (Diet A) and whilst this change was subtle, it did reach statistical significance (p=0.001). The change in EPA concentrations also confirmed that the LFn3PUFA diet was associated with a significant increase in total plasma EPA levels compared to the other three diets. (*Figure 6.4*)

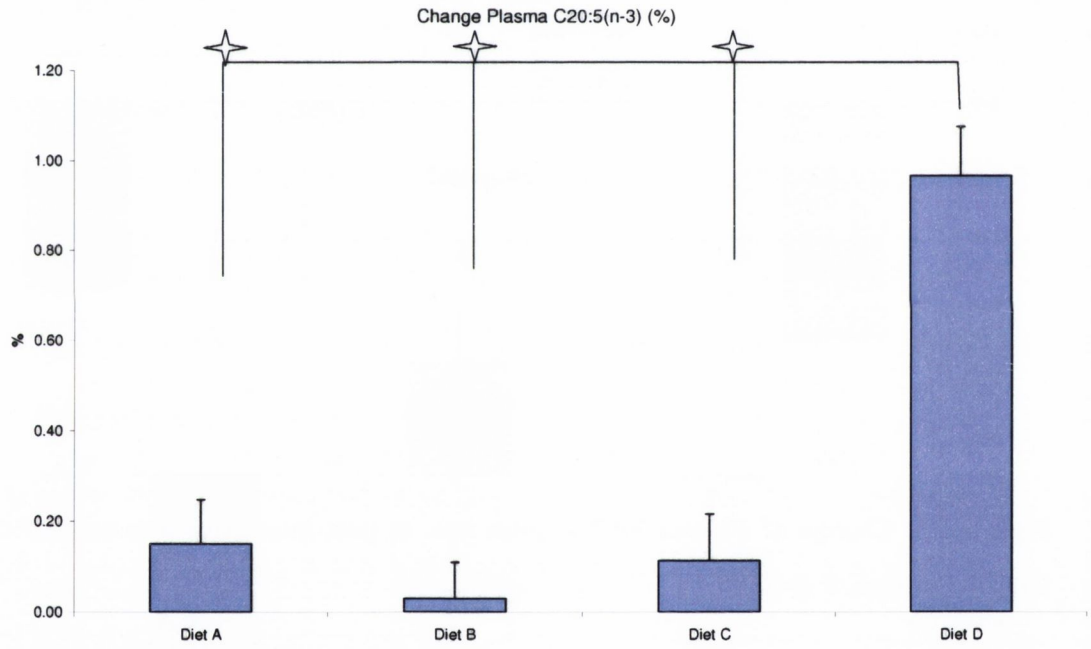


Figure 6.4: Change of Plasma C20:5:3 from pre- to post-intervention in each of the four diet groups. ✦ p<0.05

A significant time effect ($p<0.05$) demonstrated an increase in plasma DHA (C22:6:3) levels from 2.17% to 2.70% over the 12 week intervention period. Similarly to EPA, plasma DHA levels significantly increased in both Low-Fat diet groups with the greatest increase in the LFn3 PUFA diet (Diet D) ($p=0.0005$). This diet group had significantly greater percentages of plasma DHA (C22:6:3) than the HFSFA (Diet A) and the HFMUFA diets (Diet B) post-intervention ($p<0.05$). *Figure 6.5* highlights the change of plasma DHA (C22:6:3) in the four dietary groups.

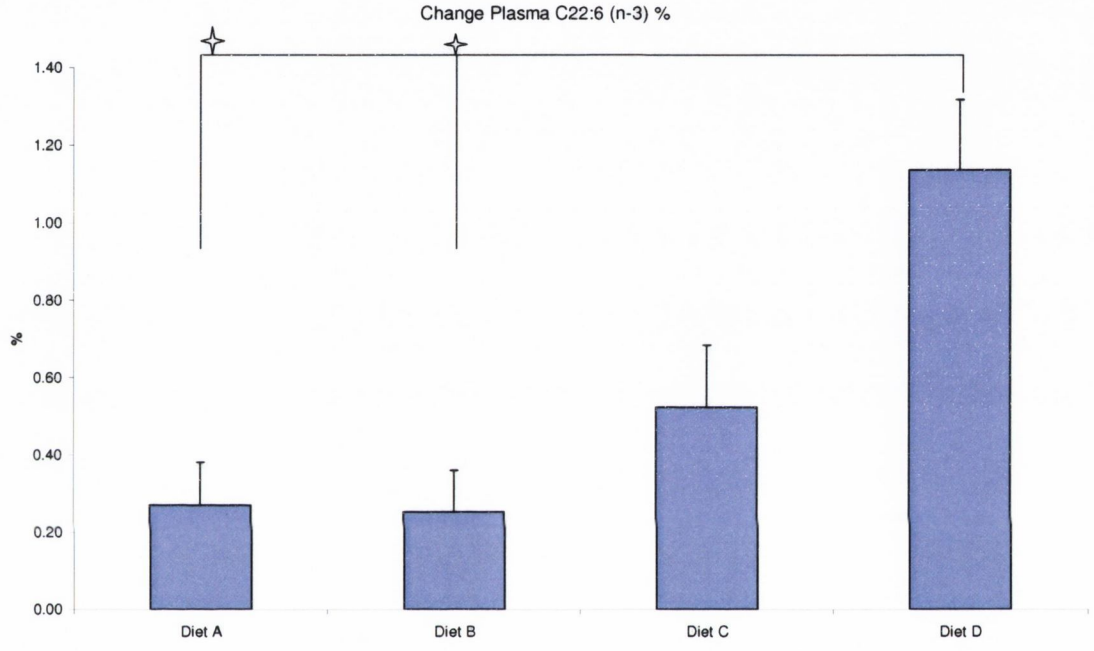


Figure 6.5: Change of Plasma C22:6:3 from pre- to post-intervention in each of the four diet groups. ✦ $p<0.05$

A time*diet effect showed increases in plasma n-3 PUFA (C18:3:3+C18:4+C20:4:3+C20:5+C22:5+C22:6) concentrations in all four diets group. However plasma concentrations of n-3 PUFA were greatly increased in the LFn3PUFA diet, (p=0.0005). Pre-intervention n-3 PUFA levels were not significantly different across the four diet groups, however, the LFn3PUFA diet had significantly greater amounts of total plasma n-3 PUFA levels as compared to the other three diet groups, post-intervention (*Figure 6.6*). A significant time effect (p=0.0005) demonstrated an increase in plasma n-3 PUFA from 3.94% to 4.99% over the intervention period (p=0.0005).

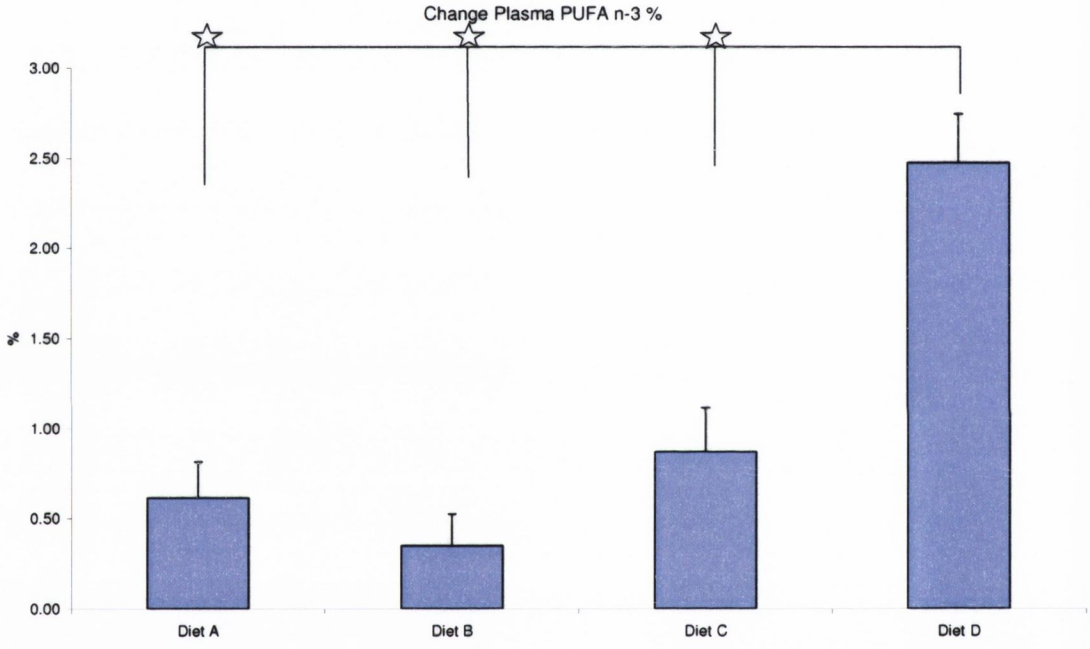


Figure 6.6: Change of Total plasma n-3 PUFA from pre- to post-intervention in each of the four diet groups. ✦p<0.05

A significant time*change in body weight interaction effect was demonstrated for plasma myristic acid (C14:0) which was the only plasma fatty acid significantly affected by a change in body weight (p=0.0006) (*Table 6.7b*). The percentage of plasma myristic acid decreased in those that lost the most weight over the intervention period i.e. between 4 and 8kg, decreasing from 1.66% to 1.27% (p=0.031). In subjects who lost or gained between 0 and 2 kg, plasma myristic acid (C14:0) increased from 1.87% to 2.74% and 1.98% to 2.76% respectively (p=0.0005).

Table 6.7c presents between and within-centre differences in plasma fatty acids (%), pre- and post-intervention. A time*centre effect highlighted an increase in plasma myristic acid (C14:0) post-intervention in TCD (p=0.0005), UoR (p=0.001), UM (p=0.0005), HURS-UCO (p=0.0005) and UU cohorts (p=0.0005). There were many between centre differences pre- and post-intervention. UoR volunteers had greater amounts of plasma myristic acid (C14:0) pre- and post-intervention as compared to UO, INSERM, HURS-UCO and JUMC and with the UU cohort pre-intervention only (p<0.05).

The percentage of plasma palmitic acid (C16:0) decreased over time from 26.48% to 24.81% (p=0.0005). The concentrations also decreased significantly in UoR (p=0.0005), UO (p=0.03) and UM (p=0.0005) cohorts. A one-way ANOVA highlighted differences between the centres pre-intervention. UM had greater concentrations of plasma palmitic acid (C16:0) pre-intervention compared to UO, HURS-UCO, JUMC and UU (26.40%, 24.38%, 23.39%, 24.63%, respectively, p<0.05). TCD had greater concentrations of plasma palmitic acid (C16:0) post-intervention (26.75%) compared to UoR and INSERM (23.71%, 23.96%, respectively, p<0.05).

The percentage of plasma palmitoleic acid (C16:1) decreased significantly in volunteers in the HURS-UCO (0.73pre, 0.66%post, p=0.022) and UU cohorts (1.86%pre, 1.38%post, p=0.0005). Again centre differences were seen for this plasma fatty acid pre- and post-intervention. UoR had greater concentrations pre- (2.11%) and post- (2.30%) intervention and were significantly higher compared to UO, UM and HURS-UCO (p<0.05). The lowest concentrations were seen in HURS-UCO and this was significantly lower than all other centres with the exception of UM (p<0.05).
A time effect was also noted for stearic acid (C18:0). When investigated, it was found that stearic acid (C18:0) decreased over time from 4.1% to 3.7%, p=0.0005. A time*centre effect (p=0.0005) showed that a decrease in plasma stearic acid (C18:0) concentrations occurred in the cohorts of UoR (p=0.016), UO (p=0.0005), INSERM (p=0.0005), UM (p=0.0005) and JUMC (p=0.027).

A time*centre interaction effect highlighted a significant decrease post-intervention in plasma oleic acid (C18:1) in the cohorts of TCD (p=0.003), UoR (p=0.039), HURS-UCO (p=0.0005) and UU (p=0.0005). In contrast, a significant increase in oleic acid (C18:1) concentrations was observed in the UM cohort (p=0.0005). Pre-intervention, plasma oleic acid (C18:1) concentrations were

significantly greater in the UU cohort (29.61%) compared to TCD, UoR, UO and UM cohorts (24.47%, 26.47%, 23.87% and 23.04%, respectively, $p<0.05$). Post-intervention, INSERM had significantly greater concentrations of plasma oleic acid (C18:1) (28.26%) compared to TCD, UoR, UO and UM (23.04%, 25.35%, 24.64% and 25.33%, respectively, $p<0.05$).

Plasma concentrations of linoleic acid (C18:2:6) significantly increased over the intervention study period in the UM cohort ($p=0.009$) and decreased in the HURS-UCO centre ($p=0.005$). UO had the highest plasma linoleic acid (C18:2:6) concentrations pre-intervention (29.56%) and this was significantly higher than in UoR (25.44%) and JUMC cohorts (26.11%), ($p<0.05$). Post-intervention, UM had the highest concentrations (30.16%) compared to UoR (26.37%) and JUMC (26.04%), ($p<0.05$).

Plasma concentrations of arachidonic acid (C20:4:6) increased significantly post-intervention in UoR ($p=0.0005$), UO ($p=0.041$) and UU ($p=0.0005$) cohorts. JUMC had significantly higher levels of plasma arachidonic acid (C20:4:6) (%) pre- and post-intervention (8.28%, 8.08%, respectively) compared to UO (6.47%, 6.87% respectively) and UU (5.70%, 6.14%, respectively) and UoR pre-intervention only (5.38%).

A significant time effect demonstrated a significant increase in plasma concentrations of EPA (C20:5:3) over the intervention period (0.94% to 1.24%. $p=0.0005$). Concentrations of EPA (C20:5:3) also increased in 5 of the 8 participating centres, TCD ($p=0.021$), UoR ($p=0.0005$), INSERM ($p=0.002$), UM ($p=0.009$) and HURS-UCO ($p=0.0005$) and decreased in JUMC ($p=0.021$). Pre-intervention, volunteers in the UO cohort had significantly greater amounts of plasma EPA (C20:5:3) (1.62%) compared to TCD, UoR, INSERM, UM and HURS-UCO (0.67%, 0.70%, 0.62%, 0.74% and 0.47%, respectively, $p<0.05$). HURS-UCO had significantly lower concentrations as compared to all other centres. Post-intervention, INSERM had lower plasma concentrations of EPA (C20:5:3) (0.85%) compared to UO and UU (1.68% and 1.85%, respectively, $p<0.05$). UU had the highest concentrations of plasma EPA (C20:5:3) post-intervention (1.85%) compared to TCD, INSERM, UM and HURS-UCO (1.02%, 0.85%, 1.05% and 0.98%, respectively, $p<0.05$).

Plasma concentrations of DHA (C22:6:3) significantly increased in all centres post-intervention with the exception of TCD. From the RM ANOVA model, a

significant centre effect established differences between the centres pre- and post-intervention for plasma concentrations of DHA (C22:6:3). UO had the highest concentrations of plasma DHA (C22:6:3) pre- (3.09%) and post- (4.13%) intervention and significantly greater compared to TCD, UoR, INSERM, UM and JUMC and UU, post-intervention only ($p<0.05$).

Total plasma saturated fat (SFA: C14:0, C16:0, C18:0) concentrations decreased over the 12-week intervention period from 32.48% at baseline to 31.10% post-intervention ($p=0.0005$). In the UoR, UO and UM centres, plasma SFA decreased also post-intervention ($p=0.0005$). In HURS-UCO and UU cohorts an increase was seen ($p<0.03$). Many centres differed to each other with respect to plasma concentrations of SFA at baseline, however only one centre difference was observed at post-intervention wherein TCD had significantly greater amounts of plasma SFA compared to INSERM ($p<0.05$).

A time*centre interaction effect demonstrated significantly lower concentrations of total plasma MUFA (C16:1, C18:1, C20:1) post-intervention in the following cohorts: TCD, HURS-UCO and UU ($p=0.0005$). In contrast UM had significantly greater plasma levels ($p=0.003$). A significant centre effect ($p=0.0005$) showed volunteers in the UU cohorts with significantly greater concentrations of plasma MUFA pre-intervention as compared to TCD, UoR, UO, UM and JUMC ($p<0.05$). INSERM had significantly greater concentrations of plasma MUFA post-intervention compared to TCD, UO and UM ($p<0.05$).

Total plasma PUFA (n-3, n-6) concentrations increased over the 12-week intervention from 39.42% at baseline to 41.37% at the end of the study period ($p=0.0005$). An increase was also seen in TCD ($p=0.038$), UoR ($p=0.0005$), UM ($p=0.003$) and UU ($p=0.003$). The UO cohort had significantly greater amounts of plasma PUFA concentrations pre- and post- intervention compared to TCD, and UoR cohorts at pre-intervention and JUMC and UU cohorts post-intervention ($p<0.05$).

A time*centre within-subject effect ($p=0.0005$) highlighted an increase in plasma n-6 PUFA (C18:2, C18:3:6, C20:3, C20:4:6, C22:4) concentrations in UoR ($p=0.0005$), UM ($p=0.008$) and UU ($p=0.035$) and a decrease in HURS-UCO ($p=0.003$) cohorts. A between-subject effect for centre demonstrated differences between UoR and UO, INSERM and HURS-UCO, pre-intervention. UM had greater plasma n-6 PUFA concentrations post-intervention compared to UU (34.71%, $p<0.05$).

A time*centre within-subject effect (p=0.0005), illustrated that TCD, UoR, UO, INSERM, UM and HURS-UCO had significantly greater concentrations of plasma n-3 PUFA (C18:3:3, C18:4, C20:4:4, C20:5, C22:5. C22:6) post-intervention compared to baseline levels. In contrast, however, JUMC had significantly lesser amounts. The plasma concentrations of n-3 PUFA in UU also increased but this did not reach the level of significance. UO, JUMC and UU cohorts had the greatest amounts of plasma n-3 PUFA concentrations pre-intervention compared to all other centres. UO had the greatest plasma n-3 PUFA concentrations post-intervention compared to TCD, INSERM, UM and JUMC (p<0.05).

Table 6.7d highlights the difference in plasma palmitoleic acid (C16:1) pre- and post-intervention between males and females. Females had higher amounts of palmitoleic acid (C16:1) in their plasma pre- (1.54%) and post- (1.45%) intervention as compared to their male counterparts (p=0.0005).

There was a significant difference between the 35-49 age group as compared to the 50-64 and the 65-70 age groups pre- and post-intervention wherein plasma EPA concentrations increased with increasing age (*Table 6.7e*).

Plasma concentrations of DHA increased in the 35-50 (p=0.007) and the 50-64 (p=0.0005) age groups over the study period. The 50-64 age group had significantly greater concentrations compared to the 35-49 age groups post-intervention ($p<0.05$). A time*age effect (p=0.009), showed an increase at post-intervention in all age categories (p<0.03) for n-3 PUFA. At both time-points the concentration of plasma n-3 PUFA in the 35-49 age group were significantly lower than the 65-70 age group.

Table 6.7a: The effects of dietary fat modification on plasma fatty acids (%) in subjects who completed the LIPGENE Human Dietary Intervention Study (n=417)

	HighFat SFA-rich Diet				High-Fat MUFA-rich Diet				Low-Fat Diet				Low-Fat Diet with 1.24g LC n3 PUFA			
	Diet A				DietB				DietC				DietD			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
Total Composition (w/w%)	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
C14:0	1.90	0.10	2.66	0.14	1.85	0.10	2.56	0.14	1.90	0.10	2.53	0.14	1.97	0.10	2.62	0.14
C16:0	26.66	0.50	25.31	0.42	26.14	0.48	24.57	0.40	27.03	0.48	25.00	0.40	26.19	0.50	24.51	0.42
C16:1	1.29	0.10	1.26	0.11	1.50	0.10	1.37	0.10	1.29	0.10	1.25	0.10	1.45	0.10	1.34	0.11
C18:0	4.21	0.21	3.92	0.19	4.17	0.20	3.81	0.18	4.08	0.20	3.51	0.18	4.00	0.21	3.67	0.19
C18:1	26.81	1.45	25.16 †	0.42	26.25	0.43	27.19 †	0.40	26.06	0.43	26.01	0.41	26.92	0.45	25.64 †	0.43
C18:2:6	27.51	0.55	28.47	0.54	27.64	0.53	27.31	0.52	27.58	0.53	27.95	0.52	27.33	0.56	27.59	0.55
C20:4:6	6.66	0.24	7.52	0.20	7.07	0.23	7.37	0.20	6.63	0.23	7.36	0.20	6.95	0.24	6.91	0.21
C20:5:3	0.82	0.09	0.97 a†	0.09	0.98	0.09	1.01 a	0.09	1.01	0.09	1.13 a†	0.09	0.98	0.09	1.95 b†	0.09
C22:6:3	2.04	0.12	2.31 a	0.16	2.20	0.12	2.45 a	0.16	2.34	0.12	2.86 ab†	0.16	2.12	0.12	3.25 b†	0.17
SFA	32.77	0.65	31.89	0.50	32.16	0.62	30.94	0.48	33.01	0.62	31.04	0.48	32.16	0.65	30.80	0.51
MUFA	28.32	0.48	26.56 a†	0.45	27.94	0.46	28.74 b	0.43	27.57	0.47	27.44 ab	0.44	28.59	0.49	27.20 a†	0.46
PUFA	38.92	0.70	41.55	0.56	39.90	0.67	40.32	0.53	39.42	0.67	41.52	0.53	39.26	0.71	41.99	0.56
n-6 PUFA	35.27	0.64	37.29	0.55	35.88	0.61	35.95	0.53	35.23	0.62	36.46	0.53	35.37	0.65	35.63	0.56
n-3 PUFA	3.65	0.20	4.27 a†	0.22	4.03	0.19	4.38 ab†	0.21	4.19	0.19	5.06 b†	0.21	3.89	0.20	6.37 c†	0.22

Values represent adjusted means and SEMs for diet*time

†significant time*diet interaction p<0.0005, post- compared to pre-intervention

abc... denotes significance across diet groups (when two superscripts contain the same letter there is no significant difference between two of the four diets)

SEM: standard error of the mean

SFA, saturated fatty acids (C14:0+C16:0+C18:0); MUFA, monounsaturated fatty acids (C16:1+C18:1+C20:1);

PUFA, polyunsaturated fatty acids (C18:2+C18:3:6+C18:3:3+C18:4+C20:3+C20:4:6+C20:4:3+C20:5+C22:4+C22:5+C22:6)

n-3 PUFA (C18:3:3+C18:4+C20:4:3+C20:5+C22:5+C22:6)

n-6 PUFA (C18:2+C18:3:6+C20:3+C20:4:6+C22:4)

Table 6.7b: Plasma C14(%) across body weight change categories, pre- and post-intervention, irrespective of dietary treatment

	Lost 4-8Kg n=14		Lost2-4Kg n=57		Lost 0-2Kg n=191		Gained 0-2Kg n=129		Gained 2-5Kg n=23	
Total Composition (w/w%)	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre C14:0	1.66	0.21	1.92	0.12	1.87	0.07	1.98	0.10	1.99	0.19
Post C14:0	1.27 †	0.08	2.22	0.18	2.74 †	0.11	2.76 †	0.12	2.26	0.24

Values represent adjusted means and SEMs for time*change in body weight interactions

†significant time*change in body weight interaction p<0.0005, post- compared to pre-intervention

SEM: standard error of the mean

Table 6.7c: Plasma Fatty Acids (%) (mean ±SEM) across each centre, in subjects who completed the LIPGENE Dietary Intervention Study, irrespective of dietary treatment

	TCD n=55		UoR n=57		UO n=54		INSERM n=38		UM n=49		HURS-UCO n=74		JUMC n=43		UU n=46	
Total Composition (w/w%)	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Pre C14:0	2.22 ^{ac}	0.13	2.68 ^{a}	0.17	1.95 ^{cd}	0.09	1.63 ^{cd}	0.09	2.14 ^{acd}	0.13	1.18 ^{b}	0.08	2.05 ^{cd}	0.18	1.59 ^{d}	0.07
Post C14:0	3.25 ^{a†}	0.20	3.42 ^{a†}	0.17	1.87 ^{bc}	0.08	1.59 ^{b}	0.10	3.21 ^{a†}	0.26	2.40 ^{c†}	0.15	2.04 ^{bc}	0.13	2.72 ^{ac†}	0.23
Pre C16:0	28.80 ^{ac}	0.72	28.86 ^{ac}	0.86	26.40 ^{ab}	0.42	24.44 ^{bde}	0.39	30.52 ^{ce}	0.71	24.38 ^{bd}	0.51	23.39 ^{d}	0.26	24.63 ^{bd}	0.42
Post C16:0	26.75 ^{a}	0.81	23.71 ^{b†}	0.48	25.44 ^{ab†}	0.52	23.96 ^{b}	0.39	25.37 ^{ab†}	0.66	24.51 ^{ab}	0.48	24.18 ^{ab}	0.48	24.36 ^{ab}	0.39
Pre C16:1	1.70 ^{ace}	0.14	2.11 ^{ae}	0.21	1.28 ^{cd}	0.12	1.53 ^{ace}	0.15	0.95 ^{bd}	0.11	0.73 ^{b}	0.05	1.26 ^{ac}	0.09	1.86 ^{e}	0.10
Post C16:1	1.75 ^{a}	0.15	2.30 ^{a}	0.24	1.13 ^{bd}	0.11	1.55 ^{ab}	0.14	0.74 ^{cd}	0.07	0.66 ^{c†}	0.05	1.36 ^{ab}	0.12	1.38 ^{ab†}	0.09
Pre C18:0	4.30	0.45	4.54	0.40	3.79	0.18	4.01	0.10	4.36	0.42	3.54	0.15	4.60	0.10	3.77	0.09
Post C18:0	4.45	0.48	3.99 †	0.33	3.02 †	0.13	3.69 †	0.10	2.82 †	0.15	3.48	0.17	4.28 †	0.09	3.95	0.15
Pre C18:1	24.47 ^{ac}	0.45	26.47 ^{ad}	0.47	23.87 ^{c}	0.50	27.75 ^{bd}	0.70	23.04 ^{c}	0.58	28.98 ^{b}	0.50	27.43 ^{bd}	0.57	29.61 ^{b}	0.50
Post 18:1	23.04 ^{a†}	0.58	25.35 ^{ac†}	0.51	24.64 ^{a}	0.48	28.26 ^{b}	0.47	25.33 ^{ad†}	0.57	26.96 ^{bcd}	0.51	28.03 ^{b}	0.61	27.36 ^{bcd†}	0.59
Pre C18:2:6	27.17 ^{ab}	0.70	25.44 ^{a}	0.64	29.56 ^{b}	0.70	28.24 ^{ab}	0.86	27.83 ^{ab}	0.94	28.76 ^{ab}	0.71	26.11 ^{b}	0.67	26.70 ^{ab}	0.61
Post C18:2:6	28.35 ^{ab}	0.87	26.37 ^{b}	0.65	29.38 ^{ab}	0.64	28.40 ^{ab}	0.69	30.16 ^{a†}	0.79	27.17 ^{ab†}	0.75	26.04 ^{b}	0.70	27.43 ^{ab}	0.59
Pre C20:4:6	7.02 ^{abc}	0.46	5.38 ^{a}	0.30	6.47 ^{b}	0.24	7.96 ^{c}	0.34	6.57 ^{abc}	0.41	7.64 ^{c}	0.18	8.28 ^{c}	0.29	5.70 ^{ab}	0.18
Post C20:4:6	7.25 ^{abc}	0.36	7.52 ^{ac†}	0.25	6.87 ^{ab†}	0.25	7.74 ^{ac}	0.27	7.11 ^{ac}	0.21	7.63 ^{ac}	0.29	8.08 ^{c}	0.23	6.14 ^{b†}	0.18
Pre C20:5:3	0.67 ^{a}	0.09	0.70 ^{a}	0.08	1.62 ^{b}	0.15	0.62 ^{a}	0.06	0.74 ^{a}	0.10	0.47 ^{c}	0.06	1.42 ^{b}	0.16	1.55 ^{b}	0.14
Post C20:5:3	1.02 ^{ac†}	0.13	1.40 ^{ab†}	0.12	1.68 ^{b}	0.15	0.85 ^{ac†}	0.08	1.05 ^{ac†}	0.14	0.98 ^{c†}	0.13	1.15 ^{abc†}	0.10	1.85 ^{b}	0.16
Pre C22:6:3	1.77 ^{a}	0.17	1.18 ^{b}	0.08	3.09 ^{c}	0.19	1.57 ^{ab}	0.10	1.64 ^{ab}	0.13	2.57 ^{c}	0.09	2.97 ^{c}	0.19	2.44 ^{c}	0.14
Post C22:6:3	1.30 ^{a†}	0.12	2.24 ^{bd†}	0.19	4.13 ^{c†}	0.22	1.80 ^{bd†}	0.12	1.98 ^{bd†}	0.15	4.07 ^{c†}	0.21	2.49 ^{bd†}	0.18	2.72 ^{d†}	0.16
Pre SFA	35.33 ^{ad}	1.10	36.09 ^{ad}	1.17	32.14 ^{ac}	0.42	30.08 ^{bc}	0.44	37.02 ^{d}	1.10	29.10 ^{b}	0.46	30.04 ^{bc}	0.36	29.99 ^{bc}	0.43
Post SFA	34.45 ^{a}	1.26	31.12 ^{ab†}	0.75	30.33 ^{ab†}	0.51	29.24 ^{b}	0.48	31.40 ^{ab†}	0.50	30.39 ^{ab†}	0.39	30.50 ^{ab}	0.52	31.04 ^{ab†}	0.36
Pre MUFA	26.43 ^{ac}	0.53	28.94 ^{ab}	0.60	25.26 ^{c}	0.57	29.46 ^{bd}	0.79	24.30 ^{c}	0.63	29.94 ^{bd}	0.50	28.81 ^{ab}	0.59	31.67 ^{d}	0.54
Post MUFA	24.95 ^{a}	0.68	28.12 ^{bc}	0.71	25.91 ^{ab}	0.47	29.97 ^{c}	0.54	26.26 ^{abd}	0.60	27.74 ^{bc}	0.51	29.54 ^{c}	0.62	28.83 ^{cd}	0.61
Pre PUFA	38.24 ^{ac}	1.08	34.98 ^{a}	0.91	42.60 ^{bd}	0.84	40.46 ^{cde}	1.03	38.68 ^{ad}	1.25	40.96 ^{cde}	0.69	41.16 ^{cde}	0.67	38.34 ^{ae}	0.73
Post PUFA	40.60 ^{ab†}	1.04	40.76 ^{ab†}	0.69	43.76 ^{a}	0.70	40.79 ^{ab}	0.74	42.34 ^{ab†}	0.78	41.87 ^{ab}	0.67	39.96 ^{b}	0.71	40.14 ^{b†}	0.65
Pre n6 PUFA	35.09 ^{ab}	0.96	31.96 ^{a}	0.81	37.10 ^{bc}	0.75	37.59 ^{bc}	1.03	35.27 ^{ab}	1.12	37.43 ^{b}	0.72	35.75 ^{ab}	0.63	33.49 ^{ac}	0.66
Post n6 PUFA	36.84 ^{ab}	1.03	35.51 ^{ab}	0.67	37.16 ^{ab}	0.67	37.42 ^{ab}	0.78	38.28 ^{a}	0.78	35.98 ^{ab}	0.70	35.36 ^{ab}	0.72	34.71 ^{b}	0.62
Pre n-3 PUFA	3.15 ^{a}	0.25	3.02 ^{a}	0.18	5.50 ^{b}	0.34	2.87 ^{a}	0.16	3.41 ^{a}	0.22	3.53 ^{a}	0.14	5.41 ^{b}	0.33	4.85 ^{b}	0.28
Post n-3 PUFA	3.76 ^{ac†}	0.24	5.25 ^{bc†}	0.27	6.60 ^{b†}	0.35	3.37 ^{a†}	0.21	4.06 ^{ad†}	0.28	5.89 ^{bd†}	0.29	4.60 ^{cd†}	0.26	5.43 ^{b}	0.31

†Significant time*centre interaction, p<0.05, post- compared to pre-intervention

$^{abc...}$ denotes significance across centres (when two superscripts contain the same letter there is no significant difference between two of the eight categories)

SEM: standard error of the mean

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

SFA, saturated fatty acids (C14:0+C16:0+C18:0); MUFA, monounsaturated fatty acids (C16:1+C18:1+C20:1);

PUFA, polyunsaturated fatty acids (C18:2+C18:3:6+C18:3:3+C18:4+C20:3+C20:4:6+C20:4:3+C20:5+C22:4+C22:5+C22:6)

n-3 PUFA (C18:3:3+C18:4+C20:4:3+C20:5+C22:5+C22:6)

n-6 PUFA (C18:2+C18:3:6+C20:3+C20:4:6+C22:4)

Table 6.7d: Plasma C16:1(%) pre-and post-intervention for males and females who completed the LIPGENE Dietary Intervention Study

	Males n=185			Females n=232		
Total Composition (w/w%)	**Mean**		**SEM**	**Mean**		**SEM**
Pre C16:1	1.23	a	0.07	1.54	b	0.07
Post C16:1	1.16	a	0.08	1.45	b	0.07

[a, b] denotes significance across gender, $p<0.05$

SEM: standard error of the mean

Table 6.7e: Plasma 20:5(n-3), C22:6(n-3) and n-3 PUFA (%) across age categories pre- and post-intervention, in subjects who completed the LIPGENE Dietary Intervention Study

	35-49 n=107			50-64 n=249			65-70 n=61		
Total Composition (w/w%)	**Mean**		**SEM**	**Mean**		**SEM**	**Mean**		**SEM**
Pre C20:5:3	0.76	a	0.08	0.94	b	0.05	1.26	b	0.14
Post C20:5:3	0.98	a	0.07	1.29	b	0.06	1.52	b	0.15
Pre C22:6:3	1.98		0.13	2.21		0.07	2.32		0.15
Post C22:6:3	2.33	a†	0.16	2.84	b†	0.11	2.80	ab	0.18
Pre n-3 PUFA	3.58	a	0.21	3.94	ab	0.12	4.56	b	0.26
Post n-3 PUFA	4.44	a†	0.20	5.12	ab†	0.15	5.42	b†	0.30

[abc...] denotes significance across age categories (when two superscripts contain the same letter there is no significant difference between two of the three categories)$p<0.05$, $p<0.017$ where bonferroni correction applied

†Significant time*age interaction, $p<0.05$, post compared to pre

n-3 PUFA (C18:3:3+C18:4+C20:4:3+C20:5+C22:5+C22:6)

6.4.2 Correlation Analysis

A range of IVGTT parameters measured at post-intervention were correlated with each other; the results are presented in *Table 6.8a*. The objective of this was to describe the strength and direction of the linear relationship between two variables, post-intervention. When significant correlations were observed, the file was split by diet and the correlations were carried out again to determine whether any diet altered the association between variables (*Table 6.8b*). All IVGTT parameters correlated significantly with each other, with the exception of S_G which only correlated significantly with DI and G0. As the main outcome variable, S_I correlated significantly with all other IVGTT parameters and measures of insulin resistance. Insulin sensitivity showed a moderately strong and negative correlation with AIRg (-0.254, $p<0.0005$), HOMA-IR (-0.373, $p<0.0005$) and first phase insulin response (-0.244, $p<0.0005$) and was positively correlated with DI (0.325, $p<0.0005$). S_I was strongly and positively correlated with P3 (0.738, $p<0.0005$) and QUICKI values (0.612, $p<0.0005$), and negatively correlated with fasting insulin concentrations (-0.584, $p<0.0005$), C-peptide concentrations (-0.512, $p<0.0005$) and insulin to C-peptide ratio (-0.491, $p<0.0005$), all post-intervention analysis. These significant correlations were seen in all diet groups and in the HFSFA and LF diets (Diets A and C) for the negative relationship with first phase insulin response. The strength of the correlations varied. Strong positive correlations were seen for S_I with P3, the strongest in the LF diet (Diet C); S_I with fasting insulin (negatively correlated), particularly in the HFSFA diet (Diet A); S_I with QUICKI values (positively correlated), C-peptide and insulin to C-peptide ratio (negatively correlated), also strongest in the HFSFA diet (Diet A). As expected, S_G and G0 were strongly and positively correlated with each other irrespective of diet and in all diet groups.

AIRg was positively and strongly correlated with DI (0.781, $p<0.0005$) which is a product of S_I and AIRg. The strong correlations were retained in each of the four diets. AIRg and fasting glucose were also strongly and negatively correlated to each other (-0.434, $p<0.0005$). AIRg and first phase insulin response were almost perfectly positively correlated (r=0.995, $p<0.0005$). This is not surprising as the two indices represent insulin action in the first 10 minutes of the IVGTT. Again, similar coefficient values were seen in all diet groups.

DI was strongly and negatively correlated with fasting glucose concentrations (-0.574, $p<0.0005$) and strongly, but, positively with first phase insulin response

(0.784, $p<0.0005$). Both parameters were significantly correlated irrespective of diet but also in each diet group. As G0 represents distributed glucose at time 0 minutes, it follows that a strong positive correlation with fasting glucose is significant and is seen in all diet groups.

As two key indices derived from the IVGTT modelling, P2 and P3 represent stages of insulin action where P2 represents the removal of insulin from the interstitial space and P3 represents the movement of insulin to the interstitial space. These parameters are highly and positively correlated with each other regardless of dietary treatment (0.727, $p<0.0005$) but also found to be strongly correlated in each of the four diets. P3 was also negatively correlated with fasting insulin (-0.565, $p<0.0005$), HOMA-IR (-0.584, $p<0.0005$) and C-peptide concentrations (-0.526, $p<0.0005$) and positively correlated with QUICKI (0.584, $p<0.0005$), and seen in all diets (Diets A, B, C and D).

In addition to being highly correlated with DI (-0.574, $p<0.0005$) and G0 (0.500, $p<0.0005$), fasting glucose post-intervention was also strongly and negatively correlated with first phase insulin response in all diets. Because post-intervention HOMA-IR and QUICKI are derived from fasting insulin, strong correlations are expected, with a positive relationship for HOMA-IR (0.963, $p<0.0005$) and a negative with QUICKI (-0.948, $p<0.0005$). The strong associations were also maintained within each diet. As expected, post-intervention fasting insulin and fasting C-peptide concentrations correlated positively with each other in all diets. HOMA-IR and QUICKI values also correlated strongly with C-peptide concentrations post-intervention (0.797, 0.-0.783, respectively, $p<0.0005$), these strong relationships were observed in all diets.

Post-intervention IVGTT parameters were correlated with the anthropometric variables also measured at post-intervention and the results presented in *Table 6.9a and Table 6.9b*. There were significant, moderate-to-strong ($r>0.250$) and negative correlations between S_I measured at the end of the intervention period and post-intervention body weight (-0.299, $p<0.0005$), BMI (-0.323, $p<0.0005$) and waist circumference (-0.399, $p<0.0005$), with increasing levels of S_I associated with lower body weight, lower BMI index and lower waist circumference measurements. These associations remained true within each dietary treatment. The strongest negative correlation was seen in the LF diet (Diet C) for S_I and body weight and in the HFSFA diet (Diet A) for S_I and BMI. In all diets, S_I and waist circumference were negatively

correlated, the strongest negative correlation in the HFMUFA diet and the LF diet (Diets B and C).

Post-intervention P3 (the movement of circulating insulin to the interstitial space) correlated negatively with body weight (-0.248, $p<0.0005$), BMI (-0.265, $p<0.0005$) and waist circumference also (-0.337, $p<0.0005$), with increasing P3 associated with decreasing weight, BMI and waist measurement, as seen for S_I. There was a negative correlation between P3 and body weight and BMI in diets HFSFA, HFMUFA and LF and in all four diet groups for waist circumference.

As anticipated, strong, positive correlations were observed between post-intervention fasting insulin concentrations and post-intervention body weight (0.346, $p<0.0005$), BMI (0.413, $p<0.0005$), waist (0.419, $p<0.0005$) and hip circumference (0.300, $p<0.0005$), diastolic blood pressure (0.222, $p<0.0005$) and body fat measured in kg (0.249, $p<0.0005$) with increasing fasting insulin concentrations associated with increasing anthropometric variables. These positive associations were observed in Diets HFSFA, HFMUFA and LF for fasting insulin with body weight and body fat mass, in Diets HFSFA and LFn3PUFA with diastolic blood pressure and in all diet groups for waist and hip circumference and BMI index.

There were significant positive correlations for post-intervention HOMA-IR with the same anthropometric variables as for post-intervention fasting insulin, with the same dietary treatment effects. Conversely, there were strong, negative correlations for QUICKI (a measure of insulin sensitivity) with these same variables. QUICKI values were negatively correlated with body weight and body fat mass in Diets HFSFA, HFMUFA and LF; with waist and hip circumference and BMI in all diets and with diastolic blood pressure in Diets HFSFA, HFMUFA and LFn3PUFA.

There were significant positive correlations of C-peptide concentrations with body weight (0.313, $p<0.0005$), BMI (0.395, $p<0.0005$), waist (0.402, $p<0.0005$) and hip circumference (0.279, $p<0.0005$), diastolic blood pressure (0.244, $p<0.0005$) and body fat mass (0.297, $p<0.0005$) with increasing concentrations of C-peptide concentrations measured at post-intervention associated with increasing weight, BMI, waist, hip, diastolic blood pressure and body fat mass as recorded at post-intervention clinical investigation day. There was a positive relationship between C-peptide concentrations and body weight, BMI and waist in all diet groups; in Diets HFSFA, HFMUFA and LFn3PUFA for hip circumference; Diets HFSFA and LFn3PUFA for diastolic blood pressure and in Diets HFSFA, HFMUFA and LF for body fat (kg).

Similar correlation strengths and directions were observed for insulin to C-peptide ratio.

Inflammatory cytokines measured post-intervention were correlated with IVGTT parameters derived from the MINMOD Millennium output at post-intervention also (this analysis was carried out by Jolene McMonagle). The strength of these associations are presented in *Table 6.10a*. CRP concentrations correlated positively, albeit weakly with fasting insulin post-intervention (0.103, $p<0.05$). *Table 6.10b* highlights this positive and significant correlation in Diets HFSFA and HFMUFA only. IL6 was not significantly associated with any of the IVGTT parameters. In contrast, post-intervention TNFα concentrations showed positive associations with fasting glucose (0.130, $p<0.05$), insulin (0.116, $p<0.05$), HOMA-IR (0.135, $p<0.05$) and negative associations with QUICKI (-0.122, $p<0.05$). Within the diet groups, these significant correlations were only apparent in Diet LFn3PUFA (*Table 6.10c*). Post-Intervention sICAM correlated negatively with S_I (-0.178, $p<0.001$) and QUICKI post-intervention (-0.151, $p<0.05$) and positively with fasting insulin (0.164, $p<0.001$) and HOMA-IR (0.163, $p<0.001$). These correlations were significant ($p<0.05$) in the LF diet for all and in the HFSFA diet for fasting insulin and HOMA-IR (*Table 6.10d*). Post-intervention adiponectin concentrations correlated positively with S_I (0.116, $p<0.05$) and QUICKI (0.109, $p<0.05$) and negatively with AIRg (-0.148, $p<0.05$), fasting insulin (-0.117, $p<0.05$) and HOMA-IR (-0.100, $p<0.05$). These significant correlations were lost when the file was split by diet group, except for fasting insulin in the LF diet (*Table 6.10e*). The most significant correlations were seen for leptin concentrations post-intervention. These concentrations correlated negatively with S_I (-0.177, $p<0.001$), DI (-0.148, $p<0.05$), P3 (-0.129, $p<0.05$) and QUICKI (-0.264, $p<0.0005$) and positively with G0 (0.197, $p<0.001$), fasting insulin (0.273, $p<0.0005$) and HOMA-IR (0.271, $p<0.0005$), post-intervention. *Table 6.10f* demonstrates these correlations across the four dietary groups. The negative and significant correlations were maintained for leptin with post-intervention S_I in the HFSFA diet only ($p<0.05$) and with post-intervention QUICKI in Diets HFSFA ($p<0.0005$), HFMUFA and LF ($p<0.05$) only. The positive and significant correlations were maintained for leptin with post-intervention G0 in the HFMUFA diet only ($p<0.05$) and with HOMA-IR in the HFSFA diet ($p<0.0005$), HFMUFA diet and LF diet only ($p<0.05$).

Correlation coefficients of IVGTT parameters with markers of coagulation (post-intervention) are shown in *Table 6.11a*. Very weak and non-significant correlations were observed with the parameters and fibrinogen. However, PAI-1 and tPA concentrations measured at post-intervention were moderately (r=0.202 to 0.407) and negatively correlated with S_I (-0.248, -0.337, respectively, $p<0.0005$), P3 (-0.235, -0.276, $p<0.0005$) and QUICKI (-0.328, -0.363, respectively, $p<0.0005$) and positively correlated with G0 (0.278, 0.202, respectively, $p<0.0005$), fasting glucose (0.271, 0.209, respectively, $p<0.0005$), fasting insulin (0.300, 0.350, respectively, $p<0.0005$), HOMA-IR (0.341, 0.375, respectively, $p<0.0005$) and C-peptide concentrations (0.381, 0.407, respectively, $p<0.0005$). With increasing S_I and P3 at post-intervention, tPA decreased in all diet groups (*Table 6.11b*). PAI-1 decreased in the HFMUFA, LF and LFn3PUFa diet with increasing S_I and in the HFSFA, HFMUFA and LFn3PUFA diets with P3. As QUICKI values increased post-intervention, both PAI-1 and tPA concentrations decreased in all diet groups. G0 (distributed glucose at time 0 minutes) was positively correlated with PAI-1 in the HFSFA, HFMUFA and LF and with tPA in the LFn3PUFA diet only. Fasting glucose was positively correlated with PAI-1 in the HFSFA, HFMUFA and LFn3PUFa diets and with tPA in the HFSFA, LF and LFn3PUFA diet. Fasting insulin, HOMA-IR and C-peptide concentrations were positively correlated with PAI-1 and tPA in all diet groups at post-intervention.

IVGTT parameters were correlated with fasting plasma TAG, cholesterol, NEFA, TRL TAG, TRL cholesterol, LDL-cholesterol, Total HDL-cholesterol and apolipoprotein concentrations measured at post-intervention and the results illustrated in *Table 6.12a*. When significant correlations were observed, they were analysed across the diets to see in which diets they existed (*Table 6.12b*). Insulin sensitivity (S_I) correlated negatively with TAG (-0.181, $p<0.001$), TRL TAG (-0.125, $p<0.05$), and apoE concentrations (-0.139, $p<0.05$), irrespective of diet. The negative relationship between S_I and TAG and TRL TAG was demonstrated in the HFSFA and LF diets (Diets A and C) and in the LF diet (Diet C) only for S_I with apoE. S_I positively correlated with Total HDL-cholesterol (0.179, $p<0.001$) and this was also seen in the HFSFA diet and more strongly in the LF diet. AIRg was significantly and negatively correlated with Total HDL-cholesterol (-0.149, $p<0.05$), apoA1 (-0.136, $p<0.05$) apoE (-0.117, $p<0.05$) and apoB48 (-0.134, $p<0.05$). These correlations were

somewhat stronger in the affected diet groups, HFSFA diet for apoB48 and in the LFn3PUFA diet for total HDL, apoA1 and apoE. P3 was negatively correlated with TAG and apoE, in the HFMUFA and LF and in the LF diet, respectively. This IVGTT parameter was positively correlated with total HDL-cholesterol concentrations in the HFSFA, HFMUFA and LF diets. There was a negative relationship between post-intervention fasting glucose concentrations and NEFA (-0.134, $p<0.05$) and TRL apoB (-0.169, $p<0.001$). This was apparent in the LFn3PUFA diet for both and in the HFMUFA diet for TRL apoB also. Fasting insulin and insulin to C-peptide ratio were positively correlated with TAG in the HFSFA and LF diets. HOMA-IR and QUICKI were correlated with TAG in the HFSFA, HFMUFA and LF diets and in all diets for C-peptide and TAG concentrations. There were significant relationships between post-intervention fasting insulin, HOMA-IR, QUICKI, C-peptide concentrations and insulin to C-peptide ratio with TRL TAG concentrations also measured after the 12-week study. These correlations manifested in the HFSFA, HFMUFA and LF diet groups and in the HFSFA and LF diets for insulin to C-peptide ratio. Fasting insulin and C-peptide concentrations significantly and positively correlated with TRL Cholesterol in the HFSFA, HFMUFA and LF diets and negatively with QUICKI in the HFMUFA and LF diets. Fasting insulin concentrations (HFSFA, HFMUFA and LF diets), HOMA-IR (HFSFA, HFMUFA and LF diets), C-peptide concentrations (HFSFA and LF diets) and insulin to C-peptide ratio (HFMUFA and LF diets) negatively correlated with total HDL-cholesterol concentrations and positively correlated with QUICKI values. Fasting insulin, QUICKI and C-peptide concentrations correlated with apoCIII in the LF diet group only, and positively correlated with apoE in the LFn3PUFA diet (Diet D) only.

Table 6.13a illustrates the correlation coefficients of the IVGTT parameters measured at post-intervention with the dietary data collected at week 12 of the intervention study from a 3-day weighed food diary. Substantial correlations are not observed between these two datasets. S_I was negatively correlated with Basal Metabolic Rate (BMR) (-0.260, $p<0.0005$), with increasing S_I at post-intervention associated with a decrease in BMR, post-intervention. There were no significant correlations observed for S_I with any of the dietary fats measured in grams or as a percentage of total energy. AIRg was positively associated and P3 negatively associated with BMR (0.211, -0.217, respectively, $p<0.0005$). Fasting glucose concentrations measured at

post-intervention were also positively, albeit weakly, associated with BMR (0.101, $p<0.05$), dietary palmitic acid (C16:0g) (0.118, $p<0.05$), dietary palmitoleic acid (C16:1g) (0.210, $p<0.0005$), Alcohol (g) and as a %energy (0.118, 0.115, respectively, $p<0.05$). Post-intervention fasting insulin concentrations and HOMA-IR values were positively correlated with BMR (0.269, 0.268, respectively, $p<0.0005$) and protein (% energy) (0.167, 0.173, respectively, $p<0.001$) and weakly ($r<0.20$) and negatively correlated with dietary lauric acid (C12g) (-0.145, -0.135, respectively, $p<0.05$) and CHO (% energy) (-0.131, -0.133, $p<0.05$). Post-intervention QUICKI values had similar correlations to HOMA-IR values being a measure of insulin sensitivity as opposed to insulin resistance, the direction of the relationships was reversed. C-peptide concentrations and insulin to C-peptide ratio calculated from post-intervention analysis were both positively correlated to BMR (0.199, 0.238, respectively, $p<0.0005$).

On splitting the dataset by diet, the correlation coefficients between IVGTT parameters and dietary variables at post-intervention were stronger (*Table 6.13b*) than the correlations irrespective of diet. S_I was negatively correlated with Basal Metabolic Rate (BMR) in the HFMUFA and LF diets. Acute insulin response to glucose was positively associated and P3 negatively associated with BMR in the LF diet and also in the HFMUFA diet for P3. Fasting glucose (post-intervention) was positively associated with BMR in the HFSFA diet only; with dietary palmitoleic acid (C16:1g) in the HFMUFA and LFn3PUFA diets and in the HFSFA diet for alcohol (g) and as a % of energy. For post-intervention BMR with fasting-insulin, a positive correlation in the HFMUFA, LF and LFn3PUFA diets were observed, and in the HFMUFA and LF diets for HOMA-IR and negatively correlated with QUICKI in both these diets also. With protein (% energy) the positive correlation effect with post-intervention fasting insulin and HOMA-IR and a negative correlation with QUICKI were apparent in the HFMUFA diet. As dietary lauric acid (C12g) increased in the diet, post-intervention, fasting insulin concentrations and HOMA-IR decreased in the HFSFA diet. CHO (% energy) was significantly and negatively related to both fasting insulin and HOMA-IR and positively related to QUICKI values post-intervention in the HFSFA diet only.

Table 6.14a presents the correlation coefficients between plasma fatty acid composition (w/w%) and IVGTT parameters, irrespective of diet post-intervention. The significant correlations, although weak, were further analysed across the four

diets, illustrated in *Table 6.14b* and revealed stronger correlations in this post-hoc analysis. There were significant positive correlations of S_I with plasma linoleic acid (C18:2:6) (0.140, $p<0.05$), total PUFA (0.143, $p<0.05$) and n-6 PUFA (0.151, $p<0.05$) post-intervention and a significant negative correlation with SFA (-0.114, $p<0.05$) although this significance was lost when the file was split by diet group (%). However, S_I was significantly positively correlated with post-intervention plasma linoleic acid (C18:2:6), total PUFA and n-6 PUFA in the HFSFA diet (Diet A) only. AIRg was negatively correlated with n-3 PUFA (%) and this was significant in the HFMUFA diet at post-intervention, but not for any of the other diets. G0 was moderately (0.332, $p<0.0005$) correlated with plasma stearic acid (C18:0) irrespective of diet. On further analysis, this was seen to be in all four diets post-intervention, however, more strongly correlated in the HFSFA diet. The most significant correlations were seen for post-intervention fasting glucose concentrations where this parameter was positively correlated with plasma stearic acid (C18:0) (0.231, $p<0.0005$), total SFA (0.173, $p<0.001$) and n-3 PUFA (0.161, $p<0.001$), irrespective of diet. These correlations were significant in the HFSFA, LF and LFn3PUFA diets with respect to fasting glucose and plasma stearic acid (C18:0); in the HFSFA and LFn3PUFA diets for SFA and in the LF diet for n-3 PUFA. Both P2 and P3 (post-intervention) were negatively correlated with plasma myristic acid (C14:0) (-0.172, -0.173, respectively, $p<0.05$) and SFA (-0.108, -0.147, respectively, $p<0.05$). This was apparent in the HFMUFA and LF diets for plasma myristic acid (C14:0%) and in the HFMUFA diet only for total plasma SFA. Post-intervention fasting insulin concentrations were positively correlated with plasma myristic acid (C14:0) (0.132, $p<0.05$) irrespective of diet. It was negatively correlated with plasma linoleic acid (C18:2:6) (-0.142, $p<0.05$), plasma EPA (C20:5:3) (-0.102, $p<0.05$), total PUFA (-0.167, $p<0.001$) and n-6 PUFA (-0.139, $p<0.05$). Within the dietary treatments, the positive correlation with plasma myristic acid (C14:0) was in the HFMUFA diet (Diet B); the negative correlations with plasma linoleic acid (C18:2:6) was in the HFSFA diet (Diet A) as was the negative relationship between fasting insulin and plasma n-6 PUFA; The LF diet (Diet C) showed a significant negative relationship with total plasma PUFA and as fasting insulin increased post-intervention in the LFn3PUFA diet (Diet D), plasma EPA (C20:5:3) decreased. As surrogate measures of insulin resistance HOMA-IR correlated negatively with plasma linoleic acid (C18:2:6) (HFSFA and LFn3PUFA diets) and positively with plasma PUFA (HFSFA and LF

diets) and n-6 PUFA (HFSFA diet). QUICKI correlated with the same plasma fatty acids, however in the opposite direction.

Table 6.8a: Correlation coefficients of IVGTT parameters post-intervention of the 12 week dietary intervention study, irrespective of dietary treatment

	Post S_G		Post AIRg		Post DI		Post G0		Post P2		Post P3		Post Fasting Glucose		Post Fasting Insulin		Post HOMA-IR		Post QUICKI		Post FPIR		Post C-Peptide		Post Insulin to C-peptide Ratio	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	0.087	ns	-0.254	***	0.325	***	-0.178	**	0.198	***	0.738	***	0.190	**	-0.584	***	-0.373	***	0.612	***	-0.244	***	-0.512	***	-0.491	***
Post S_G			0.034	ns	0.142	*	0.582	***	-0.064	ns	0.005	ns	0.006	ns	-0.072	ns	-0.073	ns	0.073	ns	0.035	ns	-0.052	ns	-0.012	ns
Post AIRg					0.781	***	-0.169	*	0.068	ns	-0.071	ns	-0.434	***	0.232	***	0.123	*	-0.123	*	0.995	***	0.137	*	0.236	***
Post DI							-0.237	***	0.200	***	0.377	***	-0.574	***	-0.139	*	-0.248	***	0.248	***	0.784	***	-0.186	**	-0.040	ns
Post G0									-0.070	ns	-0.195	***	0.500	***	0.202	***	0.282	***	-0.282	***	-0.168	*	0.233	***	0.179	**
Post P2											0.727	***	-0.124	*	-0.263	***	-0.266	***	0.266	***	0.072	ns	-0.256	***	-0.099	ns
Post P3													-0.276	***	-0.565	***	-0.584	***	0.584	***	-0.061	ns	-0.526	***	-0.332	***
Post Fasting Glucose															0.171	**	-0.238	***	-0.387	***	-0.445	***	0.255	***	0.026	ns
Post Fasting Insulin																	0.963	***	-0.948	***	0.243	***	0.805	***	0.812	***
Post HOMA-IR																			-0.985	***	0.121	*	0.797	***	0.762	***
Post QUICKI																					-0.121	*	-0.783	***	-0.747	***
Post First Phase Insulin Response																							0.146	*	0.243	***
Post C-Peptide																									0.353	***

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant
FPIR first phase insulin response

Table 6.8b: Correlation coefficients of post-intervention IVGTT parameters within each of the dietary treatments

HFSFA diet (Diet A)

	Post AIRg	Post DI	Post G0	Post P2	Post P3	Post Fasting Glucose	Post Fasting Insulin	Post HOMA	Post QUICKI	Post FPIR	Post C-Peptide	Post Insulin to C-peptide Ratio
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I	-0.318 *	0.304 *	-0.269 *	0.143 ns	0.691 ***	0.298 *	-0.719 ***	-0.493 ***	0.736 ***	-0.310 *	-0.606 ***	-0.601 ***
Post S_G		0.179 ns	0.450 ***									
Post AIRg		0.701 ***	-0.072 ns			-0.282 *	0.207 ns	0.152 ns	-0.152 ns	0.996 ***	0.163 ns	0.171 ns
Post DI			-0.259 *	0.206 ns	0.369 **	-0.529 ***	-0.290 *	-0.345 *	0.345 *	0.707 ***	-0.263 *	
Post G0					-0.304 *	0.581 ***	0.406 ***	0.486 ***	-0.486 ***	-0.079 ns	0.345 *	0.353 *
Post P2					0.690 ***	-0.105 ns	-0.269 *	-0.250 *	0.250 *	0.193 ns	-0.318 *	
Post P3						-0.302 *	-0.682 ***	-0.667 ***	0.667 ***	-0.062 ns	-0.646 ***	-0.529 ***
Post Fasting Glucose							0.462 ***	-0.333 **	-0.604 ***	-0.251 *	0.456 ***	
Post Fasting Insulin								0.978 ***	-0.978 ***	0.184 ns	0.909 ***	0.755 ***
Post HOMA-IR									-1.000 ***	0.121 ns	0.890 ***	0.732 ***
Post QUICKI										-0.121 ns	-0.890 ***	-0.732 ***
Post First Phase Insulin Response											0.180 ns	0.126 ns
Post C-Peptide												0.441 ***

HFMUFA diet (Diet B)

	Post AIRg	Post DI	Post G0	Post P2	Post P3	Post Fasting Glucose	Post Fasting Insulin	Post HOMA	Post QUICKI	Post FPIR	Post C-Peptide	Post Insulin to C-peptide Ratio
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I	-0.203 *	0.270 *	-0.184 ns	0.182 ns	0.751 ***	0.226 *	-0.507 ***	-0.311 *	0.554 ***	-0.195 ns	-0.424 ***	-0.416 ***
Post S_G		0.199 ns	0.537 ***									
Post AIRg		0.851 *	-0.185 ns			-0.484 ***	0.166 ns	0.051 ns	-0.051 ns	0.996 ***	-0.002 ns	0.193 ns
Post DI			-0.212 *	0.287 *	0.424 ***	-0.608 ***	-0.113 ns	-0.241 *	0.241 *	0.853 ***	-0.230 *	
Post G0					-0.098 ns	0.558 ***	0.157 ns	0.268 *	-0.268 *	-0.184 ns	0.207 *	0.081 ns
Post P2					0.715 ***	-0.135 ns	-0.251 *	-0.252 *	0.252 *	0.146 ns	-0.256 *	
Post P3						-0.325 **	-0.519 ***	-0.568 ***	0.568 ***	0.048 ns	-0.467 ***	-0.428 ***
Post Fasting Glucose							0.070 ns	-0.272 *	-0.286 *	-0.474 ***	0.218 *	
Post Fasting Insulin								0.972 ***	-0.918 ***	0.190 *	0.789 ***	0.786 ***
Post HOMA-IR									-0.946 ***	0.085 ns	0.800 ***	0.742 ***
Post QUICKI										-0.085 ns	-0.747 ***	-0.687 ***
Post First Phase Insulin Response											0.036 ns	0.199 *
Post C-Peptide												0.301 ***

LF diet (Diet C)

	Post AIRg	Post DI	Post G0	Post P2	Post P3	Post Fasting Glucose	Post Fasting Insulin	Post HOMA	Post QUICKI	Post FPIR	Post C-Peptide	Post Insulin to C-peptide Ratio
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I	-0.268 *	0.327 *	-0.093 ns	0.227 *	0.757 ***	0.192 ns	-0.543 ***	-0.386 ***	0.591 ***	-0.272 *	-0.451 ***	-0.505 ***
Post S_G		0.169 ns	0.738 ***									
Post AIRg		0.781 ***	-0.187 ns			-0.555 ***	0.313 *	0.186 ns	-0.186 ns	0.994 ***	0.137 ns	0.416 ***
Post DI			-0.172 ns	0.122 ns	0.354 **	-0.704 ***	-0.031 ns	-0.181 ns	0.181 ns	0.775 ***	-0.146 ns	
Post G0					-0.116 ns	0.381 ***	0.059 ns	0.124 ns	-0.124 ns	-0.184 ns	0.126 ns	0.062 ns
Post P2					0.757 ***	-0.066 ns	-0.232 *	-0.254 *	0.254 *	-0.088 ns	-0.248 *	
Post P3						-0.271 *	-0.472 ***	-0.529 ***	0.529 ***	-0.134 ns	-0.447 ***	-0.394 ***
Post Fasting Glucose							0.043 ns	-0.061 ns	-0.246 *	-0.563 ***	0.167 ns	
Post Fasting Insulin								0.968 ***	-0.968 ***	0.382 ***	0.726 ***	0.856 ***
Post HOMA-IR									-1.000 ***	0.241 *	0.722 ***	0.813 ***
Post QUICKI										-0.241 *	-0.722 ***	-0.813 ***
Post First Phase Insulin Response											0.206 *	0.461 ***
Post C-Peptide												0.326 **

LF n-3 PUFA diet (Diet D)

	Post AIRg	Post DI	Post G0	Post P2	Post P3	Post Fasting Glucose	Post Fasting Insulin	Post HOMA	Post QUICKI	Post FPIR	Post C-Peptide	Post Insulin to C-peptide Ratio
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I	-0.250 *	0.392 ***	-0.135 ns	0.241 *	0.748 ***	-0.001 ns	-0.593 ***	-0.328 *	0.574 ***	-0.202 ns	-0.556 ***	-0.431 ***
Post S_G		0.048 ns	0.577 ***									
Post AIRg		0.737 ***	-0.244 *			-0.333 *	0.247 *	0.145 ns	-0.145 ns	0.991 ***	0.278 *	0.150 ns
Post DI			-0.292 *	0.195 ns	0.353 *	-0.413 ***	-0.150 ns	-0.207 ns	0.207 ns	0.761 ***	-0.092 ns	
Post G0					-0.240 *	0.442 **	0.166 ns	0.224 *	-0.224 *	-0.243 *	0.227 *	0.113 ns
Post P2					0.729 ***	-0.255 *	-0.242 *	-0.274 *	0.274 *	0.101 ns	-0.230 *	
Post P3						-0.245 *	-0.567 ***	-0.553 ***	0.553 ***	-0.090 ns	-0.552 ***	-0.429 ***
Post Fasting Glucose							0.125 ns	-0.280 *	-0.400 ***	-0.472 ***	0.206 *	
Post Fasting Insulin								0.932 ***	-0.932 ***	0.192 ns	0.755 ***	0.835 ***
Post HOMA-IR									-1.000 ***	0.043 ns	0.746 ***	0.745 ***
Post QUICKI										-0.043 ns	-0.746 ***	-0.745 ***
Post First Phase Insulin Response											0.177 ns	0.112 ns
Post C-Peptide												0.323 **

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant
FPIR first phase insulin response

Table 6.9a: Correlation coefficients between IVGTT parameters and Anthropometric variables, post-intervention of the 12 week dietary study, irrespective of dietary treatment

	Post-Intervention Anthropometirc Variables																													
	Body Weight		BMI		Waist		Hip		Diastolic BP		Systolic BP		Body Fat %		Body Fat kg		Lean Body Mass %		Lean Body Mass kg		Body Water %		Body Water L		Impedance		Change Body Weight	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.299	***	-0.323	***	-0.399	***	-0.148	*	-0.199	***	-0.113	*	0.011	ns	-0.152	*	0.019	ns	-0.170	*	0.022	ns	-0.154	*	0.238	***	-0.071	ns
Post S_G	-0.014	ns	-0.004	ns	-0.045	ns	0.013	ns	-0.020	ns	0.055	ns	-0.036	ns	-0.059	ns	0.030	ns	0.032	ns	0.051	ns	0.040	ns	-0.029	ns	-0.025	ns
Post AIRg	0.176	**	0.107	*	0.199	***	0.048	ns	0.123	*	0.023	ns	-0.087	ns	0.015	ns	0.114	*	0.200	***	0.090	ns	0.165	*	-0.174	*	0.037	ns
Post DI	0.000	ns	-0.097	ns	-0.053	ns	-0.043	ns	0.004	ns	-0.044	ns	-0.091	ns	-0.086	ns	0.150	*	0.120	*	0.133	*	0.090	ns	-0.054	ns	0.007	ns
Post G0	0.147	*	0.236	***	0.159	*	0.192	***	0.115	*	0.081	ns	0.160	*	0.190	**	-0.116	*	0.027	ns	-0.061	ns	0.044	ns	-0.013	ns	0.118	*
Post P2	-0.107	ns	-0.107	*	-0.125	*	-0.007	ns	-0.013	ns	-0.082	ns	-0.066	ns	-0.113	*	0.039	ns	-0.021	ns	0.069	ns	0.004	ns	0.190	*	0.031	ns
Post P3	-0.248	***	-0.265	***	-0.337	***	-0.096	ns	-0.137	*	-0.139	*	-0.034	ns	-0.177	*	0.029	ns	-0.131	*	0.048	ns	-0.109	ns	0.251	***	-0.041	ns
Post Fasting Glucose	0.103	*	0.125	*	0.121	*	0.049	ns	0.100	*	0.121	*	-0.033	ns	0.005	ns	-0.030	ns	0.035	ns	-0.006	ns	0.046	ns	-0.146	*	0.047	ns
Post Fasting Insulin	0.346	***	0.413	***	0.419	***	0.300	***	0.222	***	0.091	ns	0.075	ns	0.249	***	-0.022	ns	0.191	***	-0.057	ns	0.163	*	-0.113	*	0.160	**
Post HOMA-IR	0.341	***	0.407	***	0.417	***	0.292	***	0.238	***	0.127	*	0.061	ns	0.230	***	-0.021	ns	0.185	***	-0.051	ns	0.161	*	-0.136	*	0.160	**
Post QUICKI	-0.327	***	-0.397	***	-0.410	***	-0.284	***	-0.251	***	-0.139	*	-0.061	ns	-0.230	***	0.021	ns	-0.185	***	0.051	ns	-0.161	*	0.136	*	-0.155	*
Post First Phase Insulin Response	0.164	**	0.126	*	0.176	***	0.077	ns	0.120	*	0.005	ns	-0.024	ns	0.062	ns	0.075	ns	0.160	*	0.052	ns	0.132	*	-0.123	*	0.058	ns
Post C-Peptide	0.313	***	0.395	***	0.402	***	0.279	***	0.244	***	0.126	*	0.148	*	0.297	***	-0.081	ns	0.159	*	-0.072	ns	0.145	*	-0.045	ns	0.219	***
Post Insulin to C-peptide Ratio	0.279	***	0.331	***	0.325	***	0.261	***	0.122	*	0.025	ns	0.005	ns	0.156	*	0.008	ns	0.154	*	-0.042	ns	0.127	*	-0.155	*	0.078	ns

r correlation coefficient

***$p < 0.0005$

**$p < 0.001$

*$p < 0.05$

ns non significant

Table 6.9b: Correlation coefficients between post-intervention IVGTT parameters and post-intervention anthropometric measurements within each of the dietary treatments

HFSFA diet (Diet A) Post-Intervention

	Body Weight		BMI		Waist		Hip		Dias BP		Sys BP		Body Fat %		Body Fat kg		Lean Body Mass%		Lean Body Mass kg		Body Water%		Body Water L		Impedance		Change Body Weight	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.356	*	-0.393	**	-0.450	***	-0.293	*	-0.294	*	-0.150	ns			-0.226	ns			-0.073	ns			-0.128	ns	0.165	ns		
Post AIRg	0.145	ns	0.079	ns	0.222	ns	0.102	ns	0.184	ns							-0.020	ns	0.048	ns			0.089	ns	-0.127	ns		
Post DI																	0.141	ns	0.048	ns	0.093	ns						
Post G0	0.297	*	0.375	**	0.315	*	0.325	*	0.201	ns			0.070	ns	0.157	ns	-0.021	ns									0.039	ns
Post P2			-0.083	ns	-0.049	ns									-0.098	ns									0.219	ns		
Post P3	-0.269	*	-0.333	*	-0.362	*			-0.162	ns	-0.162	ns			-0.281	*			-0.035	ns					0.238	ns		
Post Fasting Glucose	0.214	*	0.290	*	0.293	*			0.224	*	0.126	ns													-0.110	ns		
Post Fasting Insulin	0.335	**	0.520	***	0.493	***	0.421	***	0.328	**					0.362	***			0.009	ns			0.027	ns			0.316	*
Post HOMA-IR	0.339	**	0.507	***	0.485	***	0.423	***	0.344	**	0.204	*			0.329	*			0.023	ns			0.030	ns	-0.014	ns	0.305	*
Post QUICKI	-0.339	**	-0.507	***	-0.485	***	-0.423	***	-0.344	**	-0.204	*			-0.329	*			-0.023	ns			-0.030	ns	0.014	ns	-0.305	*
Post First Phase Insulin Response	0.165	ns	0.173	ns	0.197	ns			0.176	ns									0.069	ns					-0.172	ns		
Post C-Peptide	0.331	**	0.485	***	0.476	***	0.373	***	0.433	***	0.260	*	0.289	*	0.399	***			0.033	ns			0.045	ns			0.277	*
Post Insulin to C-peptide Ratio	0.284	*	0.405	***	0.356	***	0.339	**	0.060	ns					0.252	*			0.014	ns			0.028	ns	-0.084	ns		

HFMUFA diet (Diet B) Post-Intervention

	Body Weight		BMI		Waist		Hip		Dias BP		Sys BP		Body Fat %		Body Fat kg		Lean Body Mass%		Lean Body Mass kg		Body Water%		Body Water L		Impedance		Change Body Weight	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.256	*	-0.322	*	-0.354	***	-0.102	ns	-0.189	ns	-0.095	ns			-0.196	ns			-0.122	ns			-0.092	ns	0.310	*		
Post AIRg	0.109	ns	0.106	ns	0.144	ns	-0.023	ns	-0.024	ns							0.017	ns	0.068	ns			0.021	ns	0.017	ns		
Post DI																	0.121	ns	0.077	ns	0.149	ns						
Post G0	0.196	ns	0.227	*	0.141	ns	0.165	ns	0.303	*			0.113	ns	0.203	ns	-0.008	ns									0.045	ns
Post P2			-0.180	ns	-0.245	*									-0.115	ns									0.324	*		
Post P3	-0.263	*	-0.310	*	-0.383	***			-0.134	ns	-0.151	ns			-0.174	ns			-0.143	n					0.405	*		
Post Fasting Glucose	0.148	ns	0.149	ns	0.135	ns			0.225	*	0.189	*													-0.207	ns		
Post Fasting Insulin	0.386	***	0.437	***	0.384	***	0.296	*	0.175	ns					0.242	*			0.298	*			0.248	*			0.101	ns
Post HOMA-IR	0.406	***	0.450	***	0.399	***	0.302	**	0.224	*	0.020	ns			0.260	*			0.302	*			0.258	*	-0.258	*	0.119	ns
Post QUICKI	-0.352	***	-0.412	***	-0.370	***	-0.266	*	-0.269	*	-0.060	ns			-0.260	*			-0.302	*			-0.258	*	0.258	*	-0.094	ns
Post First Phase Insulin Response	0.099	ns	0.093	ns	0.124	ns			0.063	ns									0.071	n					0.051	ns		
Post C-Peptide	0.321	**	0.484	***	0.346	***	0.297	*	0.126	ns	0.002	ns	0.224	*	0.377	***			0.147	n			0.117	ns			0.175	ns
Post Insulin to C-peptide Ratio	0.324	**	0.283	*	0.314	**	0.269	*	0.104	ns					0.041	ns			0.326	**			0.277	*	-0.378	**		

LF diet (Diet C) Post-Intervention

	Body Weight		BMI		Waist		Hip		Dias BP		Sys BP		Body Fat %		Body Fat kg		Lean Body Mass%		Lean Body Mass kg		Body Water%		Body Water L		Impedance		Change Body Weight	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.493	***	-0.381	***	-0.482	***	-0.110	ns	-0.087	ns	-0.005	ns			-0.229	*			-0.450	***			-0.441	***	0.358	*		
Post AIRg	0.224	*	0.169	ns	0.177	ns	0.128	ns	0.290	*							0.208	ns	0.333	*			0.312	**	-0.263	*		
Post DI																	0.125	ns	0.065	ns	0.077	ns						
Post G0	-0.032	ns	0.126	ns	0.020	ns	0.061	ns	-0.114	ns			0.273	*	0.167	ns	-0.279	*									0.292	*
Post P2			-0.086	ns	-0.093	ns									-0.092	ns									0.121	ns		
Post P3	-0.377	***	-0.276	*	-0.355	**			-0.024	ns	0.057	ns			-0.190	ns			-0.348	*					0.228	ns		
Post Fasting Glucose	0.003	ns	0.055	ns	0.069	ns			-0.146	ns	-0.004	ns													-0.093	ns		
Post Fasting Insulin	0.444	***	0.329	**	0.408	***	0.226	*	0.154	ns					0.307	*			0.394	***			0.397	***			0.092	ns
Post HOMA-IR	0.430	***	0.343	***	0.421	***	0.221	*	0.144	ns	0.063	ns			0.288	*			0.375	***			0.384	***	-0.168	ns	0.100	ns
Post QUICKI	-0.430	***	-0.343	***	-0.421	***	-0.221	*	-0.144	ns	-0.063	ns			-0.288	*			-0.375	***			-0.384	***	0.168	ns	-0.100	ns
Post First Phase Insulin Response	0.272	*	0.185	ns	0.221	*			0.262	*									0.338	**					-0.230	*		
Post C-Peptide	0.360	***	0.311	**	0.397	***	0.202	*	0.152	ns	0.045	ns	0.062	ns	0.279	*			0.314	*			0.333	**			0.187	ns
Post Insulin to C-peptide Ratio	0.370	***	0.274	*	0.321	**	0.198	*	0.112	ns					0.267	*			0.333	**			0.326	*	-0.142	ns		

LF n-3PUFA diet (Diet D) Post-Intervention

	Body Weight		BMI		Waist		Hip		Dias BP		Sys BP		Body Fat %		Body Fat kg		Lean Body Mass%		Lean Body Mass kg		Body Water%		Body Water L		Impedance		Change Body Weight	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.090	ns	-0.199	ns	-0.322	*	-0.125	ns	-0.266	*	-0.257	*			0.028	ns			-0.013	ns			0.098	ns	0.096	ns		
Post AIRg	0.230	*	0.071	ns	0.305	*	-0.020	ns	0.055	ns							0.276	*	0.367	*			0.226	ns	-0.392	*		
Post DI																	0.230	ns	0.306	*	0.194	ns						
Post G0	0.147	ns	0.225	*	0.203	ns	0.185	ns	0.008	ns			0.225	ns	0.237	*	-0.168	ns									0.102	ns
Post P2			-0.101	ns	-0.091	ns									-0.182	ns									0.030	ns		
Post P3	-0.059	ns	-0.168	ns	-0.244	*			-0.237	*	-0.333	*			-0.101	ns			0.052	ns					0.034	ns		
Post Fasting Glucose	0.054	ns	0.022	ns	-0.010	ns			0.072	ns	0.165	ns													-0.198	ns		
Post Fasting Insulin	0.193	ns	0.356	***	0.388	***	0.250	*	0.245	*					0.066	ns			0.026	ns			-0.061	ns			0.160	ns
Post HOMA-IR	0.170	ns	0.313	*	0.346	**	0.223	*	0.261	*	0.247	*			0.045	ns			0.001	ns			-0.071	ns	-0.036	ns	0.123	ns
Post QUICKI	-0.170	ns	-0.313	*	-0.346	**	-0.223	*	-0.261	*	-0.247	*			-0.045	ns			-0.001	ns			0.071	ns	0.036	ns	-0.123	ns
Post First Phase Insulin Response	0.139	ns	0.079	ns	0.203	*			-0.029	ns									0.187	ns					-0.141	ns		
Post C-Peptide	0.260	*	0.290	*	0.404	***	0.237	*	0.279	*	0.200	ns	-0.018	ns	0.126	ns			0.167	ns			0.097	ns			0.217	*
Post Insulin to C-peptide Ratio	0.139	ns	0.388	***	0.327	**	0.270	*	0.187	ns					0.103	ns			-0.085	ns			-0.137	ns	-0.024	ns		

r correlation coefficient
***$p < 0.0005$
**$p < 0.001$
*$p < 0.05$
ns non significant

Table 6.10a: Correlation coefficients between IVGTT parameters and inflammatory cytokines, post-intervention of the 12 week dietary study, irrespective of dietary treatment

	Post-Intervention IVGTT parameters																							
	S_I		S_g		AIR_g		DI		G0		P2		P3		Fasting Glucose		Fasting Insulin		HOMA-IR		QUICKI		FPIR	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post CRP	-0.087	ns	-0.026	ns	0.079	ns	0.260	ns	0.036	ns	0.019	ns	-0.017	ns	-0.013	ns	0.103	*	0.093	ns	-0.089	ns	0.070	ns
Post IL-6	0.002	ns	-0.056	ns	0.083	ns	0.060	ns	-0.029	ns	0.007	ns	0.016	ns	0.002	ns	0.089	ns	0.077	ns	-0.083	ns	0.036	ns
Post TNFα	-0.078	ns	-0.028	ns	-0.061	ns	-0.091	ns	0.097	ns	0.089	ns	0.028	ns	0.130	*	0.116	**	0.135	*	-0.122	*	-0.055	ns
Post sICAM-1	-0.178	**	0.015	ns	0.049	ns	-0.085	ns	0.037	ns	0.059	ns	-0.059	ns	0.042	ns	0.164	**	0.163	**	-0.151	*	0.031	ns
Post sVCAM-1	-0.038	ns	-0.103	ns	0.006	ns	-0.043	ns	-0.095	ns	0.018	ns	-0.030	ns	-0.038	ns	0.067	ns	0.058	ns	-0.071	ns	0.022	ns
Post Resistin	-0.047	s	0.086	ns	0.030	ns	0.014	ns	0.101	ns	0.022	ns	-0.019	ns	0.038	ns	0.078	ns	0.080	ns	-0.072	ns	0.039	ns
Post Adiponectin	0.116	*	0.059	ns	-0.148	*	-0.046	ns	0.080	ns	0.017	ns	0.081	ns	0.050	ns	-0.117	*	-0.100	*	0.109	*	-0.064	ns
Post Leptin	-0.177	**	-0.046	ns	-0.018	ns	-0.148	*	0.197	**	-0.053	ns	-0.129	*	0.086	ns	0.273	***	0.271	***	-0.264	***	0.037	ns

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant

Table 6.10b: Correlations between post-intervention CRP and IVGTT parameters across the dietary treatments

	Post-Intervention CRP							
	HFSFA diet (Diet A)		HFMUFA diet (Diet B)		LF diet (Diet C)		LFn-3PUFA diet (Diet D)	
	r		*r*		*r*		*r*	
Post Fasting Insulin	0.214	*	0.228	*	-0.076	ns	0.045	ns

ns (non significant),* (p<0.05), ** (p<0.001), *** (p<0.0005)

Table 6.10c: Correlations between post-intervention TNFa and IVGTT parameters across the dietary treatments

	Post-Intervention TNFα							
	HFSFA diet (Diet A)		HFMUFA diet (Diet B)		LF diet (Diet C)		LFn-3PUFA diet (Diet D)	
	r		*r*		*r*		*r*	
Post Fasting Insulin	0.124	ns	0.105	ns	0.06	ns	-0.189	*
Post Fasting Glucose	0.11	ns	0.146	ns	0.012	ns	0.208	*
Post HOMA-IR	0.137	ns	0.138	ns	0.042	ns	0.229	*
Post QUICKI	-0.137	ns	-0.087	ns	-0.042	ns	-0.229	*

ns (non significant),* (p<0.05), ** (p<0.001), *** (p<0.0005)

Table 6.10d: Correlations between post-intervention sICAM and IVGTT parameters across the dietary treatments

	Post-Intervention sICAM							
	HFSFA diet (Diet A)		HFMUFA diet (Diet B)		LF diet (Diet C)		LFn-3PUFA diet (Diet D)	
	r		*r*		*r*		*r*	
Post S_I	-0.184	ns	0.000	ns	-0.298	*	-0.201	ns
Post Fasting Insulin	0.231	*	0.082	s	0.202	*	0.144	ns
Post HOMA-IR	0.26	*	0.082	ns	0.225	*	0.088	ns
Post QUICKI	-0.26	ns	-0.037	ns	0.225	*	-0.088	ns

ns (non significant),* (p<0.05), ** (p<0.001), *** (p<0.0005)

Table 6.10e: Correlations between post-intervention Adiponectin and IVGTT parameters across the dietary treatments

	Post-Intervention Adiponectin							
	HFSFA diet (Diet A)		HFMUFA diet (Diet B)		LF diet (Diet C)		LFn-3PUFA diet (Diet D)	
	r		*r*		*r*		*r*	
Post Fasting Insulin	0.04	ns	-0.127	ns	0.194	*	-0.088	ns
Post HOMA-IR	0.058	ns	-0.155	ns	-0.155	ns	-0.197	ns
Post QUICKI	-0.058	ns	0.144	ns	0.155	ns	0.197	ns

ns (non significant),* (p<0.05), ** (p<0.001), *** (p<0.0005)

Table 6.10f: Correlations between post-intervention Leptin and IVGTT parameters across the dietary treatments

	Post-Intervention Leptin							
	HFSFA diet (Diet A)		HFMUFA diet (Diet B)		LF diet (Diet C)		LFn-3PUFA diet (Diet D)	
	r		*r*		*r*		*r*	
Post S_I	-0.242	*	0.2	ns	-0.077	ns	-0.127	ns
Post G0	0.175	ns	0.284	*	0.192	ns	0.131	ns
Post P3	-0.117	ns	-0.064	ns	-0.159	ns	-0.156	ns
Post DI	-0.214	ns	-0.15	ns	-0.098	ns	-0.116	ns
Post HOMA-IR	0.398	***	0.227	*	0.23	*	0.203	ns
Post QUICKI	-0.398	***	-0.204	*	-0.23	*	-0.203	ns

ns (non significant),* (p<0.05), ** (p<0.001), *** (p<0.0005)

Table 6.11a: Correlation coefficients between IVGTT parameters and markers of coagulation, post-intervention of the 12 week dietary study, irrespective of dietary treatment

	PAI-1		tPA		Fibrinogen	
	r		*r*		*r*	
Post S_I	-0.248	***	-0.337	***	0.042	ns
Post S_G	0.051	ns	0.027	ns	-0.061	ns
Post AIRg	-0.059	ns	0.069	ns	0.049	ns
Post DI	-0.210	***	-0.167	*	0.080	ns
Post G0	0.278	***	0.202	***	-0.031	ns
Post P2	-0.122	*	-0.102	ns	0.045	ns
Post P3	-0.235	***	-0.276	***	0.077	ns
Post Fasting Glucose	0.271	***	0.209	***	-0.124	*
Post Fasting Insulin	0.300	***	0.350	***	0.014	ns
Post HOMA-IR	0.341	***	0.375	***	-0.009	ns
Post QUICKI	-0.328	***	-0.363	***	0.011	ns
Post First Phase Insulin Response	-0.030	ns	0.061	ns	0.045	ns
Post C-Peptide	0.381	***	0.407	***	0.037	ns
Post Insulin to C-peptide Ratio	0.162	**	0.207	***	-0.001	ns

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant

Table 6.11b: Correlation coefficients between post-intervention IVGTT parameters and post-intervention markers of coagulation within each of the dietary treatments

	HFSFA diet (Diet A)				HFMUFA diet (Diet B)				LF diet (Diet C)				LFn-3PUFA diet (Diet D)			
	Post PAI-1		Post tPA		Post PAI-1		Post tPA		Post PAI-1		Post tPA		Post PAI-1		Post tPA	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.194	ns	-0.570	***	-0.283	*	-0.219	*	-0.225	*	-0.290	*	-0.230	*	-0.297	*
Post DI	-0.204	ns	-0.198	ns	-0.280	*	-0.194	ns	-0.130	ns	-0.151	ns	-0.181	ns	-0.114	ns
Post G0	0.236	*	0.214	ns	0.435	***	0.133	ns	0.269	*	0.147	ns	0.203	ns	0.333	*
Post P2	-0.078	ns			-0.169	ns			0.023	ns			-0.223	ns		
Post P3	-0.233	*	-0.327	*	-0.272	*	-0.242	*	-0.160	ns	-0.281	*	-0.242	*	-0.256	*
Post Fasting Glucose	0.269	*	0.329	**	0.330	***	0.112	ns	0.158	ns	0.202	*	0.296	*	0.210	*
Post Fasting Insulin	0.317	*	0.486	***	0.396	***	0.321	**	0.284	*	0.334	***	0.218	*	0.274	*
Post HOMA-IR	0.342	**	0.508	***	0.442	***	0.328	***	0.293	*	0.372	***	0.278	*	0.310	*
Post QUICKI	-0.342	**	-0.508	***	-0.393	***	-0.285	*	-0.293	*	-0.372	***	-0.278	*	-0.310	*
Post C-Peptide	0.352	***	0.459	***	0.467	***	0.359	***	0.358	***	0.436	***	0.371	***	0.377	***
Post Insulin to C-peptide Ratio	0.164	ns	0.370	***	0.219	*	0.168	ns	0.160	ns	0.185	ns	0.107	ns	0.156	ns

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant

Table 6.12a: Correlation coefficients between IVGTT parameters and lipid fractions and apolipoproteins, post-intervention of the 12 week dietary study, irrespective of dietary treatment

	Post-Intervention Lipids and Apolipoproteins																													
	TAG		Cholesterol		NEFA		TRL TAG		TRL Cholesterol		LDL		Total HDL		Apo A1		Apo B		Apo CII		Apo CIII		Apo E		Apo B48		TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.181	**	0.019	ns	-0.039	ns	-0.125	*	0.039	ns	-0.043	ns	0.179	**	0.108	ns	-0.082	ns	-0.031	ns	-0.086	ns	-0.139	*	0.011	ns	0.056	ns
Post S_G	-0.013	ns	0.041	ns	-0.127	*	0.072	ns	0.007	ns	-0.030	ns	0.055	ns	0.106	ns	0.044	ns	0.018	ns	0.074	ns	0.010	ns	0.023	ns	-0.111	ns
Post AIRg	0.029	ns	-0.090	ns	-0.037	ns	0.045	ns	0.057	ns	-0.042	ns	-0.149	*	-0.136	*	-0.039	ns	-0.039	ns	-0.008	ns	-0.117	*	-0.134	*	0.063	ns
Post DI	-0.095	ns	-0.086	ns	-0.071	ns	-0.031	ns	0.025	ns	-0.075	ns	-0.046	ns	-0.086	ns	-0.083	ns	-0.066	ns	-0.063	ns	-0.200	***	-0.105	ns	0.082	ns
Post G0	-0.052	ns	0.030	ns	-0.062	ns	0.026	ns	-0.075	ns	-0.072	ns	0.057	ns	0.088	ns	0.058	ns	-0.064	ns	0.065	ns	0.037	ns	0.010	ns	-0.174	*
Post P2	-0.046	ns	0.021	ns	-0.096	ns	-0.016	ns	-0.005	ns	0.017	ns	0.065	ns	0.022	ns	0.005	ns	-0.003	ns	-0.010	ns	-0.077	ns	-0.042	ns	0.023	ns
Post P3	-0.169	*	0.035	ns	-0.106	ns	-0.118	*	-0.058	ns	-0.010	ns	0.163	*	0.075	ns	-0.055	ns	-0.039	ns	-0.074	ns	-0.142	*	-0.004	ns	0.042	ns
Post Fasting Glucose	0.016	ns	0.022	ns	-0.134	*	-0.010	ns	-0.039	ns	0.010	ns	-0.040	ns	0.013	ns	0.087	ns	0.016	ns	0.082	ns	0.076	ns	0.071	ns	-0.169	**
Post Fasting Insulin	0.271	***	-0.006	ns	-0.014	ns	0.269	***	0.181	***	0.034	ns	-0.215	***	-0.052	ns	0.094	ns	0.052	ns	0.101	*	0.129	*	0.063	ns	0.075	ns
Post HOMA-IR	0.243	***	-0.019	ns	-0.048	ns	0.232	***	-0.092	ns	0.021	ns	-0.205	***	-0.048	ns	0.085	ns	0.030	ns	0.094	ns	0.123	*	0.058	ns	0.028	ns
Post QUICKI	-0.249	***	0.018	ns	0.049	ns	-0.239	***	-0.144	*	-0.021	ns	0.199	***	0.049	ns	-0.081	ns	-0.036	ns	-0.102	*	-0.123	*	-0.073	ns	-0.038	ns
Post First Phase Insulin Response	0.071	ns	-0.034	ns	-0.028	ns	0.091	ns	0.107	*	-0.021	ns	-0.127	*	-0.113	*	0.013	ns	0.002	ns	0.053	ns	-0.073	ns	-0.069	ns	0.096	ns
Post C-Peptide	0.263	***	0.065	ns	0.014	ns	0.266	***	0.207	***	0.066	ns	-0.183	***	-0.056	ns	0.156	*	0.101	*	0.165	**	0.136	*	0.053	ns	0.084	ns
Post Insulin to C-peptide Ratio	0.180	***	-0.060	ns	-0.032	ns	0.170	**	0.081	ns	-0.002	ns	-0.164	**	-0.029	ns	0.009	ns	0.008	ns	0.016	ns	0.092	ns	0.055	ns	0.012	ns

r correlation coefficient

***$p<0.0005$

**$p<0.001$

*$p<0.05$

ns non significant

TAG, Triglycerides; NEFA, Non esterified fatty acids; TRL triglyceride rich lipid; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein

Table 6.12b: Correlation coefficients between post-intervention IVGTT parameters and post-intervention lipid concentrations within each of the dietary treatments

HFSFA diet (Diet A) Post-Intervention

	Post TAG		Post NEFA		Post TRL TAG		Post TRL Cholesterol		Post LDL		Post Total HDL		Post Apo A1		Post Apo B		Post Apo CII		Post Apo CIII		Post Apo E		Post Apo B48		Post TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.276	*	-0.141	ns	-0.270	*	0.074	ns	-0.051	ns	0.263	*	0.168	ns							-0.209	ns				
Post S_G			-0.128	ns																					-0.114	ns
Post AIRg											-0.113	ns	-0.006	ns							0.021	ns	-0.415	**		
Post DI	-0.077	ns																			-0.123	ns				
Post G0																									-0.293	*
Post P3	-0.208	ns			-0.233	*					0.273	*									-0.110	ns				
Post Fasting Glucose			-0.092	ns																					-0.156	ns
Post Fasting Insulin	0.292	*			0.321	*	0.246	*			-0.251	*							0.155	ns	0.177	ns				
Post HOMA-IR	0.243	*			0.285	*					-0.229	*									0.167	ns				
Post QUICKI	-0.243	*			-0.285	*	-0.196	ns			0.229	*							-0.163	ns	-0.167	ns				
Post First Phase Insulin Response							0.113	ns			-0.122	ns	-0.031	ns												
Post C-Peptide	0.246	*			0.302	*	0.252	*			-0.235	*			0.297	*	0.180	ns	0.154	ns	0.145	ns				
Post Insulin to C-peptide Ratio	0.217	*			0.240	*					-0.180	ns														

HFMUFA diet (Diet B) Post-Intervention

	Post TAG		Post NEFA		Post TRL TAG		Post TRL Cholesterol		Post LDL		Post Total HDL		Post Apo A1		Post Apo B		Post Apo CII		Post Apo CIII		Post Apo E		Post Apo B48		Post TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.098	ns	-0.047	ns	-0.082	ns	0.029	ns	0.067	ns	0.056	ns	0.091	ns							-0.126	ns				
Post S_G			-0.204	*																					-0.148	ns
Post AIRg											-0.027	ns	-0.037	ns							-0.171	ns	-0.167	ns		
Post DI	-0.199	ns																			-0.246	*				
Post G0																									-0.189	ns
Post P3	-0.224	*			-0.180	ns					0.211	*									-0.145	ns				
Post Fasting Glucose			-0.105	ns																					-0.218	*
Post Fasting Insulin	0.171	ns			0.267	*	0.211	*			-0.229	*							0.023	ns	0.093	ns				
Post HOMA-IR	0.197	*			0.265	*					-0.211	*									0.133	ns				
Post QUICKI	-0.222	*			-0.294	*	-0.227	*			0.184	ns							-0.080	ns	-0.136	ns				
Post First Phase Insulin Response							0.068	ns			-0.014	ns	-0.018	ns												
Post C-Peptide	0.235	*			0.249	*	0.245	*			-0.178	ns			0.089	ns	0.074	ns	0.125	ns	0.132	ns				
Post Insulin to C-peptide Ratio	0.071	ns			0.137	ns					-0.190	*														

LF diet (Diet C) Post-Intervention

	Post TAG		Post NEFA		Post TRL TAG		Post TRL Cholesterol		Post LDL		Post Total HDL		Post Apo A1		Post Apo B		Post Apo CII		Post Apo CIII		Post Apo E		Post Apo B48		Post TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.283	*	-0.035	ns	-0.213	*	0.036	ns	-0.112	ns	0.328	*	0.199	ns							-0.223	*				
Post S_G			-0.063	ns																					-0.115	ns
Post AIRg											-0.180	ns	-0.180	ns							-0.034	ns	0.019	ns		
Post DI	0.002	ns																			-0.149	ns				
Post G0																									-0.036	ns
Post P3	-0.226	*			-0.160	ns					0.228	*									-0.228	*				
Post Fasting Glucose			-0.027	ns																					-0.077	ns
Post Fasting Insulin	0.442	***			0.371	***	0.263	*			-0.272	*							0.240	*	0.038	ns				
Post HOMA-IR	0.396	***			0.307	*					-0.265	*									0.036	ns				
Post QUICKI	-0.396	***			-0.307	*	-0.204	*			0.265	*							-0.210	*	-0.036	ns				
Post First Phase Insulin Response							0.186	ns			-0.163	ns	-0.150	ns												
Post C-Peptide	0.350	***			0.337	**	0.241	*			-0.204	*			0.083	ns	0.087	ns	0.285	*	0.055	ns				
Post Insulin to C-peptide Ratio	0.285	*			0.239	*					-0.194	*														

LF n3 PUFA diet (Diet D) Post-Intervention

	Post TAG		Post NEFA		Post TRL TAG		Post TRL Cholesterol		Post LDL		Post Total HDL		Post Apo A1		Post Apo B		Post Apo CII		Post Apo CIII		Post Apo E		Post Apo B48		Post TRL Apo B	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.101	ns	0.045	ns	0.062	ns	0.034	ns	-0.104	ns	0.074	ns	-0.063	ns							-0.026	ns				
Post S_G			-0.119	ns																					-0.020	ns
Post AIRg											-0.275	*	-0.275	*							-0.225	*	-0.019	ns		
Post DI	-0.079	ns																			-0.205	ns				
Post G0																									-0.158	ns
Post P3	0.007	ns			0.157	ns					-0.082	ns									-0.065	ns				
Post Fasting Glucose			-0.278	*																					-0.259	*
Post Fasting Insulin	0.184	ns			0.097	ns	0.018	ns			-0.108	ns							0.022	ns	0.200	*				
Post HOMA-IR	0.131	ns			0.036	ns					-0.097	ns									0.166	ns				
Post QUICKI	-0.131	ns			-0.036	ns	0.056	ns			0.097	ns							0.028	ns	-0.166	ns				
Post First Phase Insulin Response							0.141	ns			-0.192	ns	-0.220	*												
Post C-Peptide	0.216	*			0.110	ns	0.057	ns			-0.084	ns			0.146	ns	0.058	ns	0.117	ns	0.203	*				
Post Insulin to C-peptide Ratio	0.136	ns			0.043	ns					-0.065	ns														

r correlation coefficient

***$p<0.0005$

**$p<0.001$

*$p<0.05$

ns non significant

TAG, Triglycerides; NEFA, Non esterified fatty acids; TRL triglyceride rich lipid; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein

Table 6.13a: Correlation coefficients between IVGTT parameters and dietary data, post-intervention of the 12 week dietary study, irrespective of dietary treatment

	Post-Intervention Dietary Variables																													
	Post Dietary Variables																													
	BMR		Total Energy MJ		Total fat(g)		Total fat%Energy		SAFA(g)		SAFA%Energy		MUFA(g)		MUFA%Energy		PUFA(g)		PUFA%Energy		n-3(g)		n-6(g)		C8:0(g)		C10:(g)		C12:(g)		C14:0(g)	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.260	***	-0.034	ns	-0.036	ns	-0.027	ns	-0.006	ns	0.010	ns	-0.030	ns	-0.013	ns	-0.009	ns	0.007	ns	-0.011	ns	-0.034	ns	-0.003	ns	-0.010	ns	0.053	ns	0.081	ns
Post S_G	-0.017	ns	-0.061	ns	-0.019	ns	0.003	ns	-0.025	ns	0.019	ns	0.018	ns	0.061	ns	0.008	ns	0.015	ns	-0.121	ns	-0.097	ns	-0.077	ns	-0.036	ns	-0.077	ns	-0.018	ns
Post AIRg	0.211	***	0.014	ns	0.000	ns	0.006	ns	0.008	ns	0.000	ns	-0.032	ns	-0.023	ns	-0.003	ns	-0.014	ns	-0.062	ns	-0.034	ns	-0.148	ns	-0.157	*	-0.145	*	-0.021	ns
Post DI	0.053	ns	0.001	ns	-0.010	ns	-0.006	ns	0.017	ns	0.017	ns	-0.040	ns	-0.021	ns	-0.005	ns	-0.022	ns	-0.075	ns	-0.066	ns	-0.126	ns	-0.085	ns	-0.138	*	0.029	ns
Post G0	0.047	ns	-0.095	ns	0.021	ns	0.122	*	0.021	ns	0.104	ns	0.030	ns	0.112	*	-0.012	ns	0.035	ns	-0.181	*	-0.063	ns	-0.084	ns	-0.034	ns	-0.096	ns	-0.007	ns
Post P2	-0.069	ns	0.054	ns	0.050	ns	-0.003	ns	0.036	ns	0.003	ns	0.031	ns	-0.007	ns	0.024	ns	-0.043	ns	0.024	ns	0.125	ns	0.069	ns	0.059	ns	0.053	ns	0.100	ns
Post P3	-0.217	***	0.010	ns	-0.001	ns	-0.023	ns	0.006	ns	-0.001	ns	-0.008	ns	-0.020	ns	0.009	ns	-0.013	ns	0.012	ns	0.060	ns	0.027	ns	0.037	ns	0.076	ns	0.114	ns
Post Fasting Glucose	0.101	*	0.033	ns	0.060	ns	0.057	ns	0.041	ns	0.030	ns	0.084	ns	0.063	ns	0.023	ns	-0.012	ns	0.036	ns	0.057	ns	0.025	ns	0.045	ns	-0.006	ns	-0.037	ns
Post Fasting Insulin	0.269	***	-0.037	ns	-0.018	ns	0.052	ns	0.016	ns	0.067	ns	-0.043	ns	0.016	ns	-0.075	ns	-0.031	ns	0.044	ns	0.030	ns	-0.077	ns	0.000	ns	-0.145	*	-0.014	ns
Post HOMA-IR	0.268	***	-0.024	ns	-0.008	ns	0.055	ns	0.020	ns	0.063	ns	-0.025	ns	0.022	ns	-0.074	ns	-0.045	ns	0.049	ns	0.036	ns	-0.066	ns	0.017	ns	-0.135	*	-0.022	ns
Post QUICKI	-0.255	***	0.027	ns	0.005	ns	-0.062	ns	-0.017	ns	-0.060	ns	0.014	ns	-0.036	ns	0.079	ns	0.050	ns	-0.045	ns	-0.039	ns	0.083	ns	0.001	ns	0.151	*	0.033	ns
Post First Phase Insulin Response	0.183	***	0.023	ns	0.022	ns	0.031	ns	0.035	ns	0.040	ns	-0.018	ns	-0.012	ns	0.007	ns	-0.016	ns	-0.073	ns	-0.038	ns	-0.107	ns	-0.107	ns	-0.110	ns	0.042	ns
Post C-Peptide	0.199	***	-0.021	ns	0.029	ns	0.100	*	0.052	ns	0.114	*	0.014	ns	0.064	ns	-0.024	ns	0.005	ns	0.063	ns	0.063	ns	-0.035	ns	0.065	ns	-0.087	ns	0.031	ns
Post Insulin to C-peptide Ratio	0.238	***	-0.048	ns	-0.057	ns	-0.005	ns	-0.038	ns	-0.007	ns	-0.086	ns	-0.042	ns	-0.095	ns	-0.040	ns	-0.030	ns	-0.027	ns	-0.113	ns	-0.086	ns	-0.163	*	-0.084	ns

	C16:0(g)		C16:1(g)		C18:0(g)		C18:1(g)		C18:2:6(g)		C18:3:3(g)		C20:4:6(g)		C20:5:3(g)		C22:6:3(g)		Trans fat (g)		CHO(g)		CHO%Energy		Starch(g)		Total Sugars(g)		Protein (g)		Protein%Energy	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	0.024	ns	-0.011	ns	0.025	ns	0.028	ns	0.024	ns	0.026	ns	0.050	ns	-0.032	ns	-0.016	ns	-0.073	ns	-0.004	ns	0.080	ns	-0.101	ns	-0.026	ns	-0.117	*	-0.120	*
Post S_G	-0.035	ns	-0.032	ns	-0.075	ns	-0.009	ns	-0.052	ns	-0.021	ns	0.011	ns	-0.019	ns	-0.001	ns	0.091	ns	-0.081	ns	-0.011	ns	-0.042	ns	-0.109	ns	-0.088	ns	-0.068	ns
Post AIRg	-0.067	ns	-0.072	ns	-0.055	ns	-0.082	ns	-0.042	ns	-0.056	ns	0.056	ns	-0.081	ns	-0.066	ns	-0.019	ns	0.004	ns	-0.036	ns	0.023	ns	-0.026	ns	0.036	ns	0.002	ns
Post DI	-0.046	ns	-0.077	ns	-0.041	ns	-0.070	ns	-0.045	ns	-0.065	ns	0.093	ns	-0.109	ns	-0.078	ns	-0.048	ns	0.004	ns	0.005	ns	-0.023	ns	-0.029	ns	-0.032	ns	-0.071	ns
Post G0	0.020	ns	0.013	ns	-0.013	ns	0.006	ns	-0.061	ns	-0.071	ns	-0.016	ns	-0.043	ns	-0.051	ns	0.066	ns	-0.139	*	-0.096	ns	-0.082	ns	-0.095	ns	-0.140	*	-0.100	ns
Post P2	0.093	ns	0.081	ns	0.106	ns	0.078	ns	0.043	ns	-0.014	ns	0.100	ns	0.041	ns	0.034	ns	0.074	ns	0.044	ns	-0.003	ns	-0.069	ns	0.198	*	0.059	ns	0.001	ns
Post P3	0.066	ns	0.018	ns	0.068	ns	0.055	ns	0.043	ns	0.008	ns	0.111	ns	0.030	ns	0.039	ns	0.012	ns	0.028	ns	0.046	ns	-0.109	ns	0.103	ns	-0.037	ns	-0.075	ns
Post Fasting Glucose	0.118	*	0.210	***	0.091	ns	0.119	*	0.050	ns	0.062	ns	0.033	ns	0.055	ns	0.070	ns	0.061	ns	-0.021	ns	-0.073	ns	0.041	ns	-0.054	ns	0.054	ns	0.013	ns
Post Fasting Insulin	0.024	ns	0.094	ns	0.032	ns	-0.056	ns	-0.062	ns	-0.119	*	0.145	*	0.103	ns	0.104	ns	0.021	ns	-0.068	ns	-0.131	*	0.024	ns	-0.040	ns	0.061	ns	0.167	**
Post HOMA-IR	0.040	ns	0.124	*	0.044	ns	-0.032	ns	-0.060	ns	-0.110	*	0.136	*	0.114	*	0.116	*	0.022	ns	-0.060	ns	-0.133	*	0.038	ns	-0.046	ns	0.080	ns	0.173	***
Post QUICKI	-0.039	ns	-0.123	*	-0.038	ns	0.018	ns	0.063	ns	0.110	*	-0.135	ns	-0.098	ns	-0.100	ns	0.010	ns	0.064	ns	0.137	*	-0.029	ns	0.050	ns	-0.080	ns	-0.175	***
Post First Phase Insulin Response	-0.009	ns	-0.022	ns	-0.011	ns	-0.049	ns	-0.032	ns	-0.075	ns	0.115	ns	-0.065	ns	-0.043	ns	-0.049	ns	0.006	ns	-0.056	ns	0.010	ns	-0.020	ns	0.026	ns	-0.006	ns
Post C-Peptide	0.063	ns	0.146	*	0.048	ns	0.017	ns	-0.003	ns	-0.044	ns	0.145	*	0.069	ns	0.107	ns	-0.017	ns	-0.074	ns	-0.143	*	0.017	ns	-0.038	ns	0.021	ns	0.055	ns
Post Insulin to C-peptide Ratio	-0.062	ns	-0.027	ns	-0.035	ns	-0.124	*	-0.117	*	-0.147	*	0.049	ns	0.023	ns	-0.007	ns	0.019	ns	-0.046	ns	-0.076	ns	0.017	ns	-0.052	ns	0.064	ns	0.191	***

	Alcohol(g)		Alcohol%Energy		Fibre(g)		Vitamin E		Energy Kcals	
	r		r		r		r		r	
Post S_I	0.044	ns	0.044	ns	0.082	ns	0.026	ns	-0.034	ns
Post S_G	0.032	ns	0.034	ns	-0.047	ns	0.059	ns	-0.061	ns
Post AIRg	0.006	ns	0.006	ns	-0.162	*	-0.014	ns	0.014	ns
Post DI	0.033	ns	0.032	ns	-0.111	*	0.032	ns	0.001	ns
Post G0	0.055	ns	0.060	ns	-0.079	ns	0.054	ns	-0.095	ns
Post P2	0.002	ns	0.012	ns	0.031	ns	0.074	ns	0.054	ns
Post P3	0.033	ns	0.041	ns	0.064	ns	0.056	ns	0.010	ns
Post Fasting Glucose	0.118	*	0.115	*	0.062	ns	-0.080	ns	0.033	ns
Post Fasting Insulin	-0.041	ns	-0.043	ns	-0.105	*	0.002	ns	-0.037	ns
Post HOMA-IR	-0.023	ns	-0.026	ns	-0.086	ns	-0.014	ns	-0.024	ns
Post QUICKI	0.030	ns	0.033	ns	0.080	ns	0.019	ns	0.027	ns
Post First Phase Insulin Response	-0.014	ns	-0.016	ns	-0.182	***	0.007	ns	0.023	ns
Post C-Peptide	0.026	ns	0.028	ns	-0.100	*	0.039	ns	-0.021	ns
Post Insulin to C-peptide Ratio	-0.090	ns	-0.095	ns	-0.110	*	-0.022	ns	-0.048	ns

r correlation coefficient
***$p < 0.0005$
**$p < 0.001$
*$p < 0.05$
ns non significant
BMR Basal Metabolic Rate; SAFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids

Table 6.13b: Correlation coefficients between post-intervention IVGTT parameters and post-intervention dietary variables within each of the dietary treatments

HFSFA diet (Diet A) Post Dietary Variables

	BMR		Total fat%Energy		SAFA%Energy		MUFA%Energy		n-3 (g)		C10:0 (g)		C12:0 (g)		C16:0(g)		C16:1(g)		C18:1(g)		C18:2:6(g)		C18:3:3(g)		C20:4:6(g)		C20:5:3(g)		C22:6:3(g)	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.184	ns																												
Post AIRg	0.067	ns									-0.265	ns	-0.372	ns																
Post DI													-0.221	ns																
Post G0			0.134	ns			0.141	ns	-0.202	ns																				
Post P2																														
Post P3	-0.143	ns																												
Post Fasting Glucose	0.212	*													0.045	ns	0.017	ns	0.133	ns										
Post Fasting Insulin	0.128	ns											-0.308	*									-0.148	ns	0.124	0.412				
Post HOMA-IR	0.154	ns											-0.253	*			0.115	ns					-0.113	ns	0.090	0.551	0.130	ns	0.131	ns
Post QUICKI	-0.154	ns											0.253	*			-0.115	ns					0.113	ns	-0.090	0.551				
Post First Phase Insulin Response	0.086	ns																												
Post C-Peptide	0.153	ns			-0.029	ns											0.103	ns							0.066	0.668				
Post Insulin to C-peptide Ratio	0.088	ns											-0.187	ns					-0.068	ns	-0.015	ns	-0.074	ns						

HFMUFA diet (Diet B) Post Dietary Variables

	BMR		Total fat%Energy		SAFA%Energy		MUFA%Energy		n-3 (g)		C10:0 (g)		C12:0 (g)		C16:0(g)		C16:1(g)		C18:1(g)		C18:2:6(g)		C18:3:3(g)		C20:4:6(g)		C20:5:3(g)		C22:6:3(g)	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.227	*																												
Post AIRg	0.106	ns									-0.236	ns	-0.055	ns																
Post DI													-0.027	ns																
Post G0			-0.009	ns			0.079	ns	-0.333	*																				
Post P2																														
Post P3	-0.247	*																												
Post Fasting Glucose	0.123	ns													0.139	ns	0.287	*	0.087	ns										
Post Fasting Insulin	0.301	*											-0.153	ns									-0.158	ns	0.123	ns				
Post HOMA-IR	0.315	**											-0.189	ns			0.222	*					-0.150	ns	0.112	ns	-0.033	ns	0.026	ns
Post QUICKI	-0.266	*											0.254	*			-0.206	ns					0.160	ns	-0.083	ns				
Post First Phase Insulin Response	0.102	ns																												
Post C-Peptide	0.157	ns			0.276	*											0.259	*							0.042	ns				
Post Insulin to C-peptide Ratio	0.335	***											-0.133	ns					-0.201	ns	-0.198	ns	-0.240	*						

LF diet (Diet C) Post Dietary Variables

	BMR		Total fat%Energy		SAFA%Energy		MUFA%Energy		n-3 (g)		C10:0 (g)		C12:0 (g)		C16:0(g)		C16:1(g)		C18:1(g)		C18:2:6(g)		C18:3:3(g)		C20:4:6(g)		C20:5:3(g)		C22:6:3(g)	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.475	***																												
Post AIRg	0.300	*									-0.036	ns	-0.084	ns																
Post DI													-0.107	ns																
Post G0			0.181	ns			0.079	ns	-0.222	ns																				
Post P2																														
Post P3	-0.380	***																												
Post Fasting Glucose	0.015	ns													0.095	ns	0.198	ns	0.151	ns										
Post Fasting Insulin	0.406	***											-0.070	ns									-0.154	ns	0.107	ns				
Post HOMA-IR	0.393	***											-0.079	ns			-0.002	ns					-0.149	ns	0.127	ns	0.155	ns	0.073	ns
Post QUICKI	-0.393	***											0.079	ns			0.002	ns					0.149	ns	-0.127	ns				
Post First Phase Insulin Response	0.352	***																												
Post C-Peptide	0.291	*			0.152	ns											0.026	ns							0.118	ns				
Post Insulin to C-peptide Ratio	0.346	***											-0.211	ns					-0.235	*	-0.188	ns	-0.182	ns						

LFn3PUFA diet (Diet D) Post Dietary Variables

	BMR		Total fat%Energy		SAFA%Energy		MUFA%Energy		n-3 (g)		C10:0 (g)		C12:0 (g)		C16:0(g)		C16:1(g)		C18:1(g)		C18:2:6(g)		C18:3:3(g)		C20:4:6(g)		C20:5:3(g)		C22:6:3(g)	
	r		r		r		r		r		r		r		r		r		r		r		r		r		r		r	
Post S_I	-0.096	ns																												
Post AIRg	0.355	*									-0.172	ns	-0.205	ns																
Post DI													-0.255	ns																
Post G0			0.241	*			0.236	ns	-0.061	ns																				
Post P2																														
Post P3	-0.026	ns																												
Post Fasting Glucose	0.054	ns													0.193	ns	0.281	*	0.205	ns										
Post Fasting Insulin	0.229	*											-0.188	ns									-0.007	ns	0.267	ns				
Post HOMA-IR	0.196	ns											-0.168	ns			0.202	ns					-0.009	ns	0.254	ns	0.232	*	0.288	*
Post QUICKI	-0.196	ns											0.168	ns			-0.202	ns					0.009	ns	-0.254	ns				
Post First Phase Insulin Response	0.186	ns																												
Post C-Peptide	0.226	*			0.178	ns											0.300	*							0.358	*				
Post Insulin to C-peptide Ratio	0.170	ns											-0.318	*					-0.015	ns	-0.033	ns	-0.038	ns						

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant
BMR Basal Metabolic Rate; SAFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids

Table 6.13b continued

HFSFA diet (Diet A) Post Dietary Variables																				
	CHO(g)		CHO%Energy		Total Sugars(g)		Protein (g)		Protein%Energy		Alcohol(g)		Alcohol%Energy		Fibre(g)	
	r		r		r		r		r		r		r		r	
Post S_I							-0.077	s	-0.133	ns						
Post AIRg															-0.184	ns
Post DI															-0.192	ns
Post G0	-0.127	ns					-0.096	ns								
Post P2					0.016	ns										
Post P3																
Post Fasting Glucose											0.230	*	0.232	*		
Post Fasting Insulin			-0.216	*					0.136	ns					-0.152	ns
Post HOMA			-0.242	*					0.113	ns						
Post QUICKI			0.242	*					-0.113	ns						
Post First Phase Insulin Response															-0.231	*
Post C-Peptide			-0.107	ns											-0.129	ns
Post Insulin to C-peptide Ratio									0.170	ns					-0.109	ns

HFMUFA diet (Diet B) Post Dietary Variables																
	CHO(g)		CHO%Energy		Total Sugars(g)		Protein (g)		Protein%Energy		Alcohol(g)		Alcohol%Energy		Fibre(g)	
	r		r		r		r		r		r		r		r	
Post S_I							-0.169	ns	-0.108	ns						
Post AIRg															-0.076	ns
Post DI															0.072	ns
Post G0	-0.041	ns					-0.201	ns								
Post P2					0.348	*										
Post P3																
Post Fasting Glucose											-0.034	ns	-0.037	ns		
Post Fasting Insulin			-0.148	ns					0.295	*					-0.080	ns
Post HOMA			-0.161	ns					0.292	*						
Post QUICKI			0.157	ns					-0.305	**						
Post First Phase Insulin Response															-0.098	ns
Post C-Peptide			-0.129	ns											-0.009	ns
Post Insulin to C-peptide Ratio									0.412	***					-0.198	*

LF diet (Diet C) Post Dietary Variables																
	CHO(g)		CHO%Energy		Total Sugars(g)		Protein (g)		Protein%Energy		Alcohol(g)		Alcohol%Energy		Fibre(g)	
	r		r		r		r		r		r		r		r	
Post S_I							-0.217	*	-0.086	ns						
Post AIRg															-0.244	*
Post DI															-0.218	*
Post G0	-0.176	ns					-0.049	ns								
Post P2					0.191	ns										
Post P3																
Post Fasting Glucose											0.101	ns	0.105	ns		
Post Fasting Insulin			-0.090	ns					0.062	ns					-0.047	ns
Post HOMA			-0.093	ns					0.116	ns						
Post QUICKI			0.093	ns					-0.116	ns						
Post First Phase Insulin Response															-0.190	ns
Post C-Peptide			-0.166	ns											-0.048	ns
Post Insulin to C-peptide Ratio									0.011	ns					-0.061	ns

LFn3PUFA diet (Diet D) Post Dietary Variables																
	CHO(g)		CHO%Energy		Total Sugars(g)		Protein (g)		Protein%Energy		Alcohol(g)		Alcohol%Energy		Fibre(g)	
	r		r		r		r		r		r		r		r	
Post S_I							0.046	ns	-0.092	ns						
Post AIRg															-0.202	ns
Post DI															-0.160	ns
Post G0	-0.197	ns					-0.200	ns								
Post P2					0.199	ns										
Post P3																
Post Fasting Glucose											0.173	ns	0.169	ns		
Post Fasting Insulin			-0.154	ns					0.187	ns					-0.087	ns
Post HOMA			-0.149	ns					0.194	ns						
Post QUICKI			0.149	ns					-0.194	ns						
Post First Phase Insulin Response															-0.206	*
Post C-Peptide			-0.293	*											-0.163	ns
Post Insulin to C-peptide Ratio									0.202	*					-0.046	ns

r correlation coefficient
***$p<0.0005$
**$p<0.001$
*$p<0.05$
ns non significant
BMR Basal Metabolic Rate; SAFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids

Table 6.14a: Correlation coefficients between IVGTT parameters and plasma fatty acids (%), post-intervention of the 12 week dietary study, irrespective of dietary treatment

	Post-Intervention Plasma Fatty Acids (w/w %)																													
	C14:0		C16:0		C16:1		C18:0		C18:1		C18:2:6		C20:4:6		C20:5:3		C22:6:3		SFA		MUFA		PUFA		n-6		n-3	
	r		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*		*r*	
Post S_I	-0.057	ns	-0.105	ns	0.054	ns	-0.036	ns	-0.074	ns	0.140	*	0.068	ns	0.049	ns	-0.080	ns	-0.114	*	-0.053	ns	0.143	*	0.151	*	-0.040	ns
Post S_G	-0.023	ns	-0.046	ns	-0.030	ns	0.152	*	-0.016	ns	-0.003	ns	-0.027	ns	0.042	ns	0.037	ns	-0.012	ns	-0.040	ns	0.020	ns	-0.010	ns	0.021	ns
Post AIRg	0.019	ns	-0.002	ns	-0.021	ns	-0.059	ns	0.002	ns	0.094	ns	-0.015	ns	-0.060	ns	-0.106	ns	-0.001	ns	0.009	ns	0.065	ns	0.095	ns	-0.118	*
Post DI	-0.024	ns	-0.062	ns	0.008	ns	-0.056	ns	-0.045	ns	0.158	*	0.031	ns	-0.028	ns	-0.129	*	-0.071	ns	-0.036	ns	0.100	ns	0.147	*	-0.120	*
Post G0	0.025	ns	-0.043	ns	-0.071	ns	0.332	***	-0.057	ns	-0.047	ns	-0.072	ns	0.066	ns	0.058	ns	0.056	ns	-0.103	ns	-0.041	ns	-0.060	ns	0.031	ns
Post P2	-0.172	*	-0.034	ns	-0.010	ns	-0.010	ns	-0.105	ns	0.128	*	-0.011	ns	0.056	ns	0.038	ns	-0.108	*	-0.079	ns	0.143	*	0.131	*	0.036	ns
Post P3	-0.173	*	-0.092	ns	0.035	ns	-0.053	ns	-0.108	ns	0.177	**	0.052	ns	0.082	ns	0.006	ns	-0.147	*	-0.074	ns	0.198	***	0.188	**	0.016	ns
Post Fasting Glucose	-0.037	ns	0.103	*	-0.199	***	0.231	***	-0.046	ns	-0.168	**	-0.054	ns	0.072	ns	-0.170	**	0.173	**	-0.104	*	-0.099	*	-0.162	**	0.161	**
Post Fasting Insulin	0.132	*	0.085	ns	-0.014	ns	0.039	ns	0.096	ns	-0.142	*	-0.020	ns	-0.102	*	0.021	ns	0.127	*	0.088	ns	-0.167	**	-0.139	*	-0.034	ns
Post HOMA-IR	0.014	ns	0.100	*	-0.054	ns	0.081	ns	0.077	ns	-0.173	**	-0.027	ns	-0.071	ns	0.014	ns	0.154	*	0.057	ns	-0.177	***	-0.168	**	0.007	ns
Post QUICKI	-0.131	*	-0.095	ns	0.056	ns	-0.077	ns	-0.070	ns	0.159	**	0.018	ns	0.085	ns	-0.047	ns	-0.144	*	-0.051	ns	0.165	**	0.154	*	0.008	ns
Post First Phase Insulin Response	0.028	ns	-0.015	ns	0.011	ns	-0.061	ns	0.046	ns	0.085	ns	-0.026	ns	-0.067	ns	-0.142	*	-0.007	ns	0.057	ns	0.030	ns	0.081	ns	-0.139	*
Post C-Peptide	0.134	*	0.059	ns	-0.015	ns	0.027	ns	0.060	ns	-0.118	*	-0.094	ns	0.008	ns	0.074	ns	0.111	*	0.053	ns	-0.113	*	-0.132	*	0.055	ns
Post Insulin to C-peptide Ratio	0.123	*	0.051	ns	0.007	ns	0.050	ns	0.082	ns	-0.104	ns	0.033	ns	-0.137	*	-0.024	ns	0.098	*	0.079	ns	-0.130	*	-0.081	ns	-0.080	ns

r correlation coefficient

***$p<0.0005$

**$p<0.001$

*$p<0.05$

ns non significant

SFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids

Table 6.14b: Correlation coefficients between post-intervention IVGTT parameters and post-intervention plasma fatty acids(%) within each of the dietary treatments

HFSFA diet (Diet A) Post-intervention Plasma Fatty Acids(w/w%)

	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2:6	C20:5:3	C22:6:3	SFA	MUFA	PUFA	n6	n3
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I						0.272 *			-0.160 ns		0.265 *	0.325 *	
Post S_G				0.163 ns									
Post AIRg													0.125 ns
Post DI						0.186 ns		-0.004 ns				0.216 ns	-0.001 ns
Post G0				0.434 ***									
Post P2	-0.014 ns					-0.022 ns			0.114 ns		0.033 ns	-0.036 ns	
Post P3	-0.203 ns					0.163 ns			-0.024 ns		0.197 ns	0.189 ns	
Post Fasting Glucose		0.151 ns	-0.118 ns	0.300 *		-0.258 *		-0.079 ns	0.264 *	-0.069 ns	-0.193 ns	-0.249 *	0.187 ns
Post Fasting Insulin	0.107 ns				0.175 ns	-0.219 *	0.122 ns		0.092 ns		-0.195 ns	-0.245 *	
Post HOMA-IR		0.030 ns				-0.253 *			0.128 ns		-0.206 *	-0.266 *	
Post QUICKI	-0.132 ns					0.253 *			-0.128 ns		0.206 *	0.266 *	
Post First Phase Insulin Response								0.051 ns					0.063 ns
Post C-Peptide	0.145 ns					-0.186 ns			0.027 ns		-0.078 ns	-0.156 ns	
Post Insulin to C-peptide Ratio	0.141 ns					-0.163 ns	0.059 ns		0.133 ns		-0.195 ns		

HFMUFA diet (Diet B) Post-intervention Plasma Fatty Acids(w/w%)

	C14	C16	C16:1	C18	C18:1	C18:2:6	C20:5:3	C22:6:3	SFA	MUFA	PUFA	n6	n3
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I						-0.046 ns			-0.018 ns		0.025 ns	-0.020 ns	
Post S_G				0.019 ns									
Post AIRg													-0.237 *
Post DI						0.189 ns		-0.126 ns				0.176 ns	-0.162 ns
Post G0				0.271 *									
Post P2	-0.218 *					0.221 *			-0.371 ***		0.257 *	0.241 *	
Post P3	-0.251 *					0.135 ns			-0.238 *		0.230 *	0.173 ns	
Post Fasting Glucose		0.105 ns	-0.279 *	0.166 ns		-0.039 ns		-0.246 *	0.147 ns	-0.128 ns	-0.046 ns	-0.109 ns	0.182 ns
Post Fasting Insulin	0.205 *				-0.019 ns	-0.058 ns	-0.153 ns		0.090 ns		-0.093 ns	-0.036 ns	
Post HOMA-IR		0.047 ns				-0.067 ns			0.120 ns		-0.101 ns	-0.065 ns	
Post QUICKI	-0.157 ns					0.018 ns			-0.086 ns		0.052 ns	0.014 ns	
Post First Phase Insulin Response								-0.138 ns					-0.180 ns
Post C-Peptide	0.221 *					-0.057 ns			0.147 ns		-0.100 ns	-0.074 ns	
Post Insulin to C-peptide Ratio	0.186 ns					-0.033 ns	-0.188 ns		0.039 ns		-0.021 ns		

LF diet (Diet C) Post-intervention Plasma Fatty Acids(w/w%)

	C14	C16	C16:1	C18	C18:1	C18:2:6	C20:5:3	C22:6:3	SFA	MUFA	PUFA	n6	n3
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I						0.185 ns			-0.157 ns		0.192 ns	0.172 ns	
Post S_G				0.217 *									
Post AIRg													-0.200 ns
Post DI						0.104 ns		-0.228 *				0.048 ns	-0.235 *
Post G0				0.280 *									
Post P2	-0.264 *					0.193 ns			-0.100 ns		0.187 ns	0.152 ns	
Post P3	-0.239 *					0.289 *			-0.191 ns		0.273 *	0.238 *	
Post Fasting Glucose		0.045 ns	-0.173 ns	0.247 *		-0.252 *		-0.096 ns	0.089 ns	-0.080 ns	-0.109 ns	-0.175 ns	0.203 *
Post Fasting Insulin	0.167 ns				0.183 ns	-0.148 ns	-0.126 0.202		0.166 ns		-0.264 *	-0.167 ns	
Post HOMA-IR		0.182 ns				-0.186 ns			0.165 ns		-0.265 *	-0.184 ns	
Post QUICKI	-0.157 ns					0.186 ns			-0.165 ns		0.265 *	0.184 ns	
Post First Phase Insulin Response								-0.226 *					-0.269 *
Post C-Peptide	0.155 ns					-0.174 ns			0.116 ns		-0.242 *	-0.228 *	
Post Insulin to C-peptide Ratio	0.129 ns					-0.029 ns	-0.153 0.122		0.085 ns		-0.127 ns		

LFn3PUFA diet (Diet D) Post-intervention Plasma Fatty Acids(w/w%)

	C14	C16	C16:1	C18	C18:1	C18:2:6	C20:5:3	C22:6:3	SFA	MUFA	PUFA	n6	n3
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post S_I						0.222 ns			-0.174 ns		0.182 ns	0.199 ns	
Post S_G				0.232 *									
Post AIRg													-0.179 ns
Post DI				0.174 ns				-0.128 ns				0.128 ns	-0.047 ns
Post G0				0.276 *									
Post P2	-0.104 ns					0.086 ns			-0.017 ns		0.062 ns	0.121 ns	
Post P3	0.046 ns					0.128 ns			-0.101 ns		0.090 ns	0.135 ns	
Post Fasting Glucose		0.125 ns	-0.237 *	0.235 *		-0.155 ns		-0.200 ns	0.205 *	-0.124 ns	-0.082 ns	-0.132 ns	0.103 ns
Post Fasting Insulin	0.039 ns				0.009 ns	-0.194 ns	-0.292 *		0.163 ns		-0.147 ns	-0.150 ns	
Post HOMA-IR		0.125 ns				-0.207 *			0.189 ns		-0.148 ns	-0.164 ns	
Post QUICKI	-0.073 ns					0.207 *			-0.189 ns		0.148 ns	0.164 ns	
Post First Phase Insulin Response								-0.243 *					-0.178 ns
Post C-Peptide	-0.021 ns					-0.070 ns			0.165 ns		-0.059 ns	-0.072 ns	
Post Insulin to C-peptide Ratio	0.015 ns					-0.227 *	-0.295 *		0.105 ns		-0.196 ns		

r correlation coefficient

***$p<0.0005$

**$p<0.001$

*$p<0.05$

ns non significant

SFA saturated fatty acids; MUFA monounsaturated fatty acids;PUFA polyunsaturated fatty acids

6.4.3 Linear Regression Analysis

Table 6.15a presents the independent relationship of each variable to S_I measured at post-intervention, using univariate and multivariate regression analysis. Although many variables were significant predictors of post-intervention S_I, some failed to be significant in subsequent multiple linear regression analysis, controlling for age, gender and body weight post-intervention, for example, G0 pre-intervention, body fat (kg) pre- and post-intervention, systolic blood pressure post-intervention, TRL TAG and total HDL-cholesterol concentrations, post-intervention, plasma palmitic acid (C16:0), basal metabolic rate, total fat (g/day), MUFA (g/day), dietary caprylic acid (C8:0 g/day), dietary oleic acid (C18:1 g/day), Starch (g/day) analysed at pre-intervention and basal metabolic rate, starch and protein (g/day and % Energy) post-intervention. Interestingly the intervention diets had no predictive impact on S_I measured at post-intervention.

Table 6.15b shows seven separate models for which multiple linear regression analysis was performed on each, using the dependent variable S_I post-intervention. In the IVGTT parameters model, pre-intervention S_G, DI, G0, QUICKI, Revised QUICKI and AIRg and post-intervention QUICKI were shown to be the strongest significant predictors of S_I post-intervention; increasing S_G and DI pre-intervention, were inversely associated with S_I post-intervention and increasing G0, Revised QUICKI, AIRg pre-intervention and QUICKI pre- and post-intervention were positively associated with S_I post-intervention. In the anthropometry model, increasing body weight, waist circumference and percentage body water post-intervention, were inversely associated with S_I post-intervention ($p<0.05$). In the coagulation factors model, again, post-intervention body weight and tPA concentrations were significant predictors, negatively associated with post-intervention S_I. In the inflammatory markers model, increasing age, post-intervention body weight and sICAM and in the lipid markers model, increasing age, post-intervention body weight and pre-intervention NEFA concentrations were negatively and significantly associated with S_I post-intervention ($p<0.05$). In the plasma fatty acids model, plasma EPA concentrations (C20:5:3) pre-intervention was the only significant predictor, positively associated with S_I post-intervention. Finally, from the dietary variables model, from the 3-day weighed food diaries pre- and post-intervention, body weight and *trans* fatty acids post-intervention and starch pre-intervention were the only significant predictors ($p<0.05$).

Table 6.15c illustrates the main predictors of S_I post-intervention. The model had a high adjusted r^2 (99.8%), signifying an excellent fit for the population. G0 and S_G pre-intervention were the strongest predictors for S_I post-intervention. Increasing G0 was a positive predictor whilst increasing S_G showed an inverse association with S_I post-intervention. G0 post-intervention was a significant albeit a weak predictor of S_I post-intervention. Additionally, and as expected increasing QUICKI values pre- and post-intervention and Revised QUICKI pre-intervention were associated with an increase in post-intervention S_I values. Volunteers in the UoR, UM and UU cohorts were associated with a lower S_I post-intervention, compared to volunteers in TCD ($p<0.05$). In contrast, volunteers in the UO, HURS-UCO and JUMC cohorts were associated with an increase in S_I post-intervention. Increasing age and body weight post-intervention were negatively associated with S_I at post-intervention. Gender was a significant predictor of S_I at post-intervention in the LIPGENE study. Other strong positive and significant predictors in the final model were DI post-intervention, fasting insulin, AIRg and plasma concentrations of EPA (C20:5:3) pre-intervention. However, DI pre-intervention and fasting insulin post-intervention were negatively associated with S_I post-intervention. Other IVGTT parameters inversely associated with S_I at post-intervention were P2 and fasting glucose, pre-and post-intervention. Non-smokers compared to smokers and those volunteers that did not play sport compared to those who did, were significant negative predictors of S_I at post-intervention. From the dietary variables, *trans* fatty acids (g/day) were negatively associated with S_I post-intervention.

Table 6.16 presents the results of the final models with the main predictors of S_I post-intervention, in each of the four dietary groups, controlling for age, sex and body weight post-intervention. The same process was applied as above (Tables 1 and 2 of the model process not shown). However, some variables that were not significant in the prediction of S_I post-intervention, irrespective of diet, became significant when broken down into each diet category. For example, first phase insulin response and insulin to C-peptide ratio post-intervention, were negatively associated with S_I post-intervention in the HFSFA diet (Diet A). Meanwhile tPA concentrations post-intervention were a positive predictor, although very weakly, of S_I post-intervention, irrespective of diet, tPA concentrations pre-intervention were inversely related to S_I post-intervention in the HFSFA diet (Diet A). The only plasma fatty acid positively predicting S_I post-intervention in the HFSFA diet was found to be plasma arachidonic

acid (C20:4:6). In common with the final prediction model irrespective of diet, fasting glucose pre-intervention and G0 post-intervention were negatively and positively associated with S_I post-intervention, respectively, in the HFSFA diet (Diet A). This model also had a high adjusted r^2 (74.3%). In the HFMUFA diet (Diet B), common to the final model, irrespective of diet, DI was a strong positive predictor of SI post-intervention. Interestingly G0 post-intervention showed an inverse association with S_I post-intervention when it was positively predicting S_I post-intervention, not splitting by diet. First phase insulin response pre-and post-intervention were negatively associated with S_I post-intervention whereas S_I pre-intervention and volunteers in the JUMC cohort were associated with an increase in S_I post-intervention as compared to volunteers in the TCD cohort, in the HFMUFA diet (Diet B). The adjusted r^2 of this model was 0.61. Interestingly, increasing body weight negatively associated with S_I post-intervention, which was only apparent in the final prediction model of the LF diet (Diet C). AIRg post-intervention was the strongest predictor for S_I post-intervention, not seen in the final model, irrespective of diet. DI post-intervention and revised QUICKI pre-intervention were positively associated with S_I post-intervention in the LF diet (Diet C). In the previous steps of the final process model, volunteers with a total fat intake >35.98% pre-intervention showed an inverse association with S_I post-intervention and this was also a predictor of S_I post-intervention in the final model. However, when entered univariately and multivariately, controlling for age, gender and body weight post-intervention, volunteers with a total fat intake <35.98% pre-intervention showed this to be a positive predictor of S_I post-intervention. When this was entered with the other significant predictors at the next stage, the significance was retained, however, the direction of the influence changed to a negative association which was maintained in the final prediction model. Of the dietary variables in the LF diet, CHO (% Energy) and fibre (g/day) were positively and negatively associated with S_I post-intervention, respectively. The adjusted r^2 of the model for the LF diet (Diet C) was 0.72. Finally, the main predictors for S_I post-intervention in the LFn3PUFA diet (Diet D) were decreasing age, P2 and first phase insulin response post-intervention and insulin to c-peptide ratio pre-intervention. Increasing DI, QUICKI values and Adiponectin concentration post-intervention were positively associated with S_I post-intervention. In the LFn3PUFa diet (Diet D), those volunteers who don't play sport compared to those that do is a negative predictor of S_I post-intervention.

Table 6.15a: The relationship of each of the individual variables to S_I measured at post-intervention using univariate and multivariate (controlling for age, sex and post body weight) linear regression analysis.

	Post S_I											
	Univariate		Multivariate (controlling for sex, age and body weight)			Univariate		Multivariate (controlling for sex, age and body weight)			Univariate		Multivariate (controlling for sex, age and body weight)	
	β	Sig.	β	Sig.		β	Sig.	β	Sig.		β	Sig.	β	Sig.
Age (y)	+	0.88	na	na	Pre CRP (mg/L)	-	0.03	-	0.13	Pre PUFA (%Energy)	+	0.94	+	0.50
Sex (male/female)	+	0.00	na	na	Pre IL6 (pg/ml)	+	0.43	+	0.39	Pre n-3 (g/day)	+	0.76	+	0.10
Centre*					PreTNFa (pg/ml)	+	0.31	+	0.20	Pre n-6 (g/day)	-	0.78	+	0.44
TCD v UoR	+	0.76	+	0.52	Pre sICAM (ng/ml)	-	0.01	-	0.01	Pre C8:0 (g/day)	-	0.13	-	0.32
TCD v UO	+	0.40	+	0.24	Pre sVCAM (ng/ml)	-	0.53	-	0.52	Pre C10:0 (g/day)	-	0.84	+	0.56
TCD v INSERM	+	0.44	+	0.92	Pre Resistin (mg/L)	-	0.30	-	0.37	Pre C12:0 (g/day)	+	0.28	+	0.07
TCD v UM	+	0.89	+	0.54	Pre Adiponectin (mg/L)	+	0.05	+	0.12	Pre C14:0 (g/day)	+	0.50	+	0.07
TCD v HURS-UCO	-	0.24	-	0.29	Pre Leptin (ng/ml)	-	0.02	-	0.02	Pre C16:0 (g/day)	-	0.70	+	0.17
TCD v JUMC	+	0.23	+	0.08	Post CRP (mg/L)	-	0.23	-	0.31	Pre C16:1 (g/day)	-	0.36	+	0.46
TCD v UU	+	0.10	+	0.22	Post IL6 (pg/ml)	-	0.81	-	0.87	Pre C18:0 (g/day)	-	0.66	+	0.36
Change of Body Weight (kg)	-	0.14	-	0.14	PostTNFa (pg/ml)	-	0.40	-	0.91	Pre C18:1 (g/day)	-	0.07	-	0.60
Diet*					Post sICAM (ng/ml)	-	0.00	-	0.00	Pre C18:2:6 (g/day)	-	0.90	+	0.28
Diet A v Diet B	+	0.98	+	0.84	Post sVCAM (ng/ml)	-	0.35	-	0.29	Pre C18:3:3 (g/day)	+	0.69	+	0.20
Diet A v Diet C	-	0.61	-	0.73	Post Resistin (mg/L)	-	0.18	-	0.24	PreC20:4:6 (g/day)	-	0.90	+	0.34
Diet A v Diet D	-	0.19	-	0.22	Post Adiponectin (mg/L)	+	0.03	+	0.08	Pre C20:5:3 (g/day)	-	0.71	+	0.62
Pre S_I (mU/L)-1min-1	+	0.00	+	0.00	Post Leptin (ng/ml)	-	0.01	-	0.01	Pre C22:6:3 (g/day)	-	0.91	+	0.42
Pre $S_{G\,min}^{-1}$	+	0.01	+	0.02	Pre TAG (mmol/L)	-	0.02	-	0.01	Pre Trans fatty acids (g/day)	-	0.40	-	0.53
Pre AIRg mUL^{-1} min^{-1}	-	0.00	-	0.00	Pre Cholesterol (mmol/L)	+	0.38	+	0.98	Pre CHO (g/day)	-	0.48	+	0.43
Pre DI	+	0.00	+	0.00	Pre NEFA (µmol/L)	-	0.07	-	0.01	Pre CHO (%Energy)	+	0.37	+	0.67
Pre G0 mg/dl $^{-1}$	-	0.07	-	0.31	Pre TRL TAG (mmol/L)	-	0.05	-	0.11	Pre Starch (g/day)	-	0.01	-	0.28
Pre P2 min^{-1}	+	0.01	+	0.13	Pre TRL Cholesterol (mmol/L)	-	0.39	-	0.42	Pre Total Sugars (g/day)	-	0.45	-	0.83
Pre P3 (mU/L) min^{-2}	+	0.00	+	0.00	Pre LDL (mmol/L)	+	0.52	+	0.71	Pre Protein (g/day)	-	0.21	+	0.66
Pre Fasting Glucose (mmol/l)	-	0.00	-	0.00	Pre Total HDL (mmol/L)	+	0.00	+	0.08	Pre Protein (%Energy)	-	0.82	-	0.61
Pre Fasting Insulin(uIU/ml)	-	0.00	-	0.00	Pre Apo A1(g/L)	+	0.17	+	0.79	Pre Alcohol (g/day)	+	0.42	+	0.03
Pre HOMA-IR	-	0.00	-	0.00	Pre Apo B (g/L)	-	0.44	-	0.40	Pre Alcohol (%Energy)	+	0.23	+	0.01
Pre QUICKI	+	0.00	+	0.00	Pre Apo CII (mg/L)	-	0.21	-	0.08	Pre Fibre (g)	+	0.93	+	0.31
Pre First Phase Insulin Response (uIU/ml)	-	0.00	-	0.00	Pre Apo CIII (mg/L)	-	0.09	-	0.02	Pre Vitamin E (mg/day)	-	0.20	-	0.67
Post $S_{G\,min}^{-1}$	+	0.90	-	0.64	Pre Apo E (mg/L)	-	0.29	-	0.12	Post Basal Metabolic Rate (MJ/day)	-	0.00	+	0.54
Post AIRg mUL^{-1} min^{-1}	-	0.00	-	0.00	Pre Apo B48 (mg/L)	+	0.79	+	0.77	Post Total Energy (MJ)	-	0.22	+	0.79
Post DI	+	0.00	+	0.00	Pre TRL ApoB (mg/L)	+	0.18	+	0.06	Post Total fat (g/day)	-	0.21	+	0.97
Post G0 mg/dl $^{-1}$	-	0.00	-	0.00	Post TAG (mmol/L)	-	0.01	-	0.02	Post Total fat (%Energy)	-	0.75	-	0.92
Post P2 min^{-1}	-	0.01	-	0.01	Post Cholesterol (mmol/L)	+	0.93	-	0.55	Post SAFA (g/day)	-	0.61	+	0.70
Post P3 (mU/L) min^{-2}	+	0.00	+	0.00	Post NEFA (µmol/L)	-	0.30	-	0.08	Post SAFA (%Energy)	+	0.58	+	0.52
Post Fasting Glucose (mmol/l)	-	0.00	-	0.00	Post TRL TAG (mmol/L)	-	0.06	-	0.17	Post MUFA (g/day)	-	0.26	+	0.99
Post Fasting Insulin(uIU/ml)	-	0.00	-	0.00	Post TRL Cholesterol (mmol/L)	-	0.49	-	0.70	Post MUFA (%Energy)	-	0.78	-	0.79
Post HOMA-IR	-	0.00	-	0.00	Post LDL (mmol/L)	-	0.37	-	0.37	Post PUFA (g/day)	-	0.41	+	0.68
Post QUICKI	+	0.00	+	0.00	Post Total HDL (mmol/L)	+	0.01	+	0.16	Post PUFA (%Energy)	-	0.90	+	0.95
Post First Phase Insulin Response (uIU/ml)	-	0.00	-	0.00	Post Apo A1(g/L)	+	0.15	+	0.74	Post n-3 (g/day)	+	0.96	+	0.52
Pre C-Peptide ng/ml	-	0.00	-	0.00	Post Apo B (g/L)	-	0.32	-	0.21	Post n-6 (g/day)	-	0.53	+	0.76
Post C-Peptide ng/ml	-	0.00	-	0.00	Post Apo CII (mg/L)	-	0.40	-	0.25	Post C8:0 (g/day)	+	0.63	+	0.94
Pre Insulin to C-peptide Ratio	-	0.00	-	0.00	Post Apo CIII (mg/L)	-	0.09	-	0.02	Post C10:0 (g/day)	-	0.88	+	1.00
Post Insulin to C-peptide Ratio	-	0.00	-	0.00	Post Apo E (mg/L)	-	0.24	-	0.10	Post C12:0 (g/day)	+	0.41	+	0.44
Smoke(Yes v no)	-	0.07	-	0.07	Post Apo B48 (mg/L)	+	0.95	+	0.77	Post C14:0 (g/day)	+	0.34	+	0.12
Play sport (Yes v No)	-	0.05	-	0.03	Post TRL ApoB (mg/L)	+	0.43	+	0.26	Post C16:0 (g/day)	-	0.82	+	0.34
Pre Revised QUICKI	+	0.00	+	0.00	Pre plasma C14:0	-	0.55	+	0.88	Post C16:1 (g/day)	-	0.52	+	0.47
Post Revised QUICKI	+	0.00	+	0.00	Pre plasma C16:0	-	0.12	-	0.26	Post C18:0 (g/day)	-	0.93	+	0.30
Pre Total Fat >35.98% Total Energy	-	0.65	-	0.63	Pre plasma C16:1	+	0.63	+	0.70	Post C18:1 (g/day)	+	0.93	+	0.30
Pre Total Fat <35.98% Total Energy	+	0.87	+	0.80	Pre plasma C18:0	-	0.80	-	0.48	Post C18:2:6 (g/day)	+	0.99	+	0.19
Height(m)	-	0.47	+	0.00	Pre plasma C18:1	-	0.03	-	0.03	Post C18:3:3 (g/day)	+	0.80	+	0.37
Pre BodyWeight(kg)	-	0.00	+	0.13	Pre plasma C18:2:6	+	0.01	+	0.05	Post C20:4:6 (g/day)	-	0.90	+	0.45
Pre Waist circumference(cm)	-	0.00	-	0.01	Pre plamsa C20:4:6	+	0.39	+	0.31	Post C20:5:3 (g/day)	+	0.60	+	0.52
Pre Hip circumference (cm)	-	0.01	+	0.42	Pre plasma C20:5:3	+	0.02	+	0.01	Post C22:6:3 (g/day)	+	0.39	+	0.22
Pre Diastolic BP(mmHg)	-	0.24	+	0.94	Pre plasma C22:6:3	+	0.71	+	0.62	Post Trans fatty acids (g/day)	-	0.17	-	0.07
Pre Systolic BP(mmHg)	-	0.31	-	0.59	Pre plasma SFA	-	0.17	-	0.29	Post CHO (g/day)	-	0.67	+	0.57
Pre Body Fat (%)	-	0.67	-	0.40	Pre plasma MUFA	-	0.06	-	0.06	Post CHO (%Energy)	+	0.26	+	0.66
Pre Body Fat(kg)	-	0.00	-	0.86	Pre plasma PUFA	+	0.01	+	0.02	Post Starch (g/day)	-	0.12	-	0.41
Pre Lean Body Mass(%)	+	0.39	+	0.00	Pre plasma n-6 PUFA	+	0.02	+	0.04	Post Total Sugars (g/day)	-	0.57	-	0.46
Pre Lean Mass Body(kg)	-	0.00	+	0.00	Pre plasma n-3 PUFA	+	0.12	+	0.05	Post Protein (g/day)	-	0.05	-	0.57
Pre Body Water(%)	+	0.96	+	0.12	Post plasma C14:0	-	0.08	-	0.10	Post Protein (%Energy)	-	0.13	-	0.21
Pre Body Water (L)	-	0.11	+	0.00	Post plasma C16:0	-	0.07	-	0.13	Post Alcohol (g/day)	+	0.88	+	0.28
Pre Impedance(KHz)	+	0.01	+	0.74	Post plasma C16:1	+	0.27	+	0.39	Post Alcohol (%Energy)	+	0.99	+	0.46
Post Body Weight(kg)	-	0.00	na	na	Post plasma C18:0	-	0.52	-	0.17	Post Fibre (g)	+	0.36	+	0.10
Post Waist circumference(cm)	-	0.00	-	0.00	Post plasma C18:1	-	0.37	-	0.59	Post Vitamin E (mg/day)	-	0.72	+	0.51
Post Hip circumference (cm)	-	0.00	+	0.95	Post plasma C18:2:6	+	0.05	+	0.07					
Post Diastolic BP(mmHg)	-	0.00	-	0.05	Post plamsa C20:4:6	+	0.04	+	0.04					
Post Systolic BP(mmHg)	-	0.01	-	0.22	Post plasma C20:5:3	+	0.36	+	0.51					
Post Body Fat (%)	+	0.97	-	0.80	Post plasma C22:6:3	-	0.39	-	0.53					
Post Body Fat(kg)	-	0.00	-	0.84	Post plasma SFA	-	0.03	-	0.03					
Post Lean Body Mass(%)	+	0.60	+	0.00	Post plasma MUFA	-	0.59	-	0.80					
Post Lean Mass Body(kg)	-	0.00	+	0.00	Post plasma PUFA	+	0.02	+	0.03					
Post Body Water(%)	+	0.62	+	0.01	Post plasma n-6 PUFA	+	0.01	+	0.02					
Post Body Water (L)	-	0.01	+	0.00	Post plasma n-3 PUFA	-	0.73	-	0.78					
Post Impedance(KHz)	+	0.00	+	0.17	Pre Basal Metabolic Rate (MJ/day)	-	0.00	+	0.24					
Pre BMI (kg/m^2)	-	0.00	-	0.00	Pre Total Energy (MJ)	-	0.23	+	0.36					
Post BMI (kg/m^2)	-	0.00	-	0.00	Pre Total fat (g/day)	-	0.09	+	0.93					
Pre PAI-1 ng/ml	-	0.00	-	0.00	Pre Total fat (%Energy)	-	0.14	-	0.14					
Pre tPA ng/ml	-	0.00	-	0.00	Pre SAFA (g/day)	-	0.82	+	0.18					
Pre Fibrinogen mg/dl	+	0.93	+	0.63	Pre SAFA (%Energy)	+	0.37	+	0.39					
Post PAI-1 ng/ml	-	0.00	-	0.00	Pre MUFA (g/day)	-	0.02	-	0.30					
Post tPA ng/ml	-	0.00	-	0.00	Pre MUFA (%Energy)	-	0.05	-	0.05					
Post Fibrinogen mg/dl	-	0.79	+	0.96	Pre PUFA (g/day)	-	0.50	+	0.41					

na, non applicable, Sig =Significance value, β= Beta co-efficient. *Categorical variables with ≥3 categories for which dummy variables were created

$p = 0.00 = p < 0.001$

pre= pre-intervention; post=post-intervention

Table 6.15b: Multiple linear regression predictors of S_I measured at post-intervention in LIPGENE volunteers, analysis of seven different regression models

		Post S_I			
				95% CI	
		β	Sig.	Lower	Upper
Model 1	**IVGTT Parameters and Centre** (Adjusted r^2=0.915, r^2=0.923)†				
	Age (y)	-0.13	0.00	-0.030	-0.016
	Sex (male/female)	0.16	0.00	0.376	0.648
	Post Body Weight(kg)	-0.33	0.00	-0.042	-0.032
	Change of Body Weight (kg)	0.02	0.30	-0.014	0.046
	Pre $S_{G\,min}^{-1}$	-0.80	0.00	-208.599	-173.979
	Pre DI	-0.61	0.00	-0.001	-0.001
	Pre G0 mg/dl $^{-1}$	0.82	0.00	0.029	0.034
	Pre P2 min^{-1}	-0.38	0.00	-21.981	-17.742
	Pre Fasting Glucose (mmol/l)	-0.10	0.00	-0.295	-0.089
	Pre QUICKI	0.68	0.00	2.473	2.829
	Post AIRg mUL^{-1} min^{-1}	-0.15	0.00	-0.001	0.000
	Post DI	0.42	0.00	0.001	0.001
	Post G0 mg/dl $^{-1}$	0.06	0.00	0.000	0.002
	Post P2 min^{-1}	-0.08	0.00	-1.444	-0.519
	Post Fasting Glucose (mmol/l)	-0.22	0.00	-0.393	-0.247
	Post QUICKI	0.64	0.00	6.172	7.232
	Smoke(Yes v no)	-0.20	0.00	-0.847	-0.589
	Play sport (Yes v No)	-0.05	0.01	-0.253	-0.038
	Pre Revised QUICKI	0.58	0.00	42.950	51.075
	Centre*				
	TCD v UoR	0.14	0.00	0.403	0.820
	TCD v UO	0.26	0.00	0.980	1.450
	TCD v INSERM	0.08	0.00	0.176	0.643
	TCD v UM	0.01	0.60	-0.160	0.277
	TCD v HURS-UCO	0.08	0.00	0.140	0.535
	TCD v JUMC	0.17	0.00	0.632	1.077
	TCD v UU	-0.24	0.00	-1.458	-0.993
	Pre Fasting Insulin(uIU/ml)	0.13	0.00	0.019	0.059
	Pre AIRg mUL^{-1} min^{-1}	0.48	0.00	0.002	0.003
	Post Fasting Insulin(uIU/ml)	-0.20	0.00	-0.070	-0.037
	Pre C-Peptide ng/ml	-0.06	0.06	-0.203	0.005
	Post C-Peptide ng/ml	0.04	0.21	-0.033	0.152

†variables: Pre P3, Post P3, Pre SI, Pre HOMA-IR, Post HOMA-IR, Post RQUICKI, Pre insulin to c-peptide ratio, Post insulin to c-peptide ratio, Pre and Post First Phase Insulin Response were removed from model due to their confounding effects

Model 2	**Anthropometry** (Adjusted r2=0.234, r2=0.283)*				
	Age (y)	-0.01	0.84	-0.027	0.022
	Sex (male/female)	0.12	0.38	-0.465	1.213
	Post Body Weight(kg)	-1.31	0.00	-0.230	-0.060
	Pre Waist circumference(cm)	0.03	0.80	-0.029	0.038
	Pre Impedance(KHz)	-0.07	0.54	-0.005	0.003
	Post Waist circumference(cm)	-0.34	0.02	-0.091	-0.008
	Post Hip circumference (cm)	0.03	0.88	-0.053	0.062
	Post Diastolic BP(mmHg)	-0.09	0.21	-0.038	0.008
	Post Systolic BP(mmHg)	0.01	0.85	-0.013	0.016
	Post Body Fat(kg)	0.07	0.59	-0.033	0.057
	Post Body Water(%)	-0.78	0.00	-0.300	-0.057
	Post Impedance(KHz)	0.11	0.42	-0.003	0.007
	Post BMI (kg/m^2)	-0.11	0.51	-0.157	0.078
	Pre Hip circumference (cm)	0.27	0.15	-0.015	0.100
	Post Body Water (L)	1.77	0.00	0.131	0.446

*variables: Height, Pre body weight, Pre lean body mass (%) and (kg), Pre body water (%) and (L), Post lean body mass (%) and (kg), pre body fat (kg) and Pre BMI, were removed from model due to their confounding effects

Model 3	**Coagulation Factors** (Adjusted r2=0.173, r2=0.191)				
	Age (y)	-0.06	0.26	-0.030	0.008
	Sex (male/female)	0.01	0.81	-0.320	0.410
	Post Body Weight(kg)	-0.26	0.00	-0.043	-0.015
	Pre PAI-1 ng/ml	-0.08	0.24	-0.009	0.002
	Pre tPA ng/ml	-0.04	0.60	-0.076	0.044
	Post PAI-1 ng/ml	-0.05	0.45	-0.008	0.004
	Post tPA ng/ml	-0.19	0.02	-0.131	-0.012

		Post S_I			
				95% CI	
		β	Sig.	Lower	Upper
Model 4	**Inflammatory Markers** (Adjusted r2=0.147, r2=0.173)				
	Age (y)	-0.10	0.07	-0.037	0.001
	Sex (male/female)	0.11	0.13	-0.106	0.788
	Post Body Weight(kg)	-0.28	0.00	-0.046	-0.016
	Pre CRP (mg/L)	-0.07	0.20	-0.066	0.014
	Pre sICAM (ng/ml)	0.00	0.99	-0.003	0.003
	Pre Adiponectin (mg/L)	0.04	0.59	-0.065	0.114
	Pre Leptin (ng/ml)	-0.04	0.68	-0.016	0.010
	Post sICAM (ng/ml)	-0.14	0.06	-0.006	0.000
	Post Adiponectin (mg/L)	0.07	0.31	-0.042	0.131
	Post Leptin (ng/ml)	-0.10	0.27	-0.020	0.006
Model 5	**Lipid Markers** (Adjusted r2=0.174, r2=0.218)				
	Age (y)	-0.14	0.01	-0.043	-0.005
	Sex (male/female)	0.07	0.29	-0.182	0.610
	Post Body Weight(kg)	-0.34	0.00	-0.051	-0.024
	Pre TAG (mmol/L)	-0.11	0.49	-0.752	0.364
	Pre NEFA (µmol/L)	-0.14	0.03	-0.002	0.000
	Pre TRL TAG (mmol/L)	-0.04	0.80	-0.824	0.637
	Pre Total HDL (mmol/L)	0.14	0.17	-0.350	1.941
	Pre Apo CII (mg/L)	0.04	0.66	-0.011	0.018
	Pre Apo CIII (mg/L)	-0.13	0.33	-0.012	0.004
	Pre Apo E (mg/L)	0.07	0.47	-0.012	0.026
	Pre TRL ApoB (mg/L)	0.28	0.00	0.005	0.014
	Post TAG (mmol/L)	0.00	0.97	-0.526	0.508
	Post NEFA (µmol/L)	-0.03	0.62	-0.001	0.001
	Post TRL TAG (mmol/L)	0.03	0.79	-0.565	0.743
	Post Total HDL (mmol/L)	0.02	0.86	-1.024	1.224
	Post Apo CIII (mg/L)	-0.06	0.60	-0.009	0.005
	Post Apo E (mg/L)	-0.10	0.30	-0.027	0.008
Model 6	**Plasma Fatty Acids** (Adjusted r2=0.137, r2=0.172)§				
	Age (y)	-0.14	0.02	-0.044	-0.004
	Sex (male/female)	0.02	0.77	-0.317	0.427
	Post Body Weight(kg)	-0.36	0.00	-0.053	-0.026
	Pre plasma C20:5:3	0.20	0.04	0.011	0.698
	Pre plasma MUFA	-0.08	0.25	-0.071	0.018
	Pre plasma n-6 PUFA	0.00	0.95	-0.032	0.034
	Pre plasma n-3 PUFA	-0.07	0.52	-0.215	0.108
	Post plasma C14:0	-0.06	0.50	-0.241	0.117
	Post plasma C16:0	-0.03	0.82	-0.127	0.100
	Post plamsa C20:4:6	0.08	0.15	-0.023	0.151
	Post plasma SFA	-0.08	0.60	-0.123	0.072
	Post plasma PUFA	-0.16	0.29	-0.128	0.038
	Post plasma n-6 PUFA	0.13	0.38	-0.045	0.120

§variables: Pre plasma C16:0, Pre plasma C18:1, Pre plasma C18:2:6, Post plasma C18:2:6 and Pre plasma PUFA (%), were removed from model due to their confounding effects

Model 7	**Dietary Variables** (Adjusted r2=0.133, r2=0.247)¶				
	Age (y)	-0.15	0.11	-0.057	0.006
	Sex (male/female)	0.01	0.95	-0.633	0.672
	Post Body Weight(kg)	-0.37	0.00	-0.063	-0.019
	Pre Total fat (g/day)	0.32	0.16	-0.006	0.037
	Pre Total fat (%Energy)	-0.36	0.15	-0.179	0.029
	Pre MUFA (g/day)	0.02	0.89	-0.099	0.114
	Pre n-3 (g/day)	0.00	0.99	-0.286	0.289
	Pre C12:0 (g/day)	0.20	0.14	-0.118	0.821
	Pre C14:0 (g/day)	0.03	0.85	-0.232	0.282
	Pre Starch (g/day)	-0.38	0.03	-0.019	-0.001
	Pre Alcohol (g/day)	0.07	0.77	-0.044	0.060
	Pre Alcohol (%Energy)	0.02	0.94	-0.175	0.187
	Post C14:0 (g/day)	0.11	0.26	-0.082	0.304
	Post Trans fatty acids (g/day)	-0.18	0.04	-0.376	-0.008
	Post Starch (g/day)	-0.06	0.69	-0.010	0.007
	Post Protein (g/day)	-0.07	0.67	-0.020	0.013
	Post Protein (%Energy)	0.01	0.94	-0.094	0.101
	Post Fibre (g)	0.04	0.66	-0.029	0.046

¶variables: Pre BMR, Post BMR, Pre C8 (g/day), Pre C18:1 (g/day), Pre MUFA (g/day), were removed from model due to their confounding effects

Sig. significance value, β= Beta co-efficient, CI = confidence intervals. Each model controlled for sex, age and body weight at post-intervention
*Categorical variables with ≥3 categories for which dummy variables were created. p=0.00 = p<0.001
pre= pre-intervention; post=post-intervention

Table 6.15c: Multiple linear regression model of variables predicting increasing S_I post-intervention in LIPGENE volunteers

			95% CI	
	β	Sig.	Lower	Upper
Age (y)	-0.14	0.00	-0.026	-0.023
Sex (male/female)	0.28	0.00	0.846	0.918
Post Body Weight(kg)	-0.35	0.00	-0.040	-0.037
Pre $S_{G\ min}^{-1}$	-0.90	0.00	-219.591	-213.623
Pre DI	-0.69	0.00	-0.002	-0.001
Pre G0 mg/dl $^{-1}$	0.92	0.00	0.035	0.036
Pre P2 min^{-1}	-0.44	0.00	-23.232	-22.496
Pre Fasting Glucose (mmol/L)	-0.15	0.00	-0.302	-0.267
Pre QUICKI	0.78	0.00	3.003	3.066
Post AIRg mUL^{-1} min^{-1}	-0.01	0.08	0.000	0.000
Post DI	0.37	0.00	0.001	0.001
Post G0 mg/dl $^{-1}$	0.09	0.00	0.001	0.002
Post P2 min^{-1}	-0.08	0.00	-1.124	-0.967
Post Fasting Glucose (mmol/L)	-0.20	0.00	-0.303	-0.278
Post QUICKI	0.73	0.00	7.620	7.808
Smoke(Yes v no)	-0.26	0.00	-0.987	-0.941
Play sport (Yes v No)	-0.05	0.00	-0.178	-0.141
Pre Revised QUICKI	0.64	0.00	51.333	52.995
Centre*				
TCD v UoR	-0.02	0.00	-0.140	-0.056
TCD v UO	0.15	0.00	0.655	0.737
TCD v INSERM	0.00	0.78	-0.048	0.036
TCD v UM	-0.11	0.00	-0.587	-0.504
TCD v HURS-UCO	0.16	0.00	0.615	0.687
TCD v JUMC	0.06	0.00	0.286	0.369
TCD v UU	-0.40	0.00	-2.036	-1.950
Pre Fasting Insulin(uIU/ml)	0.12	0.00	0.032	0.038
Pre AIRg mUL^{-1} min^{-1}	0.51	0.00	0.002	0.002
Post Fasting Insulin(uIU/ml)	-0.19	0.00	-0.053	-0.048
Post Waist circumference(cm)	-0.01	0.20	-0.003	0.001
Post Body Water(%)	0.08	0.00	0.015	0.020
Post tPA ng/ml	0.05	0.00	0.016	0.021
Post sICAM (ng/ml)	-0.20	0.00	-0.004	-0.004
Pre NEFA (µmol/L)	0.00	0.88	0.000	0.000
Pre TRL ApoB (mg/L)	0.26	0.00	0.009	0.009
Pre plasma C20:5:3	0.12	0.00	0.196	0.220
Post Trans fatty acids (g/day)	-0.18	0.04	-0.376	-0.008

Adjusted r^2=0.998, r^2=0.998; p=0.00 = p<0.001

Model controlled for sex, age and body weight at post-intervention.

* Categorical variables of 3 categories or more, for which dummy variables were created

Sig. significance value, β= Beta co-efficient, CI = confidence intervals.

Pre Starch (g/day) and Post Body Water (Litres) excluded due to its confounding effects on the model

pre= pre-intervention; post=post-intervention

Table 6.16: Multiple linear regression model of variables predicting increasing S_I at post-intervention in LIPGENE volunteers, randomised to four diets

Diet A (High SFA rich diet)				
			95% CI	
	β	Sig.	Lower	Upper
Age (y)	0.03	0.72	-0.030	0.043
Sex (male/female)	-0.11	0.27	-1.101	0.312
Post Body Weight(kg)	-0.09	0.56	-0.052	0.028
Pre $S_{G\,min}^{-1}$	0.12	0.21	-19.902	85.595
Pre DI	0.18	0.26	0.000	0.001
Pre Fasting Glucose (mmol/l)	-0.17	0.08	-0.786	0.052
Post G0 mg/dl $^{-1}$	0.38	0.01	0.005	0.027
Post Fasting Glucose (mmol/l)	-0.14	0.35	-0.805	0.289
Post First Phase Insulin Response (uIU/ml)	-0.36	0.01	-0.021	-0.002
Pre C-Peptide ng/ml	0.14	0.30	-0.247	0.773
Post C-Peptide ng/ml	-0.14	0.35	-0.714	0.259
Post Insulin to C-peptide Ratio	-0.27	0.01	-0.638	-0.106
Smoke(Yes v no)	-0.26	0.00	-1.859	-0.405
Pre Impedance(KHz)	0.06	0.59	-0.003	0.006
Post Waist circumference(cm)	-0.10	0.52	-0.065	0.034
Pre tPA ng/ml	-0.41	0.00	-0.276	-0.100
Post TRL TAG (mmol/L)	0.07	0.45	-0.358	0.790
Post Apo E (mg/L)	0.01	0.89	-0.014	0.016
Post plasma C20:4:6	0.24	0.00	0.056	0.285
Pre S_I (mU/L)-1min-1	0.15	0.21	-0.080	0.346

Adjusted r^2=0.743, r^2=0.835
Model controlled for sex, age and body weight at post-intervention.
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.
Pre QUICKI excluded due to its confounding effects on the model
pre= pre-intervention; post=post-intervention

Diet B (High MUFA rich diet)				
			95% CI	
	β	Sig.	Lower	Upper
Age (y)	-0.14	0.16	-0.063	0.011
Sex (male/female)	-0.03	0.74	-0.723	0.516
Post Body Weight(kg)	-0.15	0.18	-0.045	0.009
Centre*				
TCD v UoR	0.03	0.74	-0.736	1.025
TCD v UO	0.09	0.33	-0.445	1.292
TCD v INSERM	0.03	0.78	-0.909	1.212
TCD v UM	0.01	0.92	-1.010	1.121
TCD v HURS-UCO	0.01	0.92	-0.796	0.881
TCD v JUMC	0.24	0.01	0.241	2.128
Pre First Phase Insulin Response (uIU/ml)	-0.34	0.02	-0.032	-0.003
Post DI	0.76	0.00	0.001	0.002
Post G0 mg/dl $^{-1}$	-0.26	0.01	-0.018	-0.002
Post First Phase Insulin Response (uIU/ml)	-0.53	0.00	-0.039	-0.011
Pre S_I (mU/L)-1min-1	0.20	0.04	0.013	0.452
Pre C22:6:3 (g/day)	0.13	0.13	-0.191	1.463

Adjusted r^2=0.611, r^2=0.692
Model controlled for sex, age and body weight at post-intervention.
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.
Pre C10 (g/day) and Pre Starch (g/day), TCDvUU excluded due to its confounding effects on the model
* Categorical variables of 3 categories or more, for which dummy variables were created
pre= pre-intervention; post=post-intervention

Diet C (Low Fat diet)				
			95% CI	
	β	Sig.	Lower	Upper
Age (y)	-0.10	0.21	-0.042	0.010
Sex (male/female)	0.06	0.51	-0.357	0.703
Post Body Weight(kg)	-0.21	0.06	-0.039	0.000
Centre*				
TCD v UoR	-0.09	0.31	-1.107	0.354
TCD v UO	0.08	0.38	-0.409	1.058
TCD v INSERM	-0.09	0.24	-1.299	0.328
TCD v HURS-UCO	0.06	0.52	-0.439	0.859
TCD v JUMC	0.09	0.30	-0.415	1.310
TCD v UU	0.03	0.76	-0.705	0.959
Post AIRg mUL^{-1} min^{-1}	-0.57	0.00	-0.003	-0.001
Post DI	0.40	0.00	0.000	0.001
Pre Revised QUICKI	0.40	0.00	13.511	36.172
Pre Total Fat >35.98% Total Energy	-0.33	0.01	-1.734	-0.247
Pre Total Fat <35.98% Total Energy	-0.23	0.05	-1.380	-0.011
Pre Fibrinogen mg/dl	0.04	0.59	-0.002	0.003
Pre Adiponectin (mg/L)	-0.09	0.29	-0.133	0.040
Pre plasma C20:5:3	0.14	0.11	-0.051	0.481
Pre C16:0 (g/day)	0.01	0.91	-0.040	0.045
Pre CHO (%Energy)	0.30	0.00	0.024	0.092
Pre Fibre (g)	-0.23	0.02	-0.068	-0.007

Adjusted r^2=0.724, r^2=0.801
Model controlled for sex, age and body weight at post-intervention.
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.
Pre C10 (g/day) and Pre Total Sugars (g/day) and Post Trans Fatty Acids (g/day) excluded due to its confounding effects on the model
* Categorical variables of 3 categories or more, for which dummy variables were created
pre= pre-intervention; post=post-intervention

Diet D (Low Fat Diet with 1.2gLC n-3 PUFA)				
			95% CI	
	β	Sig.	Lower	Upper
Age (y)	-0.27	0.00	-0.056	-0.018
Gender	-0.02	0.80	-0.384	0.296
Post Body Weight(kg)	-0.10	0.38	-0.033	0.013
Post $S_{G\,min}^{-1}$	-0.23	0.12	-52.101	6.137
Post DI	0.69	0.00	0.001	0.001
Post G0 mg/dl $^{-1}$	0.24	0.12	-0.001	0.004
Post P2 min^{-1}	-0.20	0.00	-2.275	-0.437
Post QUICKI	0.57	0.00	4.904	9.233
Pre Insulin to C-peptide Ratio	-0.32	0.00	-0.334	-0.143
Post Insulin to C-peptide Ratio	0.16	0.08	-0.024	0.393
Play sport (Yes v No)	-0.13	0.04	0.012	0.684
Post Waist circumference(cm)	0.12	0.30	-0.014	0.046
Post Adiponectin (mg/L)	0.15	0.01	0.019	0.165
Post First Phase Insulin Response (uIU/ml)	-0.67	0.00	-0.034	-0.021

Adjusted r^2=0.780, r^2=0.822
Model controlled for sex, age and body weight at post-intervention.
Sig. significance value, β= Beta co-efficient, CI = confidence intervals.
pre= pre-intervention; post=post-intervention

6.4.4 The Effects of Four Dietary Treatments on Volunteers with a High and Low, Pre-Intervention Total Fat Intake

The median fat intake from the pre-intervention 3-day food diary records was 36%. As in the KANWU study, data analysis was performed after excluding volunteers with the 5% lowest and the highest fat intake. The effects of the four dietary treatments were analysed in volunteers with a pre-intervention total fat intake, above and below the median with a RM ANOVA model.

In those volunteers who had a pre-intervention fat intake above the median, a significant time*gender*diet group interaction effect (p=0.001) showed that female volunteers randomised to the HFMUFA diet (Diet B), had significantly greater AIRg values post- (353.92 mU/L $^{-1}$min^{-1}) compared to pre-intervention (309.36 mU/L $^{-1}$min^{-1}) (p=0.024).

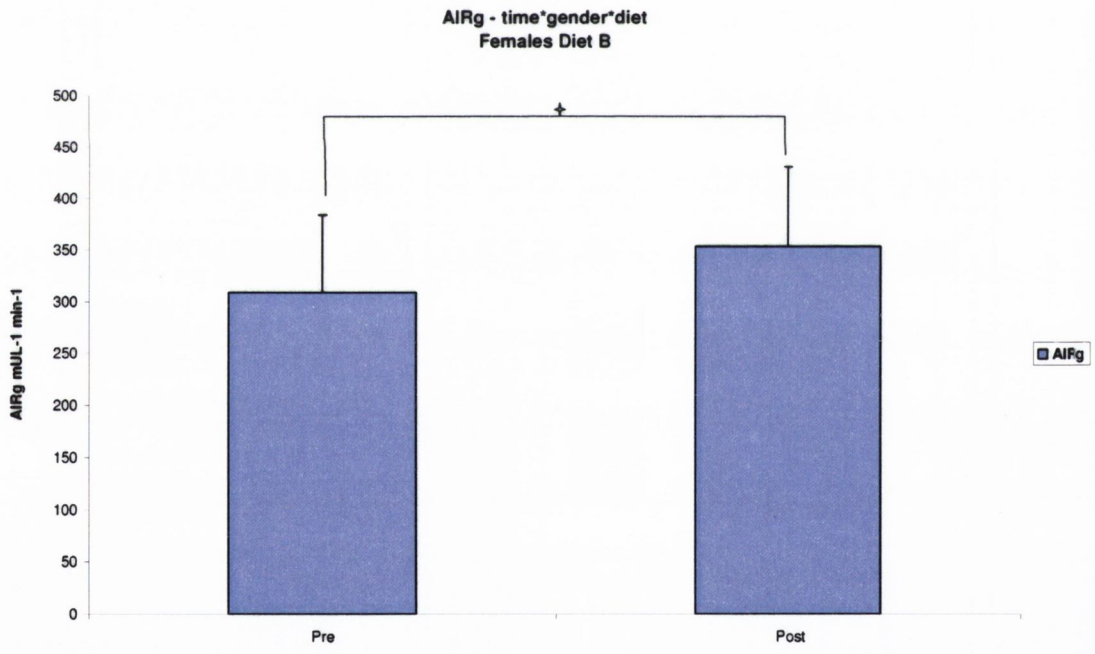


Figure 6.7: AIRg pre- and post-intervention, in females with a pre-intervention total fat intake >36%, + p<0.05

From the RM ANOVA, a significant time*centre*diet group (p=0.001) interaction effect for P2, which is the removal rate of insulin from the interstitial space, was seen in volunteers in the UO cohort, randomised to the HFMUFA diet (Diet B) and volunteers in the HURS-UCO cohort on the LF diet (Diet C) . P2 increased post-intervention in the HFMUFA diet (Diet B) (0.043 min^{-1} pre- to 0.052 min^{-1} post-intervention) and decreased in the LF diet (Diet C) (0.048 min^{-1} pre- to 0.033 min^{-1} post-intervention) (p<0.05) .

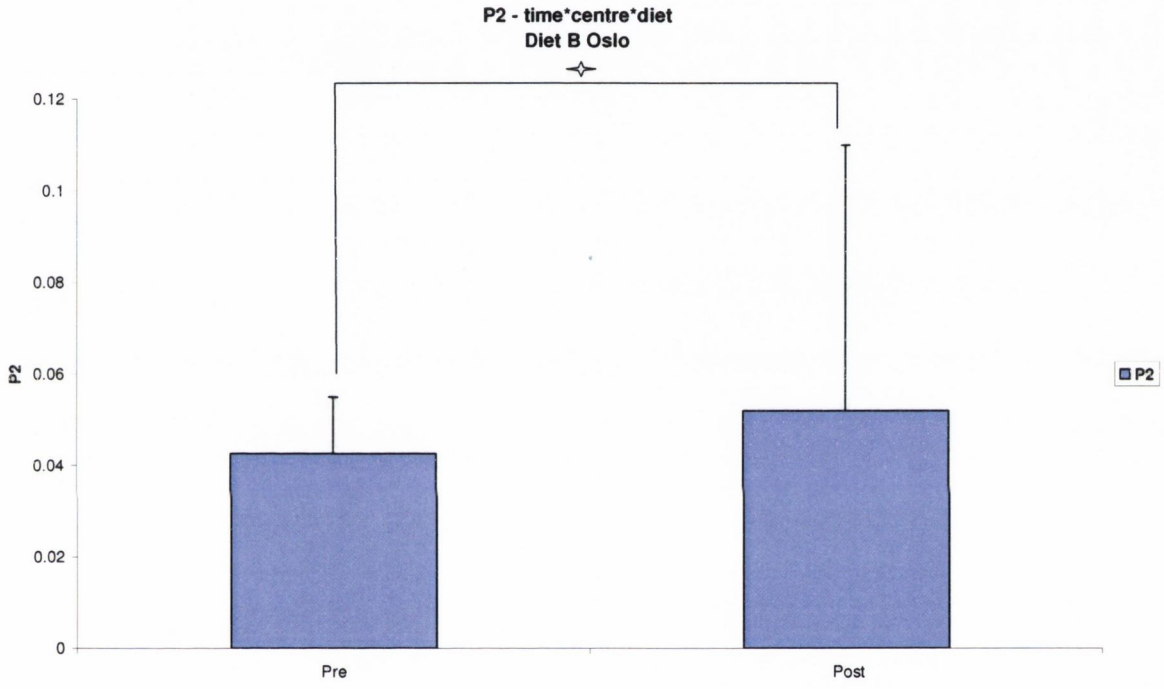


Figure 6.8: P2 pre- and post-intervention, in volunteers in the UO cohort, randomised to the HFMUFA diet (Diet B), with a pre-intervention total fat intake >36%, ✧ p<0.05

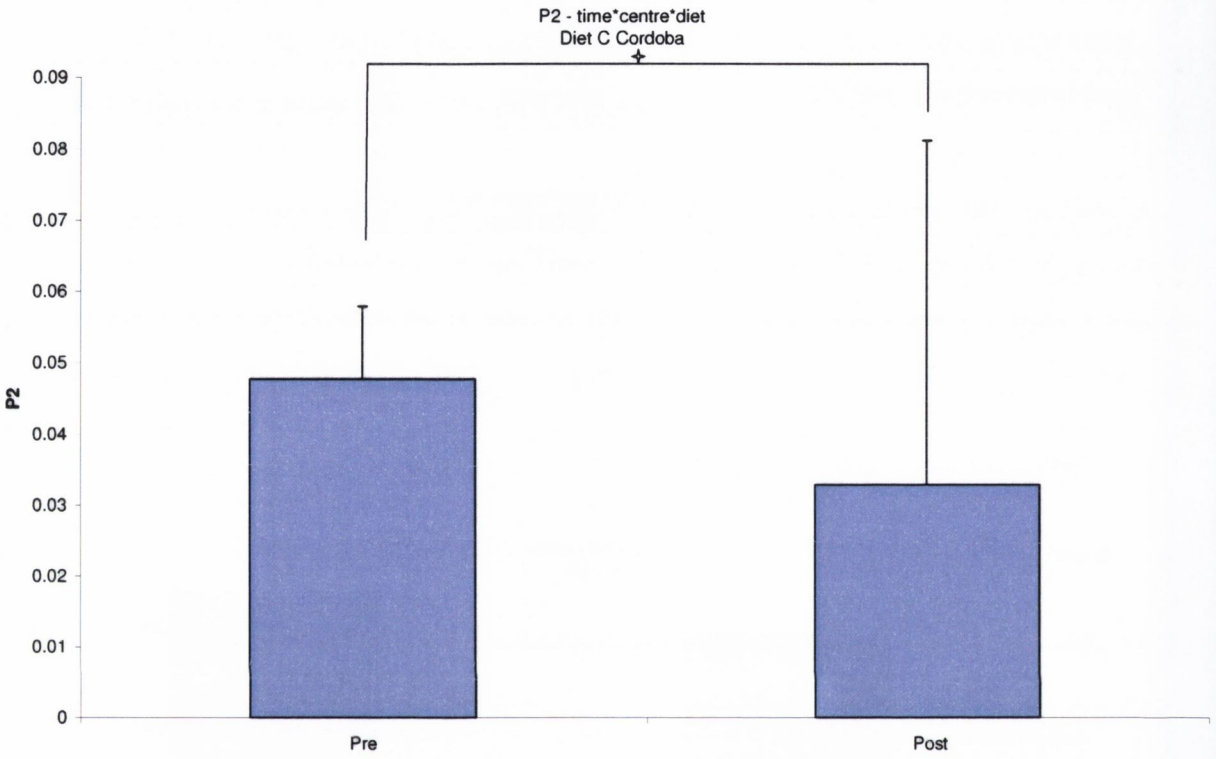


Figure 6.9: P2 pre- and post-intervention, in volunteers in the HURS-UCO cohort, randomised to the LF diet (Diet C), with a pre-intervention total fat intake >36%, ✧ $p<0.05$

Another significant interaction time*gender*centre*diet group effect (p=0.001) highlighted further similar significant effects of the HFMUFA diet (Diet B) in the UO cohort (0.043 min^{-1} pre- to 0.052 min^{-1} post-intervention) (p=0.011) and of the LF diet (Diet C) in the HURS-UCO cohort (0.052 min^{-1} pre- to 0.035 min^{-1} post-intervention) (p=0.025) on P2, in female volunteers with a pre-intervention total fat intake >36%.

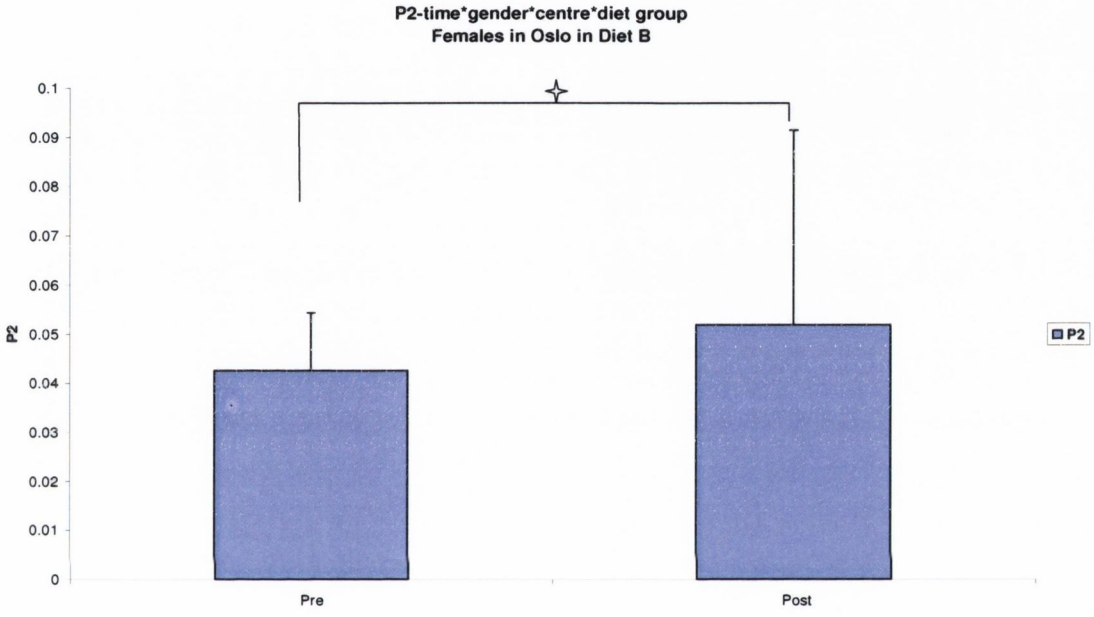


Figure 6.10: P2 pre- and post-intervention, in female volunteers in the UO cohort, randomised to the HFMUFA diet (Diet B), with a pre-intervention total fat intake >36%, ✧p<0.05

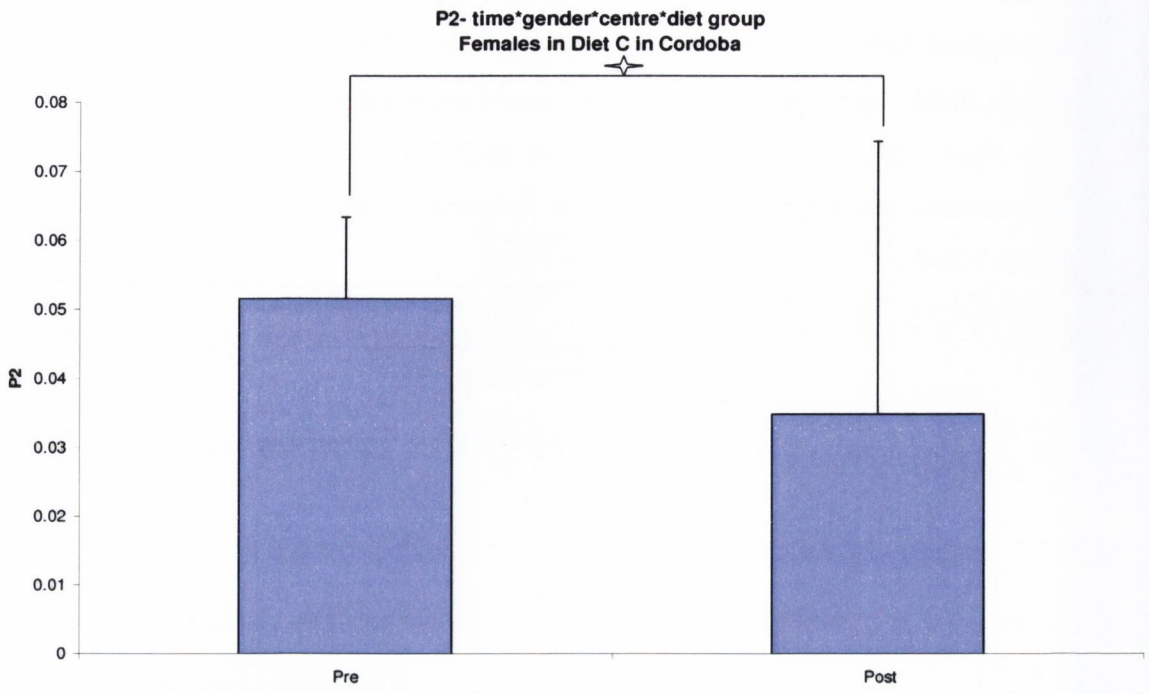


Figure 6.11: P2 pre- and post-intervention, in female volunteers in the HURS-UCO cohort, randomised to the LF diet (Diet C), with a pre-intervention total fat intake >36%, ✧ $p<0.05$

The effects of the four intervention diets were analysed in volunteers whose total fat intake at pre-intervention was below the median of 36%.

A time*diet group interaction effect (p=0.032) showed a significant decrease in insulin sensitivity (S_I) in the HFSFA diet (Diet A) (2.99 $(mU/L)^{-1}min^{-1}$ pre- to 2.54 $(mU/L)^{-1}min^{-1}$ post-intervention) (p=0.021) using the RM ANOVA model.

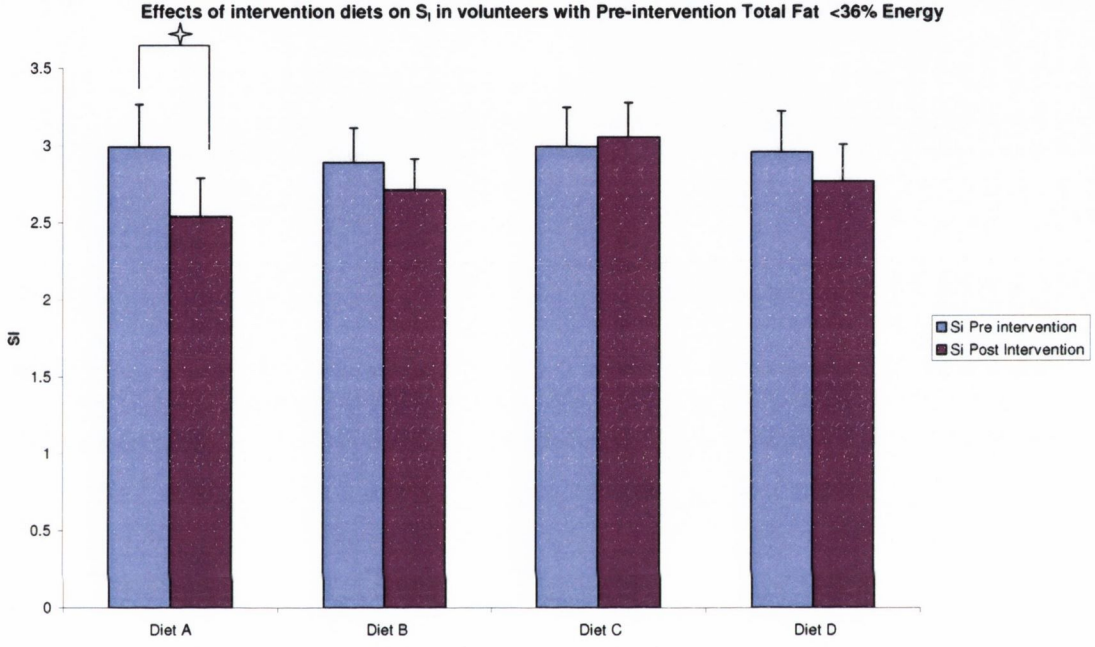


Figure 6.12: The effects of the four intervention diets on insulin sensitivity in volunteers with a pre-intervention total fat intake <36%, ✧ p<0.05

A time*centre*diet group interaction effect (p=0.026) showed an increase in S_I with volunteers in the JUMC cohort, randomised to the LF diet (Diet C) (2.83 (mU/L)$^{-1}$min^{-1} pre- to 4.02 (mU/L)$^{-1}$min^{-1} post-intervention). These volunteers had a pre-intervention total fat intake <36%.

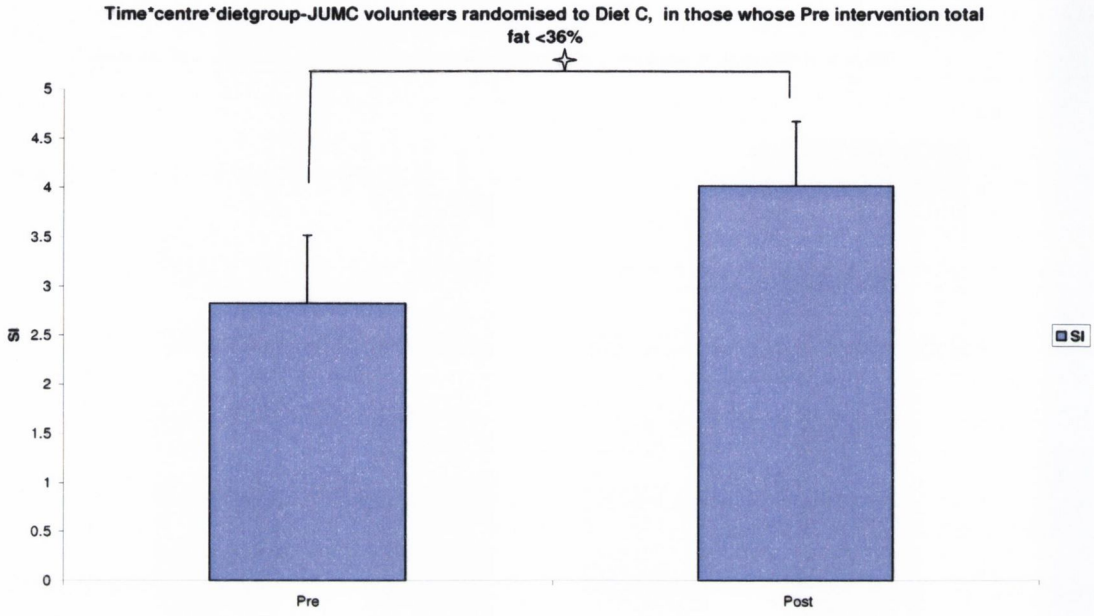


Figure 6.13: The effects of the LF diet (Diet C) on insulin sensitivity in volunteers in the JUMC cohort with a pre-intervention total fat intake <36%, ✧ p<0.05

A time*gender*diet group interaction (p=0.048) effect showed a decrease in S_I post-intervention in females on the HFSFA diet (Diet A) (3.16 $(mU/L)^{-1}min^{-1}$ pre- to 2.50 $(mU/L)^{-1}min^{-1}$ post-intervention.

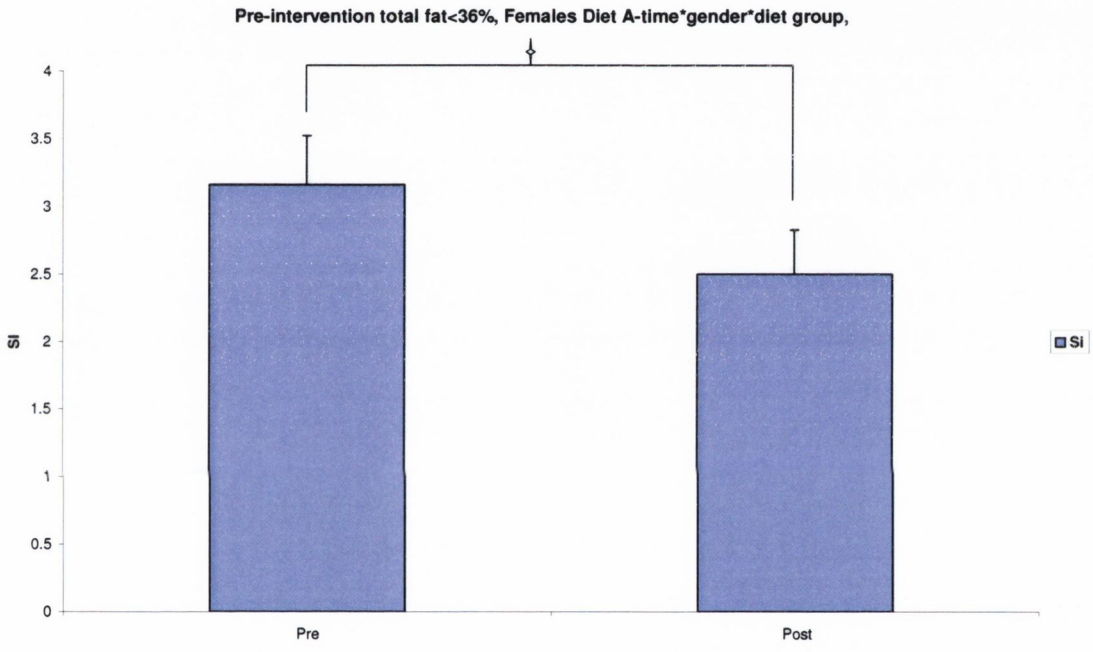


Figure 6.14: The effects of the HFSFA diet (Diet A) on insulin sensitivity in female volunteers with a pre-intervention total fat intake <36%, ✧p<0.05

Similar effects were seen for P2 in those volunteers with a pre-intervention total fat intake <36% as seen in those with pre-intervention total fat >36% wherein a time*diet group interaction effect (p=0.048) showed a decrease in P2 from pre- to post-intervention (p=0.033) in the LF diet (Diet C) group (0.044 min^{-1} pre- to 0.033 min^{-1} post-intervention).

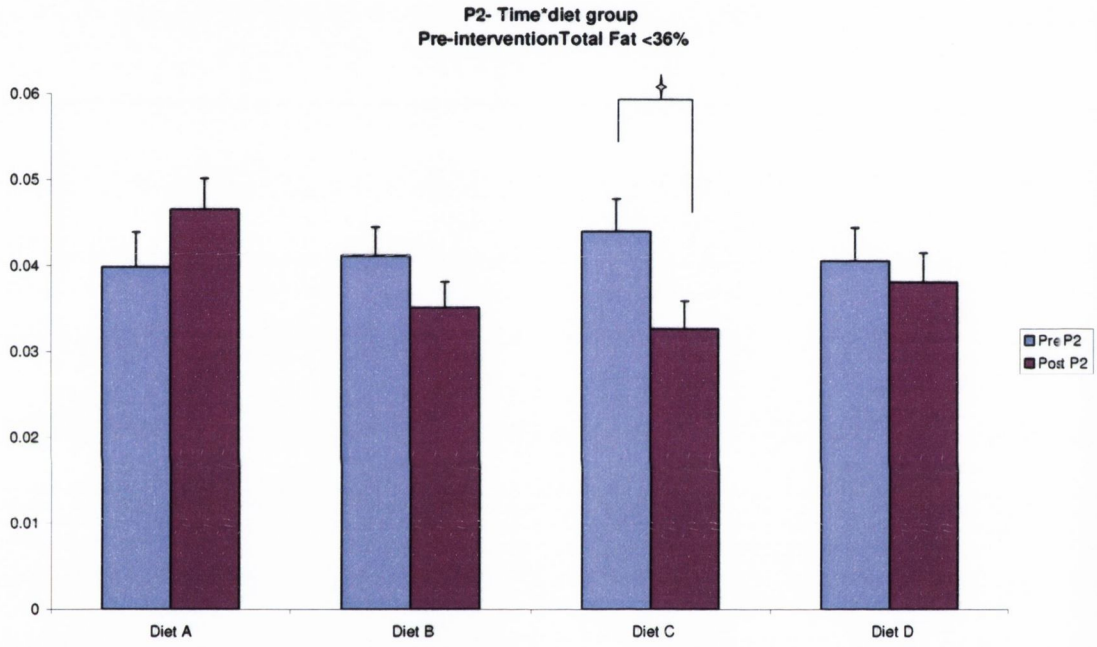


Figure 6.15: The effects of the LF diet (Diet C) on P2 in volunteers with a pre-intervention total fat intake <36%. ✧p<0.05

A time*gender*diet group interaction effect (p=0.004) demonstrated a significant decrease with HOMA-IR in female volunteers assigned to the HFMUFA diet (Diet B) (2.57 pre- to 2.12 post-intervention) (p<0.05).

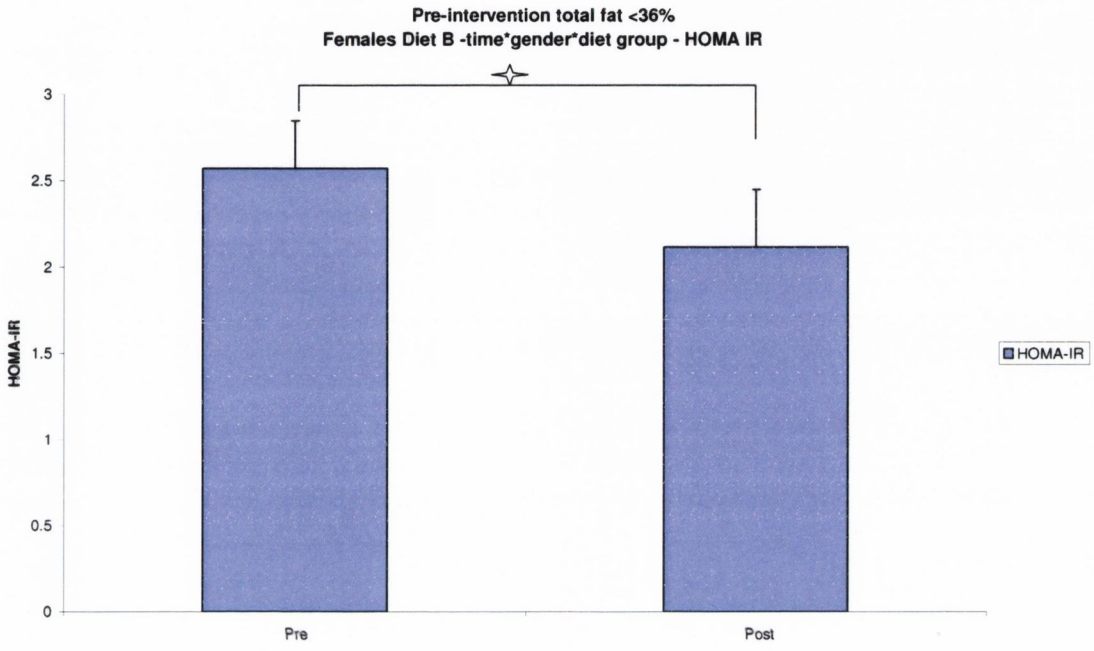


Figure 6.16: The effects of the HFMUFA diet (Diet B) on HOMA-IR in female volunteers with a pre-intervention total fat intake <36%,✧ p<0.05

A time*diet group interaction effect ($p=0.002$) demonstrated a significant reduction in the insulin to C-peptide ratio in the HFMUFA diet group (Diet B) in those whose total fat intake was <36% pre-intervention (3.68 pre- to 3.18 post-intervention).

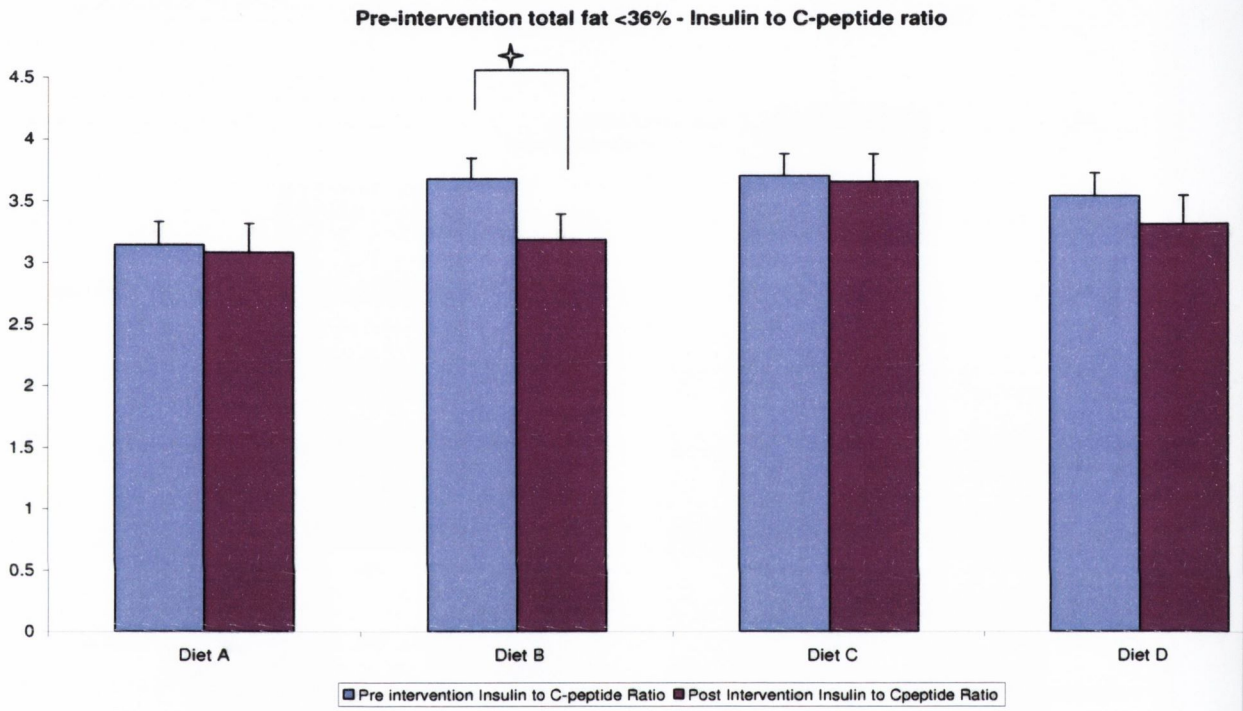
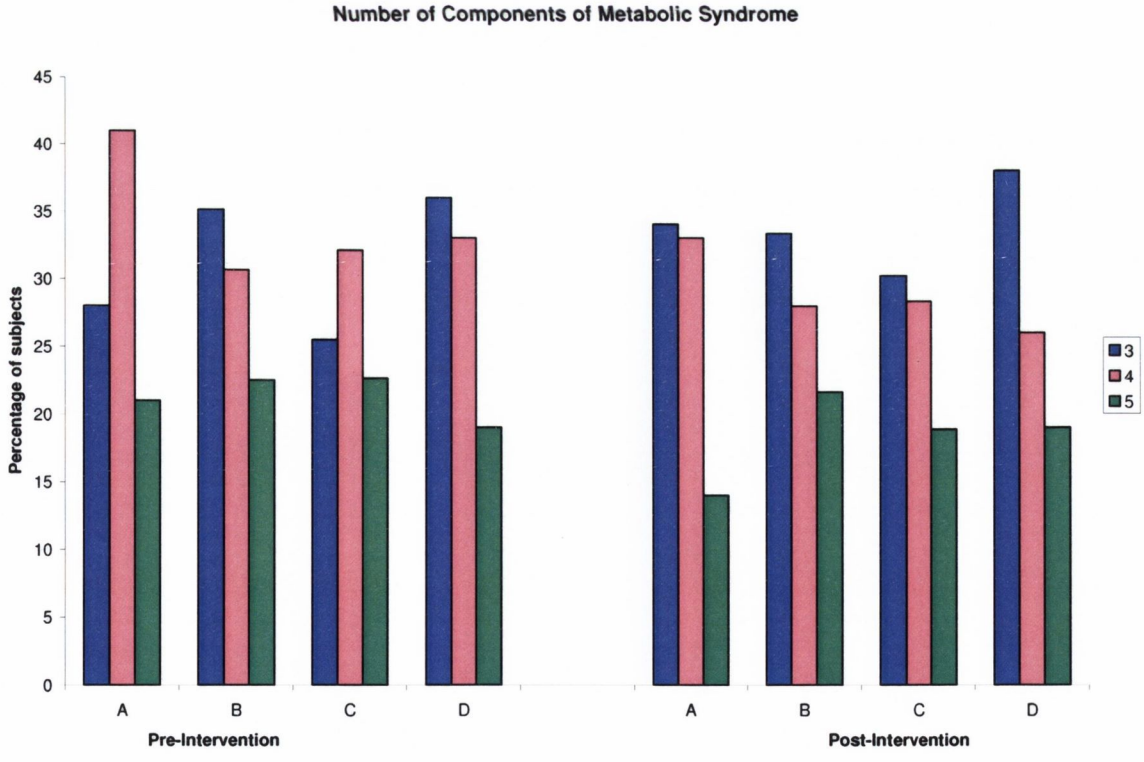


Figure 6.17: The effects of diets on insulin to C-peptide ratio in volunteers with a pre-intervention total fat intake <36%, ✧ $p<0.05$

6.4.5 MetS at post-intervention

All volunteers entering the LIPGENE dietary intervention study from screening had 3 out of 5 criteria as defined by a modified version of the NCEP ATPIII criteria. At post-intervention, 81% had the MetS (33.8% with 3; 28.8% with 4 and 18.5% with 5 criteria).

Figure 6.18 shows the percentage of volunteers with 3, 4 or 5 of the criteria post-intervention as compared to pre-intervention, where 86.6 % had the MetS. Chi Square analysis was used for comparing proportions of participants in the 2 groups with MetS before and after treatment, $p<0.05$. The total number of components of the MetS decreased in all diet groups ($p<0.05$). The number of volunteers with 3 components decreased significantly in the HFMUFA diet (Diet B) from 35% to 33% and increased in the HFSFA, LF and LFn3PUFA diet (Diets A, C and D). However, the number of volunteers with 4 criteria decreased in all diet groups and in the HFSFA, HFMUFA and LF diet groups (Diets A, B and C) for 5 criteria with no change in the HFMUFA diet group (Diet B) ($p<0.05$).



6.5 Discussion

Whilst it has been shown that saturated fat can impair insulin sensitivity in healthy cohorts (Perez-Jimenez *et al.*, 2001; Vessby *et al.*, 2001; Lovejoy *et al.*, 2002), studies demonstrating the effects of dietary fat substitution on individuals with the MetS are lacking in the literature. The LIPGENE dietary intervention study represents the largest European intervention study designed to determine the effect of dietary fat modification on insulin sensitivity and other metabolic markers associated with the MetS. To date, no study has addressed the relative efficacy of the removal of dietary SFA, either by replacement with MUFA or reduction with a low-fat, high-complex carbohydrate diet whilst maintaining an isoenergetic state in a large cohort of MetS subjects under free-living conditions. Subjects with the MetS are ideal to study the effectiveness of diet as a primary preventative measure for T2DM, as it is recognised as the prelude to the ever-growing condition and one that can be easily identified and diagnosed. This study had significant statistical power as a result of the large number of participants and the 12-week study duration. The validated and approved IVGTT method to estimate insulin sensitivity was used and considered a major strength to the study. Nutrient intake, and thus compliance, was analysed with the use of comprehensive dietary records. Considerable efforts to ensure targets were achieved was accomplished through the provision of study foods, clear and structured prescriptive advice with sample meal plans, individualised dietary advice, positive motivational strategies and subject support. As reported in Chapter 4, overall compositional targets were largely achieved with significant differences in fat quantity and quality between the four study diets.

The majority of individuals with the MetS are overweight with abdominal obesity a prerequisite of the IDF definition. Data from meta-analyses (Bray & Popkin, 1998; Yu-Poth *et al.*, 1999; Astrup *et al.*, 2000; 2005) have shown that a reduction in the percentage of dietary fat intake is associated with the amount of weight lost. Total fat intake, expressed as a % of energy, decreased significantly in both the LF and LFn3PUFA diets (Diets C and D) to 28%. This reduction in dietary fat as a % of energy, more than likely accounted for the unintentional weight loss seen in these two diet groups. In a similar group of MetS patients, overweight subjects who received an ad-libitum low-fat high-complex-carbohydrate diet lost weight and subsequently demonstrated a reduction in plasma TAG (Poppitt *et al.*, 2002). These results and results from other large prospective studies (Tuomilehto *et al.*, 2001) support the use

of low fat, high-complex carbohydrate diets for the treatment of obesity in patients with the MetS. Although weight loss was minimal (<2%), this weight loss was significant on the low-fat high CHO diets (Diets C and D) compared to the High Fat diets (Diets A and B), suggesting favourable metabolic outcomes were possible from the onset. Weight loss occurred because of a reduction in energy intake in the LF and LFn3PUFA diets (Diets C and D), as reported in Chapter 4. The reduced fat content and the lower energy density of these diets made it difficult for subjects to maintain isoenergetic conditions despite those volunteers being asked to ensure they consumed two extra portions of CHO daily (equivalent to ~ 70 g CHO) to maintain an isoenergetic state. Among overweight individuals, total fat intakes <30% total energy facilitates weight loss (Astrup *et al.*, 2000). The most profound improvements in metabolic factors associated with the MetS occur in overweight insulin-resistant or hyperinsulinemic individuals when they lose weight. However there is little evidence that low-fat high-CHO diets will improve insulin sensitivity in an isoenergetic state as compared to replacing SFA with MUFA or PUFA (Reaven, 2005). There is considerable evidence in the literature that insulin sensitivity can be improved with weight loss (Olefsky *et al.*, 1974; Su *et al.*, 1995; Reaven, 2000; Weyer *et al.*, 2000; Knowler *et al.*, 2002). Interestingly our study demonstrated a significant time*change in body weight interaction for fasting insulin, QUICKI, and C-peptide concentrations whereby fasting insulin and C-peptide concentrations decreased and QUICKI increased in subjects who lost weight. Therefore, due to the fact that body weight is intrinsically linked to insulin sensitivity, change in body weight was included as a covariate in the RM ANOVA model, which tested for significant differences in the various metabolic markers within and between the four dietary treatment groups.

With initial analysis, the use of the "gold standard" method of measuring insulin sensitivity such as the IVGTT with minimal modelling, failed to show effects of four intervention diets differing in fat quality and quantity despite establishing near isoenergetic differences in fat intake on estimates of insulin sensitivity and measures of insulin resistance. Studies using the IVGTT to measure insulin sensitivity have not demonstrated a deleterious effect of high-fat diets (Borkman *et al.*, 1991; Howard *et al.*, 1991), similar to that in LIPGENE. Fasting insulin and glucose concentrations did not significantly increase on the LF and LFn3PUFA diets (Diets C and D), a finding contradictory to other studies in T2DM patients fed isoenergetic low-fat high-CHO diets (Garg *et al.*, 1988; Garg *et al.*, 1994; Parillo *et al.*, 1996).

Controversy has for a long time surrounded the effects of LC n-3 PUFAs on insulin sensitivity and other factors of the MetS in healthy and diabetic cohorts (Vessby & Boberg, 1990; Vessby *et al.*, 2001). In LIPGENE, the beneficial effects of the 1.24g/day LC n-3 supplement were not observed despite the increase of both EPA (C20:5:3 %) and DHA (C22:6:3 %) concentrations in plasma in this diet group. It did not significantly improve insulin sensitivity or glycaemic control in this group of MetS subjects even with sufficient study duration to allow a change in cell membrane composition.

With further analysis, considering gender, centre and background fat intake, subtle changes were observed. For example, females were shown to have a significantly greater insulin sensitivity index compared to males and fasting glucose increased with increasing age pre- and post-intervention, irrespective of diet. The age effect of worsening hyperglycaemia with time has been previously reported (Hamman, 1992). As expected, there were significant differences in a number of anthropometric measurements and insulin sensitivity biomarkers, including fasting glucose and fasting insulin between the centres. These differences were not unanticipated; at the screening stage, centres employed different strategies to recruit subjects onto the study e.g. lipid clinics, blood pressure clinics, weight managements clinics, impaired fasting glucose clinics etc which could account for the differences observed.

Hyperinsulinemia is associated with hypertension, hyperlipidaemia and CHD (Stout, 1990). Diets high in total fat and SFA have been significantly associated with fasting hyperinsulinaemia with no associations for MUFA or PUFA (Marshall *et al.*, 1997). In LIPGENE, fasting insulin increased on the High-fat SFA-rich diet and decreased on the High-fat MUFA-rich and both low-fat diets, albeit, not significantly. However, a time*centre*diet group effect showed that the HFMUFA diet (Diet B) which was modelled on the Mediterranean style diet of high MUFA intakes, showed potential to improve fasting insulin and insulin sensitivity in some centres. Volunteers in the UU cohort responded to the high-fat MUFA-rich diet and the low-fat high-complex CHO diet. Also HURS-UCO volunteers responded to the low-fat high-complex CHO diet with LC n-3 PUFA supplement with respect to effects on plasma fasting insulin concentrations and QUICKI. These centres are experienced and have good track records in carrying out dietary intervention studies (UU and KANWU; HURS-UCO

and successful interventions and crossover studies). The positive and significant response seen with these two centres may be attributable to protocols being strictly followed, good deliverance of advice and therefore better compliance, or perhaps environmental factors, including habitual fat intake or genetics. As dietary targets were achieved largely by all centres, perhaps it is the latter that is culpable for little dietary responses. Further analysis is ongoing in this area with the investigation into gene-nutrient interactions wherein habitual dietary background is considered (LIPGENE WP1.1, NUGENOB). In the HURS-UCO cohort body weight decreased pre- to post-intervention. This centre also had the highest % Energy from MUFA at baseline. Within the UU centre, body weight decreased pre- to post-intervention; lower baseline waist circumference, higher plasma oleic acid (C18:1%) and total plasma MUFA concentrations pre-intervention, and higher concentrations of plasma EPA (C20:5:3 %) post- intervention, lowest energy intakes and highest CHO intake (% Energy) at baseline were significant findings that emerged with the analysis that may have a cause or effect role in the changes observed.

It is interesting to note that in the well cited KANWU study, very few differences in metabolic markers were found on first inspection. When the data was further primed, an important significant difference was noted in subjects who had a total fat intake below 37% energy wherein decreasing SFA and increasing MUFA improved insulin sensitivity (Vessby et al., 2001). Similarly, with LIPGENE, at first glance, there were no deleterious effects of the high-fat SFA-rich diet on insulin sensitivity. However, when stratifying according to background total fat intake, insulin sensitivity was reduced by 15% and 21% in all subjects and females, respectively who had a pre-intervention total fat intake <36% and were then maintained on a high-fat SFA-rich diet for 12 weeks. Again, centre differences were seen where insulin sensitivity significantly increased in the JUMC cohort in those who had a low total fat intake at baseline (<36%) and were randomised to a lower fat diet (28%) with a high-complex CHO intake (Diet C). HOMA-IR, decreased significantly in females who had <36% total fat pre-intervention and were then randomised to a high MUFA diet (Diet B). This suggests that changes in the total amount of fat in the diet from a low-fat diet (<36%) to a high-fat SFA-rich diet (>38%) can reduce insulin sensitivity. Similarly, a high-fat MUFA-rich diet can have beneficial effects on insulin resistance in subjects with a habitual low-fat diet. With the gender and centre differences observed, there is evidence of hypo- and hyper-

responders which would support the hypothesis that genetic variation may play a role in responsiveness to dietary therapy.

Dietary fatty acids have potential to modulate the inflammatory status in healthy humans (Baer et al., 2004). In LIPGENE MetS subjects, IL-6, CRP, TNFα and adiponectin decreased on the MUFA rich diet (Diet B), although not reaching significant levels. However in the cohorts of HURS-UCO (males only) and UU (females only) on the high-fat MUFA-rich diet, resistin concentrations significantly reduced. The effects of weight loss were not observed in this diet group, a factor that could confound the results through its effects on attenuating the inflammatory state (Ziccardi et al., 2002; Sharman & Volek, 2004). Adiponectin, a plasma protein, originating from the adipose tissue, is gaining considerable interest, with its potent anti-inflammatory effects. Low plasma adiponectin levels are an independent risk factor for the development of T2DM (Lindsay et al., 2002). Post-intervention, adiponectin levels correlated with post-intervention S_I and QUICKI and negatively with HOMA-IR. As a predictor of post-intervention S_I, univariately and when controlling for age, gender and body weight, adiponectin was also as a final predictor in determining S_I in the Low-fat LC n-3 PUFA diet. Other markers of inflammation (TNFα and sICAM and leptin) that correlated with and were final predictors of S_I (sICAM) support evidence of similar findings in ageing, over nourished and physically inactive patients with the MetS (Esposito & Giugliano, 2004). The lack of significant effect on inflammatory markers with the low-fat LC n-3 PUFA diet may be due to the relatively low dose of supplement. One study with 14g/day fish oil supplement showed more favourable effects (Ciubotaru et al., 2003).

The influence of dietary fat on lipoprotein metabolism is mediated by multiple mechanisms (Riccardi et al., 2004). The HFSFA rich diet (Diet A) counter intuitively decreased TAG and increased HDL concentrations. In male subjects, TAG, TRL TAG and TRL cholesterol were significantly reduced on the high-fat SFA-rich diet (Diet A) also. The HFMUFA rich diet (Diet B) showed reductions in fasting plasma TAG, total cholesterol and a significant increase in HDL-cholesterol. The significant increase seen with HDL is not consistent in the literature with some studies reporting increases and some reporting no change (Garg, 1998). While a low-fat diet is still advocated by

many to be a primary and secondary choice of prevention for treatment of T2DM, the unintended increase in carbohydrate intake may reduce HDL-cholesterol and increase TAG concentrations which are core features of the MetS (Grundy, 1999). These patterns, although non-significant, were observed in this study on the low-fat, high-carbohydrate diet (Diet C). However they were attenuated by the low-fat n-3 PUFA diet (Diet D) with the classical TAG lowering effect of LC n-3 PUFA being confirmed. Diet D also saw a reduction in TAG and TRL cholesterol and TRL TAG in males only. Plasma NEFA concentrations significantly reduced on this diet also. When the change in plasma lipids from pre- to post-intervention were analysed, plasma NEFA and cholesterol were significantly reduced in Diet D as compared to the High-fat SFA-rich diet (Diet A). The reduction in plasma NEFA is an important finding as increased concentrations have been shown to induce insulin resistance through interference of insulin signal transduction pathways and glucose transport (Grundy et al., 2004; Dandona et al., 2005). Epidemiological evidence suggests that LC n-3 PUFA have protective properties against the development of T2DM, modifying plasma lipoprotein concentrations, reducing TAG concentrations effectively (Popp-Snijders et al., 1987; Friedberg et al., 1998) and increasing HDL-cholesterol concentrations (Mori et al., 1999). Its effects on insulin sensitivity in humans, however, are not as clearly defined, with some studies showing favourable benefits (Popp-Snijders et al., 1987; Fasching et al., 1991; Delarue et al., 1996) and others showing little or no influence (Vessby et al., 2001). These inconsistencies may result from the many different study designs, patient group studied and the dose and composition of the fish oil administered. Few studies have determined the effects of fish oil supplementation in patients with the MetS. LIPGENE has shown no beneficial effects of fish oil supplementation on insulin sensitivity, and in fact, a non-significant reduction was observed. It seems from the results that CVD risk factors were more susceptible to modification as compared to IVGTT parameters and were optimised by the LC n-3 PUFA supplementation. It could be hypothesised that the relationship between the MetS and the effects on lipoproteins (TAG and HDL-cholesterol) are dependent on changes in body weight as although small, the weight reduction was a significant outcome on both low-fat diets. A diet rich in n-3 fatty acids accompanied by a weight loss factor has shown to have a more favourable outcome on CVD risk than either single component (Mori et al., 1999).

In a recent article it was postulated that fish oil was more effective in modulating insulin action than reversing insulin resistance and hence, the effects would be more evident in healthy cohorts (Giacco et al., 2007). Secondly, fish oil may be more advantageous as part of diet rich in saturated fat as seen in animal studies (Giacco et al., 2007). This same study showed that a moderate supplementation of fish oil had no effect on insulin action, insulin secretion or β-cell function in healthy volunteers, irrespective of the fatty acid composition of the diet (saturated fat or monounsaturated fat). The 1.24g/day, however, proves effective in lowering TAG levels in MetS subjects, possibly by decreasing hepatic production of VLDL TAG (Harris et al., 1990). Thus, supplementation with n-3 PUFAs may be warranted in patients with the MetS through improvement of the plasma lipoprotein profile. It may be postulated that the mechanisms by which n-3 PUFAs reduce TAGs and improve insulin sensitivity are independent of each other. This warrants further investigation.

The significant effects seen with males indicate significant gender effects of the relationship with lipid responses and dietary fat modification. Differences in age, sex, body weight, menopausal status, habitual diet, and baseline lipid values could in part explain the inconsistency in the results. However, the higher baseline values for cholesterol and TAG in males randomised to the HFSFA diet (Diet A), and therefore greater responses seen could reflect the regression to the mean phenomenon. Another potential reason is the lack of a cholesterol-raising effect seen with stearic acid compared to lauric, myristic and palmitic acids. Stearic acid was the primary saturated fatty acid in the study foods provided to subjects on the HFSFA diet (Diet A). As palmitic acid is much more abundant in the typical Western diet, it may be considered more responsible for the cholesterol-raising effect of such diets (Grande et al., 1970). The increase in HDL-cholesterol seen in the High-fat SFA-rich diet is not a new phenomenon. An increase in HDL-cholesterol concentrations with high-fat SFA-rich diets has been previously suggested (Rivellese et al., 2003). The changes seen in the lipids but not seen in glucose and insulin metabolism may indicate that the pathological mechanisms involved may be different for both and may be influenced by different factors, both genetic and environmental.

Although the 3-day weighed food diary and 24-hour recalls used to estimate volunteer's intake and thus compliance over the study period have been validated, the methods used in free living populations are never perfect. Therefore, plasma fatty acid

composition was analysed as a more reliable, objective marker of intake. Fatty acid composition of serum lipid esters (Ma et al., 1995a) or adipose tissue triglycerides (van Staveren et al., 1986; Wolk et al., 1998) reflects fatty acid intakes over several weeks and months. Due to cost and labour limitations plasma fatty acids were analysed for whole serum without standardisation for lipids and may only weakly represent dietary fat composition. This is especially true for polyunsaturated fatty acids and therefore, reflected capsule compliance. The relationship between saturated fat in the diet and in the plasma are weak, and absent with respect to monounsaturated fat (Vessby, 2003). The plasma fatty acid composition data agree largely with the subjects' reported dietary intakes. From the correlation analysis, a negative correlation was observed between the content of saturated fat in plasma and insulin sensitivity and a positive association between insulin sensitivity and total plasma PUFA and n-6 PUFA. In a Finnish prospective cohort study of middle-aged normoglycaemic men, a high proportion of linoleic acid in plasma fatty acids was associated with a lower risk of developing diabetes with lower increases in serum insulin and blood glucose over the 4 year follow-up period (Laaksonen et al., 2002).

QUICKI demonstrated a statistically strong relationship with insulin sensitivity. This has been previously observed where QUICKI demonstrated a statistically strong relationship with the minimal model (Brady et al., 2004a). Significant associations were also observed between SI and HOMA-IR, FPIR and fasting insulin. Insulin sensitivity was negatively correlated with body weight, BMI and waist circumference post-intervention in each diet group. In contrast fasting insulin concentrations were positively associated with these anthropometric measurements. This further highlights the relationship between body weight and insulin sensitivity. AIRg, a measure of insulin secretion can predict the development of T2DM in populations with impaired glucose tolerance (Bunt et al., 2007). In our study AIRg correlated with adiposity measures; body weight, waist circumference and BMI irrespective of diet and with body weight in both low fat diet groups. Greater insulin secretory responses to glucose negatively predicted SI univariately and multivariately. Also, AIRg was a final predictor in determining SI after a low-fat CHO diet (Diet C) also. With the focus mainly on insulin resistance and insulin sensitive states, more emphasis needs to be placed on insulin secretion, its determinants and the effects of varying concentrations of fat and carbohydrate. In a study of obese adolescents, a 2-fold

increase in their insulin secretion was required to maintain normal glucose and lipid concentrations on both high-fat and high-CHO diets (Sunehag et al., 2005). This demand on the pancreatic β-cells would undoubtedly contribute to early β-cell failure and progression to T2DM.

Baseline insulin sensitivity or intervention diets per se did not predict insulin sensitivity in the LIPGENE dietary intervention study. After multivariate adjustment for age, gender and body weight, dietary fats (total, saturated, monounsaturated or polyunsaturated fat) were not associated with insulin sensitivity. The only significant finding was an inverse association between *trans* fatty acids and insulin sensitivity among the LIPGENE volunteers. In the Nurses' Health Study, high intakes of *trans* fatty acids were associated with a higher risk of T2DM during the 14 years of follow up (Salmeron et al., 2001).

Poor dietary quality (lower energy intakes and higher percentages of energy from dietary lipids and alcohol) is associated with development of abdominal obesity and the MetS (Millen et al., 2006). The linear regression model used did not elucidate SFA or MUFA as predictors of insulin sensitivity. Perhaps more controlled regression analysis with a stepwise look at the individual predictors would serve to better answer a more hypothesis driven approach. Grouping together variables constituting healthy or unhealthy risk profiles might better predict insulin sensitivity. Consistent in the linear regression models, however was the inverse relationship between increasing body weight and insulin sensitivity. Univariately and in each of the seven models, increasing body weight was associated with a negative impact on insulin sensitivity. Adiposity, measured in a number of ways is associated with a reduction in insulin sensitivity (Ferrannini et al., 1997). Although waist circumference was not a significant predictor in the final model, it was a significant predictor in the anthropometry model. As men have considerably larger proportions of abdominal fat compared to women who exhibit greater amounts of total and subcutaneous fat (Pouliot et al., 1994), this may explain the lack of association between insulin sensitivity and waist circumference in the cohort as a whole. However, abdominal obesity has been identified as the most prevalent MetS risk factor during a long-term follow-up in healthy women (Millen et al., 2006). Our data was not split by gender before analysis and this could be an avenue for approach in future analysis of the LIPGENE cohort. Although lifestyle information was not assessed in detail in this chapter i.e. marriage status, occupation, sedentary behaviour; two variables, smoking

status and whether the participants played sport or not, were entered into the regression models. Smoking did not seem to have an effect on insulin sensitivity, however, not being physically active compared to those volunteers who play sport predicted a decrease in SI post-intervention. Dietary patterns rather than specific nutrients are now being studied using different statistical techniques including regression and principle component analysis to determine the components of diets that can improve insulin sensitivity and in the long term prevent the development of T2DM. Dietary patterns modelled on the Mediterranean style diet have been negatively associated with features of the MetS (Williams et al., 2000). In the Framingham Offspring study, intake of cereal fibre was associated with a 38% lower incidence of the MetS (McKeown et al., 2004). Increasing fibre intakes post-intervention was a significant determinant of SI when entered univariately whilst controlling for age, gender and body weight in the LIPGENE study. Other dietary variables predicting SI were low total fat intakes including MUFA and trans fatty acid intakes and increasing dietary n-3 PUFA. However these effects were lost when modelled with other variables which could confound effects or lose significance as compared to when entered singularly. The main findings from the linear regression model that could be related back to modifiable lifestyle determinants for improving insulin sensitivity were reducing body weight, increasing physical activity and decreasing trans fatty acids in the diet. This is consistent with current expert nutrition recommendations on the prevention of MetS and obesity risk.

It has been shown that people who followed a Mediterranean Style diet reduced the number of components of the syndrome, and after adjusting for changes in body weight the overall prevalence reduced by approximately one half (Esposito et al., 2004). This adds further weight to promoting such a dietary pattern in this group of subjects. The number of components of the MetS decreased from pre-to post-intervention in LIPGENE also. This decrease, however, was seen across all diets with the greatest decrease seen in the HFSFA diet (Diet A). The HFSFA diet also exhibited a greater decrease in the number of subjects with 4 and 5 components of the MetS at post-intervention. However, of note, subjects randomised to the HFSFA diet (Diet A), at baseline had the greatest percentage of MetS criteria and also had more people with 4 criteria than any of the other diet groups. This more than likely represents regression to the mean phenomenon.

Assessing the outcomes of dietary interventions will always be subtle. Dietary studies are particularly difficult to perform, especially in free-living subjects with respect to dietary compliance and the usual underreporting by subjects (Black & Cole, 2001). The inaccuracy of diet diaries and questionnaires may contribute to the inconsistent results in humans. Overweight and obese individuals tend to underestimate total fat and energy intake (Buzzard, 1998). So although it was shown that dietary targets were largely achieved, true compliance and underreporting are major contributors to the lack of significant findings in such a large study with diverse habitual intakes between centres. It may also be that the variability in insulin sensitivity and genetic factors can explain the inconsistencies and lack of desirable outcomes. The effects of diet on the MetS can be mediated through multiple metabolic pathways including insulin signalling, insulin secretion, lipid metabolism, inflammation, endothelial dysfunction, blood pressure and thrombotic factors. Although the MetS is thought to have a strong genetic component, central to its treatment are weight loss, physical activity and diet. Diet and exercise have previously demonstrated a reduction in insulin resistance (Uusitupa, 1996; Torjesen et al., 1997) with the combination being more effective than each isolated component (Uusitupa, 1996).

The aim of this chapter was to consider the potential role of the changes in quantity and quality of dietary fat in attenuating the manifestations of the MetS and to demonstrate that changing the macronutrient composition whilst maintaining isoenergetic conditions can result in clinically relevant metabolic changes. Variations in macronutrient content of isoeneregtic diets had little impact, if any, on insulin sensitivity when looked at first glance. It has been shown that while approximately 75% of individuals in the most insulin resistant tertile are overweight or obese, 30% in the most insulin sensitive tertile are also overweight/obese (McLaughlin et al., 2004). Weight loss strategies and intervention programmes should be aimed at this subgroup of the population who will benefit from such interventions. With no single diet emerging more advantageous over the other, perhaps the American Diabetes Association (ADA) recommendations wherein diet composition for patients with diabetes mellitus is individualised based on nutritional assessment and desired outcomes (ADA, 2007) hold same for persons with the MetS. With that, goals of therapy should be similar in that maintaining normal or near-normal blood glucose

control, optimising serum lipid and lipoprotein concentrations, attaining desirable weights and improving overall health should be of priority. Perhaps the optimal diet should include MUFA as it has no detrimental effects on insulin sensitivity. Possibly, more significant changes would have been observed if the this type of diet was for a longer duration as following a Mediterranean style diet for greater than 2 years improved endothelial function, reduced waist circumference, plasma glucose, serum insulin and HOMA score in MetS patients (Esposito et al., 2004). Due to the relatively large number of patients in our study, it will be possible to test the hypothesis that gene polymorphisms influence the response to dietary fatty acids and that genetic background is central to such responses. Further analysis will serve to look at stratifying individuals in the cohort according to BMI and to compare the effects of the intervention diets in overweight versus lean individuals. Stratifying the volunteers according to their plasma fatty acid levels (SFA, MUFA, PUFA) post-intervention and looking at the effect of the plasma fat on the biomarkers of insulin sensitivity should also be carried out to further explore the effects of fat on insulin sensitivity. As stated in Chapter 5, with any statistical analysis, it must be considered that with so many statistical tests carried out, the concept of chance findings is an issue.

In summary the LIGPENE intervention study showed that dietary fat composition can alter insulin sensitivity, wherein pre-intervention dietary environment affects responsiveness to dietary intervention. Also n-3 PUFA supplementation in combination with a low-fat diet improved the lipid related CVD risk profiles, particularly in men. Since these interventions were achieved without altered BMI it demonstrates that public health nutrition strategies to attenuate the metabolic risk profile of subjects with the MetS can be achieved, albeit modest, within the context of the overweight/obese phenotype.

ACKNOWLEDGEMENTS

- Insulin and C-peptide analysis were completed in the laboratory of St. James's Hospital, Dublin. Data preparation and statistical analysis was completed by the author.
- Lipid (TAG, HDL-cholesterol, LDL-cholesterol, Total Cholesterol, TRL TAG, TRL Cholesterol, NEFA) and glucose analysis was conducted in the University of Reading.

- Apolipoprotein (apoAI, CII, CIII, B, B48, E, TRL apoB) analysis was conducted in INSERM, Marseille, France.
- Extractions and transmethylations for the plasma fatty acid analysis was conducted in TCD by the author and Jolene McMonagle, Nutrigenomics research Group, TCD. Analysis of the data on the GC was carried out by Jane Ferguson, Nutrigenomics Research Group, TCD.
- IVGTT modelling was carried out by the author and Danielle Shaw (UoR) in the University of Reading.
- All statistical analysis for this chapter was carried out by the author.

Chapter Seven

The G972R variant of the insulin receptor substrate-1 (IRS-1) gene, insulin-sensitivity and gene-nutrient interactions in a MetS cohort.

7.1 ABSTRACT

Background: The G972R variant is a potential contributor to the insulin resistance in T2DM. The IRS-1 polymorphism appears to interact with obesity, potentiating obesity-linked insulin resistance. To date no significant gene-diet interactions in the development of T2DM in humans with IRS-1 have been found.

Objective: To explore if the IRS-1 genetic polymorphism Gly972R associated with increased risk of the MetS plays a causal role in determining an individual's responsiveness to dietary fat modification.

Design: The G972R SNP in the IRS-1 gene has been investigated in relation to its association with biomarkers of insulin resistance in a population of ~450 MetS patients recruited for the LIPGENE dietary intervention study. Gene-diet associations have been probed for using dietary record and plasma fatty acid measurements.

Results: The codon-972 aminoacid variant of IRS-1 in the heterozygous form was found in 11.7% of the WP1.2 MetS cohort. A significant difference between the GG and AG/AA genotypes with respect to Total Energy (MJ/day) (p=0.010) was observed and maintained when controlled for age, gender, centre and BMI (p=0.019). The GG homozygote group for rs1801278 in the IRS-1 gene had significantly higher QUICKI values than the AG/AA genotype (p=0.019). The effect of carrying an A allele in rs1801278 resulted in higher S_I values with plasma MUFA levels below the median of 28.2%.

Conclusion: QUICKI was significantly higher in wild-type subjects than in AG/AA carriers of codon-972 polymorphism. Independently and controlling for potentially confounding factors, total energy intake was significantly increased in A allele carriers compared to wild-type carriers. This may be linked to the finding that obese subjects heterozygous for the codon-972 variant show severe insulin resistance. Energy intake may play as yet an undefined but potentially important role in this effect.

7.2 INTRODUCTION

In light of the Human Genome Project and the rapid advances in molecular biology, a wealth of genetic information is being generated, particularly with respect to the common, polygenetic, diet-related diseases including obesity, insulin resistance and T2DM. It is becoming increasingly obvious that an individual's phenotype represents a complex interaction between the genetic background and environmental factors over the course of an individual's lifetime. Food intake and nutrient exposure are key environmental factors in the pathogenesis and progression of the common polygenic, diet-related diseases. Therefore it is time to identify the nutrient sensitive genotypes and to develop a "personalised nutrition" approach, whereby nutrient intake is manipulated/optimised based on an individual's genetic profile to reduce disease risk and/or improve the effectiveness of dietary guidelines/recommendations in general (Phillips *et al.*, 2007).

For example the "thrifty genotype" (Neel, 1962), which conferred a protective effect in times of food deprivation, promoting fat deposition, has now been proposed to explain the escalating incidence of obesity in recent times as food is more abundant and energy dense and physical activity has decreased. Insulin affects the transcription of several genes involved in energy homeostasis, glucose and lipid metabolism. Therefore the interactions between genes, dietary fat composition and insulin sensitivity are the focus of many studies investigating the molecular mechanisms that affect insulin responsive genes. However, there is a need to further substantiate research in this area.

There has been a lot of focus on proximal components of the insulin signalling pathway since functional variation of these molecules could in part explain the widespread disruption of post-receptor signalling that characterises T2DM (Zeggini *et al.*, 2004). Insulin receptor substrate–1 (IRS-1) plays a key role in proximal insulin signalling and many sequential variants of the IRS-1 gene have been identified (Zeggini *et al.*, 2004). The main focus has been on two relatively infrequent nonsynonymous variants P512A (rs1801276) and G971R (G972R, rs1801278). The combined prevalence of these variants was suggested to be increased in T2DM (Almind *et al.*, 1993). The G972R variant, however, is more common and has stronger evidence to support direct effects on gene product formation (Almind *et al.*, 1996; Hribal *et al.*, 2000). When polymorphisms

of IRS-1 were grouped together, the overall frequency of the codon 972 variant was 10.7% in T2DM patients versus 5.8% in control subjects ($p<0.02$), suggesting that this genetic variant is a potential contributor to the insulin resistance in T2DM (Hitman *et al.*, 1995). A recent augmented meta-analysis of 27 association studies indicated that carrying the G972R allele conferred a significant increase in disease risk by ~15% (Zeggini *et al.*, 2004). To test the reproducibility of this association, Florez *et al.*, (2004) recently attempted to replicate the same genetic model in a large sample of 9,000 white individuals. Despite >95% power to obtain a $p<0.05$ for the odds ratio of 1.25 that had been estimated in the meta-analysis, they did not observe an association of G972R with T2DM related traits or age of onset (Florez *et al.*, 2004).

Insulin stimulated glucose uptake is significantly decreased in skeletal muscle from the obese and in adipose tissue from rats fed a high-fat diet, with reduced protein levels of IRS-1 and PI3K (Goodyear *et al.*, 1995; Stevenson *et al.*, 1996). Mice made IRS-1 deficient by targeted gene knockout exhibit hyperinsulinaemia and glucose intolerance (Araki *et al.*, 1994; Tamemoto *et al.*, 1994). mRNA and protein levels of IRS-1 and PI3K were significantly lower in rats fed a beef tallow diet than those fed a safflower oil diet suggesting an impairment of the initial steps in insulin signal-transduction pathways contributing to the defect in insulin-stimulated glucose uptake in skeletal muscle (Kim *et al.*, 1996). The R972 allele of the IRS-1 gene inhibits IRS-1 binding to PI3K owing to the defective interaction between IRS-1 and the p85 subunit of PI3K (Almind *et al.*, 1996). In the Diabetes Prevention Study, the R972 allele predicted poor weight reduction in the intervention group and also predicted the development of T2DM (Laukkanen *et al.*, 2004).

In intervention studies aimed at dietary changes, those subjects who are more susceptible or resistant to weight changes or other changes in outcome measures may be discovered more easily (Uusitupa, 2005). Selecting individuals according to their genetic background and looking at the impact of the intervention in genetically known groups of subjects may be applied in diet studies with surrogate outcome measures like changes in insulin sensitivity, blood pressure or serum lipids. Although the IRS-1 polymorphism appears to interact with obesity, potentiating obesity-linked insulin resistance (Clausen *et al.*, 1995; Baroni *et al.*, 2001) as yet, no significant gene-diet interactions in the

development of T2DM or the MetS with IRS-1 have been found. The aim of this chapter was to explore genotype-by-nutrient interactions for the IRS1 gene in MetS subjects using the LIPGENE intervention cohort.

7.3 SUBJECTS AND METHODS

Details of subject recruitment, study design, dietary intervention methodology, collection of fasting blood samples and determination of the various metabolic makers including plasma fatty acids and IVGTT protocol have been previously described in Chapter 2.

7.3.1 DNA extraction and genotyping

Buffy coat samples were obtained from blood collected from participants of the dietary intervention study. The total number of samples was 464. The DNA was extracted using the Gentra Autopure LS system. Briefly; samples were thawed at 37° and lysed using 1ml cell lysate solution. Each sample was placed in a bar-coded tube, and these were inputted into the system using a computer and scanner, and a standard protocol was selected. Further extraction was thus carried out by the machine. DNA was left to re-hydrate for ~24hours prior to quantification and further use. The concentration of the extracted DNA was determined using Nanodrop. Samples with a concentration less than 60ng/ul were replaced by re-extracting another buffy coat sample. There were 55 samples with low DNA concentrations, i.e. ~12% of the total number. Where a replacement was not available, or the re-extraction also gave a low yield, whole genome amplification was completed using the Qiagen Repli-g midi WGA kit. Altogether, 24 samples were amplified. An input amount of DNA of 10ng or more was used in all cases (this involved using 5ul in most cases and 10ul in very low samples i.e. those below 5ng/ul to begin with). Quantification of DNA using Nanodrop recorded high yields of DNA (1000 – 2000ng/ul) but this is not a reliable indicator of actual concentration due to the presence of primers and other nucleotides that can mask an absence of amplified material. Performance was thus determined by Ilumina genotyping, and was generally good for the WGA samples.

7.3.2 Statistical Analysis

An independent samples t-test was carried out using SPSS version 14 to probe for associations between the genotypes in the IRS-1 gene, and individual biomarkers of the metabolic syndrome. The tests were carried out on the cohort at baseline. Tukey's Post Hoc test was used in all cases unless otherwise indicated (LSD for borderline significance, Tamhane for non-homogeneity of variance). ANCOVA was carried out to determine whether associations were present after controlling for age, gender, centre of origin and BMI.

Repeated Measures ANOVA was carried out to assess the effects of the SNP with the diet group assigned on each biomarker at both the pre- and post-intervention timepoints. These RM ANOVAs showed significant within-subject effects, where the SNP in question was associated with the response i.e. an interaction was present between the SNP and the timepoints for a measured variable; and between-subject effects where the SNP was associated with differences in the values of the variables between individuals. RM ANCOVA was also carried out, controlling for age, gender, centre, diet group and BMI.

Following on from the associations seen in the ANOVA analysis, the information regarding dietary fat intake from dietary records, and the composition of fatty acids as determined from plasma analysis, was added to the model. The measures used, both from dietary records and plasma, include total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA) and the calculated PUFA:MUFA (P:M), PUFA:SFA (P:S) and MUFA:SFA (M:S) ratios.

For each fatty acid total, or calculated ratio, the median was determined. New variables were thus created, where the cohort was dichotomised, scoring 1 for a value below the median, and 2 for a value above the median for each fatty acid measure. The cohort was then split based on this new dichotomous variable, and a one-way ANOVA was carried out baseline to assess whether previously significant associations showed an interaction with high or low intake of the particular fatty acid. This thus showed cases where the effect of genotype on a biomarker of insulin resistance could be altered by dietary fat intake.

7.4 RESULTS

The frequency of the IRS-1 SNP (rs1801278) among the LIPGENE WP1.2 cohort is illustrated in Figure 7.1. Of the MetS cohort, 88% had the GG genotype, 11.7% had the AG genotype and 0.23% had the AA genotype.

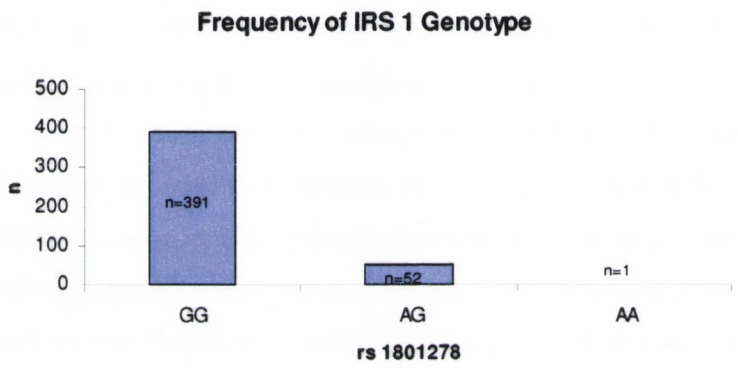


Figure 7.1: The frequency of the IRS-1 genotype in the LIPGENE WP1.2 Cohort

The only subject homozygous for AA was female, overweight (BMI 29.03kg/m^2) and met 5 out of 5 criteria for the MetS (ATPIII criteria). Her S_I at pre-intervention was 15.28 (mU/L)-1min-1 and was deemed an outlier and removed for all previous analysis and hence for further analysis to maintain consistency. As such, this subject was pooled with the AG heterozygote group for all future analysis to increase power.

The frequency of the GG and AG/AA genotypes were assessed across BMI categories, illustrated in *Figure 7.2.* For both the GG and AG/AA genotypes, the number of subjects increased with increasing BMI.

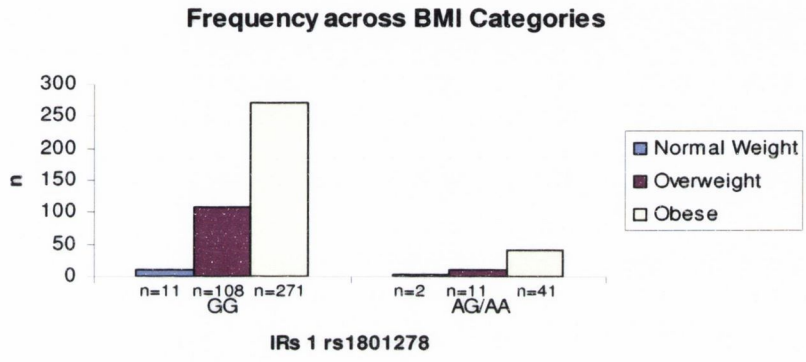


Figure 7.2: The frequency of the IRS-1 genotype in the LIPGENE WP1.2 Cohort across BMI Categories.

7.4.1 Differences at Baseline

Table 7.1 highlights the differences between anthropometric, insulin sensitivity and other variables exported from the IVGTT, lipid parameters, plasma fatty acids, dietary markers and TNFα. An independent samples t-test demonstrated significant differences between the GG and AG/AA genotypes with respect to total energy intake (MJ/day) (p=0.010) and when controlling for age, gender, centre and BMI, this significance was maintained (p=0.019). The ANCOVA also illustrated a significant difference in the QUICKI values between genotypes, with the GG homozygote group for rs1801278 in the IRS-1 gene having significantly higher QUICKI values than the AG/AA genotype (p=0.019).

Table 7.1: Anthropometirc and biochemical variables between the genotypes

		GG			AG/AA			
Baseline	n	Mean	SEM	n	Mean	SEM	Independent t-test	ANCOVA
Age	391	54.51	0.45	53	53.51	1.23		
Height (m)	390	1.68	0.00	53	1.68	0.01		
Body weight (kg)	390	91.50	0.73	53	93.41	1.72		
BMI (kg/m^2)	390	32.41	0.21	53	33.12	0.56		
Waist Circumference (cm)	390	106.13	0.57	53	107.78	1.29		
Hip Circumference (cm)	390	112.99	0.50	53	115.42	1.41		
Diastolic Blood pressure (mmHg)	389	86.14	0.51	53	86.33	1.32		
Systolic Blood Pressure (mmHg)	389	138.46	0.81	53	140.85	2.04		
Body Fat (%)	378	36.81	0.46	52	36.92	1.09		
Body fat (kg)	378	33.45	0.48	52	34.42	1.27		
Lean body mass (%)	378	61.67	0.50	52	61.37	1.17		
Lean Body Mass (kg)	378	56.39	0.71	52	57.37	1.73		
Body Water (%)	326	44.70	0.39	48	44.46	0.87		
Body Water (Litres)	313	40.96	0.58	43	41.44	1.44		
Impedance (kHZ)	273	522.47	5.50	39	519.14	10.17		

		GG			AG/AA			
Baseline	n	Mean	SEM	n	Mean	SEM		
S_I	349	2.85	0.09	45	2.85	0.24		
S_G	350	0.02	0.00	46	0.02	0.00		
AIRg	350	359.33	18.74	46	320.95	33.71		
DI	350	880.65	38.93	46	936.71	107.76		
G0	350	299.55	2.20	46	297.76	5.34		
P(2)	350	0.04	0.00	46	0.04	0.00		
P(3)	350	0.00	0.00	46	0.00	0.00		
Fasting Glucose(mmol/L)	382	5.94	0.04	53	6.00	0.09		
Fasting Insulin	382	10.14	0.30	53	10.28	0.64		
HOMA-IR	385	2.68	0.09	53	2.78	0.19		
QUICKI	382	0.61	0.02	53	0.49	0.09		p=0.019
C-peptide	379	2.62	0.05	53	2.64	0.13		

		GG			AG/AA			
Baseline	n	Mean	SEM	n	Mean	SEM		
TAG (mmol/L)	390	1.81	0.05	53	1.70	0.09		
Total Cholesterol (mmol/L)	390	5.35	0.05	53	5.34	0.12		
NEFA	390	614.71	11.63	53	606.00	32.61		
TRL-TAG	385	0.88	0.03	51	0.80	0.06		
TRL-Cholesterol	385	0.39	0.02	51	0.36	0.03		
LDL-Cholesterol	386	3.29	0.05	52	3.12	0.15		
HDL-cholesterol	390	1.10	0.01	53	1.14	0.04		

		GG			AG/AA			
Baseline	n	Mean	SEM	n	Mean	SEM		
Plasma C20:5:3	384	1.00	0.05	52	0.85	0.10		
Plasma C22:6:3	384	2.21	0.06	52	2.07	0.17		
Plasma SFA	384	32.61	0.32	52	32.47	1.01		
Plasma MUFA	384	28.08	0.24	52	27.96	0.63		
Plasma PUFA	384	39.32	0.35	52	39.56	0.99		
Plasma n-6 PUFA	384	35.28	0.32	52	35.85	0.97		
Plasma n-3 PUFA	384	4.04	0.11	52	3.72	0.26		
Plasma PUFA: SFA	384	1.27	0.02	52	1.30	0.06		
Plasma MUFA:SFA	384	0.89	0.01	52	0.91	0.04		
Plasma MUFA:PUFA	384	0.75	0.01	52	0.74	0.03		

		GG			AG/AA			
Baseline	n	Mean	SEM	n	Mean	SEM		
Basal Metabolic Rate	361	7.18	0.06	52	7.29	0.14		
Total Energy (MJ)	363	8.66	0.14	52	9.67	0.37	p=0.010	p=0.019
Total fat (% Energy)	363	35.85	0.38	52	37.45	1.12		
SFA (% Energy)	363	12.46	0.17	51	12.10	0.41		
MUFA (% Energy)	363	13.39	0.25	52	14.41	0.73		
PUFA (% Energy)	363	5.55	0.12	52	5.58	0.24		
n-3 (g/day)	253	1.45	0.07	43	1.68	0.19		
n-6 (g/day)	253	9.09	0.35	43	10.30	0.97		
CHO (% Energy)	363	43.91	0.40	52	42.39	1.12		
Protein (% Energy)	363	17.88	0.21	52	17.08	0.46		
Alcohol (% Energy)	363	2.38	0.20	52	3.11	0.64		
Dietary PUFA:SFA	363	0.47	0.01	51	0.49	0.03		
Dietary MUFA:SFA	363	1.13	0.03	51	1.22	0.06		
Dietary MUFA:PUFA	363	2.70	0.07	52	2.83	0.19		

		GG			AG/AA		
Baseline	n	Mean	SEM	n	Mean	SEM	
TNFα	389	5.13	0.26	53	5.34	0.40	

7.4.2 SNP*Fatty Acid interactions

To investigate the presence of SNP*Fatty Acid interactions in association with the biomarkers, a Univariate General Linear Model was used. Following this analysis, significant interactions were further investigated using medians, tertiles and quartiles of the fatty acids in one-way ANOVA models. In *Figure 7.3*, the effect of carrying an A allele in rs1801278 results in higher S_I values with plasma MUFA levels below the median of 28.2%.

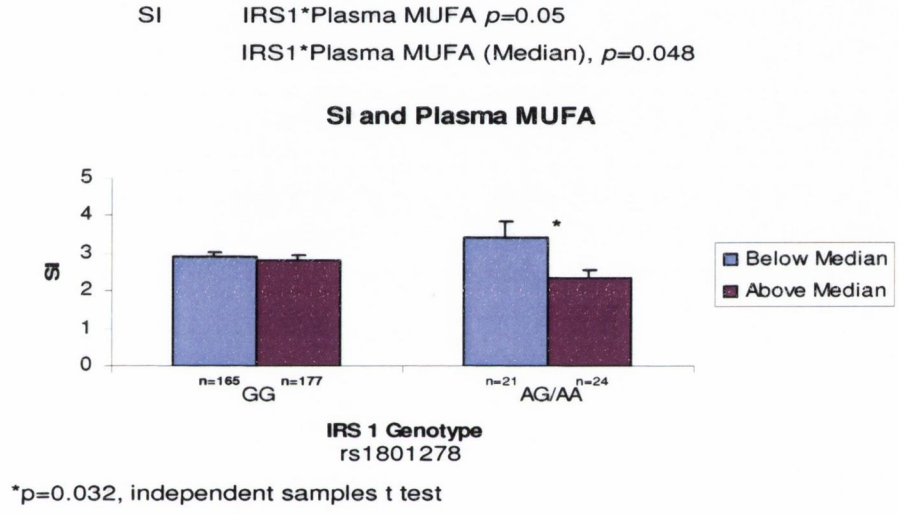
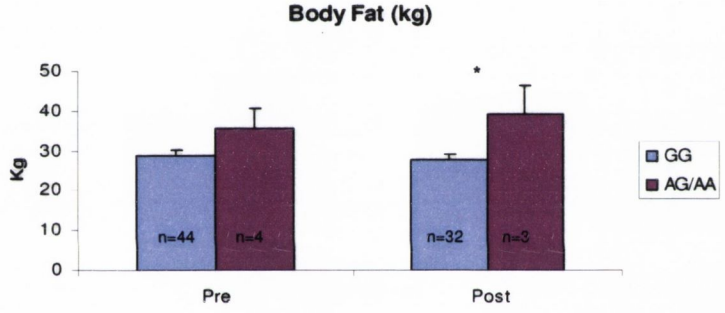


Figure 7.3: Mean pre-intervention S_I levels in the cohort split by genotype at rs1801278 in IRS-1 gene, and separated by plasma MUFA values above and below the median. While the A allele carriers show significant increases in S_I associated with lower plasma MUFA levels (p=0.032), this is not seen in the GG homozygotes, whose S_I stays constant across varying MUFA levels.

7.4.3 RM ANOVA

A repeated measures ANOVA (RM ANOVA) was carried out on the various biomarkers pre- and post-intervention (within subject variables) controlling for genotype, gender, centre and diet (between subject factors) and age and BMI at baseline (covariates) to assess time*genotype*diet group interactions and genotype*diet main effects. The figures below describe the significant interaction and main effects observed.

Genotype*Gender*Diet, p=0.029
Diet A (High Fat, High SFA diet), Post-Intervention Body Fat (kg), Males



*p=0.034

Figure 7.4: Males assigned the control high-fat, SFA rich diet (Diet A) for 12 weeks and carrying the A allele in rs1801278 had significantly higher body fat (kg) post-intervention compared to the GG homozygote group (p=0.034).

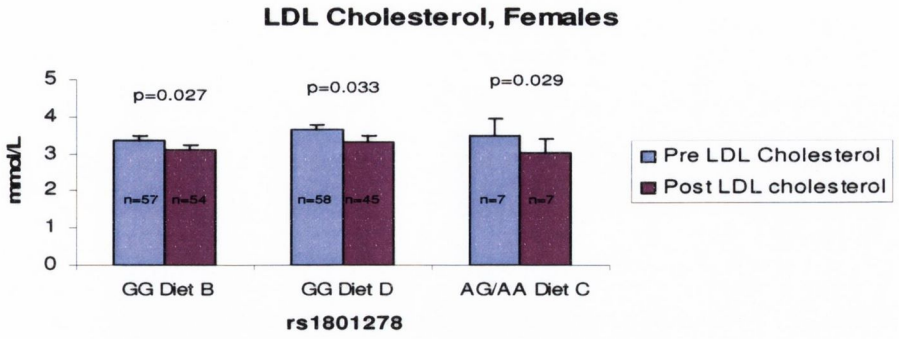
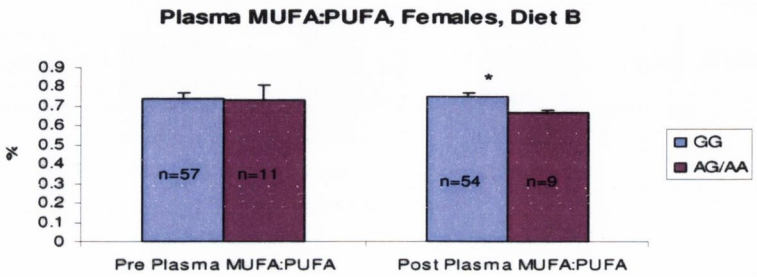


Figure 7.5: This figure highlights a significant time*genotype*gender*diet (p=0.009) effect wherein females in the GG homozygote group and randomly assigned to Diet B (High-fat, MUFA rich diet) and Diet D (Low-fat with 1.24g LC n-3 PUFA) had significantly lower LDL cholesterol concentrations post- compared to pre-intervention (p=0.027 and p=0.033, respectively). Females carrying an A allele in rs1801278 and assigned the low-fat, high-CHO diet (Diet C) also had significantly lower LDL cholesterol concentrations post-intervention (p=0.029).



*p=0.009

Figure 7.8: Females assigned to the high-fat, high MUFA diet in the GG homozygote group had a significantly higher plasma MUFA:PUFA ratio post-intervention as compared to the AG/AA genotypes (p=0.009).

7.4.4 Linear Regression

Linear regression analysis was carried out to determine if the G972R variant predicts MetS criteria or measures of insulin sensitivity. The analysis was carried out univariately and multivariately controlling for age, gender and BMI. Both univariately and multivariately, QUICKI was determined by G972R (Univariately: p=0.026, β -0.107, CI=-0.232 to -0.015, r^2 0.011, adjusted r^2 0.009; Multivariately: p=0.039, β -0.098, CI=-0.221 to -0.005, r^2 0.034, adjusted r^2 0.025). Total energy intake was also significantly associated with rs1801278 (Univariately: p=0.010, β 0.127, CI=0.244 to 1.770, r^2 0.016, adjusted r^2 0.014 and Multivariately: p=0.010, β 0.110, CI=0.206 to 1.541, r^2 0.256, adjusted r^2 0.248).

7.5 DISCUSSION

IRS-1 is a major substrate for the insulin receptor and regulates insulin signalling in skeletal muscle and adipose tissue. Of the many polymorphisms of the IRS-1 gene described, the glycine to arginine substitution at codon 972 (G972R) has been studied in conjuction with obesity, polycystic ovarian syndrome and T2DM (Almind *et al.*, 1993; Almind *et al.*, 1996; Sir-Petermann *et al.*, 2001). The R allele has been associated with impaired insulin associated signalling (Almind *et al.*, 1993; Almind *et al.*, 1996) and lower fasting plasma concentration of insulin and C-peptide (Almind *et al.*, 1993). Carriers of this polymorphism also have several features of the MetS, including high levels of total TAG, total/HDL-cholesterol ratio, FFA levels, systolic blood pressure and intima-media thickness (Marini *et al.*, 2003).

We have shown that the codon-972 amino-acid variant of IRS-1 in the heterozygous form (AG) is found in 11.7% of the WP1.2 MetS cohort. Overall, the frequency of G972R amino acid substitution is ~6% in normal populations and is increased in T2DM patients by about twofold (Hitman *et al.*, 1995). When polymorphisms of IRS-1 were grouped together, the overall frequency of the codon 972 variant was 10.7% in T2DM patients versus 5.8% in control subjects ($p<0.02$), suggesting that his genetic variant is a potential contributor to the insulin resistance in T2DM (Hitman *et al.*, 1995). In the current study only one of the 444 subjects was homozygous for the codon-972 variant. The phenotype of the subject homozygous for the codon-972 variant was interesting in that she was overweight and attained 5 out of 5 of the criteria for the MetS at baseline. It is difficult to assess whether these characteristics are as a result of the IRS-1 variant or the contributing effects of environmental or other genetic factors. However similar to the observation by Clausen *et al.,* (1995) of a single subject with the same genotype, they both had reduced glucose effectiveness and therefore both at increased risk of developing T2DM. The molecular mechanisms by which the homozygous form of the codon-972 variant of IRS-1 adds to the phenotype are unknown (Clausen *et al.*, 1995). It would be interesting and perhaps more meaningful to assess the prevalence of this codon-972 amino acid polymorphism in a random sample of control, disease-free subjects and to relate the presence of the variant to insulin sensitivity and glucose effectiveness in comparison to this MetS cohort.

In analysis of the whole study population, there were no significant differences in anthropometric measures, lipid parameters, plasma fatty acid concentrations and parameters exported from the IVGTT in carriers of the A allele. However, QUICKI calculated from fasting insulin and glucose measures were significantly higher in wild-type subjects than in AG/AA carriers of codon-972 polymorphism. The prevalence of G972R polymorphism is significantly higher in patients with T2DM than in normal subjects, and subjects who carry IRS-1 polymorphism tend to be insulin-resistant compared with those with no such mutation (Ura *et al.*, 1996). Independently and controlling for potentially confounding factors, total energy intake was significantly increased in A allele carriers compared to wild-type carriers. One could speculate that this may be linked to the finding that obese subjects heterozygous for the codon-972 variant show severe insulin resistance; their insulin sensitivity half that of obese wild-type subjects and a third of that of normal-weight codon -972 variant carriers (Clausen *et al.*, 1995) and that energy intake may play as yet an undefined but potentially important role in this effect.

Multiple linear regression analysis was used to find out whether there was an independent association of the IRS-1 codon-972 variant and insulin sensitivity after adjustment for age, gender, centre of origin and BMI. Again AG/AA genotype versus the GG homozygote group was associated with decreasing QUICKI values and increasing energy intakes. Although the adjusted r^2 was low, it nevertheless suggests that the G972R polymorphism plays a role in determining these two factors and as such further work is needed in exploring this hypothesis.

The concept of gene-diet interaction describes the modulation of the effect of a dietary component on a specific phenotype by a genetic polymorphism (Lopez-Miranda *et al.*, 2007). Many genes have been explored in this sense and the results suggest that diet plays a major role in triggering the insulin resistance syndrome by interacting with genetic variants at candidate genes. However, to my knowledge no study has looked at the G972R IRS-1 polymorphism in this regard. The results of this chapter are more a demonstration of the statistical methods and approach used in determining gene-nutrient interactions. For studies on gene-diet interactions the dietary data collected should be reliable with sound dietary methods used. Large scale studies with thousands of subjects

are needed, both cross-sectional and follow-up in nature. Interestingly, no gene*dietary nutrient effects were observed in this set of analysis. With increasing plasma MUFA levels, however, insulin sensitivity decreased in the AG/AA genotype. No such effects were observed for any other plasma fatty acid. The effects of the intervention were also analysed to investigate if genotype and changing the quality or quantity of dietary fat for 12 weeks resulted in a change in the metabolic biomarkers of the MetS (time*genotype*diet). We have shown p values that would need adjustments to compensate for multiple testing. It is also important to put emphasis on the evaluation of the size and effects assessed, taking into account the genotype-by-diet interactions are exploratory rather than confirmatory.

This study was a logical step in the exploration of the hypothesis that a common genetic variant in IRS-1 may influence insulin resistance. Other variants of IRS-1 may affect gene expression or protein function and thereby induce insulin resistance; in such a scenario varying degrees of linkage disequilibrium (LD) in different populations may give rise to discrepant association signals. A limitation of this study was the exclusion of several other variants of the IRS-1 gene. The small size of our study with the rare frequency of some alleles limits our ability to assess significantly significant associations.

This chapter served its purpose as a first glance at gene-nutrient interactions with a common variant in the IRS-1 gene. Future work will include further assessing the effects of BMI on differences between the genotypes since obesity is known to be a stressor of insulin action and pancreatic β-cell function.

Some individuals appear to be relatively insensitive to dietary intervention, whereas others have an enhanced sensitivity (Lopez-Miranda *et al.*, 2007). Identifying hypo- and hyperresponders to support the hypothesis that responsiveness is related to genetic variation is important in deciphering information important at individual and at a population level.

ACKNOWLEDGEMENTS

- DNA extraction was carried out by Jane Ferguson, Nutrigenomics Research Group, TCD.
- All analysis for this chapter was carried out by the author.

Chapter Eight

General Discussion

An important justification for the investigation of the MetS is its high-risk associated with the development of T2DM. Therefore a dietary intervention study with adequate size and duration to comprehensively determine the effect of an IVGTT and other metabolic risk factors that determine insulin sensitivity is of considerable importance. The LIPGENE Dietary Intervention Study represents the largest pan-European intervention study designed to determine the effect of dietary fat modification on insulin sensitivity and other metabolic markers associated with the MetS, including lipid metabolism, inflammation, and coagulation. To date no study has addressed the relative efficacy of the removal of dietary SFA either by replacement with MUFA or reduction with a low-fat, high-complex carbohydrate diet whilst maintaining isoenergetic conditions or without a weight loss factor in a group of subjects with the MetS. The modified version of the NCEP definition used in LIPGENE and described in Chapter 3 detected more subjects at future risk for diabetes, supporting the notion of early detection with prevalence rates rising to epidemic proportions. The NCEP definition has a clinical strategy with the objective of reducing the population burden of atherosclerosis (NCEP, 2002). In the San Antonio Heart Study, the prediction of diabetes by the MetS improved when using the fasting plasma glucose cutoff of ≥5.4mmol/L (Lorenzo *et al.*, 2003) and performed well as a predictor of diabetes.

The outcomes of LIPGENE were dependent on the successful manipulation of the subject's habitual diets to one of the four intervention diet groups. In Chapter 4 the methods employed in the design and implementation of the four experimental diets differing in saturated, monounsaturated and total fat composition were described. The results indicated that the food exchange model used was successful. The compositional objectives were largely achieved enabling the creation of two diets (HFSFA and HFMUFA) that were significantly different in terms of %E from fat than the other two diets (LF and LF n-3 PUFA), thus fulfilling the objective of the model. The HFSFA diet had significantly greater %E from SFA compared to the other three diets whereas diet HFMUFA had significantly greater %E from MUFA than the other diets. Significantly greater LC n-3 PUFA intakes in the LF n-3 PUFA diet group were

also achieved. However, mean EI/BMR values in the low-fat diet groups were lower than the high-fat diets groups, suggesting that underreporting of energy intake may have occurred. Numerous studies have shown that it is difficult to obtain a representative picture of what people usually eat, mainly because of large day-to-day variations in food intake, misreporting and changes in dietary habits during a given study. Intervention trials attempting to establish a relationship between diet and chronic disease require careful measurement of adherence to dietary protocols. As such, assessing the outcomes of dietary interventions will always be subtle. The inaccuracy of diet diaries and questionnaires may contribute to the inconsistent results in humans. Overweight and obese individuals tend to underestimate total fat and energy intake (Buzzard, 1998). So although it was shown that dietary targets were largely achieved, true compliance and underreporting are major contributors to the lack of significant findings in such a large study with diverse habitual intakes between the participating centres.

The objective of Chapter 5 was to assess, among MetS subjects, participating in the LIPGENE Dietary Intervention Study, the physiological, metabolic, biochemical and dietary characteristics between gender, age groups and BMI status as the aim of identifying subjects with the MetS is to distinguish those with an elevated risk of developing CVD. Costly efforts are now being employed to detect and treat the MetS thus reducing the risks and attempting to prevent the continuing surge of obesity, T2DM and CVD, which many populations are now experiencing. To further understand the characteristics of the MetS and population groups most at risk, attention needs to be paid to age groups and gender differences in the various abnormalities to prevent the multicomponent disorder. Although obesity is probably the major lifestyle determinant of the MetS, examining a persons education, work and living status as well as physical activity, cigarette smoking, alcohol intake and age enables public health strategists to target specific groups of the population deemed at risk. Interestingly, being married, divorced or separated and working in the home were negatively associated with insulin sensitivity when entered univariately into the model. In common, being separated and working in the home were positively associated with an increase in HOMA-IR. Perhaps underlying stresses associated with living and working experiences should be examined in further detail. Characterising a group of MetS subjects in terms of differences between countries, age, BMI and intakes of total amount and type of fat will give a better understanding of isolated

components and combined risk factors that can be better targeted. The differences in metabolic variables with increasing age and BMI, highlights the fact that the prevalence of obesity and impact of age could be main determinants of the MetS. The finding that the MetS increases with age was not apparent in this study (examining the five components) but this could be as a result of recruitment strategies or medication usage. We examined gender differences in the characteristics of the metabolic components and found differences in waist and hip circumference, diastolic blood pressure, insulin sensitivity, AIRg, fasting glucose, total cholesterol and NEFA concentrations, among others. It has previously been demonstrated that men are more insulin resistant than women (Nuutila *et al.*, 1995). Metabolic control varies among the genders (Hwang *et al.*, 2007) with molecular and cellular hormonal, sexual and body fat composition differences. The protective effect of female sex hormones and the unfavourable effects of testosterone on substrate metabolism has been suggested (Hwang *et al.*, 2007). From our analysis in Chapter 5 it would seem that males are more at risk (higher fasting glucose and insulin concentrations, blood pressure, waist circumference, TAG, lower insulin sensitivity and HDL-cholesterol concentrations). This might be explained by the more favourable gluteal fat distribution in women. Abdominal obesity is more attributable to men which confers a greater risk of developing insulin resistance.

In Chapter 5, when stratifying the group according to low, medium and high intakes of dietary SFA, an increase in fasting insulin, HOMA-IR and C-peptide concentrations was observed. In animal studies diets high in saturated fat caused distinct insulin resistance (Storlien *et al.*, 1991). Long term basal hyperinsulinaemia may eventually cause β-cell toxicity and damage (Stein *et al.*, 1997). A reduction in dietary SFA intake from <13% of total energy to <10% of total energy in our study population was associated with a reduction in fasting insulin of 21%. This finding is similar to the Normative Aging study where they experienced a reduction in fasting insulin of 26% and 32% in postprandial insulin with a reduction of SFA (Parker *et al.*, 1993). Similar to their study, family history was not accounted for in this analysis which places volunteers at further risk. However, the results suggest that a reduction in body weight and SFA intake may ameliorate fasting insulin concentrations and thus CVD risk. Fasting glucose and fasting insulin levels increased with increasing number of metabolic disorders which has been demonstrated previously (Haffner *et al.*, 1992; Mykkanen *et al.*, 1993; Mykkanen *et al.*, 1997). Subjects with 5 disorders had 28.4%

higher fasting insulin concentrations than subjects with 3. Fasting insulin and HOMA-IR was significantly associated with a clustering of metabolic disorders compared to insulin sensitivity. Reasons for this could be the close association of hyperinsulinaemia to the components that cluster in the MetS.

Central to the prevention of insulin-resistance syndrome is the avoidance and management of overweight, especially central obesity. The combination of a reduced-energy diet and increased physical activity is recommended by the US NIH (1998) on the premise that it produces weight loss and may also result in decreases in abdominal fat. In Chapter 5 we identified body weight as a re-occurring risk factor to emerge from the regression models in the determination of insulin sensitivity and resistance. In addition, body weight significantly decreased from low to high insulin sensitivity tertiles as did BMI, confirming its underlying causality in the MetS. There is considerable evidence in the literature that insulin sensitivity can be improved with weight loss (Olefsky *et al.*, 1974; Su *et al.*, 1995; Reaven, 2000; Weyer *et al.*, 2000; Knowler *et al.*, 2002). Interestingly in Chapter 6 a significant time*change in body weight interaction for fasting insulin, QUICKI, and C-peptide concentrations whereby fasting insulin and C-peptide concentrations decreased and QUICKI increased in subjects who lost weight was demonstrated. It has been shown that while approximately 75% of individuals in the most insulin resistant tertile are overweight or obese, 30% in the most insulin sensitive tertile are also overweight/obese (McLaughlin *et al.*, 2004). Weight loss strategies and intervention programmes should be aimed at this subgroup of the population who will benefit from such interventions. With initial analysis in Chapter 6, the use of the "gold standard" method of measuring insulin sensitivity such as the IVGTT with minimal modelling, failed to show effects of four intervention diets differing in fat quality and quantity despite however establishing near isoenergetic differences in fat intake on estimates of insulin sensitivity and measures of insulin resistance. With further analysis however, considering gender, centre and background fat intake, subtle changes were observed. For example, females were shown to have a significantly greater insulin sensitivity index compared to males and fasting glucose increased with increasing age pre- and post-intervention, irrespective of diet. The age effect of worsening hyperglycaemia with time has been previously reported (Hamman, 1992). At first glance, there were no deleterious effects of the high-fat SFA-rich diet on insulin sensitivity. However, when stratifying according to background total fat intake, insulin sensitivity was

reduced by 15% and 21% in all subjects and females, respectively, who had a pre-intervention total fat intake <36% and were then maintained on a high-fat SFA-rich diet for 12 weeks. HOMA-IR, decreased significantly in females who had <36% total fat pre-intervention and were then randomised to a high MUFA diet (Diet B). This suggests that changes in the total amount of fat in the diet from a low-fat diet (<36%) to a high-fat SFA-rich diet (>38%) can reduce insulin sensitivity. Similarly, a high-fat MUFA-rich diet can have beneficial effects on insulin resistance in subjects with a habitual low-fat diet.

While a low-fat diet is still advocated by many to be a primary and secondary choice of prevention for treatment of T2DM, the unintended increase in carbohydrate intake may reduce HDL-cholesterol and increase TAG concentrations which are core features of the MetS (Grundy, 1999). These patterns, although non-significant, were observed in this study on the low-fat, high-carbohydrate diet (Diet C). However they were attenuated by the low-fat n-3 PUFA diet (Diet D) with the classical TAG lowering effect of LC n-3 PUFA being confirmed. Diet D also saw a reduction in TAG and TRL cholesterol and TRL TAG in males only. Plasma NEFA concentrations significantly reduced on this diet also. When the change in plasma lipids from pre- to post-intervention was calculated, plasma NEFA and cholesterol were significantly reduced in Diet D as compared to the High-fat SFA-rich diet (Diet A). The reduction in plasma NEFA is an important finding as increased concentrations have been shown to induce insulin resistance through interference of insulin signal transduction pathways and glucose transport (Grundy *et al.*, 2004; Dandona *et al.*, 2005). Controversy has for a long time surrounded the effects of LC n-3 PUFA on insulin sensitivity and other factors of the MetS in healthy and diabetic cohorts (Vessby & Boberg, 1990; Vessby *et al.*, 2001). In LIPGENE, the beneficial effects of 1.24g/day LC n-3 supplement on insulin sensitivity were not observed despite the increase of both EPA (C20:5:3 %) and DHA (C22:6:3 %) concentrations in plasma in this diet group. It did not significantly improve insulin sensitivity or glycaemic control in this group of MetS subjects even with sufficient study duration to allow a change in cell membrane composition. It seems from the results in Chapter 6 that CVD risk factors were more susceptible to modification as compared to IVGTT parameters and were optimised by the LC n-3 PUFA supplementation. It could be hyothesised that the relationship between the MetS and the effects on lipoproteins (TAG and HDL-cholesterol) are dependent on changes in body weight as although small, the weight

reduction was a significant outcome on both low-fat diets. A diet rich in n-3 fatty acids accompanied by a weight loss factor has shown to have a more favourable outcome on CVD risk than either single component (Mori *et al.*, 1999).

With no one diet emerging more advantageous over the other, perhaps the American Diabetes Association (ADA) recommendations wherein diet composition for patients with diabetes mellitus is individualised based on nutritional assessment and desired outcomes (ADA, 2007) hold same for persons with the MetS. With that, goals of therapy should be similar in that maintaining normal or near-normal blood glucose control, optimising serum lipid and lipoprotein concentrations, attaining desirable weights and improving overall health should be of priority. Perhaps the optimal diet should include MUFA as it has no detrimental effects on insulin sensitivity. Possibly, more significant changes would have been observed if this type of diet was for a longer duration as following a Mediterranean style diet for greater than 2 years improved endothelial function, reduced waist circumference, plasma glucose, serum insulin and HOMA score in MetS patients (Esposito *et al.*, 2004). It may also be that the variability in insulin sensitivity and genetic factors can explain the inconsistencies and lack of desirable outcomes. Although the MetS is thought to have a strong genetic component, central to its treatment are weight loss, physical activity and diet. Diet and exercise have previously demonstrated a reduction in insulin resistance (Uusitupa, 1996; Torjesen *et al.*, 1997) with the combination being more effective than each isolated component (Uusitupa, 1996).

With genetics in mind, the second objective of the LIPGENE study was to explore if common genetic polymorphisms associated with increased risk of the MetS play a causal role in determining an individual's responsiveness to dietary fat modification. The codon-972 aminoacid variant of IRS-1 in the heterozygous form was found in 11.7% of the WP1.2 MetS cohort, as illustrated in Chapter 7. When polymorphisms of IRS-1 were grouped together, the overall frequency of the codon 972 variant was 10.7% in NIDDM patients versus 5.8% in control subjects ($p<0.02$), suggesting that this genetic variant is a potential contributor to the insulin resistance in NIDDM (Hitman *et al.*, 1995). This chapter served its purpose as a first glance at gene-nutrient interactions with a common variant in the IRS-1 gene. Some individuals appear to be relatively insensitive to dietary intervention, whereas others have an enhanced sensitivity (Lopez-Miranda *et al.*, 2007). Identifying hypo- and hyperresponders to support the hypothesis that responsiveness is related to genetic

variation is important in deciphering information important at an individual and at population level.

Associated with the increased prevalence of the MetS and T2DM will be a dramatic increase in healthcare and social welfare costs. These costs are expected to rise to economically unsustainable levels in Europe during the early part of this century (www.europa.eu.int/comm/eurostat). Effective strategies are therefore needed to tackle the spread of the disease and to decrease the dependency on medical management. At present, therapy for T2DM relies mainly on several approaches intended to reduce hyperglycaemia: sulphonylureas (which increase insulin release from pancreatic islets; metformin (which acts to reduce hepatic glucose production); PPARγ agonists, thiazolidinediones (which enhance insulin action); α-glucosidase inhibitors (which interfere with gut glucose absorption) and insulin itself (which suppresses glucose production and augments glucose utilisation). These therapies, whilst being continuously improved upon, have limited efficacy, limited tolerability and significant side-effects, especially weight gain. Evidence suggests that a combination of diet and exercise are more effective than drug treatment in preventing the development of T2DM in high-risk individuals (Knowler *et al.*, 2002).

The concept of a personalised, genotype-based nutrition that may provide individuals with a high risk of diabetes or CVD with specific dietary advice, which in turn increases the efficacy of the intervention is being placed at the forefront in nutritional research. It is anticipated that personalised nutrition would help fine-tune the prevention of nutrition-related diseases. Attempts, however, to derive dietary recommendations based on the genotypes of few single SNPs is largely in the experimental phase. Most of the associations between diet, risk factors for disease and genotype have come from epidemiological studies and need to be studied in intervention studies for further verification. LIPGENE is adopting an integrated approach to examine the interaction of food supply and genes in the MetS to attempt to reduce the global surge of obesity and related complications.

Chapter Nine

Bibliography

Abbasi F, McLaughlin T, Lamendola C, Lipinska I, Tofler G & Reaven GM (1999) Comparison of plasminogen activator inhibitor-1 concentration in insulin-resistant versus insulin-sensitive healthy women. *Arterioscler Thromb Vasc Biol* **19**, 2818-2821.

Ader M, Ni TC & Bergman RN (1997) Glucose effectiveness assessed under dynamic and steady state conditions. Comparability of uptake versus production components. *J Clin Invest* **99**, 1187-1199.

Aguilera AA, Diaz GH, Barcelata ML, Guerrero OA & Ros RM (2004) Effects of fish oil on hypertension, plasma lipids, and tumor necrosis factor-alpha in rats with sucrose-induced metabolic syndrome. *J Nutr Biochem* **15**, 350-357.

Aguirre V, Uchida T, Yenush L, Davis R & White MF (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* **275**, 9047-9054.

Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE & White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* **277**, 1531-1537.

Ahima RS & Flier JS (2000) Adipose tissue as an endocrine organ. *Trends Endocrinol Metab* **11**, 327-332.

Alberti KG, Zimmet P & Shaw J (2006) Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* **23**, 469-480.

Alberti KG & Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* **15**, 539-553.

Albu J, Konnarides C & Pi-Suniyer F (1995) Weight Control: metabolic and cardiovascular effects. *Diabetes Rev* **3**, 335-347.

Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB & Cohen P (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* **7**, 261-269.

Alexander CM, Landsman PB, Teutsch SM & Haffner SM (2003) NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* **52**, 1210-1214.

Almind K, Bjorbaek C, Vestergaard H, Hansen T, Echwald S & Pedersen O (1993) Aminoacid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. *Lancet* **342**, 828-832.

Almind K, Inoue G, Pedersen O & Kahn CR (1996) A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. Evidence from transfection studies. *J Clin Invest* **97**, 2569-2575.

Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L & Lander ES (2000) The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* **26**, 76-80.

American Diabetes Association.Nutrition Recommendations and Interventions for Diabetes. A position statement of the American Diabetes Association. (2007) *Diabetes Care* **30**, S48-S65.

Anand SS, Yi Q, Gerstein H, Lonn E, Jacobs R, Vuksan V, Teo K, Davis B, Montague P & Yusuf S (2003) Relationship of metabolic syndrome and fibrinolytic dysfunction to cardiovascular disease. *Circulation* **108**, 420-425.

Anderson AS (2000) How to implement dietary changes to prevent the development of metabolic syndrome. *Br J Nutr* **83 Suppl 1**, S165-168.

Anderson RL, Hamman RF, Savage PJ, Saad MF, Laws A, Kades WW, Sands RE & Cefalu W (1995) Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. The Insuln Resistance Atherosclerosis Study. *Am J Epidemiol* **142**, 724-732.

Andres R, Swerdloff R, Pozefsky T & Coleman D (1966) Manual feedback technique for the control of blood glucose concentration. In *Automation in Analytical Chemistry* [LT Skeggs, editor].

Annuzzi G, Rivellese A, Capaldo B, Di Marino L, Iovine C, Marotta G & Riccardi G (1991) A controlled study on the effects of n-3 fatty acids on lipid and glucose metabolism in non-insulin-dependent diabetic patients. *Atherosclerosis* **87**, 65-73.

Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH & Karanja N (1997) A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* **336**, 1117-1124.

Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, 3rd, Johnson RS & Kahn CR (1994) Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* **372**, 186-190.

Araujo-Vilar D, Rega-Liste CA, Garcia-Estevez DA, Sarmiento-Escalona F, Mosquera-Tallon V & Cabezas-Cerrato J (1998) Minimal model of glucose metabolism: modified equations and its application in the study of insulin sensitivity in obese subjects. *Diabetes Res Clin Pract* **39**, 129-141.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T & Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* **257**, 79-83.

Ascaso JF, Romero P, Real JT, Lorente RI, Marti, nez-Valls J & Carmena R (2003) Abdominal obesity, insulin resistance, and metabolic syndrome in a southern European population. *Eur J Intern Med* **14**, 101-106.

Asciutti-Moura LS, Guilland JC, Fuchs F, Richard D & Klepping J (1988) Fatty acid composition of serum lipids and its relation to diet in an elderly institutionalized population. *Am J Clin Nutr* **48**, 980-987.

Astrup A (2001) Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutr* **4**, 499-515.

Astrup A, Grunwald GK, Melanson EL, Saris WH & Hill JO (2000) The role of low-fat diets in body weight control: a meta-analysis of ad libitum dietary intervention studies. *Int J Obes Relat Metab Disord* **24**, 1545-1552.

Athyros VG, Ganotakis ES, Elisaf M & Mikhailidis DP (2005) The prevalence of the metabolic syndrome using the National Cholesterol Educational Program and International Diabetes Federation definitions. *Curr Med Res Opin* **21**, 1157-1159.

Aucott LS (2008) Influences of weight loss on long-term diabetes outcomes. *Proc Nutr Soc* **67**, 54-59.

Baer DJ, Judd JT, Clevidence BA & Tracy RP (2004) Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr* **79**, 969-973.

Balkau B & Charles MA (1999) Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med* **16**, 442-443.

Balkau B, Charles MA, Drivsholm T, Borch-Johnsen K, Wareham N, Yudkin JS, Morris R, Zavaroni I, van Dam R, Feskins E, Gabriel R, Diet M, Nilsson P & Hedblad B (2002) Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. *Diabetes Metab* **28**, 364-376.

Banskota NK, Taub R, Zellner K, Olsen P & King GL (1989) Characterization of induction of protooncogene c-myc and cellular growth in human vascular smooth muscle cells by insulin and IGF-I. *Diabetes* **38**, 123-129.

Baroni MG, Arca M, Sentinelli F, Buzzetti R, Capici F, Lovari S, Vitale M, Romeo S & Di Mario U (2001) The G972R variant of the insulin receptor substrate-1 (IRS-1) gene, body fat distribution and insulin-resistance. *Diabetologia* **44**, 367-372.

Bastard JP, Robert JJ, Jardel C, Bruckert E, Grimaldi A & Hainque B (2001) Is quantitative insulin sensitivity check index, a fair insulin sensitivity index in humans? *Diabetes Metab* **27**, 69-70.

Beard JC, Bergman RN, Ward WK & Porte D, Jr. (1986) The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. *Diabetes* **35**, 362-369.

Beckman JA, Creager MA & Libby P (2002) Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *Jama* **287**, 2570-2581.

Berg AH, Combs TP, Du X, Brownlee M & Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* **7**, 947-953.

Bergman RN, Finegood DT & Ader M (1985) Assessment of insulin sensitivity in vivo. *Endocr Rev* **6**, 45-86.

Bergman RN, Ider YZ, Bowden CR & Cobelli C (1979) Quantitative estimation of insulin sensitivity. *Am J Physiol* **236**, E667-677.

Bergman RN, Phillips LS & Cobelli C (1981) Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* **68**, 1456-1467.

Bergman RN, Prager R, Volund A & Olefsky JM (1987) Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest* **79**, 790-800.

Bergstrom RW, Newell-Morris LL, Leonetti DL, Shuman WP, Wahl PW & Fujimoto WY (1990) Association of elevated fasting C-peptide level and increased intra-abdominal fat distribution with development of NIDDM in Japanese-American men. *Diabetes* **39**, 104-111.

Berry EM (1997) Dietary fatty acids in the management of diabetes mellitus. *Am J Clin Nutr* **66**, 991S-997S.

Bjorntorp P, Fahlen M, Grimby G, Gustafson A, Holm J, Renstrom P & Schersten T (1972) Carbohydrate and lipid metabolism in middle-aged, physically well-trained men. *Metabolism* **21**, 1037-1044.

Bjorntorp P & Rosmond R (2000) The metabolic syndrome--a neuroendocrine disorder? *Br J Nutr* **83 Suppl 1**, S49-57.

Black AE (2000) The sensitivity and specificity of the Goldberg cut-off for EI:BMR for identifying diet reports of poor validity. *Eur J Clin Nutr* **54**, 395-404.

Black AE & Cole TJ (2001) Biased over- or under-reporting is characteristic of individuals whether over time or by different assessment methods. *J Am Diet Assoc* **101**, 70-80.

Black AE, Goldberg GR, Jebb SA, Livingstone MB, Cole TJ & Prentice AM (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* **45**, 583-599.

Black AE, Prentice AM, Goldberg GR, Jebb SA, Bingham SA, Livingstone MB & Coward WA (1993) Measurements of total energy expenditure provide insights into the validity of dietary measurements of energy intake. *J Am Diet Assoc* **93**, 572-579.

Bladbjerg EM, Tholstrup T, Marckmann P, Sandstrom B & Jespersen J (1995) Dietary changes in fasting levels of factor VII coagulant activity (FVII:C) are accompanied by changes in factor VII protein and other vitamin K-dependent proteins. *Thromb Haemost* **73**, 239-242.

Blann AD, Jackson P, Bath PM & Watts GF (1995) von Willebrand factor, a possible indicator of endothelial cell damage, decreases during long-term compliance with a lipid-lowering diet. *J Intern Med* **237**, 557-561.

Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911-917.

Blum CB (1982) Dynamics of apolipoprotein E metabolism in humans. *J Lipid Res* **23**, 1308-1316.

Blundell JE & Cooling J (2000) Routes to obesity: phenotypes, food choices and activity. *Br J Nutr* **83 Suppl 1**, S33-38.

Boberg M, Pollare T, Siegbahn A & Vessby B (1992) Supplementation with n-3 fatty acids reduces triglycerides but increases PAI-1 in non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* **22**, 645-650.

Boden G (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 3-10.

Boden G & Shulman GI (2002) Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell function. *European Journal of Clinical Investigation* **32**, 14-23.

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC & Muggeo M (2003) Metabolic syndrome: epidemiology and more extensive phenotypic

description. Cross-sectional data from the Bruneck Study. *Int J Obes Relat Metab Disord* **27**, 1283-1289.

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna RC & Muggeo M (1998) Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* **47**, 1643-1649.

Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, Corgnati A & Muggeo M (1989) Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. *J Clin Endocrinol Metab* **68**, 374-378.

Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T & Muggeo M (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* **23**, 57-63.

Borkman M, Campbell LV, Chisholm DJ & Storlien LH (1991) Comparison of the effects on insulin sensitivity of high carbohydrate and high fat diets in normal subjects. *J Clin Endocrinol Metab* **72**, 432-437.

Borkman M, Chisholm DJ, Furler SM, Storlien LH, Kraegen EW, Simons LA & Chesterman CN (1989) Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. *Diabetes* **38**, 1314-1319.

Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ & Campbell LV (1993) The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N Engl J Med* **328**, 238-244.

Braam LA, Ocke MC, Bueno-de-Mesquita HB & Seidell JC (1998) Determinants of obesity-related underreporting of energy intake. *Am J Epidemiol* **147**, 1081-1086.

Brady LM, Gower BA, Lovegrove SS, Williams CM & Lovegrove JA (2004a) Revised QUICKI provides a strong surrogate estimate of insulin sensitivity when compared with the minimal model. *Int J Obes Relat Metab Disord* **28**, 222-227.

Brady LM, Lovegrove SS, Lesauvage SV, Gower BA, Minihane AM, Williams CM & Lovegrove JA (2004b) Increased n-6 polyunsaturated fatty acids do not attenuate

the effects of long-chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians. *Am J Clin Nutr* **79**, 983-991.

Bray GA & Popkin BM (1998) Dietary fat intake does affect obesity! *Am J Clin Nutr* **68**, 1157-1173.

Briefel RR, Sempos CT, McDowell MA, Chien S & Alaimo K (1997) Dietary methods research in the third National Health and Nutrition Examination Survey: underreporting of energy intake. *Am J Clin Nutr* **65**, 1203S-1209S.

Brinton EA, Eisenberg S & Breslow JL (1990) A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J Clin Invest* **85**, 144-151.

Browning LM, Krebs JD, Moore CS, Mishra GD, O'Connell MA & Jebb SA (2007) The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. *Diabetes Obes Metab* **9**, 70-80.

Bruce A (2000) Strategies to prevent the metabolic syndrome at the population level: role of authorities and non-governmental bodies. *Br J Nutr* **83 Suppl 1**, S181-186.

Brunner EJ, Marmot MG, Nanchahal K, Shipley MJ, Stansfeld SA, Juneja M & Alberti KG (1997) Social inequality in coronary risk: central obesity and the metabolic syndrome. Evidence from the Whitehall II study. *Diabetologia* **40**, 1341-1349.

Bunt JC, Krakoff J, Ortega E, Knowler WC & Bogardus C (2007) Acute insulin response is an independent predictor of type 2 diabetes mellitus in individuals with both normal fasting and 2-h plasma glucose concentrations. *Diabetes Metab Res Rev* **23**, 304-310.

Burchfiel CM, Hamman RF, Marshall JA, Baxter J, Kahn LB & Amirani JJ (1990) Cardiovascular risk factors and impaired glucose tolerance: the San Luis Valley Diabetes Study. *Am J Epidemiol* **131**, 57-70.

Buzzard M (1998) 24-Hour dietary recall and food record methods. In *Nutritional Epidemiology*, pp. 50-73 [WC Willet, editor]. Oxford: Oxford University Press.

Calder PC (2003) N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* **38**, 343-352.

Calder PC (2004) n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci (Lond)* **107**, 1-11.

Cameron AJ, Zimmet PZ, Soderberg S, Alberti KG, Sicree R, Tuomilehto J, Chitson P & Shaw JE (2007) The metabolic syndrome as a predictor of incident diabetes mellitus in Mauritius. *Diabet Med* **24**, 1460-1469.

Carr DB, Utzschneider KM, Hull RL, Kodama K, Retzlaff BM, Brunzell JD, Shofer JB, Fish BE, Knopp RH & Kahn SE (2004) Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* **53**, 2087-2094.

Carr MC (2003) The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* **88**, 2404-2411.

Carroll S, Cooke CB & Butterly RJ (2000) Metabolic clustering, physical activity and fitness in nonsmoking, middle-aged men. *Med Sci Sports Exerc* **32**, 2079-2086.

Cefalu WT (2001) Insulin resistance: cellular and clinical concepts. *Exp Biol Med (Maywood)* **226**, 13-26.

Cefalu WT, Wang ZQ, Werbel S, Bell-Farrow A, Crouse JR, 3rd, Hinson WH, Terry JG & Anderson R (1995) Contribution of visceral fat mass to the insulin resistance of aging. *Metabolism* **44**, 954-959.

Chaput JP, Tremblay A, Rimm EB, Bouchard C & Ludwig DS (2008) A novel interaction between dietary composition and insulin secretion: effects on weight gain in the Quebec Family Study. *Am J Clin Nutr* **87**, 303-309.

Chen H, Sullivan G, Yue LQ, Katz A & Quon MJ (2003) QUICKI is a useful index of insulin sensitivity in subjects with hypertension. *Am J Physiol Endocrinol Metab* **284**, E804-812.

Childs CE, Romeu-Nadal M, Burdge GC & Calder PC (2008) Gender differences in the n-3 fatty acid content of tissues. *Proc Nutr Soc* **67**, 19-27.

Ciubotaru I, Lee YS & Wander RC (2003) Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT. *J Nutr Biochem* **14**, 513-521.

Clausen JO, Borch-Johnsen K, Ibsen H, Bergman RN, Hougaard P, Winther K & Pedersen O (1996) Insulin sensitivity index, acute insulin response, and glucose

effectiveness in a population-based sample of 380 young healthy Caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. *J Clin Invest* **98**, 1195-1209.

Clausen JO, Hansen T, Bjorbaek C, Echwald SM, Urhammer SA, Rasmussen S, Andersen CB, Hansen L, Almind K, Winther K & et al. (1995) Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. *Lancet* **346**, 397-402.

Clifton PM & Nestel PJ (1998) Relationship between plasma insulin and erythrocyte fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids* **59**, 191-194.

Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. (1998) National Institutes of Health. *Obes Res* **6 Suppl 2**, 51S-209S.

Coakes SJ & Steed LG (1999) *SPSS, Analysis without anguish.* Australia: John Wiley and Sons.

Cobelli C, Bettini F, Caumo A & Quon MJ (1998) Overestimation of minimal model glucose effectiveness in presence of insulin response is due to undermodeling. *Am J Physiol* **275**, E1031-1036.

Colditz GA, Manson JE, Stampfer MJ, Rosner B, Willett WC & Speizer FE (1992) Diet and risk of clinical diabetes in women. *Am J Clin Nutr* **55**, 1018-1023.

Combs TP, Berg AH, Obici S, Scherer PE & Rossetti L (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* **108**, 1875-1881.

Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association (1998) *Diabetes Care* **21**, 310-314.

Correia ML, Morgan DA, Sivitz WI, Mark AL & Haynes WG (2001) Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension* **37**, 936-942.

Dalla Man C, Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G & Cobelli C (2005) Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. *Diabetes* **54**, 3265-3273.

Daly ME, Vale C, Walker M, Alberti KG & Mathers JC (1997) Dietary carbohydrates and insulin sensitivity: a review of the evidence and clinical implications. *Am J Clin Nutr* **66**, 1072-1085.

Dandona P, Aljada A, Chaudhuri A, Mohanty P & Garg R (2005) Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* **111**, 1448-1454.

Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A & Wadden T (1998) Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* **83**, 2907-2910.

Day C (2007) Metabolic syndrome, or What you will: definitions and epidemiology. *Diab Vasc Dis Res* **4**, 32-38.

Daynes RA & Jones DC (2002) Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol* **2**, 748-759.

DECODE Study Group on behalf of the European Diabetes Epidemiology Study Group. Will new diagnostic criteria for diabetes mellitus change phenotype of patients with diabetes? Reanalysis of European epidemiological data. (1998) *Bmj* **317**, 371-375.

DEFRA. National Food Survey (2000) http://statistics.defra.gov.uk/esg/publications/nfs/2000/default.asp.

DeFronzo RA (1981) The effect of insulin on renal sodium metabolism. A review with clinical implications. *Diabetologia* **21**, 165-171.

DeFronzo RA, Bonadonna RC & Ferrannini E (1992) Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* **15**, 318-368.

DeFronzo RA, Simonson D & Ferrannini E (1982) Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **23**, 313-319.

DeFronzo RA, Tobin JD & Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* **237**, E214-223.

Delarue J, Couet C, Cohen R, Brechot JF, Antoine JM & Lamisse F (1996) Effects of fish oil on metabolic responses to oral fructose and glucose loads in healthy humans. *Am J Physiol* **270**, E353-362.

Despres JP, Lamarche B, Mauriege P, Cantin B, Lupien PJ & Dagenais GR (1996) Risk factors for ischaemic heart disease: is it time to measure insulin? *Eur Heart J* **17**, 1453-1454.

Desvergne B & Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* **20**, 649-688.

DiPietro L, Seeman TE, Stachenfeld NS, Katz LD & Nadel ER (1998) Moderate-intensity aerobic training improves glucose tolerance in aging independent of abdominal adiposity. *J Am Geriatr Soc* **46**, 875-879.

Dougherty RM, Galli C, Ferro-Luzzi A & Iacono JM (1987) Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *Am J Clin Nutr* **45**, 443-455.

Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF & Shulman GI (1999) Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* **103**, 253-259.

Efendic S, Grill V, Luft R & Wajngot A (1988) Low insulin response: a marker of prediabetes. *Adv Exp Med Biol* **246**, 167-174.

El-Atat F, Aneja A, McFarlane S & Sowers J (2003) Obesity and hypertension. *Endocrinol Metab Clin North Am* **32**, 823-854.

Elosua R, Ordovas JM, Cupples LA, Lai CQ, Demissie S, Fox CS, Polak JF, Wolf PA, D'Agostino RB, Sr. & O'Donnell CJ (2006) Variants at the APOA5 locus, association with carotid atherosclerosis, and modification by obesity: the Framingham Study. *J Lipid Res* **47**, 990-996.

Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB & Willett WC (2007) Dietary patterns, insulin resistance, and prevalence of the metabolic syndrome in women. *Am J Clin Nutr* **85**, 910-918.

Esposito K & Giugliano D (2004) The metabolic syndrome and inflammation: association or causation? *Nutr Metab Cardiovasc Dis* **14**, 228-232.

Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F & Giugliano D (2004) Effect of a mediterranean-style diet on

endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *Jama* **292**, 1440-1446.

European Group for the study of Insulin Resistance (EGIR) (2002) Frequency of the WHO Metabolic Syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. *Diabetes Metabolism* **28**, 364-376.

Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III).(2001) *Jama* **285**, 2486-2497.

Expert Panel on detection evaluation and treatment of high blood cholesterol in adults (2001) Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, andtreatment of high blood cholesterol in adults (Adult Treatment Panel III). *Journal of the American Medical Association* **285**, 2486-2497.

Fahlman MM, Boardley D, Lambert CP & Flynn MG (2002) Effects of endurance training and resistance training on plasma lipoprotein profiles in elderly women. *J Gerontol A Biol Sci Med Sci* **57**, B54-60.

Fasching P, Ratheiser K, Schneeweiss B, Rohac M, Nowotny P & Waldhausl W (1996) No effect of short-term dietary supplementation of saturated and poly- and monounsaturated fatty acids on insulin secretion and sensitivity in healthy men. *Ann Nutr Metab* **40**, 116-122.

Fasching P, Ratheiser K, Waldhausl W, Rohac M, Osterrode W, Nowotny P & Vierhapper H (1991) Metabolic effects of fish-oil supplementation in patients with impaired glucose tolerance. *Diabetes* **40**, 583-589.

Fernandez-Real JM, Lopez-Bermejo A, Casamitjana R & Ricart W (2003) Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *J Clin Endocrinol Metab* **88**, 2714-2718.

Ferrannini E & Camastra S (1998) Relationship between impaired glucose tolerance, non-insulin-dependent diabetes mellitus and obesity. *Eur J Clin Invest* **28 Suppl 2**, 3-6; discussion 6-7.

Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N & Mingrone G (1997) Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin Invest* **100**, 1166-1173.

Feskens EJ & Kromhout D (1990) Habitual dietary intake and glucose tolerance in euglycaemic men: the Zutphen Study. *Int J Epidemiol* **19**, 953-959.

Feskens EJ, Loeber JG & Kromhout D (1994) Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. *Am J Epidemiol* **140**, 350-360.

Feskens EJ, Virtanen SM, Rasanen L, Tuomilehto J, Stengard J, Pekkanen J, Nissinen A & Kromhout D (1995) Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* **18**, 1104-1112.

Festa A, D'Agostino R, Jr., Howard G, Mykkanen L, Tracy RP & Haffner SM (2000) Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* **102**, 42-47.

Festa A, D'Agostino R, Jr., Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP & Haffner SM (2001) The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord* **25**, 1407-1415.

Field A (2005) *Discovering Statistics Using SPSS*, 2nd edition ed. London: Sage.

Finegood DT, Hramiak IM & Dupre J (1990) A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. *J Clin Endocrinol Metab* **70**, 1538-1549.

Finegood DT & Tzur D (1996) Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal-model method. *Am J Physiol* **271**, E485-495.

Fischer S, Weber PC & Dyerberg J (1986) The prostacyclin/thromboxane balance is favourably shifted in Greenland Eskimos. *Prostaglandins* **32**, 235-241.

Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM & Altshuler D (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* **355**, 241-250.

Florez JC, Sjogren M, Burtt N, Orho-Melander M, Schayer S, Sun M, Almgren P, Lindblad U, Tuomi T, Gaudet D, Hudson TJ, Daly MJ, Ardlie KG, Hirschhorn

JN, Altshuler D & Groop L (2004) Association testing in 9,000 people fails to confirm the association of the insulin receptor substrate-1 G972R polymorphism with type 2 diabetes. *Diabetes* **53**, 3313-3318.

Folsom AR, Burke GL, Ballew C, Jacobs DR, Jr., Haskell WL, Donahue RP, Liu KA & Hilner JE (1989) Relation of body fatness and its distribution to cardiovascular risk factors in young blacks and whites. The role of insulin. *Am J Epidemiol* **130**, 911-924.

Folsom AR, Ma J, McGovern PG & Eckfeldt H (1996) Relation between plasma phospholipid saturated fatty acids and hyperinsulinemia. *Metabolism* **45**, 223-228.

Folsom AR, Qamhieh HT, Wing RR, Jeffery RW, Stinson VL, Kuller LH & Wu KK (1993) Impact of weight loss on plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. *Arterioscler Thromb* **13**, 162-169.

Ford ES (2005a) Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care* **28**, 2745-2749.

Ford ES (2005b) Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care* **28**, 1769-1778.

Ford ES, Giles WH & Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *Jama* **287**, 356-359.

Ford ES & Li C (2008) Metabolic Syndrome and Health-Related Quality of Life among U.S. Adults. *Ann Epidemiol* **18**, 165-171.

Freeman MS, Mansfield MW, Barrett JH & Grant PJ (2002) Heritability of features of the insulin resistance syndrome in a community-based study of healthy families. *Diabet Med* **19**, 994-999.

Frevert EU & Kahn BB (1997) Differential effects of constitutively active phosphatidylinositol 3-kinase on glucose transport, glycogen synthase activity, and DNA synthesis in 3T3-L1 adipocytes. *Mol Cell Biol* **17**, 190-198.

Friday KE, Childs MT, Tsunehara CH, Fujimoto WY, Bierman EL & Ensinck JW (1989) Elevated plasma glucose and lowered triglyceride levels from omega-3 fatty acid supplementation in type II diabetes. *Diabetes Care* **12**, 276-281.

Friedberg CE, Janssen MJ, Heine RJ & Grobbee DE (1998) Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care* **21**, 494-500.

Friedman M & Rosenman RH (1959) Association of specific overt behavior pattern with blood and cardiovascular findings; blood cholesterol level, blood clotting time, incidence of arcus senilis, and clinical coronary artery disease. *J Am Med Assoc* **169**, 1286-1296.

Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeing H, Muche R, Brenner H & Koenig W (2000) Association between C-reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care* **23**, 1835-1839.

Fry J & Finley W (2005) The prevalence and costs of obesity in the EU. *Proc Nutr Soc* **64**, 359-362.

Fuller JH, Shipley MJ, Rose G, Jarrett RJ & Keen H (1980) Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* **1**, 1373-1376.

Galli C, Marangoni F & Galella G (1993) Modulation of lipid derived mediators by polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* **48**, 51-55.

Garg A (1998) High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am J Clin Nutr* **67**, 577S-582S.

Garg A, Bantle JP, Henry RR, Coulston AM, Griver KA, Raatz SK, Brinkley L, Chen YD, Grundy SM, Huet BA & et al. (1994) Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *Jama* **271**, 1421-1428.

Garg A, Bonanome A, Grundy SM, Zhang ZJ & Unger RH (1988) Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* **319**, 829-834.

Garg A, Grundy SM & Unger RH (1992) Comparison of effects of high and low carbohydrate diets on plasma lipoproteins and insulin sensitivity in patients with mild NIDDM. *Diabetes* **41**, 1278-1285.

Gauderman WJ (2002) Sample size requirements for association studies of gene-gene interaction. *Am J Epidemiol* **155**, 478-484.

German JB & Dillard CJ (2004) Saturated fats: what dietary intake? *Am J Clin Nutr* **80**, 550-559.

Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P & Dandona P (2004) Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* **110**, 1564-1571.

Ghoussaini M, Meyre D, Lobbens S, Charpentier G, Clement K, Charles MA, Tauber M, Weill J & Froguel P (2005) Implication of the Pro12Ala polymorphism of the PPAR-gamma 2 gene in type 2 diabetes and obesity in the French population. *BMC Med Genet* **6**, 11.

Giacco R, Cuomo V, Vessby B, Uusitupa M, Hermansen K, Meyer BJ, Riccardi G & Rivellese AA (2007) Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? *Nutr Metab Cardiovasc Dis* **17**, 572-580.

Ginsberg HN (2006) REVIEW: Efficacy and mechanisms of action of statins in the treatment of diabetic dyslipidemia. *J Clin Endocrinol Metab* **91**, 383-392.

Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA & Prentice AM (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45**, 569-581.

Goldstein BJ & Scalia R (2004) Adiponectin: A novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab* **89**, 2563-2568.

Goodpaster BH, Thaete FL & Kelley DE (2000) Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *Am J Clin Nutr* **71**, 885-892.

Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ & Dohm GL (1995) Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin Invest* **95**, 2195-2204.

Goralski KB & Sinal CJ (2007) Type 2 diabetes and cardiovascular disease: getting to the fat of the matter. *Can J Physiol Pharmacol* **85**, 113-132.

Grande F, Anderson JT & Keys A (1970) Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *Am J Clin Nutr* **23**, 1184-1193.

Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A & Stefansson K (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* **38**, 320-323.

Gravena C, Mathias PC & Ashcroft SJ (2002) Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. *J Endocrinol* **173**, 73-80.

Greenfield MS, Doberne L, Kraemer F, Tobey T & Reaven G (1981) Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. *Diabetes* **30**, 387-392.

Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E & DeFronzo RA (1989) Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *J Clin Invest* **84**, 205-213.

Grundleger ML & Thenen SW (1982) Decreased insulin binding, glucose transport, and glucose metabolism in soleus muscle of rats fed a high fat diet. *Diabetes* **31**, 232-237.

Grundy SM (1999) Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol* **83**, 25F-29F.

Grundy SM (2006) Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. *J Am Coll Cardiol* **47**, 1093-1100.

Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr. & Lenfant C (2004) Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* **109**, 433-438.

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA & Costa F (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* **112**, 2735-2752.

Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, Schneiderman N, Skyler JS & Marks JB (2000) Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract* **47**, 177-184.

Haffner SM, Kennedy E, Gonzalez C, Stern MP & Miettinen H (1996) A prospective analysis of the HOMA model. The Mexico City Diabetes Study. *Diabetes Care* **19**, 1138-1141.

Haffner SM, Stern MP, Mitchell BD, Hazuda HP & Patterson JK (1990) Incidence of type II diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity, and body-fat distribution. *Diabetes* **39**, 283-288.

Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA & Stern MP (1992) Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes* **41**, 715-722.

Hamman RF (1992) Genetic and environmental determinants of non-insulin-dependent diabetes mellitus (NIDDM). *Diabetes Metab Rev* **8**, 287-338.

Hanley AJ, Festa A, D'Agostino RB, Jr., Wagenknecht LE, Savage PJ, Tracy RP, Saad MF & Haffner SM (2004) Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes* **53**, 1773-1781.

Hanley AJ, Wagenknecht LE, D'Agostino RB, Jr., Zinman B & Haffner SM (2003) Identification of subjects with insulin resistance and beta-cell dysfunction using alternative definitions of the metabolic syndrome. *Diabetes* **52**, 2740-2747.

Hansen BC (1999) The metabolic syndrome X. *Ann N Y Acad Sci* **892**, 1-24.

Hansen E, Hajri T & Abumrad NN (2006) Is all fat the same? The role of fat in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus. *Surgery* **139**, 711-716.

Harano Y, Hidaka H, Takatsuki K, Ohgaku S, Haneda M, Motoi S, Kawagoe K, Shigeta Y & Abe H (1978) Glucose, insulin, and somatostatin infusion for the determination of insulin sensitivity in vivo. *Metabolism* **27**, 1449-1452.

Harrington KE, Robson PJ, Kiely M, Livingstone MB, Lambe J & Gibney MG (2001) The North/South Ireland Food Consumption Survey: survey design and methodology. *Public Health Nutrition* **4**, 1037-1042.

Harris WS, Connor WE, Illingworth DR, Rothrock DW & Foster DM (1990) Effects of fish oil on VLDL triglyceride kinetics in humans. *J Lipid Res* **31**, 1549-1558.

Heine RJ, Mulder C, Popp-Snijders C, van der Meer J & van der Veen EA (1989) Linoleic-acid-enriched diet: long-term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in noninsulin-dependent diabetic patients. *Am J Clin Nutr* **49**, 448-456.

Henderson L, Gregory J, Irving K & Swan G (2003) The National Diet and Nutrition Survey: adults aged 19-64 years: HMSO, London.

Henry RR, Wallace P & Olefsky JM (1986) Effects of weight loss on mechanisms of hyperglycemia in obese non-insulin-dependent diabetes mellitus. *Diabetes* **35**, 990-998.

Hidvegi T, Hetyesi K, Biro L & Jermendy G (2001) Education level and clustering of clinical characteristics of metabolic syndrome. *Diabetes Care* **24**, 2013-2015.

Hill RJ & Davies PS (2001) The validity of self-reported energy intake as determined using the doubly labelled water technique. *Br J Nutr* **85**, 415-430.

Himsworth HP (1934) Dietetic factors influencing the glucose tolerance and the activity of insulin. *J Physiol* **81**, 29-48.

Himsworth HP (1936) Diabetes Mellitus: its differentiation into insulin-sensitive and insulin insensitive types. *Lancet* **1**, 117-121.

Himsworth HP (1939) Insulin-sensitive and insulin-insensitive types of diabetes mellitus. *Clin Sci (Lond)* **41**, 119-152.

Hirose H, Lee YH, Inman LR, Nagasawa Y, Johnson JH & Unger RH (1996) Defective fatty acid-mediated beta-cell compensation in Zucker diabetic fatty rats. Pathogenic implications for obesity-dependent diabetes. *J Biol Chem* **271**, 5633-5637.

Hitman GA, Hawrami K, McCarthy MI, Viswanathan M, Snehalatha C, Ramachandran A, Tuomilehto J, Tuomilehto-Wolf E, Nissinen A & Pedersen O (1995) Insulin receptor substrate-1 gene mutations in NIDDM; implications for the study of polygenic disease. *Diabetologia* **38**, 481-486.

Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA & Giles GG (2007) Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. *Am J Clin Nutr* **86**, 189-197.

Holman GD & Kasuga M (1997) From receptor to transporter: insulin signalling to glucose transport. *Diabetologia* **40**, 991-1003.

Holman RT, Smythe L & Johnson S (1979) Effect of sex and age on fatty acid composition of human serum lipids. *Am J Clin Nutr* **32**, 2390-2399.

Honstra G, van Houwelingen AC, Kivits GA, Fischer S & Uedelhoven W (1990) Influence of dietary fish on eicosanoid metabolism in man. *Prostaglandins* **40**, 311-329.

Hornstra G, Barth CA, Galli C, Mensink RP, Mutanen M, Riemersma RA, Roberfroid M, Salminen K, Vansant G & Verschuren PM (1998) Functional food science and the cardiovascular system. *Br J Nutr* **80 Suppl 1**, S113-146.

Hosmer DW & Lemeshow S (2000) *Applied Logistic Regression*, 2nd edition ed. New York: John Wiley and Sons.

Hossain P, Kawar B & El Nahas M (2007) Obesity and diabetes in the developing world--a growing challenge. *N Engl J Med* **356**, 213-215.

Hotamisligil GS, Budavari A, Murray D & Spiegelman BM (1994a) Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest* **94**, 1543-1549.

Hotamisligil GS, Murray DL, Choy LN & Spiegelman BM (1994b) Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A* **91**, 4854-4858.

Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF & Spiegelman BM (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* **271**, 665-668.

Hotamisligil GS, Shargill NS & Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259**, 87-91.

Howard BV, Abbott WG & Swinburn BA (1991) Evaluation of metabolic effects of substitution of complex carbohydrates for saturated fat in individuals with obesity and NIDDM. *Diabetes Care* **14**, 786-795.

Howard BV, Manson JE, Stefanick ML, Beresford SA, Frank G, Jones B, Rodabough RJ, Snetselaar L, Thomson C, Tinker L, Vitolins M & Prentice R (2006) Low-fat dietary pattern and weight change over 7 years: the Women's Health Initiative Dietary Modification Trial. *Jama* **295**, 39-49.

Howard BV, Mayer-Davis EJ, Goff D, Zaccaro DJ, Laws A, Robbins DC, Saad MF, Selby J, Hamman RF, Krauss RM & Haffner SM (1998) Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Metabolism* **47**, 1174-1179.

Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P & Bergman R (1996) Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* **93**, 1809-1817.

Howe PR (1997) Dietary fats and hypertension. Focus on fish oil. *Ann N Y Acad Sci* **827**, 339-352.

Hribal ML, Federici M, Porzio O, Lauro D, Borboni P, Accili D, Lauro R & Sesti G (2000) The Gly-->Arg972 amino acid polymorphism in insulin receptor substrate-1 affects glucose metabolism in skeletal muscle cells. *J Clin Endocrinol Metab* **85**, 2004-2013.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG & Willett WC (2001) Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* **345**, 790-797.

Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K & Pyorala K (2004) Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular

mortality in nondiabetic European men and women. *Arch Intern Med* **164**, 1066-1076.

Hwang LC, Bai CH, Chen CJ & Chien KL (2007) Gender difference on the development of metabolic syndrome: a population-based study in Taiwan. *Eur J Epidemiol.*

International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome (2005) (available from www.idf.org).

International Diabetes Federation (2001) Diabetes and cardiovascular disease: time to act. Brussles.

International Diabetes Federation (2006) Diabetes Atlas, third edition. Brussels.

Ip C, Tepperman HM, Holohan P & Tepperman J (1976) Insulin binding and insulin response of adipocytes from rats adapted to fat feeding. *J Lipid Res* **17**, 588-599.

Irwin ML, Ainsworth BE, Mayer-Davis EJ, Addy CL, Pate RR & Durstine JL (2002) Physical activity and the metabolic syndrome in a tri-ethnic sample of women. *Obes Res* **10**, 1030-1037.

Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR & Groop L (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* **24**, 683-689.

Jacobsen BK & Thelle DS (1988) Risk factors for coronary heart disease and level of education. The Tromso Heart Study. *Am J Epidemiol* **127**, 923-932.

Janssen I, Katzmarzyk PT & Ross R (2004) Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr* **79**, 379-384.

Jarrett RJ, Keen H, Fuller JH & McCartney M (1979) Worsening to diabetes in men with impaired glucose tolerance ("borderline diabetes"). *Diabetologia* **16**, 25-30.

Johansson G, Wikman A, Ahren AM, Hallmans G & Johansson I (2001) Underreporting of energy intake in repeated 24-hour recalls related to gender, age, weight status, day of interview, educational level, reported food intake, smoking habits and area of living. *Public Health Nutr* **4**, 919-927.

Jolliffe CJ & Janssen I (2007) Development of age-specific adolescent metabolic syndrome criteria that are linked to the Adult Treatment Panel III and International Diabetes Federation criteria. *J Am Coll Cardiol* **49**, 891-898.

Jones BH, Maher MA, Banz WJ, Zemel MB, Whelan J, Smith PJ & Moustaid N (1996) Adipose tissue stearoyl-CoA desaturase mRNA is increased by obesity and decreased by polyunsaturated fatty acids. *Am J Physiol* **271**, E44-49.

Jong MC, Hofker MH & Havekes LM (1999) Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol* **19**, 472-484.

Jucker BM, Cline GW, Barucci N & Shulman GI (1999) Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle: a 13C nuclear magnetic resonance study. *Diabetes* **48**, 134-140.

Kabir M, Skurnik G, Naour N, Pechtner V, Meugnier E, Rome S, Quignard-Boulange A, Vidal H, Slama G, Clement K, Guerre-Millo M & Rizkalla SW (2007) Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. *Am J Clin Nutr* **86**, 1670-1679.

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K & Tobe K (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* **116**, 1784-1792.

Kahn BB & Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* **106**, 473-481.

Karter AJ, Mayer-Davis EJ, Selby JV, D'Agostino RB, Jr., Haffner SM, Sholinsky P, Bergman R, Saad MF & Hamman RF (1996) Insulin sensitivity and abdominal obesity in African-American, Hispanic, and non-Hispanic white men and women. The Insulin Resistance and Atherosclerosis Study. *Diabetes* **45**, 1547-1555.

Katsuki A, Sumida Y, Gabazza EC, Murashima S, Urakawa H, Morioka K, Kitagawa N, Tanaka T, Araki-Sasaki R, Hori Y, Nakatani K, Yano Y & Adachi Y (2002) QUICKI is useful for following improvements in insulin sensitivity after therapy in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* **87**, 2906-2908.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G & Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* **85**, 2402-2410.

Katzmarzyk PT, Leon AS, Wilmore JH, Skinner JS, Rao DC, Rankinen T & Bouchard C (2003) Targeting the metabolic syndrome with exercise: evidence from the HERITAGE Family Study. *Med Sci Sports Exerc* **35**, 1703-1709.

Kelley DE, Goodpaster BH & Storlien L (2002) Muscle triglyceride and insulin resistance. *Annu Rev Nutr* **22**, 325-346.

Kerenyi Z, Tabak AG, Stella P, Bosnyak Z, Simon K, Karadi I & Tamas G (2000) Association between socioeconomic factors and the metabolic syndrome in women with prior gestational diabetes mellitus. *Diabetes Care* **23**, 1444-1445.

Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R & Simsolo RB (1995) The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* **95**, 2111-2119.

Keys A (1980) W. O. Atwater memorial lecture: overweight, obesity, coronary heart disease and mortality. *Nutr Rev* **38**, 297-307.

Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR & Grunfeld C (2004) Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* **45**, 1169-1196.

Kim SH & Reaven GM (2008) Isolated impaired fasting glucose and peripheral insulin sensitivity: not a simple relationship. *Diabetes Care* **31**, 347-352.

Kim YB, Nakajima R, Matsuo T, Inoue T, Sekine T, Komuro M, Tamura T, Tokuyama K & Suzuki M (1996) Gene expression of insulin signal-transduction pathway intermediates is lower in rats fed a beef tallow diet than in rats fed a safflower oil diet. *Metabolism* **45**, 1080-1088.

King H, Aubert RE & Herman WH (1998) Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* **21**, 1414-1431.

Kissebah AH, Alfarsi S, Adams PW, Seed M, Folkard J & Wynn V (1976a) Transport kinetics of plasma free fatty acid, very low density lipoprotein triglycerides and apoprotein in patients with endogenous hypertriglyceridaemia: effects of 2,2-dimethyl, 5(2, 5-xylyoxy) valeric acid therapy. *Atherosclerosis* **24**, 199-218.

Kissebah AH, Alfarsi S, Adams PW & Wynn V (1976b) Role of insulin resistance in adipose tissue and liver in the pathogenesis of endogenous hypertriglyceridaemia in man. *Diabetologia* **12**, 563-571.

Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK & Adams PW (1982) Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab* **54**, 254-260.

Kitamura T, Kitamura Y, Kuroda S, Hino Y, Ando M, Kotani K, Konishi H, Matsuzaki H, Kikkawa U, Ogawa W & Kasuga M (1999) Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. *Mol Cell Biol* **19**, 6286-6296.

Klein-Platat C, Drai J, Oujaa M, Schlienger JL & Simon C (2005) Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr* **82**, 1178-1184.

Klein S, Burke LE, Bray GA, Blair S, Allison DB, Pi-Sunyer X, Hong Y & Eckel RH (2004) Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. *Circulation* **110**, 2952-2967.

Knapp HR (1996) n-3 fatty acids and human hypertension. *Curr Opin Lipidol* **7**, 30-33.

Knapper J, Tredger J, Webb D, Culverwell C, Faulkner W, Roche HM & Williams CM (1996) Substitution of dietary monounsaturated fatty acids for saturated fatty acids in a free-living population: a feasibility study. *Journal of Human Nutrition and Dietetics* **9**, 273-282.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA & Nathan DM (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* **346**, 393-403.

Knowler WC, Pettitt DJ, Saad MF & Bennett PH (1990) Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* **6**, 1-27.

Kobayashi J, Nishimura K, Matoba M, Maekawa N & Mabuchi H (2007) Generation and gender differences in the components contributing to the diagnosis of the metabolic syndrome according to the Japanese criteria. *Circ J* **71**, 1734-1737.

Kohler HP & Grant PJ (2000) Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med* **342**, 1792-1801.

Kolterman OG, Insel J, Saekow M & Olefsky JM (1980) Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects. *J Clin Invest* **65**, 1272-1284.

Kraja AT, Borecki IB, North K, Tang W, Myers RH, Hopkins PN, Arnett D, Corbett J, Adelman A & Province MA (2006) Longitudinal and age trends of metabolic syndrome and its risk factors: the Family Heart Study. *Nutr Metab (Lond)* **3**, 41.

Krauss RM & Dreon DM (1995) Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. *Am J Clin Nutr* **62**, 478S-487S.

Kris-Etherton PM, Harris WS & Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**, 2747-2757.

Krummel D, Etherton TD, Peterson S & Kris-Etherton PM (1993) Effects of exercise on plasma lipids and lipoproteins of women. *Proc Soc Exp Biol Med* **204**, 123-137.

Kullo IJ, Hensrud DD & Allison TG (2002) Relation of low cardiorespiratory fitness to the metabolic syndrome in middle-aged men. *Am J Cardiol* **90**, 795-797.

Laaksonen DE, Lakka TA, Lakka HM, Nyyssonen K, Rissanen T, Niskanen LK & Salonen JT (2002) Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med* **19**, 456-464.

Lafay L, Mennen L, Basdevant A, Charles MA, Borys JM, Eschwege E & Romon M (2000) Does energy intake underreporting involve all kinds of food or only specific food items? Results from the Fleurbaix Laventie Ville Sante (FLVS) study. *Int J Obes Relat Metab Disord* **24**, 1500-1506.

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J & Salonen JT (2002) The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *Jama* **288**, 2709-2716.

Lamarche B, Uffelman KD, Carpentier A, Cohn JS, Steiner G, Barrett PH & Lewis GF (1999) Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J Clin Invest* **103**, 1191-1199.

Larsson H, Elmstahl S, Berglund G & Ahren B (1999) Habitual dietary intake versus glucose tolerance, insulin sensitivity and insulin secretion in postmenopausal women. *J Intern Med* **245**, 581-591.

Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M & Laakso M (2004) Common polymorphisms in the genes regulating the early insulin signalling pathway: effects on weight change and the conversion from impaired glucose tolerance to Type 2 diabetes. The Finnish Diabetes Prevention Study. *Diabetologia* **47**, 871-877.

Lawlor DA, Smith GD & Ebrahim S (2006) Does the new International Diabetes Federation definition of the metabolic syndrome predict CHD any more strongly than older definitions? Findings from the British Women's Heart and Health Study. *Diabetologia* **49**, 41-48.

Le Good JA, Ziegler WH, Parekh DB, Alessi DR, Cohen P & Parker PJ (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* **281**, 2042-2045.

Leon AS & Sanchez OA (2001) Response of blood lipids to exercise training alone or combined with dietary intervention. *Med Sci Sports Exerc* **33**, S502-515; discussion S528-509.

Lerman-Garber I, Ichazo-Cerro S, Zamora-Gonzalez J, Cardoso-Saldana G & Posadas-Romero C (1994) Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. *Diabetes Care* **17**, 311-315.

Lerner RL & Porte D, Jr. (1971) Relationship between intravenous glucose loads, insulin responses and glucose disappearance rate. *J Clin Endocrinol Metab* **33**, 409-417.

Lewis GF & Steiner G (1996) Hypertriglyceridemia and its metabolic consequences as a risk factor for atherosclerotic cardiovascular disease in non-insulin-dependent diabetes mellitus. *Diabetes Metab Rev* **12**, 37-56.

Lewis RA, Austen KF & Soberman RJ (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med* **323**, 645-655.

Lichtenstein AH & Schwab US (2000) Relationship of dietary fat to glucose metabolism. *Atherosclerosis* **150**, 227-243.

Lindsay RS, Funahashi T, Hanson RL & etal (2002) Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* **360**, 57-58.

Liu S, Baracos VE, Quinney HA & Clandinin MT (1994) Dietary omega-3 and polyunsaturated fatty acids modify fatty acyl composition and insulin binding in skeletal-muscle sarcolemma. *Biochem J* **299 (Pt 3)**, 831-837.

Liu S & Manson JE (2001) Dietary carbohydrates, physical inactivity, obesity, and the 'metabolic syndrome' as predictors of coronary heart disease. *Curr Opin Lipidol* **12**, 395-404.

Lopes SM, Trimbo SL, Mascioli EA & Blackburn GL (1991) Human plasma fatty acid variations and how they are related to dietary intake. *Am J Clin Nutr* **53**, 628-637.

Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC & Hu FB (2004) Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr* **134**, 1806-1811.

Lopez-Miranda J, Perez-Martinez P, Marin C, Fuentes F, Delgado J & Perez-Jimenez F (2007) Dietary fat, genes and insulin sensitivity. *J Mol Med* **85**, 213-226.

Lopez-Segura F, Velasco F, Lopez-Miranda J, Castro P, Lopez-Pedrera R, Blanco A, Jimenez-Pereperez J, Torres A, Trujillo J, Ordovas JM & Perez-Jimenez F (1996) Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arterioscler Thromb Vasc Biol* **16**, 82-88.

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR & Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897-901.

Lorec AM, Juhel C, Pafumi Y, Portugal H, Pauli AM, Lairon D & Defoort C (2000) Determination of apolipoprotein B-48 in plasma by a competitive ELISA. *Clin Chem* **46**, 1638-1642.

Lorenzo C, Okoloise M, Williams K, Stern MP & Haffner SM (2003) The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. *Diabetes Care* **26**, 3153-3159.

Louheranta AM, Sarkkinen ES, Vidgren HM, Schwab US & Uusitupa MI (2002) Association of the fatty acid profile of serum lipids with glucose and insulin metabolism during 2 fat-modified diets in subjects with impaired glucose tolerance. *Am J Clin Nutr* **76**, 331-337.

Lovegrove JA, Isherwood SG, Jackson KG, Williams CM & Gould BJ (1996) Quantitation of apolipoprotein B-48 in triacylglycerol-rich lipoproteins by a specific enzyme-linked immunosorbent assay. *Biochim Biophys Acta* **1301**, 221-229.

Lovejoy J & DiGirolamo M (1992) Habitual dietary intake and insulin sensitivity in lean and obese adults. *Am J Clin Nutr* **55**, 1174-1179.

Lovejoy JC, Champagne CM, Smith SR, DeLany JP, Bray GA, Lefevre M, Denkins YM & Rood JC (2001) Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. *Metabolism* **50**, 86-92.

Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J & Bray GA (2002) Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care* **25**, 1283-1288.

Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK & Wareham NJ (2001) Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes* **50**, 686-689.

Luc G, Fievet C, Arveiler D, Evans AE, Bard JM, Cambien F, Fruchart JC & Ducimetiere P (1996) Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. *J Lipid Res* **37**, 508-517.

Luo J, Rizkalla SW, Boillot J, Alamowitch C, Chaib H, Bruzzo F, Desplanque N, Dalix AM, Durand G & Slama G (1996) Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. *J Nutr* **126**, 1951-1958.

Luo J, Rizkalla SW, Vidal H, Oppert JM, Colas C, Boussairi A, Guerre-Millo M, Chapuis AS, Chevalier A, Durand G & Slama G (1998) Moderate intake of n-3 fatty acids for 2 months has no detrimental effect on glucose metabolism and could ameliorate the lipid profile in type 2 diabetic men. Results of a controlled study. *Diabetes Care* **21**, 717-724.

Lyon CJ, Law RE & Hsueh WA (2003) Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* **144**, 2195-2200.

Ma J, Folsom AR, Eckfeldt JH, Lewis L & Chambless LE (1995a) Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Clin Nutr* **62**, 572-578.

Ma J, Folsom AR, Shahar E & Eckfeldt JH (1995b) Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Clin Nutr* **62**, 564-571.

Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE & Fogo AB (2004) Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* **53**, 336-346.

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y & Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* **221**, 286-289.

Mann CJ, Troussard AA, Yen FT, Hannouche N, Najib J, Fruchart JC, Lotteau V, Andre P & Bihain BE (1997) Inhibitory effects of specific apolipoprotein C-III isoforms on the binding of triglyceride-rich lipoproteins to the lipolysis-stimulated receptor. *J Biol Chem* **272**, 31348-31354.

Mann JI (2002) Diet and risk of coronary heart disease and type 2 diabetes. *Lancet* **360**, 783-789.

Mann JI, De Leeuw I, Hermansen K, Karamanos B, Karlstrom B, Katsilambros N, Riccardi G, Rivellese AA, Rizkalla S, Slama G, Toeller M, Uusitupa M & Vessby

B (2004) Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. *Nutr Metab Cardiovasc Dis* **14**, 373-394.

Marckmann P, Sandstrom B & Jespersen J (1993) Favorable long-term effect of a low-fat/high-fiber diet on human blood coagulation and fibrinolysis. *Arterioscler Thromb* **13**, 505-511.

Maresca G, Di Blasio A, Marchioli R & Di Minno G (1999) Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. *Arterioscler Thromb Vasc Biol* **19**, 1368-1377.

Marini MA, Frontoni S, Mineo D, Bracaglia D, Cardellini M, De Nicolais P, Baroni A, D'Alfonso R, Perna M, Lauro D, Federici M, Gambardella S, Lauro R & Sesti G (2003) The Arg972 variant in insulin receptor substrate-1 is associated with an atherogenic profile in offspring of type 2 diabetic patients. *J Clin Endocrinol Metab* **88**, 3368-3371.

Maron DJ, Fair JM & Haskell WL (1991) Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. *Circulation* **84**, 2020-2027.

Marshall JA, Bessesen DH & Hamman RF (1997) High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. *Diabetologia* **40**, 430-438.

Marshall JA, Hoag S, Shetterly S & Hamman RF (1994) Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. The San Luis Valley Diabetes Study. *Diabetes Care* **17**, 50-56.

Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS & Kahn CR (1992a) Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* **340**, 925-929.

Martin BC, Warram JH, Rosner B, Rich SS, Soeldner JS & Krolewski AS (1992b) Familial clustering of insulin sensitivity. *Diabetes* **41**, 850-854.

Matsuda M & DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22**, 1462-1470.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419.

Mayer-Davis EJ, D'Agostino R, Jr., Karter AJ, Haffner SM, Rewers MJ, Saad M & Bergman RN (1998) Intensity and amount of physical activity in relation to insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *Jama* **279**, 669-674.

Mayer-Davis EJ, Levin S, Bergman RN, D'Agostino RB, Jr., Karter AJ & Saad MF (2001) Insulin secretion, obesity, and potential behavioral influences: results from the Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes Metab Res Rev* **17**, 137-145.

Mayer-Davis EJ, Monaco JH, Hoen HM, Carmichael S, Vitolins MZ, Rewers MJ, Haffner SM, Ayad MF, Bergman RN & Karter AJ (1997) Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS). *Am J Clin Nutr* **65**, 79-87.

Mayer EJ, Newman B, Quesenberry CP, Jr. & Selby JV (1993) Usual dietary fat intake and insulin concentrations in healthy women twins. *Diabetes Care* **16**, 1459-1469.

McAuley K & Mann J (2006) Thematic review series: patient-oriented research. Nutritional determinants of insulin resistance. *J Lipid Res* **47**, 1668-1676.

McGarry JD (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* **51**, 7-18.

McGarry JD & Dobbins RL (1999) Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* **42**, 128-138.

McGarry JD, Mannaerts GP & Foster DW (1977) A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *J Clin Invest* **60**, 265-270.

McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW & Jacques PF (2004) Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care* **27**, 538-546.

McLaughlin T, Allison G, Abbasi F, Lamendola C & Reaven G (2004) Prevalence of insulin resistance and associated cardiovascular disease risk factors among normal weight, overweight, and obese individuals. *Metabolism* **53**, 495-499.

McManus RM, Jumpson J, Finegood DT, Clandinin MT & Ryan EA (1996) A comparison of the effects of n-3 fatty acids from linseed oil and fish oil in well-controlled type II diabetes. *Diabetes Care* **19**, 463-467.

Meigs JB (2002) Epidemiology of the metabolic syndrome, 2002. *Am J Manag Care* **8**, S283-292; quiz S293-286.

Meirhaeghe A, Cottel D, Amouyel P & Dallongeville J (2005) Association between peroxisome proliferator-activated receptor gamma haplotypes and the metabolic syndrome in French men and women. *Diabetes* **54**, 3043-3048.

Mensink RP & Katan MB (1992) Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb* **12**, 911-919.

Millen BE, Pencina MJ, Kimokoti RW, Zhu L, Meigs JB, Ordovas JM & D'Agostino RB (2006) Nutritional risk and the metabolic syndrome in women: opportunities for preventive intervention from the Framingham Nutrition Study. *Am J Clin Nutr* **84**, 434-441.

Miller WC, Lindeman AK, Wallace J & Niederpruem M (1990) Diet composition, energy intake, and exercise in relation to body fat in men and women. *Am J Clin Nutr* **52**, 426-430.

Mills GW, Avery PJ, McCarthy MI, Hattersley AT, Levy JC, Hitman GA, Sampson M & Walker M (2004) Heritability estimates for beta cell function and features of the insulin resistance syndrome in UK families with an increased susceptibility to type 2 diabetes. *Diabetologia* **47**, 732-738.

Mimura A, Kageyama S, Maruyama M, Ikeda Y & Isogai Y (1994) Insulin sensitivity test using a somatostatin analogue, octreotide (Sandostatin). *Horm Metab Res* **26**, 184-187.

Mittendorfer B (2005) Insulin resistance: sex matters. *Curr Opin Clin Nutr Metab Care* **8**, 367-372.

Moilanen T, Rasanen L, Viikari J, Akerblom HK, Ahola M, Uhari M, Pasanen M & Nikkari T (1985) Fatty acid composition of serum cholesteryl esters in 3- to 18-year-old Finnish children and its relation to diet. *Am J Clin Nutr* **42**, 708-713.

Moller DE & Kaufman KD (2005) Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med* **56**, 45-62.

Montori VM, Farmer A, Wollan PC & Dinneen SF (2000) Fish oil supplementation in type 2 diabetes: a quantitative systematic review. *Diabetes Care* **23**, 1407-1415.

Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM & Heine RJ (1996) Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. *Diabetologia* **39**, 298-305.

Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF & Beilin LJ (1999) Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am J Clin Nutr* **70**, 817-825.

Morris MC, Sacks F & Rosner B (1993) Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* **88**, 523-533.

Mortensen JZ, Schmidt EB, Nielsen AH & Dyerberg J (1983) The effect of N-6 and N-3 polyunsaturated fatty acids on hemostasis, blood lipids and blood pressure. *Thromb Haemost* **50**, 543-546.

Muniyappa R, Lee S, Chen H & Quon MJ (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* **294**, E15-26.

Muscelli E, Camastra S, Catalano C, Galvan AQ, Ciociaro D, Baldi S & Ferrannini E (1997) Metabolic and cardiovascular assessment in moderate obesity: effect of weight loss. *J Clin Endocrinol Metab* **82**, 2937-2943.

Mykkanen L, Haffner SM, Ronnemaa T, Bergman RN & Laakso M (1997) Low insulin sensitivity is associated with clustering of cardiovascular disease risk factors. *Am J Epidemiol* **146**, 315-321.

Mykkanen L, Laakso M & Pyorala K (1993) High plasma insulin level associated with coronary heart disease in the elderly. *Am J Epidemiol* **137**, 1190-1202.

National Centre for Health Statistics Prevalence of overweight among children and adolescents: United States 2003-2004 (www.cdc.gov/nccdphp/dnpa/obesity).

Neel JV (1962) Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet* **14**, 353-362.

Nieves DJ, Cnop M, Retzlaff B, Walden CE, Brunzell JD, Knopp RH & Kahn SE (2003) The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. *Diabetes* **52**, 172-179.

Nilsson PM, Engstrom G & Hedblad B (2007) The metabolic syndrome and incidence of cardiovascular disease in non-diabetic subjects--a population-based study comparing three different definitions. *Diabet Med* **24**, 464-472.

Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A & Chen RS (2004) Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation* **109**, 42-46.

Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H, Matsuda M, Kondo H, Furuyama N, Kihara S, Nakamura T, Tochino Y, Funahashi T & Matsuzawa Y (2002) Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes* **51**, 2734-2741.

Nissen SE & Wolski K (2007) Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* **356**, 2457-2471.

Nuutila P, Knuuti MJ, Maki M, Laine H, Ruotsalainen U, Teras M, Haaparanta M, Solin O & Yki-Jarvinen H (1995) Gender and insulin sensitivity in the heart and in skeletal muscles. Studies using positron emission tomography. *Diabetes* **44**, 31-36.

Nydahl MC, Smith RD, Kelly CN, Fielding BA & Williams CM (2003) Achievement of dietary fatty acid intakes in long-term controlled intervention studies: approach and methodology. *Public Health Nutr* **6**, 31-40.

Ohlson LO, Larsson B, Svardsudd K, Welin L, Eriksson H, Wilhelmsen L, Bjorntorp P & Tibblin G (1985) The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes* **34**, 1055-1058.

Olefsky J, Reaven GM & Farquhar JW (1974) Effects of weight reduction on obesity. Studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J Clin Invest* **53**, 64-76.

Olefsky JM, Kolterman OG & Scarlett JA (1982) Insulin action and resistance in obesity and noninsulin-dependent type II diabetes mellitus. *Am J Physiol* **243**, E15-30.

Olefsky JM & Saekow M (1978) The effects of dietary carbohydrate content on insulin binding and glucose metabolism by isolated rat adipocytes. *Endocrinology* **103**, 2252-2263.

Onat A, Hergenc G, Sansoy V, Fobker M, Ceyhan K, Toprak S & Assmann G (2003) Apolipoprotein C-III, a strong discriminant of coronary risk in men and a determinant of the metabolic syndrome in both genders. *Atherosclerosis* **168**, 81-89.

Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P & Garg A (2002) Leptin-replacement therapy for lipodystrophy. *N Engl J Med* **346**, 570-578.

Orchard TJ, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S & Fowler S (2005) The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann Intern Med* **142**, 611-619.

Ordovas JM & Corella D (2004) Nutritional genomics. *Annu Rev Genomics Hum Genet* **5**, 71-118.

Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T & Matsuzawa Y (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* **100**, 2473-2476.

Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH & Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**, 457-461.

Pacini G & Bergman RN (1986) MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* **23**, 113-122.

Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M & Scherer PE (2003) Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem* **278**, 9073-9085.

Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA & Scherer PE (2004) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* **279**, 12152-12162.

Palaniappan L, Carnethon MR, Wang Y, Hanley AJ, Fortmann SP, Haffner SM & Wagenknecht L (2004) Predictors of the incident metabolic syndrome in adults: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* **27**, 788-793.

Pan DA, Lillioja S, Milner MR, Kriketos AD, Baur LA, Bogardus C & Storlien LH (1995) Skeletal muscle membrane lipid composition is related to adiposity and insulin action. *J Clin Invest* **96**, 2802-2808.

Panagiotakos DB & Polychronopoulos E (2005) The role of Mediterranean diet in the epidemiology of metabolic syndrome; converting epidemiology to clinical practice. *Lipids Health Dis* **4**, 7.

Paniagua JA, de la Sacristana AG, Sanchez E, Romero I, Vidal-Puig A, Berral FJ, Escribano A, Moyano MJ, Perez-Martinez P, Lopez-Miranda J & Perez-Jimenez F (2007) A MUFA-Rich Diet Improves Posprandial Glucose, Lipid and GLP-1 Responses in Insulin-Resistant Subjects. *J Am Coll Nutr* **26**, 434-444.

Parikh NI, Pencina MJ, Wang TJ, Lanier KJ, Fox CS, D'Agostino RB & Vasan RS (2007) Increasing trends in incidence of overweight and obesity over 5 decades. *Am J Med* **120**, 242-250.

Parillo M, Giacco R, Ciardullo AV, Rivellese AA & Riccardi G (1996) Does a high-carbohydrate diet have different effects in NIDDM patients treated with diet alone or hypoglycemic drugs? *Diabetes Care* **19**, 498-500.

Parillo M, Rivellese AA, Ciardullo AV, Capaldo B, Giacco A, Genovese S & Riccardi G (1992) A high-monounsaturated-fat/low-carbohydrate diet improves peripheral

insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism* **41**, 1373-1378.

Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR & Heymsfield SB (2003) The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med* **163**, 427-436.

Parker DR, Weiss ST, Troisi R, Cassano PA, Vokonas PS & Landsberg L (1993) Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: the Normative Aging Study. *Am J Clin Nutr* **58**, 129-136.

Parks EJ & Hellerstein MK (2000) Carbohydrate-induced hypertriacylglycerolemia: historical perspective and review of biological mechanisms. *Am J Clin Nutr* **71**, 412-433.

Pei D, Jones CN, Bhargava R, Chen YD & Reaven GM (1994) Evaluation of octreotide to assess insulin-mediated glucose disposal by the insulin suppression test. *Diabetologia* **37**, 843-845.

Pekkanen J, Tuomilehto J, Uutela A, Vartiainen E & Nissinen A (1995) Social class, health behaviour, and mortality among men and women in eastern Finland. *Bmj* **311**, 589-593.

Perez-Jimenez F, Lopez-Miranda J, Pinillos MD, Gomez P, Paz-Rojas E, Montilla P, Marin C, Velasco MJ, Blanco-Molina A, Jimenez Pereperez JA & Ordovas JM (2001) A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* **44**, 2038-2043.

Perseghin G, Caumo A, Caloni M, Testolin G & Luzi L (2001) Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *J Clin Endocrinol Metab* **86**, 4776-4781.

Peyron-Caso E, Quignard-Boulange A, Laromiguiere M, Feing-Kwong-Chan S, Veronese A, Ardouin B, Slama G & Rizkalla SW (2003) Dietary fish oil increases lipid mobilization but does not decrease lipid storage-related enzyme activities in adipose tissue of insulin-resistant, sucrose-fed rats. *J Nutr* **133**, 2239-2243.

Phillips C, Lopez-Miranda J, Perez-Jimenez F, McManus R & Roche HM (2006) Genetic and nutrient determinants of the metabolic syndrome. *Curr Opin Cardiol* **21**, 185-193.

Phillips C, Tierney AC & Roche HM (2007) Gene-Nutrient Interactions in the Metabolic Syndrome. *Journal of Nutrigenetics and Nutrigenomics* **In Press**.

Pi-Sunyer FX (1993) Medical hazards of obesity. *Ann Intern Med* **119**, 655-660.

Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC & Rimm EB (2003) Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation* **108**, 155-160.

Popp-Snijders C, Schouten JA, Heine RJ, van der Meer J & van der Veen EA (1987) Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. *Diabetes Res* **4**, 141-147.

Poppitt SD, Keogh GF, Prentice AM, Williams DE, Sonnemans HM, Valk EE, Robinson E & Wareham NJ (2002) Long-term effects of ad libitum low-fat, high-carbohydrate diets on body weight and serum lipids in overweight subjects with metabolic syndrome. *Am J Clin Nutr* **75**, 11-20.

Poppitt SD, Swann D, Black AE & Prentice AM (1998) Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes Relat Metab Disord* **22**, 303-311.

Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, Nadeau A & Lupien PJ (1994) Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* **73**, 460-468.

Poulsen P, Levin K, Petersen I, Christensen K, Beck-Nielsen H & Vaag A (2005) Heritability of insulin secretion, peripheral and hepatic insulin action, and intracellular glucose partitioning in young and old Danish twins. *Diabetes* **54**, 275-283.

Poulsen P, Vaag A, Kyvik K & Beck-Nielsen H (2001) Genetic versus environmental aetiology of the metabolic syndrome among male and female twins. *Diabetologia* **44**, 537-543.

Prentki M & Corkey BE (1996) Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* **45**, 273-283.

Price GM, Paul AA, Cole TJ & Wadsworth ME (1997) Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Br J Nutr* **77**, 833-851.

Qiao Q (2006) Comparison of different definitions of the metabolic syndrome in relation to cardiovascular mortality in European men and women. *Diabetologia* **49**, 2837-2846.

Quon MJ, Cochran C, Taylor SI & Eastman RC (1994) Non-insulin-mediated glucose disappearance in subjects with IDDM. Discordance between experimental results and minimal model analysis. *Diabetes* **43**, 890-896.

Rabasa-Lhoret R, Bastard JP, Jan V, Ducluzeau PH, Andreelli F, Guebre F, Bruzeau J, Louche-Pellissier C, MaItrepierre C, Peyrat J, Chagne J, Vidal H & Laville M (2003) Modified quantitative insulin sensitivity check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. *J Clin Endocrinol Metab* **88**, 4917-4923.

Radziuk J (2000) Insulin sensitivity and its measurement: structural commonalities among the methods. *J Clin Endocrinol Metab* **85**, 4426-4433.

Randle PJ, Newsholme EA & Garland PB (1964) Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. *Biochem J* **93**, 652-665.

Rasmussen BM, Vessby B, Uusitupa M, Berglund L, Pedersen E, Riccardi G, Rivellese AA, Tapsell L & Hermansen K (2006) Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects. *Am J Clin Nutr* **83**, 221-226.

Ravaja N, Keltikangas-Jarvinen L & Viikari J (1996) Life changes, locus of control and metabolic syndrome precursors in adolescents and young adults: a three-year follow-up. *Soc Sci Med* **43**, 51-61.

Reaven G (1988a) Banting Lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37**, 1595-1607.

Reaven G (2004) The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinol Metab Clin North Am* **33**, 283-303.

Reaven GM (1988b) Banting Lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37**, 1595-1607.

Reaven GM (1995) Pathophysiology of insulin resistance in human disease. *Physiol Rev* **75**, 473-486.

Reaven GM (1997) Do high carbohydrate diets prevent the development or attenuate the manifestations (or both) of syndrome X? A viewpoint strongly against. *Curr Opin Lipidol* **8**, 23-27.

Reaven GM (2000) Diet and Syndrome X. *Curr Atheroscler Rep* **2**, 503-507.

Reaven GM (2005) The insulin resistance syndrome: definition and dietary approaches to treatment. *Annu Rev Nutr* **25**, 391-406.

Reaven GM (2006) The metabolic syndrome: is this diagnosis necessary? *Am J Clin Nutr* **83**, 1237-1247.

Reaven GM, Lerner RL, Stern MP & Farquhar JW (1967) Role of insulin in endogenous hypertriglyceridemia. *J Clin Invest* **46**, 1756-1767.

Reaven GM, Lithell H & Landsberg L (1996) Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* **334**, 374-381.

Reaven GM & Olefsky JM (1974a) Increased plasma glucose and insulin responses to high-carbohydrate feedings in normal subjects. *J Clin Endocrinol Metab* **38**, 151-154.

Reaven GM & Olefsky JM (1974b) Relationship between insulin response during the intravenous glucose tolerance test, rate of fractional glucose removal and the degree of insulin resistance in normal adults. *Diabetes* **23**, 454-459.

Reaven GM & Olefsky JM (1977) Relationship between heterogeneity of insulin responses and insulin resistance in normal subjects and patients with chemical diabetes. *Diabetologia* **13**, 201-206.

Reaven PD, McPhillips JB, Barrett-Connor EL & Criqui MH (1990) Leisure time exercise and lipid and lipoprotein levels in an older population. *J Am Geriatr Soc* **38**, 847-854.

Regitz-Zagrosek V, Lehmkuhl E & Weickert MO (2006) Gender differences in the metabolic syndrome and their role for cardiovascular disease. *Clin Res Cardiol* **95**, 136-147.

Rensen PC & van Berkel TJ (1996) Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chylomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. *J Biol Chem* **271**, 14791-14799.

Resnick HE, Jones K, Ruotolo G, Jain AK, Henderson J, Lu W & Howard BV (2003) Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic american indians: the Strong Heart Study. *Diabetes Care* **26**, 861-867.

Riboli E, Ronnholm H & Saracci R (1987) Biological markers of diet. *Cancer Surv* **6**, 685-718.

Riccardi G, Giacco R & Rivellese AA (2004) Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* **23**, 447-456.

Riccardi G & Rivellese AA (1991) Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. *Diabetes Care* **14**, 1115-1125.

Ridker PM, Vaughan DE, Stampfer MJ, Manson JE & Hennekens CH (1993) Endogenous tissue-type plasminogen activator and risk of myocardial infarction. *Lancet* **341**, 1165-1168.

Rivellese A (2000) Effects of omega-3 fatty acids on carbohydrate and lipid metabolism in healthy people: the KANWU study. *Diabetologia* **43**.

Rivellese AA, Maffettone A, Iovine C, Di Marino L, Annuzzi G, Mancini M & Riccardi G (1996) Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. *Diabetes Care* **19**, 1207-1213.

Rivellese AA, Maffettone A, Vessby B, Uusitupa M, Hermansen K, Berglund L, Louheranta A, Meyer BJ & Riccardi G (2003) Effects of dietary saturated,

monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis* **167**, 149-158.

Rizza RA, Mandarino LJ & Gerich JE (1981) Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* **240**, E630-639.

Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, Gingerich R, Goldstein D, Widemeyer HM, Haffner S, Hales CN, Jarett L, Polonsky K, Porte D, Skyler J, Webb G & Gallagher K (1996) Report of the American Diabetes Association's Task Force on standardization of the insulin assay. *Diabetes* **45**, 242-256.

Rocchini AP, Moorehead C, DeRemer S, Goodfriend TL & Ball DL (1990) Hyperinsulinemia and the aldosterone and pressor responses to angiotensin II. *Hypertension* **15**, 861-866.

Roche HM (2005) Fatty acids and the metabolic syndrome. *Proc Nutr Soc* **64**, 23-29.

Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW & Shulman GI (1996) Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* **97**, 2859-2865.

Rogers S, James KS, Butland BK, Etherington MD, O'Brien JR & Jones JG (1987) Effects of a fish oil supplement on serum lipids, blood pressure, bleeding time, haemostatic and rheological variables. A double blind randomised controlled trial in healthy volunteers. *Atherosclerosis* **63**, 137-143.

Rosell MS, Hellenius ML, de Faire UH & Johansson GK (2003) Associations between diet and the metabolic syndrome vary with the validity of dietary intake data. *Am J Clin Nutr* **78**, 84-90.

Rosengren A & Wilhelmsen L (1997) Physical activity protects against coronary death and deaths from all causes in middle-aged men. Evidence from a 20-year follow-up of the primary prevention study in Goteborg. *Ann Epidemiol* **7**, 69-75.

Ross R (2003) Does exercise without weight loss improve insulin sensitivity? *Diabetes Care* **26**, 944-945.

Rossi AS, Lombardo YB, Lacorte JM, Chicco AG, Rouault C, Slama G & Rizkalla SW (2005) Dietary fish oil positively regulates plasma leptin and adiponectin levels in

sucrose-fed, insulin-resistant rats. *Am J Physiol Regul Integr Comp Physiol* **289**, R486-R494.

Ruan H & Lodish HF (2003) Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev* **14**, 447-455.

Ruan H & Lodish HF (2004) Regulation of insulin sensitivity by adipose tissue-derived hormones and inflammatory cytokines. *Curr Opin Lipidol* **15**, 297-302.

Ryan M, McInerney D, Owens D, Collins P, Johnson A & Tomkin GH (2000) Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *Qjm* **93**, 85-91.

Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YD, Sands RE, Pei D, Savage PJ & Bergman RN (1994) A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. *Diabetes* **43**, 1114-1121.

Saad MF, Steil GM, Kades WW, Ayad MF, Elsewafy WA, Boyadjian R, Jinagouda SD & Bergman RN (1997) Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. *Diabetes* **46**, 1167-1171.

Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pfeffer MA & Braunwald E (2000) VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation* **102**, 1886-1892.

Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL & Willett WC (1997) Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* **20**, 545-550.

Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB & Willett WC (2001) Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* **73**, 1019-1026.

Salomaa V, Ahola I, Tuomilehto J, Aro A, Pietinen P, Korhonen HJ & Penttila I (1990) Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population-based study. *Metabolism* **39**, 1285-1291.

Saltiel AR (2000) Series introduction: the molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. *J Clin Invest* **106**, 163-164.

Sampath H & Ntambi JM (2004) Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* **62**, 333-339.

Sarkkinen E, Schwab U, Niskanen L, Hannuksela M, Savolainen M, Kervinen K, Kesaniemi A & Uusitupa MI (1996) The effects of monounsaturated-fat enriched diet and polyunsaturated-fat enriched diet on lipid and glucose metabolism in subjects with impaired glucose tolerance. *Eur J Clin Nutr* **50**, 592-598.

Schaefer EJ (2002) Lipoproteins, nutrition, and heart disease. *Am J Clin Nutr* **75**, 191-212.

Schaefer EJ, Ordovas JM, Law SW, Ghiselli G, Kashyap ML, Srivastava LS, Heaton WH, Albers JJ, Connor WE, Lindgren FT & et al. (1985) Familial apolipoprotein A-I and C-III deficiency, variant II. *J Lipid Res* **26**, 1089-1101.

Scheffer PG, Henry RM, Wever EJ, van Rooij GJ, Bos G, Heine RJ, Dekker JM, Diamant M, Stehouwer CD, Nijpels G, Blankenstein MA & Teerlink T (2004) LDL oxidative modifications in well- or moderately controlled type 2 diabetes. *Diabetes Metab Res Rev* **20**, 298-304.

Schinner S, Scherbaum WA, Bornstein SR & Barthel A (2005) Molecular mechanisms of insulin resistance. *Diabet Med* **22**, 674-682.

Schwab US, Niskanen LK, Maliranta HM, Savolainen MJ, Kesaniemi YA & Uusitupa MI (1995) Lauric and palmitic acid-enriched diets have minimal impact on serum lipid and lipoprotein concentrations and glucose metabolism in healthy young women. *J Nutr* **125**, 466-473.

Sehayek E & Eisenberg S (1991) Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem* **266**, 18259-18267.

Semenkovich CF (2006) Insulin resistance and atherosclerosis. *J Clin Invest* **116**, 1813-1822.

Serne EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJ & Gans RO (1999) Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* **99**, 896-902.

Shafrir E (1996) Development and consequences of insulin resistance: lessons from animals with hyperinsulinaemia. *Diabetes Metab* **22**, 122-131.

Shahar E, Folsom AR, Wu KK, Dennis BH, Shimakawa T, Conlan MG, Davis CE & Williams OD (1993) Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypocoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb* **13**, 1205-1212.

Sharman MJ & Volek JS (2004) Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men. *Clin Sci (Lond)* **107**, 365-369.

Shaw DI, Hall WL & Williams CM (2005) Metabolic syndrome: what is it and what are the implications? *Proc Nutr Soc* **64**, 349-357.

Shaw JE, Zimmet PZ, de Courten M, Dowse GK, Chitson P, Gareeboo H, Hemraj F, Fareed D, Tuomilehto J & Alberti KG (1999) Impaired fasting glucose or impaired glucose tolerance. What best predicts future diabetes in Mauritius? *Diabetes Care* **22**, 399-402.

Shen J, Arnett DK, Peacock JM, Parnell LD, Kraja A, Hixson JE, Tsai MY, Lai CQ, Kabagambe EK, Straka RJ & Ordovas JM (2007) Interleukin1beta genetic polymorphisms interact with polyunsaturated fatty acids to modulate risk of the metabolic syndrome. *J Nutr* **137**, 1846-1851.

Shen SW, Reaven GM & Farquhar JW (1970) Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J Clin Invest* **49**, 2151-2160.

Shepherd PR & Kahn BB (1999) Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *N Engl J Med* **341**, 248-257.

Shepherd PR, Withers DJ & Siddle K (1998) Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem J* **333 (Pt 3)**, 471-490.

Shulman GI (2000) Cellular mechanisms of insulin resistance. *J Clin Invest* **106**, 171-176.

Sierra-Johnson J, Johnson BD, Allison TG, Bailey KR, Schwartz GL & Turner ST (2006) Correspondence between the adult treatment panel III criteria for metabolic syndrome and insulin resistance. *Diabetes Care* **29**, 668-672.

Sikaris KA (2007) The clinical biochemistry of obesity-more than skin deep. *Heart Lung Circ* **16 Suppl 3**, S45-50.

Simopoulos AP (1999) Essential fatty acids in health and chronic disease. *Am J Clin Nutr* **70**, 560S-569S.

Simpson HC, Simpson RW, Lousley S, Carter RD, Geekie M, Hockaday TD & Mann JI (1981) A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. *Lancet* **1**, 1-5.

Sir-Petermann T, Perez-Bravo F, Angel B, Maliqueo M, Calvillan M & Palomino A (2001) G972R polymorphism of IRS-1 in women with polycystic ovary syndrome. *Diabetologia* **44**, 1200-1201.

Sniderman AD & Faraj M (2007) Apolipoprotein B, apolipoprotein A-I, insulin resistance and the metabolic syndrome. *Curr Opin Lipidol* **18**, 633-637.

Sniderman AD, Scantlebury T & Cianflone K (2001) Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med* **135**, 447-459.

Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K & Gold H (1998) Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with nondiabetic patients: a potential factor predisposing to thrombosis and its persistence. *Circulation* **97**, 2213-2221.

Somova L, Moodley K, Channa ML & Nadar A (1999) Dose-dependent effect of dietary fish-oil (n-3) polyunsaturated fatty acids on in vivo insulin sensitivity in rat. *Methods Find Exp Clin Pharmacol* **21**, 275-278.

Sparks JD & Sparks CE (1994) Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochim Biophys Acta* **1215**, 9-32.

Stamler J, Caggiula AW & Grandits GA (1997) Relation of body mass and alcohol, nutrient, fiber, and caffeine intakes to blood pressure in the special intervention and usual care groups in the Multiple Risk Factor Intervention Trial. *Am J Clin Nutr* **65**, 338S-365S.

Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y & Tataranni PA (2002) Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *J Clin Endocrinol Metab* **87**, 4652-4656.

Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD & McGarry JD (1997) The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. *J Clin Invest* **100**, 398-403.

Steinbaum SR (2004) The metabolic syndrome: an emerging health epidemic in women. *Prog Cardiovasc Dis* **46**, 321-336.

Stettler R, Ith M, Acheson KJ, Decombaz J, Boesch C, Tappy L & Binnert C (2005) Interaction between dietary lipids and physical inactivity on insulin sensitivity and on intramyocellular lipids in healthy men. *Diabetes Care* **28**, 1404-1409.

Stevenson RW, McPherson RK, Persson LM, Genereux PE, Swick AG, Spitzer J, Herbst JJ, Andrews KM, Kreutter DK & Gibbs EM (1996) The antihyperglycemic agent englitazone prevents the defect in glucose transport in rats fed a high-fat diet. *Diabetes* **45**, 60-66.

Storlien LH, Baur LA, Kriketos AD, Pan DA, Cooney GJ, Jenkins AB, Calvert GD & Campbell LV (1996) Dietary fats and insulin action. *Diabetologia* **39**, 621-631.

Storlien LH, James DE, Burleigh KM, Chisholm DJ & Kraegen EW (1986) Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol* **251**, E576-583.

Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S & Kraegen EW (1991) Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* **40**, 280-289.

Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG & Pascoe WS (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* **237**, 885-888.

Storlien LH, Oakes ND, Pan DA, Kusunoki M & Jenkins AB (1993) Syndromes of insulin resistance in the rat. Inducement by diet and amelioration with benfluorex. *Diabetes* **42**, 457-462.

Stout RW (1990) Insulin and atheroma. 20-yr perspective. *Diabetes Care* **13**, 631-654.

Stubbs CD & Smith AD (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim Biophys Acta* **779**, 89-137.

Stumvoll M, Wahl HG, Machicao F & Haring H (2002) Insulin sensitivity of glucose disposal and lipolysis: no influence of common genetic variants in IRS-1 and CAPN10. *Diabetologia* **45**, 651-656.

Su HY, Sheu WH, Chin HM, Jeng CY, Chen YD & Reaven GM (1995) Effect of weight loss on blood pressure and insulin resistance in normotensive and hypertensive obese individuals. *Am J Hypertens* **8**, 1067-1071.

Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR & Frayn KN (2002) Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* **45**, 369-377.

Sundstrom J, Riserus U, Byberg L, Zethelius B, Lithell H & Lind L (2006) Clinical value of the metabolic syndrome for long term prediction of total and cardiovascular mortality: prospective, population based cohort study. *Bmj* **332**, 878-882.

Sunehag AL, Toffolo G, Campioni M, Bier DM & Haymond MW (2005) Effects of dietary macronutrient intake on insulin sensitivity and secretion and glucose and lipid metabolism in healthy, obese adolescents. *J Clin Endocrinol Metab* **90**, 4496-4502.

Swinburn BA, Boyce VL, Bergman RN, Howard BV & Bogardus C (1991a) Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians. *J Clin Endocrinol Metab* **73**, 156-165.

Swinburn BA, Nyomba BL, Saad MF, Zurlo F, Raz I, Knowler WC, Lillioja S, Bogardus C & Ravussin E (1991b) Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest* **88**, 168-173.

Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S & et al. (1994) Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* **372**, 182-186.

Tanti JF, Gremeaux T, Grillo S, Calleja V, Klippel A, Williams LT, Van Obberghen E & Le Marchand-Brustel Y (1996) Overexpression of a constitutively active form of phosphatidylinositol 3-kinase is sufficient to promote Glut 4 translocation in adipocytes. *J Biol Chem* **271**, 25227-25232.

Taskinen MR (2003) Diabetic dyslipidaemia: from basic research to clinical practice. *Diabetologia* **46**, 733-749.

Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. (2002) *Circulation* **106**, 3143-3421.

Tierney AC, Lovegrove JA, Gulseth HL, Defoort C, Blaak E, Marin C, Partyka L, Karlstrom B, Vessby B, Dembinska-Kiec A, Miranda JL, Saris WH, Lairon D, Drevon CA, Williams CM, Gibney MJ & Roche HM (2007) LIPGENE dietary intervention: Cohort details and study design. *Proc Nutr Soc* **66**.

Tierney AC & Roche HM (2007) The potential role of olive oil-derived MUFA in insulin sensitivity. *Mol Nutr Food Res* **51**, 1235-1248.

Toft I, Bonaa KH, Ingebretsen OC, Nordoy A & Jenssen T (1995) Effects of n-3 polyunsaturated fatty acids on glucose homeostasis and blood pressure in essential hypertension. A randomized, controlled trial. *Ann Intern Med* **123**, 911-918.

Tong P, Thomas T, Berrish T, Humphriss D, Barriocanal L, Stewart M, Walker M, Wilkinson R & Alberti KG (1995) Cell membrane dynamics and insulin resistance in non-insulin-dependent diabetes mellitus. *Lancet* **345**, 357-358.

Torjesen PA, Birkeland KI, Anderssen SA, Hjermann I, Holme I & Urdal P (1997) Lifestyle changes may reverse development of the insulin resistance syndrome. The Oslo Diet and Exercise Study: a randomized trial. *Diabetes Care* **20**, 26-31.

Tremblay AJ, Despres JP, Piche ME, Nadeau A, Bergeron J, Almeras N, Tremblay A & Lemieux S (2004) Associations between the fatty acid content of triglyceride, visceral adipose tissue accumulation, and components of the insulin resistance syndrome. *Metabolism* **53**, 310-317.

Trevisan M, Krogh V, Freudenheim J, Blake A, Muti P, Panico S, Farinaro E, Mancini M, Menotti A & Ricci G (1990) Consumption of olive oil, butter, and vegetable

oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. *Jama* **263**, 688-692.

Trevisan M, Liu J, Bahsas FB & Menotti A (1998) Syndrome X and mortality: a population-based study. Risk Factor and Life Expectancy Research Group. *Am J Epidemiol* **148**, 958-966.

Tripathy D, Carlsson M, Almgren P, Isomaa B, Taskinen MR, Tuomi T & Groop LC (2000) Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. *Diabetes* **49**, 975-980.

Trischitta V, Frittitta L & Vigneri R (1997) Early molecular defects in human insulin resistance: studies in healthy subjects with low insulin sensitivity. *Diabetes Metab Rev* **13**, 147-162.

Tsunehara CH, Leonetti DL & Fujimoto WY (1991) Animal fat and cholesterol intake is high in men with IGT progression to NIDDM. *Diabetes* **40**, 427A.

Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V & Uusitupa M (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* **344**, 1343-1350.

Unger RH & Zhou YT (2001) Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* **50 Suppl 1**, S118-121.

Ura S, Araki E, Kishikawa H, Shirotani T, Todaka M, Isami S, Shimoda S, Yoshimura R, Matsuda K, Motoyoshi S, Miyamura N, Kahn CR & Shichiri M (1996) Molecular scanning of the insulin receptor substrate-1 (IRS-1) gene in Japanese patients with NIDDM: identification of five novel polymorphisms. *Diabetologia* **39**, 600-608.

Uusitupa M (2005) Gene-diet interaction in relation to the prevention of obesity and type 2 diabetes: evidence from the Finnish Diabetes Prevention Study. *Nutr Metab Cardiovasc Dis* **15**, 225-233.

Uusitupa M, Schwab U, Makimattila S, Karhapaa P, Sarkkinen E, Maliranta H, Agren J & Penttila I (1994) Effects of two high-fat diets with different fatty acid compositions on glucose and lipid metabolism in healthy young women. *Am J Clin Nutr* **59**, 1310-1316.

Uusitupa MI (1996) Early lifestyle intervention in patients with non-insulin-dependent diabetes mellitus and impaired glucose tolerance. *Ann Med* **28**, 445-449.

Valsta LM, Jauhiainen M, Aro A, Katan MB & Mutanen M (1992) Effects of a monounsaturated rapeseed oil and a polyunsaturated sunflower oil diet on lipoprotein levels in humans. *Arterioscler Thromb* **12**, 50-57.

van Staveren WA, Deurenberg P, Katan MB, Burema J, de Groot LC & Hoffmans MD (1986) Validity of the fatty acid composition of subcutaneous fat tissue microbiopsies as an estimate of the long-term average fatty acid composition of the diet of separate individuals. *Am J Epidemiol* **123**, 455-463.

Vanhala M, Vanhala P, Kumpusalo E, Halonen P & Takala J (1998) Relation between obesity from childhood to adulthood and the metabolic syndrome: population based study. *Bmj* **317**, 319.

Velez-Carrasco W, Lichtenstein AH, Welty FK, Li Z, Lamon-Fava S, Dolnikowski GG & Schaefer EJ (1999) Dietary restriction of saturated fat and cholesterol decreases HDL ApoA-I secretion. *Arterioscler Thromb Vasc Biol* **19**, 918-924.

Vessby B (2000) Dietary fat and insulin action in humans. *Br J Nutr* **83 Suppl 1**, S91-96.

Vessby B (2003) Dietary fat, fatty acid composition in plasma and the metabolic syndrome. *Curr Opin Lipidol* **14**, 15-19.

Vessby B, Aro A, Skarfors E, Berglund L, Salminen I & Lithell H (1994a) The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. *Diabetes* **43**, 1353-1357.

Vessby B & Boberg M (1990) Dietary supplementation with n-3 fatty acids may impair glucose homeostasis in patients with non-insulin-dependent diabetes mellitus. *J Intern Med* **228**, 165-171.

Vessby B, Gustafsson IB, Tengblad S, Boberg M & Andersson A (2002) Desaturation and elongation of Fatty acids and insulin action. *Ann N Y Acad Sci* **967**, 183-195.

Vessby B, Tengblad S & Lithell H (1994b) Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. *Diabetologia* **37**, 1044-1050.

Vessby B, Unsitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen

E, Gustafsson IB & Storlien LH (2001) Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* **44**, 312-319.

Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, Portugal H, Planells R, Grolier P, Amiot-Carlin MJ, Vague P & Lairon D (2005) The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. *Am J Clin Nutr* **82**, 964-971.

Wahrenberg H, Hertel K, Leijonhufvud BM, Persson LG, Toft E & Arner P (2005) Use of waist circumference to predict insulin resistance: retrospective study. *Bmj* **330**, 1363-1364.

Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* **21**, 697-738.

Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R & Kadowaki T (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem* **278**, 40352-40363.

Wallace TM & Matthews DR (2002) The assessment of insulin resistance in man. *Diabet Med* **19**, 527-534.

Wamala SP, Lynch J, Horsten M, Mittleman MA, Schenck-Gustafsson K & Orth-Gomer K (1999) Education and the metabolic syndrome in women. *Diabetes Care* **22**, 1999-2003.

Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS & Lau J (2006) n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* **84**, 5-17.

Wang CS, McConathy WJ, Kloer HU & Alaupovic P (1985) Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J Clin Invest* **75**, 384-390.

Wang J, Kuusisto J, Vanttinen M, Kuulasmaa T, Lindstrom J, Tuomilehto J, Uusitupa M & Laakso M (2007) Variants of transcription factor 7-like 2 (TCF7L2) gene

predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. *Diabetologia* **50**, 1192-1200.

Wang L, Folsom AR, Zheng ZJ, Pankow JS & Eckfeldt JH (2003) Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* **78**, 91-98.

Wannamethee SG & Shaper AG (1999) Weight change and duration of overweight and obesity in the incidence of type 2 diabetes. *Diabetes Care* **22**, 1266-1272.

Wannamethee SG, Shaper AG & Whincup PH (2006) Modifiable lifestyle factors and the metabolic syndrome in older men: Effects of lifestyle changes. *J Am Geriatr Soc* **54**, 1909-1914.

Warensjo E, Ohrvall M & Vessby B (2006) Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. *Nutr Metab Cardiovasc Dis* **16**, 128-136.

Warensjo E, Riserus U & Vessby B (2005) Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia* **48**, 1999-2005.

Watarai T, Kobayashi M, Takata Y, Sasaoka T, Iwasaki M & Shigeta Y (1988) Alteration of insulin-receptor kinase activity by high-fat feeding. *Diabetes* **37**, 1397-1404.

Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS & Caprio S (2004) Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* **350**, 2362-2374.

Wellen KE & Hotamisligil GS (2005) Inflammation, stress, and diabetes. *J Clin Invest* **115**, 1111-1119.

Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE & Tataranni PA (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**, 1930-1935.

Weyer C, Hanson K, Bogardus C & Pratley RE (2000) Long-term changes in insulin action and insulin secretion associated with gain, loss, regain and maintenance of body weight. *Diabetologia* **43**, 36-46.

White MF (1998) The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Recent Prog Horm Res* **53**, 119-138.

White MF (2002) IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* **283**, E413-422.

WHO. (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: World Health Organisation.

Williams DE, Prevost AT, Whichelow MJ, Cox BD, Day NE & Wareham NJ (2000) A cross-sectional study of dietary patterns with glucose intolerance and other features of the metabolic syndrome. *Br J Nutr* **83**, 257-266.

Wilson PW, D'Agostino RB, Parise H, Sullivan L & Meigs JB (2005) Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* **112**, 3066-3072.

Wilson PW, Kannel WB, Silbershatz H & D'Agostino RB (1999) Clustering of metabolic factors and coronary heart disease. *Arch Intern Med* **159**, 1104-1109.

Wolk A, Vessby B, Ljung H & Barrefors P (1998) Evaluation of a biological marker of dairy fat intake. *Am J Clin Nutr* **68**, 291-295.

Wood DA, Butler S, Riemersma RA, Thomson M, Oliver MF, Fulton M, Birtwhistle A & Elton R (1984) Adipose tissue and platelet fatty acids and coronary heart disease in Scottish men. *Lancet* **2**, 117-121.

www.europa.eu.int/comm/eurostat.

www.lipgene.tcd.ie.

Yalow RS & Berson SA (1960) Plasma insulin concentrations in nondiabetic and early diabetic subjects. Determinations by a new sensitive immuno-assay technic. *Diabetes* **9**, 254-260.

Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY & Chuang LM (2001) Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* **86**, 3815-3819.

Yang YJ, Youn JH & Bergman RN (1987) Modified protocols improve insulin sensitivity estimation using the minimal model. *Am J Physiol* **253**, E595-602.

Ybarra J, Blanco-Vaca F, Fernandez S, Castellvi A, Bonet R, Palomer X, Ordonez-Llanos J, Trius A, Vila-Rovira R & Perez A (2008) The Effects of Liposuction Removal of Subcutaneous Abdominal Fat on Lipid Metabolism are Independent of Insulin Sensitivity in Normal-Overweight Individuals. *Obes Surg* **18**, 408-414.

Yeni-Komshian H, Carantoni M, Abbasi F & Reaven GM (2000) Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. *Diabetes Care* **23**, 171-175.

Yin MJ, Yamamoto Y & Gaynor RB (1998) The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* **396**, 77-80.

Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S & Kris-Etherton PM (1999) Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. *Am J Clin Nutr* **69**, 632-646.

Yudkin JS, Stehouwer CD, Emeis JJ & Coppack SW (1999) C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* **19**, 972-978.

Zeggini E, Parkinson J, Halford S, Owen KR, Frayling TM, Walker M, Hitman GA, Levy JC, Sampson MJ, Feskens EJ, Hattersley AT & McCarthy MI (2004) Association studies of insulin receptor substrate 1 gene (IRS1) variants in type 2 diabetes samples enriched for family history and early age of onset. *Diabetes* **53**, 3319-3322.

Zhu S, St-Onge MP, Heshka S & Heymsfield SB (2004) Lifestyle behaviors associated with lower risk of having the metabolic syndrome. *Metabolism* **53**, 1503-1511.

Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, D'Andrea F, Molinari AM & Giugliano D (2002) Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* **105**, 804-809.

Zierath JR, He L, Guma A, Odegoard Wahlstrom E, Klip A & Wallberg-Henriksson H (1996) Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia* **39**, 1180-1189.

Zimmet P, Boyko EJ, Collier GR & de Courten M (1999) Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. *Ann N Y Acad Sci* **892**, 25-44.

Zimmet PZ (1995) The pathogenesis and prevention of diabetes in adults. Genes, autoimmunity, and demography. *Diabetes Care* **18**, 1050-1064.