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OBESITY, INSULIN RESISTANCE AND TYPE 2 DIABETES IN YOUNG IRISH PEOPLE.

A Thesis Submitted By

FRANCIS FINUCANE

For The Degree Of

DOCTOR IN MEDICINE (M.D.)

At

The University of Dublin, Trinity College.

2008
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2. The work submitted has not been submitted as an exercise for a degree at this or any other university.

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DATE: 9th March 2008
To My Father
Acknowledgements

There are many individuals who helped me to complete this work, and it was a privilege to work with each of them. I am grateful to my supervisor, Professor John J. Nolan, for advice and constructive criticism over the past three years. I thank the nurses at the Metabolic Research Unit, Geraldine Neary and Ciara De Long, for their help and support. Thanks must also go to Nicole Burns, the exercise physiologist who devised and delivered the exercise intervention for these studies. In particular I would like to thank Dr. Mensud Hatunic for his support, advice and patience over the past three years. I thank Dr. Vivion Crowley, Dr. Kheng Teong and Declan Gasparro, from the Department of Clinical Biochemistry in St. James’s Hospital for their help with study design, patient assessment and sample analysis, respectively. In addition Andrea Mari and Amalia Gastaldelli from the Institute of Biomedical Engineering in Padova, Italy helped with mathematical modelling, interpretation of data and tracer analysis for which I am grateful. More recently, Nita Forouhi, Stephen Sharp and Ken Ong at the MRC Epidemiology Unit in Cambridge provided valuable instruction during the writing of this manuscript.

I am grateful for statistical advice and input from Elaine Hand and Tony Fitzgerald from the Haughton Institute. I would also like to thank the staff at Our Lady’s Hospital for Sick Children in Crumlin for their help and support. Dr. Siobhan Pittock, Dr. Colm Costigan and Dr. Nuala Murphy lent invaluable
clinical guidance there. Special thanks must also go to Miriam Fallon, the endocrine nurse specialist, whose help and support were greatly appreciated. I would also like to thank the staff of the medical records department in the hospital. They were helpful on several occasions.

It would be remiss not to mention the help of my colleagues in higher specialist training who offered useful guidance whenever I needed it. In particular I would like to thank Dr. Diarmuid Smith and Dr. Mark Sherlock for helpful advice. I would also like to thank Dr. Chris Thompson for his valuable mentorship and support throughout the past ten years.

I am deeply indebted to the patients (and their parents) who so generously and selflessly took part in these studies. I have learned a great deal from my interactions with them, and I hope that their generosity will serve to inform the wider scientific community in its efforts to better understand obesity and diabetes. I would like to thank my family for their unconditional love and support throughout the years. Finally I would like to thank my wife Naadia and my daughter Ruby, for their love and for their patient acceptance of prolonged neglect. I thank God for all these blessings.
Summary

The epidemic of obesity in Irish youth, one of the highest ranking internationally, represents a major threat to public health. However, little is known of the metabolic and clinical characteristics of this cohort. I performed a retrospective observational study of a clinic based cohort of obese Irish children. Analysis of data relating to age, body mass index and blood pressure showed that younger patients tended to have a higher degree of obesity at initial presentation, while half of the cohort had initial blood pressure measurements in the hypertensive range. There was a correlation between the degree of obesity and blood pressure elevation.

In a smaller, prospective follow up study from this cohort, more detailed physiological characterisation showed that there were significant correlations between the degree of obesity and insulin resistance, dyslipidaemia and suspected fatty liver disease. Subsequently, I conducted detailed metabolic assessments of young obese people with type 2 diabetes and demonstrated marked defects in beta-cell function in addition to severe insulin resistance. Somewhat surprisingly, these parameters did not improve after twelve weeks of fully supervised aerobic exercise. Young obese Caucasians with type 2 diabetes carry an enormous cardiovascular risk burden and may be resistant to the beneficial effects of aerobic exercise.
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<td>ADA</td>
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<tr>
<td>AIR</td>
<td>Acute Insulin Response (to Glucose)</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Amino Transferase</td>
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<tr>
<td>AST</td>
<td>Aspartate Amino Transferase</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<td>CDC</td>
<td>Centre for Disease Control (Atlanta)</td>
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<td>Co A</td>
<td>Coenzyme A</td>
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<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorbtometry</td>
</tr>
<tr>
<td>EDEG</td>
<td>European Diabetes Epidemiology Group</td>
</tr>
<tr>
<td>EGIR</td>
<td>European Group for the study of Insulin Resistance</td>
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<tr>
<td>FBC</td>
<td>Full Blood Count</td>
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<tr>
<td>FTO</td>
<td>Fused Toe Mouse Obesity Candidate Gene</td>
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<tr>
<td>GAD</td>
<td>Glutamic Acid Decarboxylase</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography/ Mass Spectrometry</td>
</tr>
<tr>
<td>GDR</td>
<td>Glucose Disposal Rate</td>
</tr>
<tr>
<td>GIR</td>
<td>Glucose Infusion Rate</td>
</tr>
<tr>
<td>GLUT 4</td>
<td>Glucose Transporter 4</td>
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<tr>
<td>GOOS</td>
<td>Genetics of Obesity Study (Cambridge, UK)</td>
</tr>
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<td>GWA</td>
<td>Genome Wide Association Study</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated Haemoglobin</td>
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<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<td>HGO(b)</td>
<td>Hepatic Glucose Output (Basal)</td>
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<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>HGO(ss)</td>
<td>Hepatic Glucose Output (Steady State)</td>
</tr>
<tr>
<td>HNF</td>
<td>Hepatocyte Nuclear Factor</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
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<tr>
<td>IGF-1</td>
<td>Insulin Like Growth Factor 1</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin Receptor Substrate 1</td>
</tr>
<tr>
<td>IVGTT</td>
<td>Intravenous Glucose Tolerance Test</td>
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<tr>
<td>LBT / LFT</td>
<td>Liver Blood Tests / Liver Function Tests</td>
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<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<tr>
<td>MC4R</td>
<td>Melanocortin 4 Receptor</td>
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<tr>
<td>MIRIAM</td>
<td>Metabolism, Insulin Resistance and Initial Anthropometric Measurements in Obese Irish Children</td>
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<tr>
<td>MODY</td>
<td>Maturity Onset Diabetes in the Young</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSH</td>
<td>Melanocyte Stimulating Hormone</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass: Charge Ratio</td>
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<tr>
<td>NAFLD</td>
<td>Non-alcoholic Fatty Liver Disease</td>
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<tr>
<td>NASH</td>
<td>Non-alcoholic Steatohepatitis</td>
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<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program (US)</td>
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<tr>
<td>NEAT</td>
<td>Non- Exercise Associated Thermogenesis</td>
</tr>
<tr>
<td>NGT</td>
<td>Normal Glucose Tolerance</td>
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<tr>
<td>NIDDM</td>
<td>Non-insulin Dependant Diabetes (Type 2 Diabetes)</td>
</tr>
<tr>
<td>OGIS</td>
<td>Oral Glucose Insulin Sensitivity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>PC</td>
<td>Prohormone Convertase</td>
</tr>
<tr>
<td>PC</td>
<td>Pearson Correlation</td>
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<tr>
<td>PCOS</td>
<td>Polycystic Ovary Syndrome</td>
</tr>
<tr>
<td>POMC</td>
<td>Proopiomelanocortin</td>
</tr>
<tr>
<td>PPAR γ</td>
<td>Peroxisome Proliferator- Activated Receptor Gamma</td>
</tr>
<tr>
<td>RISC</td>
<td>Relationship between Insulin Sensitivity and Cardiovascular Risk (EGIR Study)</td>
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<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard Deviation Score</td>
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<tr>
<td>SE</td>
<td>Standard Error</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>SIM</td>
<td>Secondary Ion Mass (spectrometry)</td>
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<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>Transcription Factor 7-Like 2 Gene</td>
</tr>
<tr>
<td>TFT</td>
<td>Thyroid Function Test</td>
</tr>
<tr>
<td>TTR</td>
<td>Tracer: Tracee Ratio</td>
</tr>
<tr>
<td>VO₂max</td>
<td>Maximal Oxygen Uptake (per unit time)</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist: Hip Ratio</td>
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<td>YT2</td>
<td>Young type 2 diabetes subjects</td>
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**Original Papers:**

"Early Onset Type 2 Diabetes in Obese Caucasians is Characterised by a Marked Defect in Beta-Cell Insulin Secretion, Severe Insulin Resistance and a Lack of Response to Aerobic Exercise Training."


PMID 17393133.

"Adverse Metabolic Profiles in a Cohort of Obese Irish Children."

Finucane FM, Teong L, Pittock S, Fallon M, Hatunic M, Costigan C, Murphy N, Crowley VEF, Nolan JJ.


"Elevated Blood Pressure in a Clinic- Based Cohort of Obese Irish Children."

Finucane FM, Pittock S, Fallon M, Hatunic M, Teong L, Burns N, Costigan C, Murphy N, Crowley VEF, Nolan JJ.

Submitted to *Irish Journal of Medical Science*
"Plasma Visfatin is Reduced after Aerobic Exercise in Early Onset Type 2 Diabetes Mellitus." [Letter]
*Diabetes, Obesity and Metabolism*. In press.

"Vascular Inflammatory Markers in Early Onset Obese and Type 2 Diabetes Subjects before and after Three Months Aerobic Exercise Training."
Hatunic M, Finucane FM, Burns N, Gasparro D, Nolan J.
*Diab Vasc Dis Res*. 2007 Sep;4(3):231-4. PMID: 17907114

**Abstracts:**
"Comparison of anthropometric and metabolic measurements in young obese people with type 2 diabetes and those with normal glucose tolerance."
Finucane F, Burns N, Murphy M, Hatunic M, Nolan, JJ.
Print abstract number 2570-PO, ADA Scientific Meeting, San Diego, 2005.

"Metabolic changes after an exercise programme in young obese subjects with and without type 2 diabetes."
Burns N., Finucane F., Murphy M., Hatunic M., Nolan JJ.
Print abstract number 2478-PO, ADA Scientific Meeting, San Diego, 2005.
"Clinical Characteristics of Young Obese Caucasian Irish Subjects Attending a Paediatric Endocrine Service."

Finucane F, Pittock S, Fallon M, Hatunic M, Burns N, Murphy N, Costigan C, Nolan JJ.


"Metabolic Features, Insulin Resistance and Initial Anthropometric Measurements in Obese Caucasian Irish Children: Preliminary Findings from the MIRIAM Study."

Finucane F, Teong L, Pittock S, Fallon M, Hatunic M, Costigan C, Murphy N, Crowley V, Nolan JJ.


"Elevated Blood Pressure in a Clinic-Based Cohort of Obese Irish Children."

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Introduction
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The worldwide epidemic of type 2 diabetes has been paralleled by a marked increase in the prevalence of obesity. This will contribute significantly to the global burden of cardiovascular disease. Obesity induced insulin resistance and diabetes are now manifesting in younger age groups, including children. Those from ethnic minorities tend to have a higher risk of diabetes than Caucasians. Thus, most studies of type 2 diabetes in the young have involved subjects from non-white ethnic backgrounds. However the pathophysiology of insulin resistance varies in distinct ethnic groups. Data are lacking in Caucasian populations, particularly in Ireland. The objective in conducting the studies outlined below was to describe the clinical and metabolic characteristics of a cohort of Irish children and adolescents, and to examine these further in a separate cohort of young Irish adults with type 2 diabetes. A further objective was to assess the effects of exercise intervention on physiological and cellular mechanisms in this cohort.

1.2: Epidemiology of Obesity and Type 2 Diabetes:

Between 1990 and 2000, the prevalence of obesity in Ireland increased by 67% (IUNA 2001). This was putatively related to significant changes in physical activity and diet in the population (NHLS 2003). The Behavioural Risk Factor Surveillance Study confirmed a trebling of obesity prevalence rates in the US between 1985 and 2001, where more than 30% of the adults in some states are now obese (Ahluwalia, Mack et al. 2003). In Greece, 23% of adults are obese and 35% overweight (Kapantais, Tzotzas et al. 2006). In
Greek boys, obesity prevalence rates doubled in the twenty years to 2002 (Magkos, Manios et al. 2005). In the 1960’s, approximately 4% of American children were obese. In the 1990’s the figure had risen to 15% (Bloomgarden 2004). In Hong Kong, a recent study of children aged 9-12 years showed that 38% of girls and 57% of boys were overweight (Sung, Tong et al. 2003). These children were shown to have higher systolic blood pressure, fasting triglycerides, insulin and lower HDL cholesterol than normal weight children.

The best current estimate of obesity prevalence rates in Irish children was derived by Whelton et al (2005). They measured body mass index (BMI) in about 3,000 children from Co. Cork aged between four and sixteen years, attending routine dental screening in 2002. Overall obesity prevalence was 6%. Girls tended to be more obese than boys- 11% of girls aged seven were obese. More recent work compared obesity rates in almost 30,000 children aged 13-15 years from Israel, the US and thirteen European countries, including Ireland (Lissau, Overpeck et al. 2004). Irish adolescent boys and girls ranked third and second highest respectively in obesity prevalence rates in this study. While these results are striking, little is known of the clinical and metabolic characteristics of this group.

Obese children tend to become obese adults. In Singapore, longitudinal data from over 12,000 individuals born in 1958 and followed up for 42 years showed that BMI at age 11 years strongly predicted subsequent adult BMI
Another prospective study in the US followed children aged 8-15 years for twelve years and found that girls in the high normal weight range (between the 75th and 84th centiles for age and gender) were twenty times more likely to be overweight adults compared to those whose weight was below the 50th centile (Field, Cook et al. 2005). Also, parental obesity has been shown to more than double the risk of adult obesity among both obese and nonobese children under 10 years of age (Whitaker, Wright et al. 1997). However childhood obesity is associated with substantial morbidity in its own right and is not merely a predictor of adult obesity (Pontiroli 2004). Also, there is evidence that overweight and obesity are under-recognised and under-diagnosed in children, particularly those under age 5 years, even in paediatric centres (Riley, Bass et al. 2005).

In 2000, 26% of US adults aged 45-74 years had “pre-diabetes” (Benjamin, Valdez et al. 2003). Thus, 12 million Americans fulfil the ADA criteria for entry into some sort of diabetes prevention strategy. At the same time a study of nine hundred US adolescents aged 12-19 years showed an overall prevalence of impaired fasting glucose (IFG) of 7% (Williams, Cadwell et al. 2005). 17.8% of obese adolescents in that study had IFG. There were far higher overall rates of IFG in Mexican Americans (13%) compared to black (4.2%) and white (7%) subjects. In Mexico itself, in a cohort of 965 children aged 10-18 years, 26.1% of overweight subjects fulfilled (modified) diagnostic criteria for the metabolic syndrome (Rodriguez-Moran, Salazar-Vazquez et al. 2004). Similar results were found in a study of more than
76,000 seventeen year old Israeli conscripts, where hypertension and type 2 diabetes were more prevalent in overweight subjects (Bar Dayan, Elishkevits et al. 2005).

The prevalence of type 2 diabetes is now 7.4% in Australian adults. An additional 16.4% have pre-diabetes. Even in those aged 25-34 years, 5.7% have some form of abnormal glucose metabolism, which is double the prevalence from 1981 (Dunstan, Zimmet et al. 2002). Pima Indians in the US aged between 15-19 years have seen a six-fold increase in type 2 diabetes prevalence in the past forty years (Fagot-Campagna, Pettitt et al. 2000). Among black and white subjects aged 10-19 years in the state of Ohio, one third of incident diabetes cases are “type 2”. Socio-economic factors play a role in the development of diabetes in youth, such that the rich in poor countries and the poor in rich countries tend to develop the disease. A study of 3,189 children aged 9-15 years from three different European countries found that in children from Estonia and Portugal, insulin resistance correlated with family income and parental education, whereas in Denmark, an inverse correlation was found between these variables (Lawlor, Harro et al. 2005).

Recent predictions for future diabetes prevalence rates are disturbing. Based on UN population estimates from 191 countries and diabetes epidemiological data from a few of those, the worldwide prevalence is set to rise from 2.8% in 2000 to 4.4% in 2030 (Wild, Roglic et al. 2004). Thus, the number of
people with diabetes will rise from 171 to 366 million in thirty years. This analysis did not allow for a further rise in obesity prevalence rates and so may have underestimated the true future prevalence.

That diabetes carries an increased cardiovascular risk burden has been known for some time. In 1993, the Multiple Risk Factor Intervention Trial (MRFIT) examined the effect of diabetes on cardiovascular mortality in 348,000 men aged 35-57, who were followed up for 12 years. Essentially there was a three-fold increased mortality in men with diabetes, irrespective of the presence or absence of other risk factors (Stamler, Vaccaro et al. 1993). A recent analysis of the burden of mortality attributable to diabetes calculated 2.9 million excess deaths in 2000 from the condition, which was 5.2% of all deaths in the world that year (Roglic, Unwin et al. 2005). The calculated excess mortality was lower in developing countries (2%) compared to the US and the Middle East (8%). The authors conclude that globally, diabetes is the fifth leading cause of death. However information about type 2 diabetes prevalence in young people is lacking because of the scarcity of chronic disease surveillance systems (Wareham and Forouhi 2005).

1.3: Morbidity in Obese Youth:

Many of the co-morbid diseases associated with adult obesity are now manifesting in children. Metabolic complications include dyslipidaemia, insulin resistance, type 2 diabetes, endothelial dysfunction and fatty liver
disease. In addition psychiatric, musculoskeletal and respiratory disorders are common in obese children (Ebbeling, Pawlak et al. 2002). Non-alcoholic steatohepatosis is now the commonest cause of chronic liver disease in children (Marion, Baker et al. 2004; Schwimmer, McGreal et al. 2005). Obese children are at approximately a threefold higher risk for hypertension than nonobese children (Sorof and Daniels 2002). Endocrine disorders such as premature adrenarche and polycystic ovary syndrome are also occurring more frequently in obese children (Dunger, Ahmed et al. 2005). Obesity associated diseases such as diabetes and hypertension are more prevalent within ethnic minorities than in Caucasians. For example, obesity related hypertension is more common in African-Americans while obesity related diabetes is more common in Mexican-Americans (Cossrow and Falkner 2004).

About a third of obese American adolescents have a metabolic syndrome phenotype (Duncan, Li et al. 2004; Sinaiko, Steinberger et al. 2005; Liu, Wade et al. 2006), with similar findings reported in Brazil (Alvarez, Vieira et al. 2006), Mexico (Cisneros-Tapia, Navarrete et al. 2005) and Turkey (Atabek, Pirgon et al. 2006). However, difficulty arises when describing this syndrome: several organisations have developed diagnostic criteria which differ slightly. This gives rise to differences in reported prevalence rates, depending on the criteria used (NCEP 2002; de Ferranti, Gauvreau et al. 2004; Adams, Appleton et al. 2005). Nonetheless the core features of the syndrome are similar in ADA, IDF, NCEP, WHO and EGIR guidelines. These
include dyslipidaemia, hyperglycaemia, central overweight/obesity and hypertension. While there may be differences in thresholds and emphasis of these parameters between different groups, there is unanimous agreement that they are important predictors of insulin resistance and cardiovascular disease, even at an early age. However there are no guidelines for paediatric populations, so that many studies report findings based on local modifications to adult reference ranges (Jones 2006).

Factors such as birth history and family history of diabetes have been shown to be important predictors of the metabolic syndrome in obese children (Sabin, Ford et al. 2006). Waist hip ratio, a marker of visceral adiposity, predicts metabolic syndrome in adolescents (Rodriguez, Moreno et al. 2004). Prospective data from the Early Bird study in the UK has shown that overweight in five year old children is a predictor of insulin resistance (Wilkin, Voss et al. 2004). A Dutch longitudinal study of 364 individuals who were followed up from age 13 to 36 years confirmed that body fatness, physical activity, fitness and energy consumption were all important determinants of metabolic risk in early adulthood (Ferreira, Twisk et al. 2005). Other studies have confirmed that childhood cardiovascular risk factors persist into late adolescence (Reaven, Traustadottir et al. 2005). In addition, brachial artery distensibility is reduced in children with metabolic syndrome, suggesting that pathological vascular changes are established early in obese children (Whincup, Gilg et al. 2005).
Ethnicity has significant implications for metabolic risk in obese children. A study of glucose tolerance status in 167 markedly obese American children and adolescents found IGT in 25% of those aged 4-10 years and 21% of those aged 11-18 years. Across the cohort, IGT prevalence rates were 16% in white children compared to 26 and 27% in Hispanic and black children, respectively. Four percent of the adolescents had type 2 diabetes and all of these were from ethnic minorities (Sinha, Fisch et al. 2002). Indeed, up to 94% of cases of childhood type 2 diabetes in the US occur in ethnic minorities (Fagot-Campagna, Pettitt et al. 2000). A recent British study found that 56% of children aged 10-19 years with type 2 diabetes were from ethnic minorities (Ehtisham, Hattersley et al. 2004). In Argentina, only 7% of a cohort of obese children had IGT, and 1.6% had diabetes (Mazza, Ozuna et al. 2005). In a cohort of 710 severely obese Italian children, only 4.5% had IGT (Invitti, Guzzaloni et al. 2003). While there are clear ethnic and racial differences in the prevalence of insulin resistance in different populations, important confounders such as age and degree of obesity, in addition to differing diagnostic criteria, need to be taken into account. For example, one recent report of IGT prevalence rates in children and adolescents in Israel included subjects aged 22 years (Shalitin, Abrahami et al. 2005).

1.4: Overview of the Physiology of Normal Glucose Metabolism:

Insulin is the principal anabolic hormone of the body, which affects fuel metabolism in several different ways. It is a 51 amino acid molecule
consisting of two chains, A and B. It is secreted by beta cells in the Islets of Langerhans, in the pancreas. Its biosynthetic precursor is pre-pro-insulin, which is converted to pro-insulin and then insulin, the latter stage by cleavage of C-peptide by endoproteases. Thus insulin and C-peptide are produced in equimolar amounts. Secretion of insulin from the beta cell is stimulated by glucose and other secretagogues such as amino acids. Secretion is inhibited by some other hormones, notably adrenaline and somatostatin. In contrast, insulin secretion is enhanced by other hormones, e.g. glucagon. Insulin may also exert autocrine effects, inhibiting its own secretion. Insulin action in target tissues may be antagonised by counter-regulatory hormones such as catecholamines, glucagon, growth hormone and cortisol.

The main physiologic effect of insulin is to lower blood glucose. It does this in a number of ways. It inhibits hepatic gluconeogenesis and glycogenolysis. It stimulates glucose uptake by muscle and adipose tissue, and promotes glycogen synthesis. Insulin also suppresses adipocyte lipolysis and hepatic ketogenesis. In addition it regulates protein turnover and electrolyte balance, and regulates the expression of certain genes. While most cells express specific insulin receptors, the most important target cells are hepatocytes, skeletal myocytes and adipocytes. An overview of the insulin receptor and the biochemical effects of insulin on these cells is shown in figure 1.1.
Figure 1.1: Cellular Binding of Insulin to its Receptor and Post-Binding Events.

Cell Membrane

Insulin Receptor

Insulin Receptor

α

β

Glucose

AUTOPHOS­PHORYLATION

GLUT 4

Golgi Complex

GLUT 4

Translocation of GLUT 4 from Golgi to Cell Membrane

Translocation of GLUT 4 from Golgi to Cell Membrane

GLUT 4

GLUT 4

Golgi Complex

Gene Transcription

Protein Synthesis

Lipid Metabolism

Insulin Receptor Substrates

Glucose-6-phosphatase

Glycolysis

Glycogen Synthesis

PI3 Kinase Activation

PI3 Kinase Activation

Nucleus

Key:

GLUT 4 = Glucose Transporter 4.

PI3 = Phosphatidylinositol 3 Kinase

Insulin Receptor

Key:

GLUT 4 = Glucose Transporter 4.

PI3 = Phosphatidylinositol 3 Kinase
When insulin binds to the alpha subunits of the insulin receptor, conformational changes occur which allow the autophosphorylation of tyrosine residues in the intracellular domain of the beta subunit. This leads to a cascade of post-receptor signalling events, some of which have not been fully elucidated. The best characterised substrate for the insulin receptor is insulin receptor substrate-1 (IRS-1). Further phosphorylation/dephosphorylation events occur, leading ultimately to the activation of enzymes such as glycogen synthase. Insulin resistance, where insulin action is impaired, may be caused by receptor defects or more commonly by defects in the post-receptor signalling cascade. Receptor defects may be due to a reduced number of receptors, or to down-regulation of the receptors, where their affinity for insulin is reduced. Down-regulation can occur with chronic hyperinsulinaemia seen in obesity and lesser degrees of hyperglycaemia. Genetically inherited receptor defects have also been described, but are rare. Defects in intracellular events distal to the binding of insulin to its receptor usually account for the insulin resistance seen in type 2 diabetes, as described in the next section.

Secretion of insulin is very tightly matched to circulating glucose concentrations. Small amounts of insulin are secreted continuously, even during fasting, throughout the day – this background or basal insulin accounts for about half of the total daily insulin secretion in physiological conditions. The remainder is secreted at meal times, in close temporal association with the rise in portal plasma glucose that occurs post-
prandially. In health, venous plasma glucose concentrations are maintained strictly in the range 5-7 mmol/l. At any given time the plasma glucose concentration reflects the net sum of the rate of appearance of glucose in the circulation, and the rate of glucose disappearance from the circulation. Insulin lowers circulating glucose concentrations by suppressing hepatic glucose production and by stimulating glucose uptake in skeletal muscle, adipocytes and the liver. In the fasting state the principal determinant of plasma glucose concentration is hepatic glucose output. This glucose is derived from the breakdown of stored glycogen (glycogenolysis) and the de novo production of glucose from 3-carbon precursors (gluconeogenesis). As glycogen stores become depleted, gluconeogenesis assumes a quantitatively greater role. Suppression of hepatic glucose production is a major regulatory action of insulin.

Stimulation of glucose uptake (and subsequent metabolism or storage as glycogen) requires higher circulating plasma insulin concentrations than are necessary for suppression of hepatic glucose production. A major action of insulin is to stimulate the translocation of facilitative glucose transporters (GLUT 4) to the cell membrane for this purpose (see figure 1.1). Other isoforms of glucose transporters (e.g. GLUT 1 at the blood brain barrier and GLUT 2 in islet beta cells) do not require insulin to transfer glucose into cells. Insulin also stimulates glucose oxidation and glycogen synthesis in skeletal muscle.
Insulin also has a critical role in inhibiting the breakdown of fat stores to free or non-esterified fatty acids (NEFA) and glycerol, the gluconeogenic precursor. Fatty acids are the principal substrate for ketogenesis within the liver. Both fatty acids and ketones can be used as alternative fuels to glucose during prolonged exercise or starvation. Insulin is a major regulator of ketogenesis, and ketosis is a sign of absolute (as opposed to relative) insulin deficiency.

Insulin sensitivity is a critical factor determining the magnitude of the insulin response to beta cell stimulation. Thus, when beta cell function is assessed, obese individuals who are insulin resistant manifest greater responses than age-matched lean subjects (Tura, Ludvik et al. 2001). A feedback loop between insulin sensitive tissues and the beta cell determines the magnitude of this response. The nature of this relationship is such that insulin sensitivity and beta cell function are inversely and proportionally related so that the product of these two parameters is always a constant, referred to as the disposition index (Bergman 1989).

1.5: Aetiology of obesity, insulin resistance and type 2 diabetes:
Bodyweight is regulated by numerous complex physiological mechanisms that maintain balance between energy intake and energy expenditure. Any factor that increases energy intake or decreases energy expenditure by even a small amount will eventually cause weight gain and obesity. Thus, dietary intake and levels of physical activity are the principal determinants of weight
status. However perinatal, genetic and socio-economic factors are also critical in determining body weight. The interactions between all of these factors are both extremely complex and incompletely elucidated.

1.5.1: Environmental factors:

Data reported by the (Irish) National Taskforce on Obesity suggested that in 2002, 51% of Irish children consumed sweets, 37% drank fizzy drinks, 27% ate crisps, 12% ate chips and 7% ate hamburgers at least once a day (2005). These worrying dietary patterns account for the increased intake of energy-dense nutrients which underlie the obesity epidemic in Irish children. Previous work has shown that children who drink one regular carbonated beverage a day have on average a 10% higher energy intake than those who don’t (Harnack, Stang et al. 1999). Thus, for every can of soft drink consumed by a child in a day, the relative risk of being obese has been estimated to increase by sixty percent (Mattes 1996).

While total energy intake in children has increased in recent years, the proportion of calories from fat has decreased, while that from refined carbohydrates has increased (Ebbeling, Pawlak et al. 2002). Refined carbohydrates cause a rapid rise in post-prandial blood glucose, and are said to have a high “glycaemic index”. Consumption of high glycaemic index foods has been shown to induce hunger and cause over-eating in adolescents (Ludwig, Majzoub et al. 1999). Average “junk” food portion sizes have increased substantially in the past three decades (Nielsen and Popkin 2003).
A prospective longitudinal cohort study of three thousand young adults in the US confirmed a correlation between the frequency of fast food restaurant use and subsequent weight gain and insulin resistance. In this study, those who ate fast food more than twice a week were twice as insulin resistant and 4.5 kg heavier than those who ate fast food less than once a week (Pereira, Kartashov et al. 2005).

Energy expenditure in the form of physical activity is a critical determinant of bodyweight. High levels of sedentary behaviour have been reported in children aged 7-9 years living in Dublin (Hussey, Gormley et al. 2001). This study found that 39% of a cohort of 786 children reported participating in vigorous physical activity for at least twenty minutes three or more times a week. However, as this was a questionnaire based study, physical activity may have been over reported. Indeed 78% of children in the same study reported watching television for more than three hours each day. A smaller study in a rural Irish district (Carlow) confirmed sedentary behaviour in a cohort of five year old children (Kelly, Reilly et al. 2005), using accelerometers to quantify objectively activity levels. Similar findings have been reported in Scottish children (Montgomery, Reilly et al. 2004; Reilly, Jackson et al. 2004). In fact physical activity is a difficult variable to quantify accurately. Recent innovations such as the "Actiheart ®"- a combined accelerometer and heart rate monitor which can record several thousand data points continuously over seven days - are likely to improve...
measurements of physical activity in future studies (Brage, Brage et al. 2005).

An American study of eight year old children in the highest quartile of body weight recently confirmed the association between physical inactivity and increased visceral adiposity, using DEXA and MRI scans, with accelerometry (Saelens, Seeley et al. 2007). However another recent longitudinal study in young adults suggested that excess dietary intake is the major contributor to a positive energy balance over time, while confirming the association with activity levels and body fatness (Ekelund, Sarnblad et al. 2007). It is likely that there is substantial inter-individual variation in the relative contributions of inactivity and over-eating to excess body weight. It is important to note that energy expenditure is achieved not just through purposeful exercise but also through changes in posture and movement that are associated with the routines of daily life. Such "non-exercise associated thermogenesis" (NEAT) has been shown to differ significantly in lean and obese individuals, and may be biologically determined to some extent (Levine, Lanningham-Foster et al. 2005). The contribution of overall physical activity to total energy expenditure is highly variable. In sedentary individuals, physical activity still accounts for about twenty percent of daily energy expenditure, while thermogenesis accounts for approximately ten percent. Thus basal metabolic rate accounts for about two thirds of daily energy expenditure in sedentary individuals. Indeed, increased body weight actually increases basal
metabolic rate, primarily because of the increased lean body mass that develops in obese individuals (Ravussin, Burnand et al. 1982).

Aside from diet and physical activity, an individual's birth weight is a critical determinant of body fatness and insulin sensitivity later in life. This is regardless of whether the infant is small for gestational age or premature (Hofman, Regan et al. 2004). The association between low birth weight and the risk of disease in adulthood was first recognised by Barker who proposed a "thrift phenotype" hypothesis (Barker 1992). This theory proposes that low birth weight is a marker of an adverse intrauterine environment and foetal malnutrition, which leads to permanent changes in the structure, distribution and function of tissues such as fat and skeletal muscle (Barker 1992). These tissue changes are thought to be "programmed" during a plastic period of early life development, where the foetus is sensitive to environmental influences. The effects of these influences are then fixed some time after birth and have permanent effects on body composition and metabolism (Barker 1998). Thus environmental insults need to occur during sensitive periods in order to affect later development (Bateson, Barker et al. 2004).

Studies in individuals exposed to the Dutch famine (1944-45) showed that babies born immediately after the famine (and thus exposed to an adverse intrauterine environment) had lower birthweight than those born in subsequent years, and were more likely to be obese adults (Ravelli, Stein et
Follow up studies in the same cohort found that these small babies were more insulin resistant in later life, especially if they developed obesity in adulthood (Ravelli, van der Meulen et al. 1998). Thus the emergence of adverse metabolic changes that were programmed in early life is dependent upon adult life factors such as obesity and ageing (Hales and Barker 2001). For example, studies have shown that women who are born small and subsequently become obese adults have the worst cardiovascular risk profiles (Fall, Osmond et al. 1995). Conversely, studies in rural Gambia showed that even severe childhood malnutrition caused no effect on insulin sensitivity or cardiovascular risk in adults who were lean, fit and eating a low fat diet (Moore, Halsall et al. 2001). Therefore it is important to consider the impact of perinatal growth patterns on disease risk in later life in the context of adult environmental and behavioural factors.

1.5.2: Genetic factors:

Although the current rise in diabetes prevalence is almost certainly driven by lifestyle changes, the inherent susceptibility to the condition is widely considered to be attributable to complex genetic determinants. Also, while environmental factors may differ in distinct ethnic groups, they do not entirely account for the disparity in obesity and diabetes prevalence rates seen in different races (outlined on page 28), which suggests that genetic factors critically influence disease expression (Fagot-Campagna, Pettitt et al. 2000; Sinha, Fisch et al. 2002; Invitti, Guzzaloni et al. 2003; Ehtisham, Crabtree et al. 2005; Mazza, Ozuna et al. 2005).
Genetic influences are suggested by the fact that a positive family history of type 2 diabetes confers a substantially increased risk of an individual developing the disease. There is a high rate of disease concordance in monozygotic twins, with reported rates ranging from 60 to 90% (Medici, Hawa et al. 1999; Poulsen, Kyvik et al. 1999). Studies in normal glucose tolerant offspring of patients with type 2 diabetes have shown they have reduced skeletal muscle oxygen uptake in spite of similar physical activity levels to those without a family history (Thamer, Stumvoll et al. 2003).

Impaired mitochondrial function has been demonstrated in those who have a parent with type 2 diabetes (Petersen, Dufour et al. 2004). An Australian study of young non-obese normoglycaemic subjects who had a first degree relative with type 2 diabetes showed a twenty percent reduction in insulin sensitivity compared to those without a family history (Kriketos, Greenfield et al. 2004). The offspring of mothers with young onset type 2 diabetes have also been shown to have reduced beta cell function (Singh, Pearson et al. 2006).

Identifying the specific genetic determinants of type 2 diabetes has become a scientific priority over the past twenty years. Rather than being a homogenous condition with a common molecular pathogenesis, type 2 diabetes arises from defects in one or more diverse molecular pathways which ultimately lead to a combination of insulin resistance and beta cell failure. Several Mendelian disorders with diabetes as a major phenotypic feature have been characterised at the molecular level. The best known
A subgroup of these disorders is Maturity Onset Diabetes of the Young (MODY) which is caused by defects in beta cell insulin secretion (Stride and Hattersley 2002). Such defects arise from mutations in genes encoding glucokinase (an important glucose "sensing" enzyme in the beta cell) or hepatocyte nuclear factors (e.g. HNF4A and HNF1A). Note that MODY is a distinct entity from young type 2 diabetes, the former being characterised by a strong familial tendency, normal weight and normal insulin sensitivity. Aside from MODY, the rare monogenic disorders that give rise to type 2 diabetes tend to involve molecules in the insulin signalling cascade (figure 1.1) or lead to abnormalities of fat tissue development, with secondary metabolic derangements leading to insulin resistance (O'Rahilly 2002). An excellent example is the common missense variant in the gamma-2 isoform of peroxisome proliferator-activated receptor gamma (PPARγ) [Pro 12 Ala]. This mutation is associated with type 2 diabetes development, with meta-analyses suggesting that the common allele is associated with a 25% increased risk of diabetes (Altshuler, Hirschhorn et al. 2000). Major missense mutations in the gene result in severe, dominantly inherited insulin resistance and diabetes as well as partial lipodystrophy and hypertension.

A major challenge has been the elucidation of more subtle genetic determinants of diabetes risk. Candidate gene approaches have had limited success in identifying more common polymorphisms associated with diabetes. For example, association testing of common variants in the IRS-1 gene (Florez, Sjogren et al. 2007) and the PPAR-Delta gene (Grarup,
Albrechtsen et al. 2007) found no association with diabetes risk, in spite of high biological plausibility. Only in very recent times has technology become available to allow the examination of several hundred thousand single nucleotide polymorphisms (SNPs) in large populations, using high density genotyping arrays – so called genome-wide association (GWA) studies. A recent French case-control study constituted proof of principle for this approach. The workers identified four loci containing variants that confer type 2 diabetes risk, in addition to confirming known associations with the TCF7L2 gene (Sladek, Rocheleau et al. 2007). This gene is known is associated with impaired beta cell function but not reduced insulin sensitivity, and has been shown to predict progression to type 2 diabetes in individuals with IGT (Florez, Jablonski et al. 2006). More recently the Wellcome Trust Case Control Consortium conducted GWA studies for seven major diseases, including type 2 diabetes in a British Europid population (2007). This study has helped to validate the GWA approach and confirms that large sample sizes are required to observe modest effects. Further studies in different populations are likely to yield important information on the genetic determinants of diabetes risk.

GWA studies have recently identified a variant in the FTO gene (which causes fused toe in mice) associated with moderately increased BMI across 39,000 individuals in 13 separate cohorts (Frayling, Timpson et al. 2007). Given that 16% of adults are homozygous for the allele, FTO is the first common genetic lesion implicated in the development of obesity. Several much rarer
pleiotropic genetic syndromes exist, in which obesity is only one of a constellation of physical and developmental abnormalities. Examples include Prader-Willi and Bardet-Biedl syndromes, and their association with obesity has long been recognised. Over the last decade, a number of major missense and nonsense mutations have been identified which result in severe, early onset obesity that is usually associated with disruption of normal appetite control mechanisms (O'Rahilly, Farooqi et al. 2003).

The exhaustive phenotypic and metabolic characterisation of such "extreme phenotypes" has led to significant progress in the understanding of the pathophysiology of obesity. Congenital leptin deficiency was the first of these monogenic syndromes to be described (Montague, Farooqi et al. 1997). This was discovered in two consanguineous kindreds of Pakistani origin and one kindred of Turkish origin. Each affected individual carried an identical frameshift mutation (ΔG133) and had early onset obesity, hyperphagia, hypogonadotrophic hypogonadism, hyperinsulinaemia, defective function of the hypothalamic-pituitary-thyroidal axis and defects in T-cell number and function (O'Rahilly 2002). By administering leptin to affected individuals, these abnormalities were reversed, the major effect of leptin being the normalisation of hyperphagia. Congenital leptin deficiency is the exception to the rule in being the only monogenic form of obesity currently amenable to mechanism based therapy, i.e. recombinant leptin hormone.
In addition to leptin deficiency, the leptin receptor itself may be truncated by gene mutations such that its transmembrane domain disappears, resulting in a similar phenotype to that described above (O'Rahilly, Farooqi et al. 2003). However children with leptin receptor mutations also have impaired secretion of growth hormone, insulin-like growth factor 1 (IGF-1), as well as evidence of central hypothyroidism. Leptin acts in the arcuate nucleus of the hypothalamus where its first order neuronal targets include proopiomelanocortin (POMC) - containing neurons. POMC is sequentially cleaved by prohormone convertases (PC 1, PC 2) to yield peptides such as melanocyte stimulating hormone (MSH) which have a critical role in the control of appetite. They act through a family of five G-protein coupled receptors, MC1R - MC5R. MC3R and MC4R are both highly expressed in the central nervous system and play an important role in the regulation of food intake and energy balance (Coll, Farooqi et al. 2004).

Studies of extreme obesity phenotypes such as the Genetics of Obesity Study (GOOS) cohort in Cambridge, UK have identified monogenic disorders which result in POMC deficiency or loss of function of MC4R and lead to hyperphagia (Farooqi, Keogh et al. 2003). In Denmark, a population based study in 750 men showed a carrier frequency of 2.5% for pathogenic mutations in the MC4R gene (Larsen, Echwald et al. 2005). In the GOOS cohort of extremely obese children, 5.8% had mutations in MC4R. While the elucidation of the mechanistic basis for these monogenic forms of obesity is fascinating, the vast majority of obese individuals do not have an identifiable genetic lesion.
With progress in large scale, population based genetic studies, more subtle genetic influences will undoubtedly be revealed in the future.

1.6: Pathophysiology of type 2 diabetes:

Glucose homeostasis depends on the balance between insulin secretion by the beta cell and insulin action at target tissues. For hyperglycaemia to develop, insulin resistance alone is not sufficient and a degree of beta cell failure to compensate for this resistance must also be present, as shown in figure 1.2. The *sine qua non* for type 2 diabetes is loss of the first phase response to hyperglycaemia from the beta cell. This first phase response normally peaks within 2-5 minutes after intravenous glucose loading, and lasts for approximately ten minutes. The second phase response commences shortly afterwards and normally lasts for the duration of hyperglycaemia.

**Figure 1.2: The natural history of type 2 diabetes.**
This response is also diminished in type 2 diabetes, partly because of the glucose-toxic effect of hyperglycaemia on the beta cell (Polonsky, Sturis et al. 1996). Furthermore, in type 2 diabetes there are higher proportions of fasting and post-load proinsulin, due to interruption of normal insulin biosynthetic processes and attenuation of endoprotease activity (Kahn 2001).

The ratio of proinsulin to insulin has also been shown to be elevated in children, adolescents and adults with insulin resistance and pre-diabetes (Ravelli, van der Meulen et al. 1998; Hirschler, Aranda et al. 2005; Weiss, Caprio et al. 2005). The study by Weiss et al showed that obese youths with impaired glucose tolerance have a significant defect in first phase insulin secretion, while a defect in second phase secretion and proinsulin processing was specific for type 2 diabetes, in the children they studied. The same group has also demonstrated more pronounced early insulin secretory responses to maintain euglycaemia in insulin resistant obese youths from ethnic minorities, which may partially explain their greater tendency to develop type 2 diabetes (Weiss, Dziura et al. 2006). Similar findings have been reported elsewhere (Saad, Danadian et al. 2002).

The Weiss group also highlighted the importance of fat distribution in the pathogenesis of insulin resistance in obese children (Weiss, Dufour et al. 2003). They demonstrated that children with impaired glucose tolerance had a higher ratio of visceral to subcutaneous abdominal fat, using transverse abdominal sections obtained with MRI. Similarly these patients had a higher
proportion of intramyocellular to extramyocellular lipid deposition, measured using MR spectroscopy and were more insulin resistant during hyperinsulinaemic euglycaemic clamp studies, in spite of having similar levels of total body fat.

Polycystic ovary syndrome (PCOS) is associated with obesity, insulin resistance, hirsutism, hyperandrogenaemia and anovulatory infertility. Clamp studies have previously confirmed that women with PCOS have significant insulin resistance independent of obesity (Dunaif, Segal et al. 1989). At least 70% of women with PCOS are obese. The hyperinsulinaemia associated with the condition acts synergistically with luteinizing hormone to enhance the androgen production of theca cells. In addition, by reducing sex hormone binding globulin production in the liver, circulating free testosterone levels rise (Ehrmann 2005). Given that 30- 40% of women with PCOS have impaired glucose tolerance (and thus a degree of beta cell failure), it is likely that genetic components of the two disorders (PCOS and type 2 diabetes) overlap significantly, though these have not yet been defined.

The hyperinsulinaemia associated with insulin resistance leads to increased fatty acid synthesis in liver and fat cells. A compensatory increase in glucose oxidation, as well as increased malonyl coenzyme A signaling, diverts fatty acid oxidation to compensatory increases in malonyl Co A and triglyceride synthesis in the liver (Ten and Maclaren 2004). The increased levels of malonyl Co A cause hepatic insulin resistance and lead to triglyceride
accumulation in muscle and liver, thus disrupting the insulin signaling cascade. These processes also induce downstream beta cell apoptosis and accelerate the decline in glucose tolerance. Severely obese adolescents with hyperinsulinaemia have also been shown to have reduced hepatic insulin extraction (Cerutti, Sacchetti et al. 1998). Significant ethnic variations exist in patterns of hepatic insulin extraction and insulin secretion, even in normal glucose tolerant obese children (Goran, Bergman et al. 2002). Skeletal muscle has a key role in determining insulin sensitivity because, under insulin stimulated conditions, a major proportion of glucose utilization occurs in muscle. During periods of reduced energy intake (fasting) or increased energy expenditure (sustained exercise), skeletal muscle increases its reliance on fat oxidation as a source of energy. This capacity to oxidize fat under appropriate physiological conditions is related to leanness, aerobic fitness and insulin sensitivity (Kelley 2005). In insulin resistant individuals who have increased deposition of intramyocellular fat, the ability to change from glucose to fat oxidation is diminished (Blaak, van Aggel-Leijssen et al. 2000), and this "metabolic inflexibility" is a key determinant of insulin resistance at the level of skeletal muscle.

The exact sequence of events leading to type 2 diabetes in youth has not yet been fully elucidated. Previous studies from Ireland have shown that younger Irish adults with type 2 diabetes (aged less than forty years) are more obese, more dyslipidaemic and have worse initial and ongoing glycaemic control than older adults with type 2 diabetes in spite of similar
treatment protocols (Hatunic, Burns et al. 2005). In a separate study of younger Caucasian Irish subjects with type 2 diabetes (mean age 22 years), an insulin modified intravenous glucose tolerance test was used to demonstrate severe insulin resistance and loss of first-phase insulin secretion (McQuaid, O’Gorman et al. 2005). This cohort had evidence of high risk for cardiovascular complications, with elevated markers of endothelial dysfunction. The findings suggest a disproportionate cardiovascular risk burden associated with type 2 diabetes in younger patients. To date there have been only limited data relating to the underlying pathophysiology of type 2 diabetes in young obese Caucasians, in particular using the gold standard technique of the hyperinsulinaemic- euglycaemic clamp.

1.7: Effects of Exercise:

1.7.1: Population-Based Studies of Exercise Effects:

Observational studies have confirmed that individuals who are more physically active are less likely to develop diabetes. In 1991 a retrospective, questionnaire based study of 5990 male university alumni in Pennsylvania noted a negative correlation between physical activity levels and prevalence of diabetes (Helmrich, Ragland et al. 1991). The same year a prospective study of 87,000 women who were followed up over eight years confirmed the protective effect of physical activity on diabetes risk (Manson, Rimm et al. 1991). More recently, compelling epidemiological evidence has emerged of the beneficial effects of exercise interventions to prevent or delay the progression to type 2 diabetes, in at-risk populations. The first major
lifestyle intervention study was conducted in China. Of almost 111,000 adults who attended for diabetes screening in the city of Da Qing, 577 were recruited to lifestyle intervention with diet and/or exercise and followed up for six years. There was approximately a forty percent relative risk reduction in the lifestyle intervention group compared to controls (Pan, Li et al. 1997). Thereafter the Finnish Diabetes Prevention Study demonstrated a 58% relative risk reduction in progression to diabetes in 522 individuals with IGT who entered a three year lifestyle intervention (Tuomilehto, Lindstrom et al. 2001). A year later the Diabetes Prevention Program in the US confirmed similar risk reduction in a cohort of over 3,000 individuals with IGT (Knowler, Barrett-Connor et al. 2002).

1.7.2: Physiological Effects of Exercise:
More detailed physiological studies in smaller cohorts have examined the mechanistic basis for improvements in glucose metabolism after exercise. Six weeks of moderate intensity exercise has been shown to reduce hepatic glucose output and increase peripheral glucose disposal in obese subjects with normal glucose tolerance (DeFronzo, Sherwin et al. 1987). A study of the effects of twelve weeks of aerobic exercise in subjects with impaired glucose tolerance demonstrated improvements in insulin sensitivity and glucose disposal, despite no changes in body composition. These subjects had increased glycogen stores and GLUT 4 concentrations in skeletal muscle biopsy specimens after the exercise intervention (Hughes, Fiatarone et al. 1993). Work from our group and others has confirmed that exercise induces
GLUT 4 expression in skeletal muscle of subjects with type 2 diabetes (Holten, Zacho et al. 2004; O'Gorman D, Karlsson et al. 2006). Exercise also decreases intramyocellular lipid stores (Tamura, Tanaka et al. 2005). This leads to enhanced skeletal muscle fat oxidation, and reduces the "metabolic inflexibility" associated with insulin resistance (Goodpaster, Katsiaras et al. 2003; Mensink, Blaak et al. 2005; Shojaee-Moradie, Baynes et al. 2007). It is interesting to note that any of the reported metabolic improvements induced by exercise tend to result from improvements in insulin sensitivity rather than beta cell function (Chiasson and Rabasa-Lhoret 2004). Fewer studies of exercise effects have been performed in young people. One German study of obese fourteen year olds with normal glucose tolerance showed improvements in vascular inflammatory markers and carotid intima-medial thickness after a six month exercise intervention, in addition to improvements in weight, body composition and fasting insulin levels (Meyer, Kundt et al. 2006). There is evidence that obese adolescents with the worst metabolic profiles tend to improve the most after exercise (Kang, Gutin et al. 2002). Similar findings have been reported in adults (Snowling and Hopkins 2006).

1.7.3: Effects of Exercise Modality:
The type of exercise undertaken may be an important determinant of metabolic improvement. While several studies have confirmed the beneficial effects of both aerobic and resistance training, there is some evidence that resistance training is superior to aerobic training in improving insulin.
sensitivity (Eriksson, Tuominen et al. 1998; Cauza, Hanusch-Enserer et al. 2005). Proponents of resistance training have suggested that it may be more feasible in frail, elderly or very obese patients (Willey and Singh 2003). Recently, a comprehensive meta-analysis of 27 studies in 1003 diabetes patients examined the effects of different training modalities on glucose control and risk factors for diabetes complications. Differences between aerobic, resistance and combined training effects on HbA1c were trivial, although combined was generally superior in reducing fasting and post-prandial glucose levels (Snowling and Hopkins 2006). In their consensus statement regarding exercise for people with type 2 diabetes in 2006, the ADA recommended that "in the absence of contraindications, [they] should be encouraged to perform resistance exercise three times a week, targeting all major muscle groups, progressing to three sets of 8-10 repetitions at a weight that cannot be lifted more than 8-10 times" (Sigal, Kenny et al. 2006). Regardless of this relatively recent emphasis on resistance training, most research still tends to focus on aerobic exercise and its metabolic effects.

1.7.4: Exercise Duration and Timing:

The duration and intensity of aerobic exercise training are both important determinants of improvements in insulin sensitivity (Houmard, Tanner et al. 2004). However, several studies have confirmed that the beneficial effects of exercise, in terms of metabolic measurements, are short lived in adults (Heath, Gavin et al. 1983; Schneider, Amorosa et al. 1984; Burstein,
Polychronakos et al. 1985; Dela, Larsen et al. 1995) and in children (Ferguson, Gutin et al. 1999). A major challenge in physiological experiments has been differentiating between the acute and chronic effects of exercise: With acute muscle contraction, GLUT4 locates to the cell membrane through an insulin-independent mechanism, and this mediates acute changes in glucose disposal rather than any alterations in the expression of insulin signaling cascade molecules, which occur later (Tomas, Sevilla et al. 2001). Within hours of the acute exercise bout, GLUT4 levels will have returned to pre-exercise levels (Kraniou, Cameron-Smith et al. 2006).

When assessing later, chronic changes that occur with repeated exercise, measurements of glucose metabolism taken too soon after the final exercise bout may simply reflect the acute changes induced by that particular bout. Conversely, any chronic changes induced by exercise might have disappeared completely within days of finishing an exercise programme, so prolonged rest after completion of the exercise programme is not appropriate. Estimates of the duration of “chronic” exercise-induced changes vary, depending on the outcome measure, but are typically about 72 hours (Tomas, Zorzano et al. 2002; Zierath 2002). This has important implications in terms of optimising the timing of measurements within an exercise intervention programme.
Longer-term exercise stimulates insulin action and glycaemic control through several different mechanisms. Enzymes such as hexokinase, citrate synthase and glycogen synthase are up-regulated, and these are the rate limiting enzymes in Glycolysis, Kreb's cycle and glycogen synthesis, respectively. Thus, glucose is either oxidized or stored as glycogen within the cell, maintaining a concentration gradient in spite of glucose influx through GLUT4 transporters. Chronic exercise may also influence insulin sensitivity through reductions in triglyceride and free fatty acid levels. Accumulation of these lipids has been shown to impair the function of proteins within the insulin signaling cascade (Zierath, Houseknecht et al. 1997; Boden and Shulman 2002; Greco, Mingrone et al. 2002).

Body composition is an important determinant of insulin sensitivity (Ludvik, Nolan et al. 1995), and the metabolic improvements induced by exercise are mediated, at least in part, by changes in lean muscle mass and fat mass (Meyer, Kundt et al. 2006). Observational longitudinal studies have confirmed that increases in physical activity energy expenditure are associated with improvements in metabolic parameters, independent of changes in fatness and fitness (Ekelund, Franks et al. 2007). However, in many exercise intervention studies, the confounding effects of changes in body composition are not examined in detail (DeFronzo, Sherwin et al. 1987; Ferguson, Gutin et al. 1999). In some instances only relatively crude measures of body fatness are available, such as BMI and waist: hip ratio. Where body composition has been assessed, metabolic improvements have
been shown to occur independently of changes in fat mass and lean mass (Houmard, Tanner et al. 2004).

1.8: Aims and objectives:
The overall objective was to examine the clinical and metabolic features of obese Irish youth, in particular those “extreme phenotypes” who have severe insulin resistance and early onset type 2 diabetes. While there are good population based data on the prevalence of overweight and obesity in Irish children and adolescents, to date none of the clinical or metabolic characteristics of this group have been examined. The specific objectives were as follows:

1. To describe the clinical characteristics of a cross-sectional, retrospective, hospital clinic-based cohort of obese children, through an analysis of data pertaining to age, body mass index and blood pressure. A further objective was to measure the correlation between the degree of obesity at referral and blood pressure elevation in this group. **Hypothesis:** The degree of body fatness predicts higher blood pressure elevation in obese Irish children.

2. To conduct more detailed prospective studies in a subgroup of patients recruited from this cohort. Specifically the aim was to examine metabolic characteristics which are known to be associated with
disease in adulthood, and examine their relationship with the degree of body fatness in younger patients. **Hypothesis:** The degree of body fatness predicts worse metabolic risk profiles in obese Irish children.

3. Thereafter to define precisely the metabolic derangements associated with type 2 diabetes in adolescents and young adults. While several studies have confirmed beta cell failure and insulin resistance in similar cohorts, data from Caucasian subjects are lacking, especially using the hyperinsulinaemic clamp technique. **Hypothesis:** Type 2 diabetes in young Caucasians is associated with more severe insulin resistance than diabetes in older adults.

4. To characterise the mechanisms underlying the improvements in glucose metabolism that are induced by exercise. Specifically, we sought to measure changes in beta cell function and insulin sensitivity that would occur after participation in a fully supervised and individually tailored twelve week aerobic exercise intervention. **Hypothesis:** Patients with early onset type 2 diabetes are resistant to the effects of aerobic exercise compared to normal glucose tolerant controls.
Chapter Two:

Methods
2.1: Populations studied:

Three distinct groups of subjects were studied during completion of the work for this thesis. The Crumlin Cohort and the MIRIAM Cohort are described in Chapter Three, while the Exercise Cohort has baseline characteristics described in Chapter Four and exercise effects (or lack thereof) described in Chapter Five.

The Crumlin Cohort:

This cohort consisted of patients who attended the paediatric endocrine clinic led by Dr. Colm Costigan at Our Lady’s Hospital for Sick Children in Crumlin, which is a paediatric tertiary referral centre. Frequently there was a multidisciplinary clinic held specifically for assessment of obese children. This clinic was attended by specialist registrars in adult endocrinology, as part of their clinical training. The endocrine nurse specialist at this clinic, Miriam Fallon, had made a record of the names, dates of birth and hospital numbers of each child referred for assessment of obesity between 1990 and 2004. There were 249 children referred in that time. A detailed retrospective analysis of each set of available medical records was conducted. The age, weight, height and blood pressure at presentation were recorded. Complete medical records were available in 206 children, and these formed the basis of the Crumlin Cohort described later.
**The MIRIAM Cohort:**

Patients from the Crumlin Cohort were invited to participate in a new, prospective study examining "Metabolism, Insulin Resistance and Initial Anthropometric Measurements in obese Irish children" – the MIRIAM study. Of the 206 patients in the Crumlin Cohort, 120 were invited to return for repeat assessment. The remaining 86 were excluded on the basis of age (>18 years), or for other clinical reasons. A recruitment letter was posted to each eligible patient, and a toll-free telephone number was made available for potential study participants or their parents to discuss the study further. There was a relatively low level of recruitment to this study, such that 18 of the 120 participants invited (15%) were recruited, as reported in Chapter Three. Each of the patients was Caucasian and had no ongoing medical problems other than obesity.

**The Exercise Cohort:**

This cohort was entirely separate from the first two. Participants were slightly older, aged between fifteen and thirty years. They were attending either the endocrinology or diabetes services at St. James’s Hospital, Dublin, with obesity or with type 2 diabetes. Those with co-existing illnesses or secondary forms of diabetes were excluded. Using the computerized diabetes database, Diamond®, potential recruits were identified and the study was then discussed with them at their scheduled appointment time in the Diabetes Day Centre. In addition, the exercise physiologist discussed the study with patients in the obesity outpatient clinic. Together, we recruited
18 obese (control) patients with normal glucose tolerance and 13 obese young type 2 diabetes subjects. Each of the controls was recruited into the exercise programme. However, four of the thirteen diabetic patients volunteered for baseline assessment only and did not take part in the exercise programme.

2.2: Ethics:
Prior to the start of any study related activity, ethics committee approval was obtained from the St. James’s Hospital and Federated Dublin Hospitals Joint Research Ethics Committee, as well as the Ethics Committee at Our Lady’s Hospital for Sick Children in Crumlin.

2.3: Diagnostic Criteria:

Diabetes Mellitus
The criteria for the diagnosis of diabetes mellitus set out by the World Health Organisation were used (WHO 1999). These are based on the plasma glucose levels obtained during a standard two hour, 75g oral glucose tolerance test, with measurements taken at time zero (fasting) and two hours after administration of the glucose load. Diagnostic criteria are shown in Table 2.1. The American Diabetes Association (ADA) diagnostic criteria were revised in 2003. They are similar, except for a lower threshold for impaired fasting glucose of ≥ 5.6 mmol/l (ADA 2006). However the revision of this threshold caused some controversy. For example, the European Diabetes Epidemiology Group (EDEG) recently stated that the threshold was
too low and that the WHO criteria should remain (Forouhi, Balkau et al. 2006). Hence these criteria have been used throughout the study.

Table 2.1: WHO Diagnostic Criteria for Diabetes Mellitus, 1999.

<table>
<thead>
<tr>
<th></th>
<th>Fasting Glucose (mmol/l)</th>
<th>2-Hour Glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Glucose Tolerance</td>
<td>&lt; 6.0</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>Impaired Fasting Glucose</td>
<td>6.0 - 6.9</td>
<td>&lt; 7.8</td>
</tr>
<tr>
<td>Impaired Glucose Tolerance</td>
<td>&lt; 7.0</td>
<td>7.8 - 11.0</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>≥ 7.0</td>
<td>≥ 11.1</td>
</tr>
</tbody>
</table>

Type 2 Diabetes

The confirmation of a diagnosis of type 2 (versus type 1) diabetes was made after careful consideration of a number of factors, on an ad hoc, case by case basis. One factor was the absence of GAD (glutamic acid decarboxylase) antibodies, which might suggest immune mediated beta cell destruction, or type 1 diabetes. A second factor was the mode of initial presentation with diabetes. The presence of ketonaemia, ketonuria and significant weight loss at presentation are suggestive of insulinopenia and type 1 diabetes. Each of the patients in the exercise cohort had a clinical presentation consistent with type 2 diabetes and was negative for GAD antibodies. Each patient had
diabetes for at least six months prior to enrolment into the study. None of the patients in the Crumlin or MIRIAM cohorts had diabetes.

**Obesity**

In adults, the simplest and most widely used tool to screen for obesity is the body mass index (BMI). This is calculated by dividing the weight (in kilograms) by the height (in metres), squared. Normal range for BMI in an adult is 18.5 – 25 kgm⁻². A value below 18.5 kgm⁻² is considered underweight. A BMI between 25 and 30 kgm⁻² is overweight and a BMI over 30 kgm⁻² defines obesity. Varying degrees of obesity have been proposed, ranging from moderate, through severe to morbid obesity in those with a BMI over 40 kgm⁻².

In children, diagnosing and quantifying the degree of obesity is more complex. This is because BMI changes substantially throughout childhood (between two and twenty years of age), as shown in the CDC growth chart in Figure 2.1. This chart illustrates how BMI in boys decreases between two and seven years of age and then increases steadily until age twenty years. Similar patterns are seen in girls. Thus, BMI in children is age- and gender-specific. Obesity is defined as a BMI- for- age above the 95th centile, while overweight is defined as a BMI- for- age above the 85th centile (Speiser, Rudolf et al. 2005). Thus, a four year old boy with a BMI of 17.8 kgm⁻² would lie above the 95th centile on the chart and would be classified as obese, whereas an adult with a similar BMI would be classified as...
underweight. The reference population for these growth charts was taken from several large, multiracial and multiethnic cohorts in the United States, with factors such as birth weight and method of newborn feeding taken into account. These measurements were taken in the 1960s and 70s, when the prevalence of childhood overweight and obesity was significantly lower than it is now. The charts are designed to be universally applicable. Similar normative data do not exist for Irish children and while there are BMI-for-age charts available in the UK, they do not have accompanying statistical software for the more detailed analyses described below. Prof. Venkat Narayan, Chief of the Diabetes Epidemiology and Statistics Branch of the CDC, advised that the use of CDC growth charts in a Caucasian Irish population was scientifically valid.

While the charts categorise a child’s BMI as normal, overweight or obese, they do not allow precise quantification of the degree of obesity. Rather, this is done by calculating a standard deviation score (SDS) for a given BMI. The standard deviation score is obtained by subtracting the mean BMI from the measured BMI, and dividing by the standard deviation (for age and gender). Thus, the 95th percentile of BMI is equivalent to a SDS of 1.645, which is obese. The 85th percentile of BMI is equivalent to a SDS of 1.04, which is overweight. Statistical software available from the CDC website was used to calculate BMI SDS values in the Crumlin and Miriam cohorts (CDC 2007).
**Hypertension**

As with obesity, defining and quantifying the degree of blood pressure elevation in children is more complex than it is in adults. In addition to being age- and gender-specific, height must also be taken into account. This
precludes the use of simple charts to plot blood pressure readings against centiles. Rather, lengthy reference tables with threshold values for systolic and diastolic blood pressure based on gender, age and height are available. These were published in a report by the National High Blood Pressure Education Program Working Group in the US (NHBPEP 2004). However the report also provided a mathematical formula to calculate SDS for systolic and diastolic blood pressure, with hypertension defined as greater than the 95th centile and pre-hypertension defined as greater than the 90th centile for gender, age and height.

2.4: Crumlin Cohort Data (retrospective):
The data used in describing the Crumlin Cohort consists of measurements taken over a fifteen year period by two paediatric endocrine nurse specialists. Each of the measurements was taken at the time of the patient's first visit to the endocrine clinic in Crumlin. Weight was measured using a Seca® scale and height with a Holtain® stadiometer. Blood pressure was measured using a standardised protocol, with the patient sitting comfortably for five minutes, from the right arm, using a Critikon Dynamap® oscillometric device. These data were then recorded pro forma in the medical notes. In addition, the patient's name, medical record number and date of birth were recorded in a separate logbook maintained by one of the endocrine nurses. This allowed the rapid and efficient identification of patients and collection of data from the charts during this study.
2.5: Screening:
All of the investigations in subjects recruited to the MIRIAM and Exercise studies were performed at the Metabolic Research Unit in St. James’s Hospital. On the morning they attended, they were given a detailed patient information leaflet for the relevant study. For children and teenagers, written information appropriate to their level of understanding was supplied. Written informed consent was obtained from all subjects or from the parents of those under sixteen years of age. (Minors, aged under sixteen years, signed an assent form.)

Each subject had a detailed medical history and physical examination performed. Particular attention was paid to past medical history, concomitant medications, family history of diabetes and obesity and current dietary and exercise patterns. In subjects with diabetes, the duration of diabetes, mode of clinical presentation and type of treatment were recorded. All of the subjects were well at the time of assessment. On examination, particular note was made of acanthosis nigricans and axillary skin tags, which are markers of insulin resistance (Hermanns-Le, Scheen et al. 2004; Erdogan, Aktan et al. 2005). In recruits to the MIRIAM study, Tanner staging was used to measure pubertal development (Tanner 1981).

Routine bloods were taken for full blood count, renal, liver, bone profile and thyroid function tests. Routine dipstick urinalysis was performed. Also, a urinary beta-HCG test to outrule pregnancy was performed in female
subjects over sixteen years of age. A standard twelve lead ECG was taken. All of these tests were negative or normal in each subject.

2.6: Anthropometric Measurements:
Waist circumference was measured using a fibreglass, non-stretch D-loop measuring tape, at a level half way between the lower costal margin and the iliac crest. Hip circumference was measured at the level of the anterior superior iliac spine. The waist: hip ratio was calculated by dividing the waist-by hip- circumferences. Whole body weight was measured using a Seca® scale. Height was measured using a Holtain® stadiometer. Blood pressure was measured using the right arm after the subject had been sitting comfortably for five minutes, using an oscillometric device (Omron® 705 CP). Three consecutive readings were recorded and the lowest one was used for subsequent analysis. Body composition was assessed using an electrical impedance device (Tanita® TBF-300 Body Composition Analyser). This allowed an estimation of fat mass, fat free mass, percentage body fat and total body water, corrected for age, gender and height.

2.7: OGTT:
A standard two-hour 75g oral glucose tolerance test was performed. (In younger subjects weighing less than 43kg, a glucose load of 1.75 g/kg was used.) Subjects fasted overnight for ten hours before the test. 113ml of Polycal® solution (containing 75g of anhydrous glucose) was diluted to 200ml and this was ingested with 100ml of water, in less than one minute.
stopwatch was used to record accurately the time. Glucose levels at time zero and at 120 minutes were used to confirm each subject's glucose tolerance status, as per WHO criteria.

2.8: OGIS:

By taking additional samples during the standard OGTT, a mathematical model can be used to estimate insulin sensitivity, called Oral Glucose Insulin Sensitivity (OGIS) (Mari, Pacini et al. 2001). Samples were taken for glucose, insulin and C-Peptide measurements at thirty minute intervals over two hours. This allowed an assessment of the dynamic beta cell response to oral glucose loading (insulin secretion) as well as insulin sensitivity. Other model based approaches to measurement of insulin sensitivity exist, including the Homeostasis Model Assessment (HOMA) (Matthews, Hosker et al. 1985). HOMA quantifies insulin resistance based on the product of the fasting glucose and insulin concentrations, without regard to changes in glucose and insulin levels after a glucose load (Pacini and Mari 2003). It is useful in determining "basal" insulin sensitivity in larger, population-based studies (Bonora, Targher et al. 2000), while OGIS has been shown to be a superior measure of "stimulated" insulin sensitivity (Mari, Pacini et al. 2005). Furthermore, one study suggested that HOMA may not accurately reflect insulin sensitivity in peri-pubertal children (Brandou, Brun et al. 2005), though this finding was refuted elsewhere (Keskin, Kurtoglu et al. 2005). Thus, we used OGIS measurements in our studies.
OGIS Mathematical Modelling

The specific and very complex mathematical methods by which the OGIS model describes the relationship between insulin secretion and glucose concentration have been described in detail (Mari, Pacini et al. 2001; Mari, Schmitz et al. 2002; Mari, Tura et al. 2002). The model expresses insulin secretion as a sum of two components. The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point, and is characterised by a dose-response function relating the two variables. The characteristic parameter of the dose-response is the mean slope within the observed glucose range, denoted as "beta cell glucose sensitivity". The dose-response is modulated by a potentiation factor, which accounts for several other factors (e.g. prolonged exposure to hyperglycaemia, non-glucose substrates, gastrointestinal hormones and neurotransmitters). The potentiation factor is set to be a positive function of time and to average one during the experiment. It thus expresses a relative potentiation of the secretory response to glucose. Previous studies (Mari, Schmitz et al. 2002; Mari, Tura et al. 2002; Ferrannini, Gastaldelli et al. 2005) have found that insulin secretion at the end of an OGTT or meal is relatively higher than at the beginning for comparable glucose levels (i.e., when glucose returns to the basal level, insulin secretion remains higher). Potentiation thus increases during the test. This increase has been quantified as the ratio between the potentiation factor value at 2 hours after the OGTT and that at time zero.
The second insulin secretion component represents a dynamic dependence of insulin secretion on the rate of change of glucose concentration. This component is termed the derivative component, and is determined by a single parameter, denoted as "rate sensitivity". Rate sensitivity is related to early insulin release (Mari, Schmitz et al. 2002; Mari, Tura et al. 2002; Ferrannini, Gastaldelli et al. 2005).

The model parameters were estimated from glucose and C-peptide concentrations by regularised least-squares, as previously described (Mari, Schmitz et al. 2002; Mari, Tura et al. 2002). Regularisation involves the choice of smoothing factors that were selected to obtain glucose and C-peptide model residuals with standard deviations close to the expected measurement error (~1% for glucose and ~4% for C-peptide). Basal and total insulin secretion during the OGTT were calculated from the estimated model parameters. Total insulin secretion was calculated as the integral of insulin secretion during the OGTT. Insulin secretion was expressed in pmol.min\(^{-1}\).m\(^{-2}\) of body surface area.

2.9: Hyperinsulinaemic, euglycaemic clamp study:
On a separate morning (within seven days of the OGTT), subjects attended the Metabolic Research Unit for a four-hour hyperinsulinaemic, euglycaemic clamp study. Only those recruited to the exercise cohort underwent clamping. Subjects fasted for twelve hours prior to the clamp. During a two-hour basal phase, deuterated glucose tracer (Cambridge Isotopes®) was
infused through an intravenous cannula in the right antecubital fossa. 

Subsequently a two-hour insulin infusion (Actrapid®, Novo Nordisk, 
Bagsvaerd, Denmark) was commenced through an intravenous cannula in 
the left antecubital fossa at a rate of 40 mU m⁻² min⁻¹. Simultaneously a 
deuterated glucose solution was infused in order to maintain euglycaemia. 
Arterialised venous blood was taken at five-minute intervals, in order to 
monitor blood glucose levels, from a retrograde cannula on the dorsum of the 
right hand, which was warmed. The mean glucose infusion rate between 80 
and 120 minutes after commencement of the insulin infusion was calculated. 
The glucose disposal rate was calculated after correction for residual hepatic 
glucose production using the deuterated glucose tracer (Nolan, Freidenberg 

Appendix 1 contains a detailed description of the clamp and IVGTT protocols.

2.9.1: Isotopic Tracer Studies:

Background:
Stable isotopes of glucose contain a carbon or hydrogen atom with an altered 
atomic number. For example, Carbon-13 is a naturally occurring isotope 
present at 1.1% of the level of the major isotope species, Carbon-12. 
Deuterium, which has a natural abundance of 0.015% of hydrogen species, 
gives rise to deuterated glucose when incorporated into that molecule. After 
enrichment, this can be used as a stable isotope tracer in complete safety as 
it is non- radioactive and is metabolised in exactly the same way as normal 
glucose. Using mass spectrometry it is possible to differentiate precisely 
between deuterated and non- deuterated glucose concentrations. Thus, with
administration of intravenous deuterated glucose tracer, endogenous glucose production and tissue glucose uptake can be quantified under a range of conditions, i.e. the rates of glucose appearance \( (R_a) \) and disappearance \( (R_d) \) can be determined. This requires that a steady state of "isotopic equilibrium" between the intravenous infusate and the plasma pool is reached. When this equilibrium occurs, the tracer will be lost at the same rate that it appears, and no further changes in the relative concentrations will occur provided the infusion rate remains constant. Where deuterated glucose is the tracer, non-deuterated glucose is the "tracee". Thus, when steady state is reached, the tracer: tracee ratio (TTR) will be constant. As early as 1954 the first description emerged of using a priming dose of tracer in conjunction with a constant infusion of tracer to measure glucose kinetics in dogs (Searle, Strisower et al. 1954). This technique substantially reduced the time required to reach isotopic equilibrium in the plasma glucose. The primed-continuous infusion of tracers has become standard procedure for measurement of the kinetics of other substances also, such as lactate (Kreisberg, Pennington et al. 1970) and glycerol (Bortz, Paul et al. 1972). In measuring glucose kinetics in humans, approximately two hours of continuous infusion of tracer is usually adequate time to reach steady state both physiologically and isotopically, if a priming bolus is used. The TTR is measured frequently during the steady state period (typically every 5-10 minutes) and the mean TTR value is then applied for the calculation of glucose kinetics.

**Analytical Procedures:**
Plasma samples obtained during the hyperinsulinaemic- euglycaemic clamp were immediately centrifuged and frozen at -80°C. After study completion all of the samples were sent for batch analysis by Dr. Amalia Gastaldelli at the Institute of Clinical Physiology, CNR, Pisa, Italy. Deuterated glucose enrichment was determined in plasma according to validated gas chromatography/ mass spectrometry (GC-MS) methods (Wolfe 1984). All samples were analysed on a Trace DSQ GC/MS machine (Thermo-Fisher...
Scientific). For each sample, measures of the SIM (secondary ion mass spectrometry) peak areas were taken at three separate mass: charge ratios (m/z): 200, 201 and 202. Thereafter, the SIM peak area ratios of m/z 201/200 (denoted $R_{201}$) and m/z 202/200 (denoted $R_{202}$) were calculated. Enrichment was expressed as the tracer-to-tracee ratio (TTR) at time $t$ and calculated according to the formula below, where Bk denotes a blank sample:

$$TTR_{201}(t) = \frac{R_{201}(t)}{R_{201}(Bk)}$$
$$TTR_{202}(t) = \frac{R_{202}(t)}{R_{202}(Bk)} - R_{202}(Bk) \times TTR_{201}(t)$$

Calibration by auto-tuning of the GCMS machine was performed before each analysis. The quantification limit for this assay was determined to be $\sim$50 µg/ml. Quality control plasma samples were analysed at four different enrichments (A = 0%, B = 1%, C = 3% and D = 5%) with each assay batch to check instrument precision and linearity. Results from study samples were accepted if fewer than 25% of the quality control samples within each assay batch had an inaccuracy $\geq$20%.

**Calculations of tracer infusion rate:**

The tracer infusion rate (IR) at baseline was calculated by multiplying the tracer concentration (determined by the glucose oxidase method, Beckman II Glucose Analyzer, Fullerton, CA) by the pump rate, divided by total body weight, as shown here:

$$IR_{\text{baseline}} \, (\mu\text{mol/min \times kg}) = [\text{tracer}] (\mu\text{mol/ml}) \times \text{pump rate (ml/min)} / \text{body weight (kg)}$$

The tracer infusion rate (IR) during step one of the clamp was calculated by multiplying the tracer concentration by the pump rate, divided by total body weight and then adding the tracer infused with the exogenous glucose (calculated as the percentage of tracer in the glucose infusion (GINF) x glucose infusion rate (GIR)), as shown below:

$$IR_{\text{step1}} \, (\mu\text{mol/min \times kg}) = [\text{tracer conc}] (\mu\text{mol/ml}) \times \text{pump rate (ml/min)} / \text{body weight (kg)} + TTR_{\text{GINF}} / (1 + TTR_{\text{GINF}}) \times GIR_{\text{step1}} \, (\mu\text{mol/min \times kg})$$
This may be expressed alternatively as:

\[
IR_{\text{step}} = \frac{[NINF_{\text{step}} \times 0.083]}{\text{Body \_ weight}} + GIR_{\text{step}} \times \left( \frac{TTR_{\text{GINF}}}{1 + TTR_{\text{GINF}}} \right)
\]

**Calculations of glucose production and disposal:**

During the last twenty minutes of the basal tracer equilibration period, plasma glucose concentrations and 6,6-[\(^2\)H]glucose enrichment are stable and isotopic equilibrium is present. Therefore, total endogenous glucose production (hepatic glucose output, HGO) is calculated as the ratio of 6,6-[\(^2\)H]glucose infusion rate to the plasma tracer enrichment (tracer-to-tracee ratio, TTR\(_{6,6}\), mean of 3 determinations) (Wolfe 1984). At baseline, the rate of hepatic glucose output is equal to the rate of glucose appearance (Ra), which is also equal to the rate of disappearance (Rd). Thus:

\[
HGO_{\text{baseline}} = \frac{IR_{\text{baseline}}}{TTR_{\text{baseline}}}
\]

Where the TTR is stable at each step of the euglycemic clamp, the rate of glucose appearance (Ra) may be calculated using the steady state equation:

\[
Ra_{\text{step}} = \frac{IR_{\text{step}}}{TTR_{\text{step}}}
\]

Here, TTR is calculated as the average of the last thirty minutes sampling, and the infusion rate is calculated as described above. The HGO during the clamp can be calculated by subtracting the GIR (after adjusting for tracer concentration) from Ra as follows:

\[
HGO_{\text{step}} = Ra_{\text{step}} - GIR_{\text{step}} \times \left(1 - \frac{TTR_{\text{GINF}}}{1 + TTR_{\text{GINF}}} \right)
\]

The rate of glucose disappearance (Rd), which is equivalent to glucose disposal, is calculated using the following formula, where dC/dt is the derivative of plasma glucose concentration and V is the volume of distribution (165 ml/kg):
2.10: Modified Intravenous Glucose Tolerance Test:
At the end of the clamp study (at 240 minutes), a rapid bolus of intravenous glucose was administered (0.3g/ Kg) over 20 seconds and serial blood samples taken every two minutes for eight minutes to measure insulin, C-peptide and glucose. The IVGTT has been used in similar cohorts to assess beta-cell function in the past (McQuaid, O’Gorman et al. 2005). The combination of the modified IVGTT with the hyperinsulinaemic-euglycaemic clamp was based on the methodology used in the EGIR - RISC study (Hills, Balkau et al. 2004).

IVGTT Mathematical Modelling
The acute insulin response (AIR) was calculated as the mean incremental insulin concentration during the eight-minute IVGTT, where the mean was determined by trapezoidal integration. Analogous indices were obtained using C-peptide concentrations and insulin secretion, which was calculated by deconvolution from C-peptide concentrations (Van Cauter, Mestrez et al. 1992).

2.11: Laboratory Analysis:
All of the samples taken during the studies were analysed in the Department of Biochemistry in St. James’s Hospital. Serum insulin and C-peptide were
both measured using commercially available fluoroimmunoassays (Auto-DELFIA®). Plasma total cholesterol and triglycerides were measured using enzymatic methods (Human liquicolor kits/Hitachi Modular). Plasma HDL-cholesterol and LDL-cholesterol were measured directly with enzymatic methods (Randox direct kits/Hitachi Modular). Plasma glucose was measured using a glucose oxidase method (bio Merieux kit/Hitachi Modular). Serum alanine aminotransferase (ALT) was measured using an enzymatic method on a Roche® Automated Hitachi Modular-P system. HbA1c was measured using a Hi-Auto A1c analyser (Menarini HA 8140). Antibodies to glutamic acid decarboxylase were measured using a direct radioligand assay.

2.12: VO_{2max} testing:
VO_{2max} testing was performed at the Department of Physiology in Trinity College, Dublin. The tests were conducted by the exercise physiologist under medical supervision. Each subject performed a stepwise incremental exercise test on a bicycle ergometer (Excalibur, Groningen, The Netherlands). This involved a progressive increase in workload, from 25 watts for the first three minutes to 50 watts for the second three and 75 watts for the subsequent three minute period. Thereafter, the power output was increased by 10 watts per minute. Oxygen consumption and carbon dioxide excretion were measured throughout the test using an airtight facemask attached to a Metalizer® measurement apparatus, which analysed the concentrations of oxygen and CO₂ in inspired and expired air. Heart rate and blood pressure were also monitored continuously.
As the test progressed, subjects were asked to maintain a comfortable cycling rhythm of 60-70 rpm. The test ended when the subject fell below this cadence, when they were exhausted, or if the exercise physiologist felt they would not complete a subsequent increment in power output. \( \text{VO}_2 \) was calculated from the difference between inspired and expired oxygen. The level at which the \( \text{VO}_2 \) ceased to increase was taken as the \( \text{VO}_2 \text{max} \). After the test, subjects had a five minute cool down period and blood pressure and pulse rate were rechecked before they left the department. Fitness testing was repeated after completion of the exercise intervention.

2.13: Exercise Protocol:
The exercise intervention was conducted entirely at the Metabolic Research Unit in St. James's Hospital, in a purpose-built gymnasium. This involved the acquisition of two heavy duty treadmills, three upright exercise bikes and one reclined exercise bike. Participants had the choice of running or cycling, or interchanging these modes of aerobic exercise as they wished. Each subject was prescribed twelve weeks of aerobic exercise training, with four sessions per week. Each session lasted sixty minutes, with an additional five minutes each for warm-up and cool-down. The exercise sessions were always fully supervised. The target intensity for exercise was set at 70% of the subject's measured \( \text{VO}_2 \text{max} \), while warm-up and cool-down intensity was set at 50% of \( \text{VO}_2 \text{max} \). During each session, subjects wore a heart rate monitor, which transmitted a continuous reading to the researcher supervising the session.
By correlating these readings with the heart rate measurements from the initial exercise test, adequate and appropriate exercise intensity was maintained in each subject throughout the exercise programme.

2.14: Statistical Analysis:

JMP® statistical software (SAS Institute, Version 5.1) was used in all statistical calculations throughout the study. Descriptive statistics are presented as percentages and means ± standard deviation for the Crumlin and Miriam Cohorts, and means ± standard error of the mean in the Exercise Cohort, unless otherwise stated in the accompanying tables. When examining pairwise correlations between data that were not normally distributed, Spearman’s rank correlation was used. Correlations between data that were normally distributed were calculated using Pearson’s correlation. In the Miriam Cohort, associations between variables were calculated using multiple linear regression analysis, in each case adjusted for pubertal stage and where appropriate further adjusted for gender, age and height. 95% Confidence intervals for each regression coefficient are also reported. In the Exercise Cohort, comparisons between normal glucose tolerant obese controls and young obese type 2 diabetes subjects at baseline (before exercise) used a Mann-Whitney U test. Comparisons of both of these groups before and after exercise used the Wilcoxon Signed Rank test. Comparisons of differences in exercise responses between the two groups were made using multiple linear regression analysis. Statistical significance was set at p< 0.05.
Chapter Three:

Characteristics of a Clinic Based Cohort of Obese Caucasian Irish Children.
3.1: BACKGROUND:

While there are good population based data on the prevalence of overweight and obesity in young Irish people, as outlined in section 1.2, no analysis has been performed of the clinical or metabolic characteristics of this group. At the paediatric endocrine clinic in Our Lady's Hospital for Sick Children in Crumlin, the endocrine nurse specialist had maintained a log of all of the patients referred there for evaluation of obesity, over a fifteen year period. No analysis had previously been undertaken in this cohort. Each patient had weight and height recorded by a trained member of staff, while most also had blood pressure measurements recorded.

Thus, the opportunity arose to retrospectively collate these data, calculate individual BMIs and quantify the degree of obesity in clinic attendees, at their initial presentation. Decimal age was calculated based on the date of attendance and date of birth. We sought to measure the prevalence of high blood pressure readings within the group and to examine the relationship, if any, between the severity of obesity and the degree of blood pressure elevation. A further objective was to examine the relationship between the age of first referral to the clinic and the severity of obesity.

While there were comprehensive cross-sectional data relating to individuals at their initial clinic visit, the recording in the medical notes of follow-up clinical data from subsequent clinic visits was inconsistent. Thus, a
retrospective longitudinal cohort study was not feasible. Many patients did not request follow-up appointments. In those patients who did have a documented follow-up appointment noted in the chart, 50% failed either to attend or to cancel the appointment.

Rather, the objective was to conduct more detailed prospective metabolic studies in a subgroup of patients from the cohort. This involved the establishment of the MIRIAM study, examining Metabolism, Insulin Resistance and Initial Anthropometric Measurements in obese Irish youth.

3.2: METHODS:

The retrospective analysis of clinical data from obese children referred to the clinic between 1990 and 2004 was conducted in the medical records department of the hospital, or at the off-site chart storage facility. Data collated included the decimal age at presentation, gender, weight, height, BMI and blood pressure at the time of the first clinic visit. This was recorded on a password protected Microsoft Excel® spreadsheet. Thereafter, BMI SDS was calculated based on the growth charts published in 2000 by the CDC. Statistical software available on the CDC website was used to calculate BMI SDS as outlined in section 2.3. Standard deviation scores and percentile values for systolic and diastolic blood pressure (SBP and DBP) were also calculated, using formulae published in the most recent report from the
National High Blood Pressure Education Program Working Group, as outlined in section 2.3.

Patients from this cohort were then invited to take part in the prospective study, which was conducted entirely at the Metabolic Research Unit in St. James’s Hospital. Those with co-existing illnesses were excluded. Written informed consent was obtained (assent for minors, with consent from a parent or guardian).

Subjects attended on one morning after an overnight fast. A full history and physical examination were performed, including Tanner staging for pubertal development. Waist: hip ratio, weight, height and body mass index were re-measured. Routine bloods were drawn including full blood count, renal, liver and bone profiles, thyroid function tests and fasting lipids. A standard oral glucose tolerance test was performed (1.75 g/kg to a maximum of 75g) with glucose, insulin and C-Peptide levels measured at thirty minute intervals over two hours. Insulin sensitivity was measured using a mathematical model, OGIS (Mari, Pacini et al. 2001) as described in section 2.8. Laboratory and statistical analytical methods are outlined in sections 2.11 and 2.14, respectively.
3.3: RESULTS:

3.3.1: Crumlin Cohort BMI

Two hundred and forty nine children were referred to the paediatric endocrine clinic in Crumlin between 1990 and 2004 for assessment of obesity. Charts were available for review (after up to three separate attempts were made to recover each chart) in 206 children (97 males and 109 females). The mean age of referral was 9.7 ± 3.7 years (range 2 – 18 years). Ninety three percent of all patients referred (96% of girls and 92% of boys) were obese with a BMI > 95th percentile for age and gender (or BMI SDS >1.645). The remaining patients were classified as overweight (BMI >85th percentile for age and gender or BMI SDS >1.04). The mean BMI at referral was 29.2 ± 7.2 kg/m² and the mean BMI SDS was 2.49 ± 0.71. Younger patients had significantly worse obesity at referral. Spearman’s correlation for age and BMI SDS was -0.51 in boys, -0.64 in girls and -0.59 in both groups combined (all P<0.0001) as shown in Figure 3.1.

3.3.2: Crumlin Cohort BP

Blood pressure measurements were performed on 176 subjects at their initial presentation to the clinic (88 boys and 88 girls). Of these 51% of girls and 49% of boys had systolic BP readings in the hypertensive range (>95th percentile for age, sex and height, or SDS >1.645). A further 9% of boys and 7% of girls had SBP in the pre-hypertensive range (>90th percentile). Fewer patients had diastolic hypertension, with 8% of boys and 5% of girls
exceeding the 95th percentile for DBP. A relatively weak but statistically very significant correlation was found between BMI SDS and SBP SDS in boys (Pearson’s Correlation (PC) = 0.27, P = 0.007) but not in girls (PC = 0.16, P = 0.13). For both groups combined, there was a PC of 0.23 (P = 0.003), as shown in Figure 3.2. Diastolic blood pressure standard deviation scores did not correlate with BMI. I performed a subgroup analysis of the cohort based on age and gender. In the 54 children aged between two and eight years old, there was a significant correlation between BMI SDS and SBP SDS (PC = 0.37, P = 0.006). This was most pronounced in the 29 girls within that age group (PC = 0.47, P = 0.01) and was not significant in the males (PC = 0.24, P = 0.25). Analyses of children aged 8–12 years (n = 60) and 12–18 years (n = 62) showed no significant correlations, whether genders were separated or combined.

**Figure 3.1: Age versus Degree of Obesity at Referral**

![Figure 3.1: Age versus Degree of Obesity at Referral](image-url)
3.3.3: MIRIAM Cohort

Eighteen participants (6 male, 12 female) were recruited and their characteristics are summarised in table 3.1. All of the participants were well at the time of assessment, with no underlying syndrome or chronic disease. They all had normal full blood count, renal, liver and bone profiles and thyroid function tests. Each subject had normal glucose tolerance confirmed during the OGTT. All but the youngest child had started puberty (Tanner stage II to IV), while five participants had reached Tanner stage V pubertal development. Of note one subject, an 18 year old male, had marked hyperinsulinaemia with a fasting insulin of 1050 pmol/l and a 2-hour insulin of 726 pmol/l, although he had normal glucose tolerance. These two outlying values were excluded from table 1 and from statistical analyses.
Associations between various participant characteristics, insulin sensitivity and ALT are summarised in table 3.1. Insulin sensitivity was significantly associated with crude and standardised BMI, body weight and hip circumference, with a tendency towards significance for waist circumference. For example, an increase in BMI SDS of 1 would account for a reduction in OGIS of 70.1 ml m\(^{-2}\) min\(^{-1}\). There were no associations between insulin sensitivity and waist: hip ratio (WHR), height or blood pressure. There were also significant associations with insulin sensitivity, fasting and 2-hour glucose levels and total cholesterol levels. The associations between insulin sensitivity, total: HDL and LDL: HDL cholesterol ratios approached statistical significance.

There were significant associations between serum ALT levels and standardised BMI, waist circumference, body weight, height and fasting insulin levels. For example, a 1 pmol/l rise in fasting insulin accounted for a 0.22 iu/l rise in ALT.
**Table 3.1:** Participant characteristics and associations with insulin sensitivity (OGIS) and serum ALT.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>Association with OGIS</th>
<th>Confidence Interval</th>
<th>P-Value</th>
<th>Association with ALT</th>
<th>Confidence Interval</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.5</td>
<td>2.8</td>
<td>8.6 - 18.9</td>
<td>-8.3</td>
<td>[-26.3, 9.7]</td>
<td>0.34</td>
<td>2.1</td>
<td>[-3, 7.1]</td>
<td>0.39</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>110.8</td>
<td>15.2</td>
<td>91 - 134</td>
<td>* -1.9</td>
<td>[-3.9, 0.1]</td>
<td>0.057</td>
<td>* 0.6</td>
<td>[0.1, 1]</td>
<td>0.025</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>117.4</td>
<td>13.4</td>
<td>96 - 148</td>
<td>* -2</td>
<td>[-3.9, -0.03]</td>
<td>0.047</td>
<td>* 0.4</td>
<td>[-0.04, 0.9]</td>
<td>0.07</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94</td>
<td>0.08</td>
<td>0.78 - 1.11</td>
<td>* -39.3</td>
<td>[-585.5, 506.9]</td>
<td>0.88</td>
<td>* 25.2</td>
<td>[-90.4, 140.7]</td>
<td>0.64</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>105.1</td>
<td>22.7</td>
<td>61.2 - 141.8</td>
<td>* -1.7</td>
<td>[-2.9, -0.5]</td>
<td>0.011</td>
<td>* 0.4</td>
<td>[0.2, 0.7]</td>
<td>0.003</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69</td>
<td>0.09</td>
<td>1.47 - 1.86</td>
<td>* -168.8</td>
<td>[-616, 278.3]</td>
<td>0.43</td>
<td>* 111.3</td>
<td>[46.2, 176.4]</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI (kg m^{-2})</td>
<td>36.5</td>
<td>6</td>
<td>25.7 - 49.1</td>
<td>* -5.1</td>
<td>[-9.3, -0.8]</td>
<td>0.024</td>
<td>* 0.8</td>
<td>[-0.4, 1.9]</td>
<td>0.16</td>
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<tr>
<td>BMI SDS</td>
<td>2.27</td>
<td>0.42</td>
<td>1.32 - 2.77</td>
<td>-70.1</td>
<td>[-126.3, -14]</td>
<td>0.018</td>
<td>20.7</td>
<td>[6.9, 34.6]</td>
<td>0.007</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110.7</td>
<td>17.2</td>
<td>76 - 141</td>
<td>** -0.4</td>
<td>[-2.3, 1.4]</td>
<td>0.64</td>
<td>** 0.02</td>
<td>[-0.3, 0.3]</td>
<td>0.86</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>66.4</td>
<td>10.4</td>
<td>41 - 82</td>
<td>** -1.5</td>
<td>[-4.4, 1.4]</td>
<td>0.29</td>
<td>** 0.3</td>
<td>[-0.2, 0.8]</td>
<td>0.2</td>
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<tr>
<td>Test</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
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<td>Value 6</td>
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<tr>
<td><strong>Fasting Glucose (mmol/l)</strong></td>
<td>4.74</td>
<td>0.48</td>
<td>4.1 - 6.0</td>
<td>-60.5</td>
<td>[-113.7, -7.2]</td>
<td>0.029</td>
<td>13.1</td>
<td>[-1.8, 28]</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>2- Hour Glucose (mmol/l)</strong></td>
<td>5.5</td>
<td>0.95</td>
<td>3.9 - 7.4</td>
<td>-41.1</td>
<td>[-60.6, -21.6]</td>
<td>0.0005</td>
<td>5.5</td>
<td>[-2.2, 13.2]</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Fasting Insulin (pmol/l)</strong></td>
<td>102</td>
<td>44</td>
<td>45 - 198</td>
<td>-0.4</td>
<td>[-1.1, 0.4]</td>
<td>0.33</td>
<td>0.2</td>
<td>[0.1, 0.4]</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>2- Hour Insulin (pmol/l)</strong></td>
<td>308</td>
<td>246</td>
<td>52 - 960</td>
<td>-0.1</td>
<td>[-0.2, 0.02]</td>
<td>0.09</td>
<td>0.02</td>
<td>[-0.02, 0.05]</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>OGIS (ml min⁻¹ m⁻²)</strong></td>
<td>428</td>
<td>48</td>
<td>332 - 515</td>
<td></td>
<td></td>
<td>-0.1</td>
<td>[-0.3, 0.02]</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td><strong>ALT (iu/l)</strong></td>
<td>23.2</td>
<td>12.6</td>
<td>8 - 51</td>
<td>-1.8</td>
<td>[-3.9, 0.3]</td>
<td>0.084</td>
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</tr>
<tr>
<td><strong>Total Cholesterol (mmol/l)</strong></td>
<td>3.95</td>
<td>0.77</td>
<td>2.43 - 5.2</td>
<td>-35.5</td>
<td>[-66.6, -4.4]</td>
<td>0.028</td>
<td>-1.8</td>
<td>[-12.8, 9.1]</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>HDL-Cholesterol (mmol/l)</strong></td>
<td>1.06</td>
<td>0.22</td>
<td>0.78 - 1.45</td>
<td>-33.8</td>
<td>[-174.1, 106.4]</td>
<td>0.61</td>
<td>-22</td>
<td>[-60, 15.9]</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>LDL-Cholesterol (mmol/l)</strong></td>
<td>2.32</td>
<td>0.64</td>
<td>1.33 - 3.42</td>
<td>-38.2</td>
<td>[-80, 3.6]</td>
<td>0.07</td>
<td>-4.5</td>
<td>[-18.3, 9.4]</td>
<td>0.5</td>
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<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.26</td>
<td>0.78</td>
<td>0.29 – 3.11</td>
<td>-19.6</td>
<td>[-56, 16.9]</td>
<td>0.27</td>
<td>4.2</td>
<td>[-5.5, 13.9]</td>
<td>0.37</td>
</tr>
<tr>
<td>Total: HDL Cholesterol Ratio</td>
<td>3.82</td>
<td>0.82</td>
<td>2.11 – 5.05</td>
<td>-34.1</td>
<td>[-70.2, 2]</td>
<td>0.062</td>
<td>4.2</td>
<td>[-6.5, 14.9]</td>
<td>0.41</td>
</tr>
<tr>
<td>LDL: HDL Cholesterol Ratio</td>
<td>2.24</td>
<td>0.59</td>
<td>0.94 – 3.06</td>
<td>-50.7</td>
<td>[-102.8, 1.5]</td>
<td>0.056</td>
<td>1.7</td>
<td>[-13.9, 17.3]</td>
<td>0.81</td>
</tr>
</tbody>
</table>

† Adjusted for pubertal status

* Adjusted for pubertal status and gender

** Adjusted for pubertal status, gender, age and height
**Simple pairwise correlations:** There were moderately strong and significant pairwise correlations between the degree of obesity and insulin sensitivity ($r=-0.51$, $P=0.04$ – Figure 3.3), fasting insulin ($r=0.55$, $P=0.03$ – Figure 3.4) and serum ALT ($r=0.64$, $P=0.006$ – Figure 3.5). Insulin sensitivity correlated strongly with fasting glucose ($r=-0.54$, $P=0.03$ – Figure 3.6) and two-hour glucose ($r=-0.79$, $P=0.0002$ – Figure 3.7) though these two variables are used in the mathematical model to measure insulin sensitivity, which might artificially strengthen the correlations. Insulin sensitivity also correlated with total cholesterol ($r=-0.54$, $P=0.03$ – Figure 3.8) though not with HDL-, LDL- cholesterol or triglycerides. The strongest pairwise correlation occurred between fasting insulin and ALT ($r=0.8$, $P=0.0006$ – Figure 3.9). Waist: hip ratio also correlated with fasting insulin ($r=0.55$, $P=0.03$ – Figure 3.10) and to a greater extent with two-hour insulin levels ($r=0.65$, $P=0.006$ – Figure 3.11).

**Figure 3.3: BMI SDS versus Insulin Sensitivity**
**Figure 3.4:** BMI SDS versus Fasting Insulin

![Graph showing the relationship between BMI SDS and fasting insulin](image)

**Figure 3.5:** BMI SDS versus ALT

![Graph showing the relationship between BMI SDS and ALT](image)
**Figure 3.6: Insulin Sensitivity versus Fasting Glucose**

Glucose (mmol/l) vs. OGIS (ml/min/m²)

**Figure 3.7: Insulin Sensitivity versus 2-Hour Glucose**

2-Hour Glucose (mmol/l) vs. OGIS (ml/min/m²)
Figure 3.8: Insulin Sensitivity versus Total Cholesterol

Figure 3.9: ALT and Fasting Plasma Insulin
Figure 3.10: W:H Ratio and Fasting Plasma Insulin

Figure 3.11: W:H Ratio and 2-Hour Insulin
3.4: DISCUSSION:

While childhood obesity is recognised as a significant public health problem in Ireland, very little is known about the clinical and metabolic features of these children. Recent data from the National Survey of Children’s Dental Health showed obesity prevalence rates ranging from 4% to 11% in children between four and sixteen years old, with the highest rates in young adolescent girls (2005). The retrospective data from Crumlin suggest that very young Irish children are currently referred to an obesity clinic only when their obesity is very severe, as evidenced by the higher BMI SDS seen in the younger patients in the cohort. This might indicate a higher threshold for referral of younger children by general practitioners, or a failure to recognise obesity in children of this age. Previous studies have confirmed that overweight and obesity are frequently under-recognised in children: In one UK study of parental perceptions of overweight and obesity in children aged between three and five years, only 1.9% of parents of overweight children and 17.1% of parents of obese children described their child as overweight (Carnell, Edwards et al. 2005). While paediatric obesity may be under-recognised, several studies have confirmed a clustering of cardiovascular risk factors in affected children. These include the Belgian Luxembourg Child Study (Guillaume, Lapidus et al. 1996) and the (Chinese) Taipei Children Heart Study (Chu, Rimm et al. 1998). Data from the US suggest that adolescents deemed to have the metabolic syndrome have elevated levels of
C-reactive protein, a marker of vascular disease risk (Ford, Ajani et al. 2005).

The association between paediatric obesity and hypertension has been reported in numerous studies among a variety of ethnic and racial groups and virtually all have reported higher prevalence rates of hypertension in obese compared with lean children (Verma, Chhatwal et al. 1994; Macedo, Trigueiros et al. 1997; Sorof and Daniels 2002). In one American study involving 2460 students aged between twelve and sixteen years, 33% of obese subjects were hypertensive compared with 11% of lean subjects (Sorof, Poffenbarger et al. 2002). In a school-based survey of a representative sample of Canadian children aged 9, 13 and 16 years, BMI correlated with SBP and DBP in all age groups, and systolic hypertension was prevalent in obese subjects from all age groups while diastolic hypertension was not (Paradis, Lambert et al. 2004). These findings are replicated in our study. Probably the strongest evidence confirming the association between childhood obesity and hypertension comes from a sample consisting of over nine thousand American children examined in seven cross-sectional sub-studies conducted by the Bogalusa Heart Study between 1973 and 1994. These data showed that overweight children were 4.5 times more likely to have systolic hypertension compared to lean children (Freedman, Dietz et al. 1999).
The present study has demonstrated a very high prevalence of blood pressure measurements in the hypertensive range in a hospital clinic-based cohort of obese Irish children. These results cannot be directly translated into rates of hypertension, as this diagnosis requires elevated blood pressure readings on at least three occasions. Blood pressure readings, in children as in adults, tend to be lower after repeated measurements: A study of over five thousand obese American children found that the prevalence of high systolic blood pressure readings dropped from 38% on the first reading to 21% and 11% on second and third readings, respectively (Sorof, Lai et al. 2004). The same author has shown that "white coat” hypertension in children is uncommon in those with moderate to severe ambulatory blood pressure elevation, but common where values are borderline (Sorof, Poffenbarger et al. 2001). Therefore it is likely that the prevalence rate of true hypertension in this cohort would be lower than the initial 50% measured. Nonetheless, these are the first data confirming a relationship between obesity and hypertension in Irish children. This relationship clearly warrants further study. There may be important ethnic and racial differences in the interaction between body weight and haemodynamic state. For example, in another large retrospective cohort study of over 47,000 American children, black children were found to have higher blood pressure than white children at lower levels of BMI, whereas at the highest levels of BMI, Whites had greater blood pressure elevation (Rosner, Prineas et al. 2000).
It is worth noting the variation in methodologies used to measure blood pressure in different centres. While the gold standard of measurement is the auscultatory method, several large epidemiological studies employ oscillometric devices which are automated and easier to use. Several types of these devices are now available but most have not been evaluated independently for accuracy. The nurses in the current study used the Critikon Dynamap®, an automated oscillometric device widely used in clinical practice internationally. However a recent clinical review singled out this device as being potentially inaccurate, in spite of its popularity (O'Brien, Waeber et al. 2001). This may have affected the reliability of our data and also highlights the importance of using an appropriate device in such studies.

The prospective MIRIAM study clearly involved a small number of subjects. Nonetheless, there are some very significant correlations between obesity, insulin resistance, dyslipidaemia and possible fatty liver disease within the group. Non-alcoholic steatohepatosis (NASH) is the commonest form of chronic liver disease in children, and within a few decades may become the commonest indication for liver transplantation in adults in the US (Wieckowska and Feldstein 2005). ALT has been shown to correlate strongly with the degree of hepatic steatosis in children (Fishbein, Miner et al. 2003). Indeed, one recent American study used ALT as a marker of NASH and showed that in a school based sample of obese adolescents, boys were six times more likely than girls to have an elevated ALT (Schwimmer, McGreal et al. 2005). This study also demonstrated a higher prevalence of hepatic
steatosis in Hispanic compared to white children. Another study found elevated ALT levels in 77% of a cohort of obese Chinese children (Chan, Li et al. 2004), while an association between ALT and surrogate markers of insulin resistance has been demonstrated in Japanese children (Kawasaki, Hashimoto et al. 1997). There are likely to be ethnic and racial differences in the relationship between obesity and hepatic steatosis; thus further studies in Irish children are warranted.

The correlations between ALT, obesity and fasting insulin levels in our study are suggestive of hepatic fatty infiltration and inflammation proportional to body fatness, even at an early age. While most of the subjects we studied had normal ALT levels, it is worth noting that reference ranges for ALT have not been defined in children. It was recently shown that deterioration in glucose and lipid metabolism was associated with even modest ALT elevations, in a large multiethnic cohort of American adolescents (Burgert, Taksali et al. 2006).

The onset of puberty causes a significant reduction in insulin sensitivity: One study used the euglycaemic clamp technique in 357 children to show that insulin sensitivity drops immediately at the onset of puberty, is lowest at Tanner Stage three and gradually returns to normal by Tanner Stage 5 (Moran, Jacobs et al. 1999). A study of black American children showed a 30% reduction in insulin sensitivity with the onset of puberty, which was not associated with increased insulin secretion (Saad, Danadian et al. 2002).
Also, a younger age of onset of puberty has been shown to predict central adiposity in young adult males. This was confirmed in the Gothenburg Osteoporosis and Obesity Study, a retrospective longitudinal cohort study, which derived data on the age of peak height velocity from detailed growth charts of 579 men, who then had body composition measured with DEXA (Kindblom, Lorentzon et al. 2006).

Our finding that waist: hip ratio was a strong predictor of fasting and 2-hour insulin levels is consistent with results from other studies. Waist circumference has been shown to predict insulin resistance in Argentinian children (Hirschler, Aranda et al. 2005), and to correlate with abdominal fat measurements using DEXA in a multi-ethnic cohort of American children (Lee, Bacha et al. 2006). Certainly if our findings were confirmed in larger follow-up studies, the measurement of waist and hip circumference in the obese child would be warranted.

The fact that our cohort was clinic based might suggest that it is not representative of the overall population of obese Irish children. Only larger studies will allow an assessment of a more representative population. The types of large, longitudinal and cross-sectional cohort studies outlined above have not previously been conducted in Ireland but are long overdue. Of note the subjects who volunteered for the Miriam study were all otherwise well and had no underlying medical problems. Indeed few studies have been conducted in Caucasian populations outside of the US. One recently
published French study conducted similar measurements to our own, in a large cohort of 308 overweight and obese children, 72% of whom were Caucasian, from three different paediatric clinics (Druet, Dabbas et al. 2006). While the authors describe a high prevalence of the metabolic syndrome and insulin resistance in the cohort, their diagnostic criteria were defined arbitrarily, based on local modifications to adult diagnostic criteria. There is no international consensus for the diagnosis of the metabolic syndrome in children. It may be more useful to examine individual components of the syndrome, as we have done. Much work, such as that by Druet et al, compares categorical variables of “obese” versus “non-obese” or “prehypertensive” versus “hypertensive”. Expressing our own measurements as continuous variables, in a universally acceptable way, has allowed us to elucidate more discrete correlations using fewer subjects.

In conclusion, these findings demonstrate a clear and continuous increase in systolic blood pressure with increasing standardised BMI, particularly in boys, which suggests that obesity does not have a simple threshold effect on systolic blood pressure. Furthermore, a very high prevalence of hypertensive range systolic blood pressure was noted at presentation, which may represent a significant future cardiovascular risk. The prospective data show that the degree of obesity in Irish children predicts insulin resistance and possible liver steatosis. The effects of important confounders such as family history need to be assessed in future studies. Obesity in Irish children is not a benign entity. Nor is it simply a predictor of adult obesity and co-morbidity.
Rather, these data suggest that some of the pathological processes which are known to lead to diabetes and cardiovascular disease later in life are already established in obese Irish children.

The following chapters describe more detailed physiological characterisation of a slightly older cohort of obese individuals with normal glucose tolerance and with type 2 diabetes, in whom the adverse metabolic consequences of obesity are more apparent.
Chapter Four:

Anthropometric and Metabolic Characteristics of Young Obese People with Type 2 Diabetes
4.1: BACKGROUND:

The increased prevalence of type 2 diabetes in young people is a growing public health problem, described recently as an "evolving epidemic" (Bloomgarden 2004). Obesity is the most obvious risk factor, but the natural history of type 2 diabetes in younger people has not been fully elucidated. We sought to conduct detailed metabolic measurements in young obese people with type 2 diabetes and in normal glucose tolerant controls, to better define the underlying pathophysiology of the condition in this group. While several studies have examined beta cell function and insulin sensitivity in similar cohorts, they tend to be from non-white ethnic groups, in whom type 2 diabetes is far more prevalent, as outlined in section 1.3. In particular there are very limited data on insulin resistance in young Caucasians with type 2 diabetes, measured using the hyperinsulinaemic-euglycaemic clamp technique. One recently published German study examined the baseline clinical characteristics of sixteen Caucasian subjects from five different centres, and noted reduced insulin sensitivity as measured by HOMA, without further metabolic characterisation (Reinehr, Andler et al. 2005). Studies of type 2 diabetes in young Caucasians have tended to be small, with as few as four subjects in one report from the UK (Drake, Smith et al. 2002).

The Metabolic Research Unit in St. James's Hospital was ideally suited to this work. It is closely linked to a very busy diabetes centre from which to recruit potential subjects, and has the resources and support necessary to conduct
these complex studies. Indeed, my predecessors in the unit had conducted measurements in a similar (but separate) cohort to my own, using the frequently sampled intravenous glucose tolerance test. They reported that young patients with type 2 diabetes (mean age 22 years) are typically obese and severely insulin resistant, with well established loss of beta-cell insulin secretion at the time of clinical presentation (McQuaid, O'Gorman et al. 2005). This cohort had evidence of high risk for accelerated cardiovascular complications, with elevated markers of endothelial dysfunction. More recently, our group reported that younger Irish adults with type 2 diabetes (aged less than forty years) were more obese, more dyslipidaemic and had worse initial and ongoing glycaemic control than older adults with type 2 diabetes in spite of similar treatment protocols (Hatunic, Burns et al. 2005). These data suggested an unequal and additional cardiovascular risk burden associated with type 2 diabetes in younger patients. However to date there are very limited data in this population using the gold standard clamp technique utilised here.

4.2: METHODS:

Each of the participants in this study was recruited from the diabetes clinic in St. James’s Hospital. The clinic utilizes a computerized patient database, Diamond®. This allowed me to identify patients aged between fifteen and thirty years with a diagnosis of type 2 diabetes. When these young type 2
(YT2) patients attended their routine appointments, a member of staff in the diabetes centre provided written and verbal information about the study and invited them to participate. I then reviewed the clinical notes of the patient to confirm the diagnosis of type 2 diabetes, as outlined in section 2.3. Obese "control" patients with normal glucose tolerance were recruited from the endocrine clinic.

Subjects attended the unit after an overnight fast on two separate occasions within the same week. YT2 subjects taking insulin omitted the basal dose on the night before the studies as well as the dose that morning. Subjects taking metformin stopped this one week before the studies, while subjects taking sulphonylureas stopped these three days beforehand. During the first visit, consent was obtained and a full history and examination performed. Routine bloods were taken for full blood count, renal, liver, bone profile, thyroid function tests and fasting lipids. Waist: hip ratio, weight, height and body mass index were measured. Body composition was assessed using an electrical impedance device. An oral glucose tolerance test was performed, with mathematical modelling applied to measurements taken at thirty minute intervals to estimate insulin sensitivity (OGIS), as outlined in section 2.8.

On the second visit, subjects attended the unit for a four-hour hyperinsulinaemic, euglycaemic clamp study (section 2.9 and appendix 1) for a more detailed assessment of insulin sensitivity. Following this a modified IVGTT was performed to assess first phase beta cell insulin secretion (section
2.10). On a separate day within the same week, subjects attended the Department of Physiology in Trinity College, Dublin where their aerobic fitness (VO2 max) was measured using a bicycle ergometer, as outlined in section 2.12. Laboratory analytical methods are outlined in section 2.11. All data are presented as means with standard error of the mean in parenthesis. All statistical comparisons between controls and young type 2 diabetes subjects used the Mann-Whitney U test. In order to examine potential confounding effects on observed differences between the two groups, multiple linear regression analysis was performed.
4.3: RESULTS:

4.3.1: Clinical Characteristics

Thirteen YT2 subjects and 18 normal glucose tolerant controls were recruited. A summary of the individual characteristics of each of the YT2 subjects is shown in table 4.1. Eleven of these were Caucasian (as were all of the controls). The minimum duration of diabetes was six months. Of note, none of the diabetic subjects had a background of major psychiatric illness or use of psychotropic medications, nor were any on treatment with steroids. In addition to dietary modification and oral antidiabetic agents, three subjects required insulin therapy to control their diabetes. The clinical characteristics of both groups are shown in Table 4.2. They had similar age, body mass index, body fat and physical fitness. There was a higher proportion of males in the YT2 group, which accounted for a non-significant difference in weight, height, body surface area and fat free mass. None of these differences were significant when genders were analysed separately (not shown). The YT2 subjects had a much higher waist circumference, waist to hip ratio and slightly higher systolic blood pressure than the obese controls.

4.3.2: Lipid Profiles

The YT2 group had significantly higher fasting triglycerides than obese controls, as shown in Table 4.3. There were no statistically significant differences in total-, HDL- or LDL- cholesterol between the groups. However there was a tendency towards an adverse lipid profile in the diabetic group in each of these parameters.
<table>
<thead>
<tr>
<th>No.</th>
<th>Age (Years)</th>
<th>Ethnicity</th>
<th>Duration of DM (Years)</th>
<th>Gender</th>
<th>BMI (kgm$^{-2}$)</th>
<th>Treatment</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>15</td>
<td>Caucasian</td>
<td>1</td>
<td>M</td>
<td>35.5</td>
<td>Sulphonylurea</td>
<td>9.5</td>
</tr>
<tr>
<td>02</td>
<td>27</td>
<td>Caucasian</td>
<td>2</td>
<td>F</td>
<td>34.4</td>
<td>Metformin</td>
<td>8.5</td>
</tr>
<tr>
<td>03</td>
<td>22</td>
<td>Caucasian</td>
<td>3</td>
<td>F</td>
<td>31.4</td>
<td>Metformin</td>
<td>10.6</td>
</tr>
<tr>
<td>04</td>
<td>24</td>
<td>Caucasian</td>
<td>5</td>
<td>M</td>
<td>37.7</td>
<td>Metformin + Insulin</td>
<td>9.2</td>
</tr>
<tr>
<td>05</td>
<td>28</td>
<td>Caucasian</td>
<td>3</td>
<td>M</td>
<td>36</td>
<td>Metformin</td>
<td>10.9</td>
</tr>
<tr>
<td>06</td>
<td>26</td>
<td>Caucasian</td>
<td>1</td>
<td>F</td>
<td>39.9</td>
<td>Metformin</td>
<td>7.4</td>
</tr>
<tr>
<td>07</td>
<td>26</td>
<td>Chinese</td>
<td>0.8</td>
<td>M</td>
<td>38.6</td>
<td>Metformin</td>
<td>7.3</td>
</tr>
<tr>
<td>08</td>
<td>28</td>
<td>Caucasian</td>
<td>0.5</td>
<td>F</td>
<td>35.2</td>
<td>Diet</td>
<td>6.2</td>
</tr>
<tr>
<td>09</td>
<td>23</td>
<td>Caucasian</td>
<td>6</td>
<td>M</td>
<td>32.1</td>
<td>Metformin</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>Caucasian</td>
<td>0.5</td>
<td>M</td>
<td>29</td>
<td>Insulin</td>
<td>8.3</td>
</tr>
<tr>
<td>11</td>
<td>31</td>
<td>Indian</td>
<td>2</td>
<td>F</td>
<td>45.4</td>
<td>Diet</td>
<td>8.1</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>Caucasian</td>
<td>2</td>
<td>M</td>
<td>27.5</td>
<td>Insulin</td>
<td>10.6</td>
</tr>
<tr>
<td>13</td>
<td>26</td>
<td>Caucasian</td>
<td>7</td>
<td>M</td>
<td>30.3</td>
<td>Metformin + Sulphonylurea</td>
<td>7.6</td>
</tr>
</tbody>
</table>
Table 4.2: Comparison of clinical data from obese controls and obese type 2 diabetes subjects. Figures in brackets represent the standard error of the mean.

|                               | Control | Type 2 Diabetes (YT2) | P Value  
|-------------------------------|---------|-----------------------|-----------
| N                             | 18      | 13                    |           |
| M:F Ratio                     | 4:14    | 8:5                   |           |
| Mean Age (Years)              | 23.7 (0.9) | 25.8 (1.2) | 0.1024   |
| Weight (Kg)                   | 99.6 (4.4) | 109 (5) | 0.3073   |
| Height (m)                    | 1.68 (0.02) | 1.77 (0.04) | 0.0884   |
| B.M.I. (Kgm⁻²)                | 35.2 (1.4) | 34.9 (1.4) | 0.9521   |
| Systolic BP (mmHg)            | 114.9 (3.3) | 124.9 (3.4) | *0.0452  |
| Diastolic BP (mmHg)           | 72.9 (2.1) | 77.5 (2.5) | 0.1859   |
| Waist circumference (cm)      | 102.1 (2.72) | 117.2 (3.8) | *0.0063  |
| Hip circumference (cm)        | 116.4 (3.3) | 115.3 (2.8) | 0.6208   |
| Waist: Hip Ratio              | 0.88 (0.02) | 1.02 (0.03) | *0.0017  |
## Table 4.3: Comparison of lipid profiles from obese controls and obese type 2 diabetes subjects. Figures in brackets represent the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 2 Diabetes (YT2)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>4.42 (0.2)</td>
<td>4.69 (0.24)</td>
<td>0.3468</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/l)</td>
<td>1.09 (0.07)</td>
<td>0.94 (0.04)</td>
<td>0.1279</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/l)</td>
<td>2.44 (0.17)</td>
<td>2.67 (0.2)</td>
<td>0.2996</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.49 (0.19)</td>
<td>2.9 (0.37)</td>
<td>*0.001</td>
</tr>
</tbody>
</table>
4.3.3: Oral Glucose Tolerance Test

As expected, there were significant differences in glucose levels during the OGGTT between the two groups, as shown in figure 4.1. All of the controls had normal glucose tolerance, as per WHO criteria outlined in section 2.3. Mean fasting glucose was 4.94 ± 0.38 in controls and 9.99 ± 0.44 in the diabetic group (P<0.0001). Mean 2-hour glucose was 6.01 ± 0.5 in controls and 17.27 ± 0.59 in the diabetic group (P<0.0001). The diabetic group tended towards higher fasting insulin levels than controls (84.9 ± 9.9 v 65.7 ± 8.1) but the difference was not statistically significant. Insulin and C-peptide levels were significantly lower in the diabetic group after oral glucose loading, as shown in figures 4.2 and 4.3.

![Figure 4.1: Glucose levels after standard 75g OGGTT](image)
Figure 4.2: Insulin levels after standard 75g OGTT

Figure 4.3: C-Peptide levels after standard 75g OGTT
4.3.4: Oral Glucose Insulin Sensitivity (OGIS)

The glucose and insulin measurements outlined above were used to estimate insulin sensitivity using OGIS, as described in section 2.8. The diabetic subjects were much more insulin resistant (OGIS 265±10 ml.min\(^{-1}\).m\(^{-2}\)) compared with obese controls (OGIS 407±13 ml.min\(^{-1}\).m\(^{-2}\)), \(P<0.0001\). Beta-cell function during the OGTT was markedly impaired in the diabetic group compared to obese controls, as shown by the model-derived data in table 4.4. This modeling allowed a formal quantification of the insulin secretory defect that is apparent in figure 4.2. The "beta cell glucose sensitivity" confirmed a marked defect in insulin secretion relative to absolute glucose concentrations in the diabetic group. Also, the "rate sensitivity" confirmed this defect relative to the rate of change of glucose concentration. These findings were reflected in a lower "potentiation factor ratio" in the diabetic group, demonstrating an attenuated insulin secretory response to glucose. Glucose sensitivity was strongly correlated with mean glucose levels during the OGTT in the whole group (\(r=0.95\) \(P<0.0001\), after log-transformation). The OGTT modeling was performed by Andrea Mari from the Institute of Biomedical Engineering in Padova, Italy.
Table 4.4: Comparison of metabolic data derived from the O.G.T.T. related to beta cell function, between obese controls and obese type 2 diabetes subjects. Figures in brackets represent the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 2 Diabetes (YT2)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta cell glucose sensitivity</td>
<td>187.9 (44.8)</td>
<td>16.6 (3.3)</td>
<td>*&lt;0.0001</td>
</tr>
<tr>
<td>(pmol min⁻¹ m⁻² mM⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate sensitivity</td>
<td>758.9 (165)</td>
<td>175.3 (60.7)</td>
<td>*0.0322</td>
</tr>
<tr>
<td>(pmol m⁻² mM⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentiation factor ratio</td>
<td>1.892 (0.44)</td>
<td>1.117 (0.111)</td>
<td>*0.0192</td>
</tr>
<tr>
<td>Insulin secretion at time 0 OGTT</td>
<td>107.1 (10.5)</td>
<td>141.7 (13.4)</td>
<td>*0.0443</td>
</tr>
<tr>
<td>(pmol min⁻¹ m⁻²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integral of total insulin secretion OGTT (nmol m⁻²)</td>
<td>52.7 (6.9)</td>
<td>30.9 (3.6)</td>
<td>*0.0006</td>
</tr>
<tr>
<td>OGIS (ml min⁻¹ m⁻²)</td>
<td>407 (13)</td>
<td>265 (10)</td>
<td>*&lt;0.0001</td>
</tr>
</tbody>
</table>
4.3.5: Modified Intravenous Glucose Tolerance Test

During the IVGTT, glucose levels were significantly higher in the YT2 group compared to controls, as shown in figure 4.4. They had markedly reduced beta cell response to the glucose bolus compared to controls, with an absence of any significant rise in insulin and C-peptide over eight minutes, as shown in figures 4.5 and 4.6. Both AIR insulin (389 ± 62 vs 29 ± 71 pmol/l) and the equivalent C-peptide measurements (997 ± 126 vs 45 ± 80 pmol/l) were reduced in the diabetic subjects, as was the calculated incremental area of insulin secreted after the intravenous glucose bolus (435 ± 62 vs 19 ± 49 pmol.min⁻¹.m⁻²).

![Figure 4.4: Glucose Response During Modified IVGTT](image-url)
4.3.6: Hyperinsulinaemic Euglycaemic Clamp Studies

At the start of the clamp studies, basal glucose was higher in the diabetic group than in controls (9.9 ± 0.43 v 5.05 ± 0.36 mMol/l, P < 0.0001). The diabetic group also had higher basal insulin concentrations (104 ± 14 v 60 ±
11 pMol/l, \( P = 0.02 \), and higher basal hepatic glucose output \((2.6 \pm 0.21 \text{ v } 1.72 \pm 0.18 \text{ mg min}^{-1} \text{ kg}^{-1}, P = 0.004)\). "Steady state" calculations were made from measurements during the last forty minutes of the insulin infusion, as outlined in section 2.9.1. During each step of the clamp studies, the average tracer: tracee ratio (TTR) was found to be stable. The average coefficient of variation of the TTR in the last forty minutes of the clamp was <6\%, and so use of the steady state equations was appropriate. The individual TTR values at 80, 100 and 120 minutes for each clamp study are shown in appendix 2. While steady state glucose had normalised somewhat in the diabetic group, it remained significantly elevated compared to controls \((6.27 \pm 0.23 \text{ v } 4.84 \pm 0.19 \text{ mMol/l}, P < 0.0001)\). The controls required significantly more glucose to maintain euglycaemia during the steady state phase of the clamp than the diabetic group, as shown in figure 4.7 (glucose infusion rate \(3.89 \pm 0.43 \text{ v } 1.06 \pm 0.5 \text{ mg min}^{-1} \text{ kg}^{-1}, P = 0.0002)\). The diabetic group had a lower glucose disposal rate than controls \((2.15 \pm 0.69 \text{ v } 4.09 \pm 0.5 \text{ mg min}^{-1} \text{ kg}^{-1}, P = 0.03)\). Glucose production was suppressed to a similar degree by the insulin infusion in both groups \((0.66 \pm 0.11 \text{ v } 0.4 \pm 0.08 \text{ mg min}^{-1} \text{ kg}^{-1}, P = 0.07)\).

In order to determine whether the differences in baseline measurements between controls and the diabetic group were confounded by differences in gender, body composition or age, a linear regression model was used to examine correlations (\( \beta \) coefficients) between study outcomes and glucose tolerance status, after adjusting for gender, age and fat-free mass. These
supplementary results are presented in the table in appendix 2, page 164. The findings were similar to those described here. For example, waist circumference was 12.5cm higher in the diabetic subjects. However, there was no significant difference in systolic blood pressure between the groups after adjusting for these confounders, while the diabetic study participants had a statistically significantly lower VO$_2$max and higher triglyceride levels. Some of the observed differences in parameters of $\beta$-cell function (derived from the OGTT) lost statistical significance after adjusting for these confounders.

4.4: DISCUSSION:

Both groups of subjects were well matched for age, degree of obesity and body fatness. There was a preponderance of females in the control group,
which represented the demographic of patients attending the endocrine clinic; results were not affected when genders were analysed separately, except for aerobic fitness which was lower in the diabetic group after adjusting for gender, age and lean mass. A previous population-based study of 380 young healthy Caucasians in Denmark suggested that metabolic profiles were better in women, who had lower fasting glucose and better insulin responses to hyperglycaemia compared to men (Clausen, Borch-Johnsen et al. 1996). This study also noted that oral contraceptive pill use was an important determinant of insulin sensitivity in women. Our own study did not take account of contraceptive use, which is a potential limitation. The Euro Heart Survey (Dotevall, Rosengren et al. 2007) showed that when cardiovascular disease is present, the prevalence of abnormal glucose metabolism is actually higher in women than in men, suggesting that dysglycaemia confers a worse cardiovascular risk burden on women than on men. Smaller physiological studies have examined differences in glucose and fat metabolism between men and women, and have shown that these differences persist even after adjusting for differences in body composition (Mittendorfer, Horowitz et al. 2001). While we have adjusted for gender and body composition in our own analyses, these studies lack adequate statistical power to elucidate gender-based differences in metabolic traits.

The diabetic group had higher systolic blood pressure and triglyceride levels, consistent with increased cardiovascular risk, although after adjusting for age, gender and lean mass, blood pressure was similar in both groups. The
waist: hip ratio, a marker of visceral adiposity, was also significantly higher in the diabetes group compared to controls, illustrating the importance of fat distribution in determining insulin sensitivity.

During the OGTT the diabetic group had higher glucose levels and a markedly impaired insulin secretory response. They were significantly more insulin resistant than obese controls, with lower OGIS values. Results derived from OGTT modeling confirm the classical marked decrease in glucose sensitivity, rate sensitivity and potentiation factor ratio seen in type 2 diabetes. In addition, the typical associations between mean or 2-hour glucose and both beta-cell glucose sensitivity and insulin sensitivity were observed in the diabetic group. However, in our cohort the association with glucose tolerance was stronger for beta cell glucose sensitivity than for insulin sensitivity, suggesting that in this very insulin resistant population the primary factor leading to glucose intolerance is beta cell dysfunction. The modified IVGTT data supported this observation.

The use of deuterated glucose tracer during the hyperinsulinaemic clamp allowed the estimation of whole body and hepatic glucose turnover. This is the first study in a predominantly Caucasian cohort of this size and age to generate such data (86% of the YT2 group were Caucasian). Whole body glucose disposal was dramatically reduced and hepatic glucose production was elevated under fasting conditions in the diabetic group. During the insulin infusion, suppression of hepatic glucose production was similar in both
groups, in contrast to whole body glucose disposal, which was significantly reduced in the diabetic group.

The insulin resistance and beta-cell dysfunction in our diabetic subjects are similar to those described in a larger study which used the same methodology (Ferrannini, Gastaldelli et al. 2005). Using OGTT modelling and clamp data, their study described the progressive loss of beta cell function with rising insulin resistance in a cross sectional cohort spanning the range from normal glucose tolerance through to type 2 diabetes. However, the diabetic subjects in that study were predominantly Mexican-American (76%) with a mean age was 52 years. In addition to the work done previously in our unit using the frequently sampled IVGTT (McQuaid, O'Gorman et al. 2005), others have examined younger subjects with type 2 diabetes. A recent study of a group of sixteen American adolescents (mean age 17.9 years) with type 2 diabetes showed they had significant insulin resistance when compared with non-diabetic subjects of similar obesity and body fatness, and impaired insulin secretion relative to their degree of insulin resistance (Elder, Prigeon et al. 2006). Their study used oral and intravenous glucose tolerance tests, and fewer than half of the subjects were Caucasian. Interestingly, they noted that diabetic subjects retained a first phase insulin response to glucose that was comparable to lean controls, a finding that was not reproduced in our study. This might be because of chronic poor glycaemic control in our subjects who had a mean HbA1c of 8.8%. In the Elder study the mean HbA1c was 6.8%, such that their
participants may have been less prone to the beta cell toxicity associated with chronic hyperglycaemia.

More recently a group reported findings from hyperinsulinaemic euglycaemic clamp studies in six French adolescents with type 2 diabetes and a median age of 15.4 years (Druet, Tubiana-Rufi et al. 2006). These subjects were morbidly obese with a mean BMI SDS of 4.4, and only two of the six were Caucasian. They demonstrated marked insulin resistance and beta cell failure in spite of subjects having very good chronic glycaemic control, with a median HbA1c of 6.0%. There was no control group in that study, and it is possible that many of their diabetic subjects were diagnosed through involvement in a larger study of obese adolescents who underwent OGTT in the same centre (Druet, Dabbas et al. 2006). If this is the case, then it is likely that such “screen detected” cases might have had a less severe metabolic derangement than those presenting with symptoms. Certainly, none of the subjects in our study had been diagnosed with diabetes by screening and all of them had presented with symptomatic hyperglycaemia. Thus, our diabetic subjects may represent a group with worse metabolic derangement than screen detected subjects.

Two possibilities exist - one that early onset type 2 diabetes is identical in pathogenesis and clinical course to type 2 diabetes of later onset, or the second, that type 2 diabetes of early onset is a more extreme phenotype with even greater cardiovascular and lifelong risk of complications than in
older subjects. We have previously shown that patients in our clinic with onset of diabetes under forty years are significantly more obese at an earlier age, have worse cardiovascular risk profiles than their older counterparts, and have worse initial and ongoing glycaemic control, despite exactly similar care (Hatunic, Burns et al. 2005). Clearly early onset diabetes increases the lifelong duration of the disease. The combination of these factors is likely to translate into excess cardiovascular morbidity and mortality in the future. Given the evolving epidemic of type 2 diabetes in young people it is important to prioritise optimal methods for disease prevention. One potential prevention strategy is the use of aerobic exercise, and the next chapter describes the effects of an aerobic exercise programme on parameters of glucose metabolism in a subgroup from the cohort just described.
Chapter Five:

Metabolic Changes after a Supervised Aerobic Exercise Programme in Young Obese Subjects with and without Type 2 Diabetes.
5.1: BACKGROUND:

There is an abundance of evidence from large intervention studies that exercise delays or prevents the development of type 2 diabetes in at risk populations, as outlined in section 1.7 (Helmrich, Ragland et al. 1991; Manson, Rimm et al. 1991; Pan, Li et al. 1997; Tuomilehto, Lindstrom et al. 2001; Knowler, Barrett-Connor et al. 2002). Physiological studies have confirmed the beneficial effects of exercise in improving insulin sensitivity and glucose metabolism (DeFronzo, Sherwin et al. 1987; Hughes, Fiatarone et al. 1993). Such improvements are proportional to the duration and intensity of the exercise regime (Houmard, Tanner et al. 2004), but tend to be short lived (Heath, Gavin et al. 1983; Schneider, Amorosa et al. 1984; Burstein, Polychronakos et al. 1985; Dela, Larsen et al. 1995). This has important implications for designing appropriate exercise intervention programmes that will lead to long term health benefits in participants.

The aim of this study was to examine the effects of a fully supervised three-month aerobic exercise training programme in young obese subjects with type 2 diabetes and to compare their responses to those of normal glucose tolerant controls. We hypothesized that exercise would lead to an improvement in insulin sensitivity in these severely insulin resistant subjects, as well as improved beta cell function. In addition we anticipated an improvement in lipid profiles, BMI and body fat and systolic blood pressure after exercise.
5.2: METHODS:

A subgroup of participants in the baseline studies described in chapter 4 was recruited to the exercise study. This group included all eighteen of the normal glucose tolerant obese controls and nine of the thirteen subjects with type 2 diabetes. The remaining four diabetic subjects agreed to baseline studies only. The exercise programme was conducted entirely at the Metabolic Research Unit gym and involved one hour of exercise training, four times per week for twelve consecutive weeks. Each exercise session was supervised by the exercise physiologist or one of two clinical research fellows, on a rota basis. Subjects completed a five-minute warm-up, followed by sixty minutes of aerobic exercise at 70% VO$_{2\text{max}}$ and a five-minute cool down at each session. Cycle ergometers and treadmills were used, depending on the preference of the participant at each session. Continuous heart rate monitoring was used to ensure an adequate intensity of exercise throughout each session. After the completion of the twelve week intervention, subjects returned to the unit for repeat testing, exactly as outlined in section 4.2. They underwent repeat anthropometric and body composition measurements and OGTT with OGIS. Thereafter they had a repeat clamp study with a modified IVGTT, and repeat VO$_{2\text{max}}$ measurement. In statistical comparisons of the two groups pre- and post-exercise, the Wilcoxon Signed Rank test was used in all cases. In order to determine whether there was a difference between controls and diabetic subjects in their response to exercise, multiple linear regression analysis was
used, with the outcome variable being the relevant post-exercise measurement, adjusted for the pre-exercise value of that variable, gender, pre-exercise fat free mass, the time interval in days between the last exercise session and the clamp study (or OGTT where clamp was not done) and also for control/diabetes group.
5.3: RESULTS:

Of the 18 obese controls who started the exercise programme, 14 completed it. In the YT2 group, 7 of the 9 subjects completed the programme. Thus, completion rates in both groups were very similar. No follow-up measurements were taken in subjects who defaulted from the programme and so no “intention to treat” analysis was performed. Attendance rates for those who completed the study were excellent in both groups; 96% for controls (range 88- 100%) and 91% for YT2 (range 81- 100%). The baseline differences between controls and YT2 were similar to those described in the larger group in chapter 4, such that both groups remained well matched for age, BMI and physical fitness at the start of the exercise programme, with a higher proportion of males in the YT2 group.

The target interval between the last exercise session and the follow-up clamp study was two days. Given the logistical challenges associated with scheduling three separate study visits after completion of the exercise, there was some variation in this interval such that some participants were tested four days after completing the exercise intervention. In the control group, the mean interval between the last exercise session and the clamp study was $2.93 \pm 0.92$ days (range 2-4 days) while in the diabetes group the mean interval was $3.43 \pm 0.98$ days (range 2-4 days). The difference between the groups was not statistically significant ($P=0.25$). Given the importance of this interval in determining the response to exercise, we have adjusted for the time interval in multiple linear regression modeling.
5.3.1: Changes in Clinical Characteristics after Exercise:

There were no statistically significant changes in BMI, total weight or body fat percentage in either group after the twelve week aerobic exercise programme, as shown in table 5.1. Waist circumference was significantly reduced in the YT2 group (115.2 ± 5.5 v 110.7 ± 5.1 cm, P=0.031), with a similar but not significant trend in obese controls (100.8 ± 3.3 vs 96.7 ± 2.9 cm, P=0.075). VO\textsubscript{2} max increased more than 20% in the obese controls (from 2.77 ± 0.24 to 3.36 ± 0.41 ml/min/kg, P<0.0001) but did not improve significantly in the YT2 group (2.48±0.31 to 2.72±0.35 ml/min/kg, P=0.078) as shown in Figure 5.1. There were no exercise induced changes in blood pressure in either group.

![Figure 5.1: Fitness Pre- and Post Exercise](image-url)
Table 5.1: Comparison of clinical data from obese non-diabetic controls and young type 2 diabetes subjects pre and post exercise. Figures in brackets represent the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Controls Pre Ex</th>
<th>Controls Post Ex</th>
<th>P Value</th>
<th>YT2 Pre Ex</th>
<th>YT2 Post Ex</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>14</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M:F Ratio</td>
<td>4:10</td>
<td></td>
<td>3:4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>25.6 (0.9)</td>
<td>26.1 (0.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 (0.03)</td>
<td>1.73 (0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>97.5 (4.65)</td>
<td>95.6 (4.54)</td>
<td>0.07</td>
<td>108.3 (8.7)</td>
<td>109.4 (8.4)</td>
<td>0.438</td>
</tr>
<tr>
<td>B.M.I. (Kgm⁻²)</td>
<td>34.3 (1.44)</td>
<td>33.6 (1.34)</td>
<td>0.104</td>
<td>35.6 (1.23)</td>
<td>36 (1.17)</td>
<td>0.375</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>116.1 (4)</td>
<td>112.8 (4)</td>
<td>0.364</td>
<td>119 (4.69)</td>
<td>121 (4.15)</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>Controls Pre Ex</td>
<td>Controls Post Ex</td>
<td>P Value</td>
<td>YT2 Pre Ex</td>
<td>YT2 Post Ex</td>
<td>P Value</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-----------------</td>
<td>---------</td>
<td>------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.3 (2.4)</td>
<td>69.6 (2.3)</td>
<td>0.316</td>
<td>77 (2.5)</td>
<td>81 (2.3)</td>
<td>0.203</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>100.8 (3.3)</td>
<td>96.7 (2.9)</td>
<td>0.075</td>
<td>115.2 (5.5)</td>
<td>110.7 (5.1)</td>
<td>0.031</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>113.3 (3.4)</td>
<td>107.1 (3)</td>
<td>&lt;0.001</td>
<td>116.6 (4.3)</td>
<td>115.3 (3.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Waist: Hip Ratio</td>
<td>0.89 (0.02)</td>
<td>0.9 (0.02)</td>
<td>0.505</td>
<td>0.99 (0.05)</td>
<td>0.96 (0.03)</td>
<td>0.156</td>
</tr>
<tr>
<td>Fat free mass (Kg)</td>
<td>58.9 (3.3)</td>
<td>58.6 (3.3)</td>
<td>0.237</td>
<td>65.5 (7.2)</td>
<td>66.4 (7.1)</td>
<td>0.063</td>
</tr>
<tr>
<td>Body fat %</td>
<td>39 (2.4)</td>
<td>38.4 (2.3)</td>
<td>0.289</td>
<td>39.9 (3.1)</td>
<td>39.7 (3.1)</td>
<td>0.797</td>
</tr>
<tr>
<td>Fat mass (Kg)</td>
<td>38.2 (3.4)</td>
<td>37 (3.1)</td>
<td>0.188</td>
<td>42.8 (4.4)</td>
<td>43.1 (4.3)</td>
<td>0.813</td>
</tr>
<tr>
<td>Body Surface Area (m²)</td>
<td>2.16 (0.06)</td>
<td>2.14 (0.06)</td>
<td>0.068</td>
<td>2.3 (0.12)</td>
<td>2.32 (0.12)</td>
<td>0.469</td>
</tr>
<tr>
<td>VO₂ max (ml min⁻¹ kg⁻¹)</td>
<td>2.77 (0.24)</td>
<td>3.36 (0.41)</td>
<td>&lt;0.001</td>
<td>2.48 (0.31)</td>
<td>2.72 (0.35)</td>
<td>0.078</td>
</tr>
</tbody>
</table>
Table 5.2: Comparison of lipid profiles from obese non-diabetic controls and young type 2 diabetes subjects pre and post exercise. Figures in brackets represent the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Controls Pre Ex</th>
<th>Controls Post Ex</th>
<th>P Value</th>
<th>YT2 Pre Ex</th>
<th>YT2 Post Ex</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>4.47 (0.26)</td>
<td>4.25 (0.26)</td>
<td>0.078</td>
<td>4.47 (0.3)</td>
<td>4.52 (0.24)</td>
<td>0.438</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/l)</td>
<td>1.12 (0.09)</td>
<td>1.11 (0.06)</td>
<td>0.903</td>
<td>0.96 (0.05)</td>
<td>0.95 (0.05)</td>
<td>0.938</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/l)</td>
<td>2.46 (0.22)</td>
<td>2.83 (0.25)</td>
<td>0.173</td>
<td>2.6 (0.26)</td>
<td>2.88 (0.18)</td>
<td>0.375</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.37 (0.2)</td>
<td>1.19 (0.13)</td>
<td>0.235</td>
<td>2.63 (0.39)</td>
<td>2.26 (0.29)</td>
<td>0.219</td>
</tr>
</tbody>
</table>

5.3.2: Changes in Lipid Profiles after Exercise:
There were no changes in total-, LDL- or HDL- cholesterol or triglyceride levels in either group after the exercise programme, as shown in table 5.2. However in the control group there was a non-significant tendency to reduced total cholesterol. In the YT2 group there were non-significant increases in total- and LDL- cholesterol.

5.3.3: Changes in OGTT after Exercise:
There were no significant changes in glucose levels during the OGTT induced by exercise in either group, as shown in figure 5.2. While there was a non-significant tendency towards lower mean glucose in the control group, the YT2 group tended towards a higher mean glucose after the exercise.
intervention. Similarly, there were no significant changes induced by exercise in insulin and C-peptide levels in either group, as shown in figures 5.3 and 5.4, respectively.
5.3.4: Changes in OGIS after Exercise:

Metabolic data derived from OGTT modelling are shown in table 5.3. All measures of insulin secretion remained unchanged in either group after exercise. Insulin sensitivity, calculated from OGIS did not improve in either group after exercise intervention - in fact there was a slight but statistically significant deterioration in sensitivity in the YI2 group after exercise (417 ± 14 ml min⁻¹ m⁻² v 420 ± 16 in the obese, P=NS; 281 ± 16 v 260 ± 21 in the YI2, P=0.047) as shown in Figure 5.5.
**Table 5.3:** Comparison of metabolic data derived from the OGTT from obese non-diabetic controls and young type 2 diabetes subjects pre and post exercise. Figures in brackets represent the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Controls Pre Ex</th>
<th>Controls Post Ex</th>
<th>P Value</th>
<th>YT2 Pre Ex</th>
<th>YT2 Post Ex</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta cell glucose sensitivity (pmol min⁻¹ m⁻² mM⁻¹)</td>
<td>145.2 (24.7)</td>
<td>128.8 (17.4)</td>
<td>1.0</td>
<td>13.3 (2.9)</td>
<td>21 (7.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Rate sensitivity (pmol m⁻² mM⁻¹)</td>
<td>795.6 (195.4)</td>
<td>1095.9 (267.6)</td>
<td>0.3</td>
<td>241.4 (109)</td>
<td>196 (56.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>Potentiation factor ratio</td>
<td>1.44 (0.14)</td>
<td>1.57 (0.19)</td>
<td>1.0</td>
<td>1.29 (0.18)</td>
<td>1.1 (0.12)</td>
<td>0.58</td>
</tr>
<tr>
<td>Insulin secretion at time 0 OGTT (pmol min⁻¹ m⁻²)</td>
<td>100.1 (12.6)</td>
<td>102.1 (9.5)</td>
<td>0.76</td>
<td>146.4 (15.2)</td>
<td>140.6 (12.4)</td>
<td>0.69</td>
</tr>
<tr>
<td>Integral of total insulin secretion OGTT (nmol m⁻²)</td>
<td>43.4 (2.1)</td>
<td>44.1 (3.1)</td>
<td>0.54</td>
<td>29.3 (2.6)</td>
<td>34.3 (6.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>OGIS (ml min⁻¹ m⁻²)</td>
<td>417 (14)</td>
<td>420 (16)</td>
<td>0.5</td>
<td>281 (16)</td>
<td>260 (21)</td>
<td>0.047</td>
</tr>
</tbody>
</table>
5.3.5: Changes in Modified IVGTT after Exercise:

Data relating to glucose, insulin and C-peptide levels during the IVGTT are shown in Figures 5.6, 5.7 and 5.8, respectively. There were no significant changes in any of these measurements after exercise in either group.

5.3.6: Changes in Hyperinsulinaemic Clamp Studies after Exercise:

The mean glucose infusion rates during the last forty minutes of the clamp studies are shown in Figure 5.9. There was no statistically significant exercise induced change in either group, though there was a tendency to a higher glucose infusion rate in controls and a lower glucose infusion rate in the YT2 group after exercise. Basal hepatic glucose output was not changed in either group at the end of the exercise intervention, as shown in Figure
5.10. Suppression of hepatic glucose output during the clamp remained similar in both groups at baseline and was unchanged after the exercise intervention, as shown in Figure 5.11. Exercise intervention led to no significant change in whole body glucose disposal in either group (4.31±0.71
vs 5.33±0.7 mg/kg/min, P=NS in the obese, and 2.5±0.3 vs 2.57±0.42 mg/kg/min, P=NS in the YT2) as shown in Figure 5.12. None of the findings in the clamp studies were altered when measurements were corrected for fat free mass, as shown in the supplementary data table in appendix 5, page 167.
5.3.7: Differences in Exercise Response Between Controls and Young Type 2 Diabetes Subjects.

Using multiple linear regression analysis, differences in post-exercise measurements between controls and diabetic subjects were examined, after adjusting for potential confounders including age, gender, baseline lean body mass, the baseline measure of the variable of interest and the interval between the last exercise session and the follow-up clamp study. These results are presented in appendix 3 on page 165. Some differences were noted in exercise responses between control and diabetic subjects. For example, BMI was 0.69 kg m\(^{-2}\) lower after exercise in the controls compared to the diabetic subjects, after adjusting for baseline BMI and the other
confounders outlined above. Weight, hip circumference, triglyceride levels, diastolic BP and \( \beta \)-cell function were all significantly better after exercise in the control group, after adjusting for variations in these variables at baseline.

5.4: DISCUSSION:

Aerobic exercise has been shown to be an effective treatment to improve insulin sensitivity in patients with obesity, pre-diabetes and type 2 diabetes (DeFronzo, Sherwin et al. 1987; Hughes, Fiatarone et al. 1993; Houmard, Tanner et al. 2004). The landmark diabetes prevention studies have proven the efficacy of exercise intervention in the prevention of progression from impaired glucose tolerance to diabetes (Pan, Li et al. 1997; Tuomilehto, Lindstrom et al. 2001; Knowler, Barrett-Connor et al. 2002). Previous work from the Metabolic Research Unit demonstrated that just seven days of exercise training led to increased glucose disposal and muscle GLUT 4 protein content in middle aged, obese subjects with type 2 diabetes (mean age 45 years, BMI 36 kg m\(^{-2}\)) (O'Gorman D, Karlsson et al. 2006).

Since early onset type 2 diabetes is characterised by obesity and severe insulin resistance, we chose to investigate the effect of exercise intervention in these subjects. We hypothesised that exercise alone, while maintaining a stable diet, should improve insulin sensitivity. The advantage of the study design was that all exercise sessions took place at our research unit and each
session was supervised by either the exercise physiologist or study physician. The intensity of the exercise was constantly scrutinised using continuous heart rate monitoring, at five minute intervals. In both groups, 78% of subjects completed the exercise study and attendance rates for these during the study were extremely good. We compared measurements only in subjects who completed the study. We were surprised to find that there were essentially no metabolic improvements in either study group at the end of the twelve week programme. Subjects in the obese control group had a 20% increase in VO$_2$max, in contrast to the diabetic group, despite an identical training programme and documented attendance and compliance.

This raises interesting new questions concerning the pathogenesis and treatment of early onset type 2 diabetes in obese young people. Twelve weeks of exercise at the frequency and intensity chosen for this study may simply have been of insufficient duration for the improvements that were expected. However, previous studies including our own in equally obese subjects, have shown improvements in insulin sensitivity and glycaemic control with similar or even lesser exercise regimes in middle-aged and older subjects with obesity and/or type 2 diabetes (DeFronzo, Sherwin et al. 1987; Hughes, Fiatarone et al. 1993). Another question is whether it would have been preferable to combine the exercise regime with a weight losing hypocaloric diet. Subjects in the current study were instructed to maintain a stable diet during the three-month exercise programme, and compliance with this diet regime was good. We chose to study the effect of exercise alone,
not least because it was possible to document accurately exercise compliance, but also because we have found that exercise is an attractive behavioural approach to improving overall lifestyle in these subjects. In terms of understanding the beneficial physiological effects of exercise, a strict dietary regime may have confounded some of the anticipated changes in physiological measurements.

In 2005 Boulé et al published data from the Heritage family study on the effects of exercise training on glucose homeostasis in almost 600 subjects, using a standard IVGTT (Boule, Weisnagel et al. 2005). While they observed a mean increase of 10% in insulin sensitivity after 20 weeks of aerobic training, there were large variations between subjects in their metabolic response to exercise. For example, most subjects in that study showed deterioration in the acute insulin response to glucose. More recently a group in the UK studied the effects of six months aerobic exercise in 22 middle-aged subjects with well controlled diabetes, and found no improvements in physical fitness or insulin sensitivity after their intervention (Middlebrooke, Elston et al. 2006).

Perhaps the exercise modality is important - there is evidence that progressive resistance training confers greater metabolic benefits than aerobic training. One Canadian study of 28 post-menopausal women with well controlled type 2 diabetes compared responses to sixteen weeks of either aerobic exercise alone or in combination with resistance training (Cuff,
Meneilly et al. 2003; Willey and Singh 2003). They found no significant differences in insulin sensitivity (measured with clamp) or abdominal fat distribution (measured with CT) in the group who performed only aerobic exercise. Benefits were seen only in subjects who combined the two exercise modalities. However, most published studies describe the effects of aerobic exercise training.

We may not have been able to demonstrate differences in post-exercise clamp data between the two groups because of underpowering. However, there was a clear trend towards a disimprovement in glucose infusion rates in the diabetic group after exercise, which would not be explained by an inadequate number of study participants. Possibly the difference in basal hepatic glucose output may have reached statistical significance in a larger cohort, but again this would not explain the clear trend towards increased hepatic glucose production during clamp steady state seen in both groups after exercise. Clearly, larger studies would be very valuable in exploring these issues further.

In a previously sedentary cohort of individuals who completed a twelve week exercise programme, during which time they maintained a stable diet and yet did not improve their fitness or lose weight, another possibility exists - that they were less active at other times of the day when they were not exercising. We did not use physical activity questionnaires to measure energy expenditure as they tend to be valid only in much larger,
epidemiological studies. While devices are being developed to measure physical activity continuously over several days (Brage, Brage et al. 2005) we did not use these in our study. Certainly measurement of overall physical activity levels of subjects engaged in an exercise programme would be worthwhile.

More basic physiologic mechanisms might explain the non-response to exercise in these patients. For example, certain diabetes susceptibility genotypes might predispose individuals to respond or not to respond to exercise. It is possible that subjects with early onset Type 2 diabetes (and severe obesity) have either genetic or acquired factors that confer resistance at the level of skeletal muscle to the expected benefits of aerobic exercise training. Further studies will be required to address these questions that are so important for the development of effective treatments for these high-risk patients.
Chapter Six

Conclusions
6.1: General Discussion and Conclusions:

Given the high prevalence of obesity in Irish children and adults, the findings from these studies are cause for concern. Our data suggest that obesity in young children is under-recognised and its consequences are not fully appreciated. We have demonstrated for the first time that obesity in Irish children and adolescents is associated with worse blood pressure elevation and increased metabolic risk. These relatively small studies with preliminary findings will hopefully prompt further studies in larger cohorts of Irish children.

The majority of these obese children are not currently referred for medical investigation. The development of such a service has significant cost implications (HampI, Carroll et al. 2007), and given a total national budget of three million euro to combat obesity in 2005, current resources are inadequate. Furthermore, there is no coherent strategy to confront this evolving epidemic, in spite of the warnings contained within the report from the National Taskforce on Obesity (2005). However, the implementation of childhood obesity screening initiatives has, in some instances, been mired in controversy, poor leadership and ultimately failure (Hawkes 2007). Regardless, recent predictions suggest that one in three American children born in 2000 will develop diabetes (Narayan, Boyle et al. 2003) and Ireland may be following a similar course. The absence of a comprehensive, national screening programme to measure body weight and height in children makes
it difficult to quantify accurately the scale of the obesity problem or target interventions where they will be most effective.

The benefits of interventions to combat obesity and diabetes are not as apparent as they might at first seem. Certainly there is strong epidemiological evidence that preventing weight gain reduces diabetes prevalence: One study suggested that preventing Caucasian adults from becoming overweight would result in a 74% reduction in the incidence of type 2 diabetes (Burke, Williams et al. 2003). While there are clearly proven benefits of lifestyle interventions to reduce diabetes prevalence in at-risk adults, the efficacy of such interventions in children is less well established. A recent systematic review found no evidence for the use of low fat, energy-restricted diets in the treatment of childhood obesity and cited a paucity of well designed studies in this field (Gibson, Peto et al. 2006). Furthermore, a recent large cluster randomised control trial of physical activity to prevent obesity in nursery school children found no reduction in body mass index after twelve months of intervention (Reilly, Kelly et al. 2006).

In older adults, recent diabetes prevention efforts have explored pharmacological interventions in at-risk individuals. Certainly, metformin was shown to reduce diabetes incidence in people with impaired glucose tolerance in the Diabetes Prevention Programme, but was not as effective as lifestyle intervention (Knowler, Barrett-Connor et al. 2002). More recently rosiglitazone was shown to reduce the incidence of diabetes in people at risk
of the disease (Gerstein, Yusuf et al. 2006). However some have argued that the apparent reduction in diabetes incidence was due entirely to the glucose lowering effect of the drug, and that such a prevention strategy might ultimately do more harm than good (Montori, Isley et al. 2007). Furthermore, rosiglitazone has been associated with increased cardiovascular morbidity and mortality (Nissen and Wolski 2007). When drugs are promoted for prevention, and the number of people at risk of the target condition is very large, the expanded exposure to the drug may lead to unacceptably high co-morbidity. Thus, pharmacotherapy and the “medicalisation” of pre-disease states may not be the optimal approach to diabetes prevention in the future, especially in younger populations.

While further studies examining the response to lifestyle intervention in young people are needed, better assessments of the co-morbidities associated with obesity in this group are also required. The merits of comprehensive metabolic screening in obese children were highlighted recently (Jones 2002). Firstly (as we have shown,) obesity in children is not a benign entity but is associated with early pathological changes that ultimately lead to hepatic steatosis, diabetes and vascular disease. Secondly, the pathogenesis of these conditions in children has not been fully elucidated, and the optimal timing of interventions to prevent or reverse these conditions has not been defined. Also, by examining clearly defined subgroups of children (based on specific genetic, environmental or perinatal factors, for example) more subtle metabolic lesions may be uncovered. Such studies
may yield novel insights into the underlying cellular and molecular mechanisms that account for obesity induced morbidity.

Indeed major advances are underway in elucidating the genetic influences on diabetes and obesity risk, as described in chapter one. Genome-wide analysis studies have recently started to identify obesogenic genes, at significantly less cost than was previously thought (Fisler and Warden 2007). An important factor determining the scientific yield from such studies is the degree to which participants are phenotypically characterised. Thus there is scope for future overlap between detailed phenotyping studies such as ours and high throughput genotyping. It is plausible that genetic variants which determine an individual’s response to certain therapies will soon be identified and herald the era of “pharmacogenomics”. Studies of specific gene interactions with diet, exercise and other lifestyle factors in the control of intermediary metabolism would undoubtedly improve our understanding of the pathophysiology of obesity and diabetes. They may account for some of the interindividual variation in response to exercise noted in our study, for example. Significant progress in the elucidation of environmental determinants of disease risk continues to be made. For example it has been recently suggested that gut bacteria are important determinants of body weight, due to altered metabolism of otherwise indigestible polysaccharides in the distal gut (Turnbaugh, Ley et al. 2006). This discovery offers a fascinating insight into the pathogenesis of obesity and may lead to novel therapeutic interventions to reduce body weight.
Our detailed metabolic characterisations of young adults with type 2 diabetes yield especially worrying results. We have confirmed they are severely insulin resistant using the clamp technique. We confirmed marked beta cell failure in the diabetic group which may have reflected the effect of glucose toxicity, given the overall poor glycaemic control in the group. Other studies suggest some preservation of beta cell function in well controlled type 2 diabetic populations of this age. However our subjects were representative of typical young type 2 diabetes patients in our clinic. These patients have been shown to carry an increased cardiovascular risk burden, in spite of intensive treatment.

The fact that this group appears to be very resistant to the metabolic benefits of exercise is a key new finding given the fact that compliance with the intervention was excellent. It may be that early onset obesity and type 2 diabetes shifts the metabolic equilibrium beyond the reach of activity-based improvement. This casts doubt on the assumption that an exercise training protocol can improve insulin sensitivity in every metabolic situation. It is also possible that this cohort would have responded better had a more aggressive training protocol been followed. Clearly our findings should not detract from the overwhelming evidence that exercise has important benefits in the prevention and management of diabetes. Indeed a meta-analysis of the metabolic effects of exercise in adults with prevalent type 2 diabetes found that interventions which focussed only on exercise were more successful in improving metabolic parameters than those focussing on
multiple health behaviours (Conn, Hafdahl et al. 2007). Conversely a systematic review of prevention strategies in those with pre-diabetes found that exercise alone only had "equivocal" effects on outcome, in the absence of dietary intervention (Yates, Khunti et al. 2007). Regardless, as the prevalence of type 2 diabetes in youth increases (refractory to intensive treatment and lifestyle intervention) an enormous burden will be placed on health care resources and on the patients themselves.

Recent estimates suggest that the direct cost of diabetes to the healthcare system in Ireland is very significant: In 1999-2000, the annual total healthcare costs were 377.2 million euro for diagnosed diabetes, and 580.2 million for both diagnosed and undiagnosed diabetes (Nolan, O'Halloran et al. 2006). Most of these costs arose from treating the complications of the disease. Clearly these costs will escalate dramatically in the coming years. Notwithstanding the absence of any coordinated strategy to deal with the diabesity epidemic, there is a growing awareness that preventative strategies require urgent investment and public support and also that care for individuals with diabetes mellitus needs to be better resourced. The four-pronged approach advocated in a recent American Diabetes Association presidential address succinctly defines how we should tackle the increasing burden of diabetes: to invest in research, to adopt a chronic care model for the treatment of diabetes, to focus on the development of affordable and effective prevention strategies, and to control and prevent obesity (Cherrington 2006). We must all rise to this challenge.
Appendices.
Appendix 1:

Protocol for Hyperinsulinaemic-Euglycaemic Clamp Study and Modified IVGTT.

Peripheral and hepatic insulin sensitivity are both measured by the hyperinsulinaemic- euglycaemic clamp technique while insulin secretion is evaluated using the modified IVGTT. Determination of first phase insulin secretion is highly informative because it allows the detection of one of the earliest defects in beta cell function. (Insulin secretion is impaired in the offspring of those with diabetes and in impaired glucose tolerant individuals) and the IVGTT shows a strong correlation with other indices of insulin secretion.

Materials

- Clamp CRF and graph paper to plot record of clamp stability and level of euglycaemia
- Laptop with excel spreadsheet
- Infusion pump for insulin administration (infusion range: 15-60 ml/h)
- Infusion pump for 20% glucose solution (range of infusion rate 1-500 ml/h) with the possibility to make changes as small as 1 ml/h
- Pump for infusion of [6-6^2H_2]-glucose tracer
- YSI® STAT 2300 Glucose oxidase-based analyser (automatically calibrated every fifteen minutes for continuous glucose monitoring)
- Electric heat pad
- Clock for timing clamp
**Consumables**

3 x infusion administration set  
10 Blue cap aliquots  
2 Neutral cap aliquots  
Cotton wool  
Extension tubing  
1, 2, 10 and 20ml syringes

3 x cannulae  
3 x 3-way stopcocks  
sterets (alcohol)  
gauze (non-sterile)  
needles  
50ml syringes (for driver)

**Solutions for infusion**

Intravenous glucose 20%  
Intravenous glucose 50%  
Regular insulin  
[6-6²H₂]-glucose solution (10g/100ml 0.9% saline)  
0.9% sodium chloride (normal saline)

- 500ml bags  
- 50ml vials  
- Actrapid 100 u/ml  
- 100ml bags

**Methods**

1. Start exam at 8am, with subject fasting for 12 hours (i.e. no caloric or caffeinated food, no nicotine). Subject should not travel to the visit by bicycle or have taken part in heavy or unusual physical activity for the two days preceding study.

2. Subject should void urine before starting the clamp and then remain on the bed for the four hour duration of the clamp study. Tanita® body composition analysis should be performed to measure the fat free mass.

3. YSI® STAT 2300 glucose analyzer should be started and calibrated. The heating pad should be applied to the right forearm, to warm the hand and thus “arterialise” the peripheral venous blood.
Deuterated [6-6²H₂]-glucose infusion – (at Time -120 min before hyperinsulinaemic clamp operation)

4. Cannulate the antecubital vein in the right arm for infusion of [6-6²H₂]-glucose during the run-in phase of the study. Note at times -120, 0, +80, +100 and +120 minutes blood samples must be taken for deuterated tracer, glucose, insulin and C-peptide.

[6-6²H₂]-glucose infusion format:

Time -120 min: Give [6-6²H₂]-glucose bolus (4mg/kg lean body mass).

From -120 min to +15 min give constant infusion of [6-6²H₂]-glucose (in 50 ml 0.9% NaCl) at rate of 0.04 mg/min/kg lean body mass. This infusion stopped at time +15 min. This 0.9% NaCl based solution is termed NINF.

Formula to calculate lean body mass (if measured lean body mass not available from Tanita®)

Female = 0.296 x weight (kg) + 0.418 x height (cm) - 43.3
Male = 0.328 x weight (kg) + 0.339 x height (cm) - 29.5

[6-6²H₂]-glucose added to 20% glucose solution (to make up to a final concentration of [6-6²H₂]1.6% glucose/glucose) to make up an enriched glucose solution (GINF). (In 500 ml of 20% glucose there are 100g glucose; thus, 1.6g [6-6²H₂]-glucose should be added to 500 ml of glucose 20% solution).

REMEMBER - Take 1ml sample of the enriched 20% glucose infusion (GINF) and 1ml sample of the 0.9% NaCl solution (NINF) used for the constant infusion for later analysis. Label both samples with initials, NINF/GINF, and date store in neutral top aliquots at -80°C.
Euglycaemic clamp phase

5. Draw 6 ml 0.9% NaCl from 100ml bag for insulin infusion. Add 6 ml of subject's own blood to 100 ml bag and mix well. This prevents insulin adhering to the plastic tubing during the infusion.

6. Add to this bag the required dose of insulin, according to body surface area (40mU.min^{-1}.m^{-2}).

<table>
<thead>
<tr>
<th>i) calculate body surface area (BSA) in m^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ S = W^{0.425} \times H^{0.725} \times 71.84 \times 10^{-4} ]</td>
</tr>
<tr>
<td>S=body surface area (cm^2), W=weight (kg), H=height (cm)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ii) calculate for amount of insulin to be added to solution e.g.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ = \frac{BSA \times 2.4}{\text{constant infusion rate (15 ml/h)}} \times \text{volume (e.g. 100 ml)} ]</td>
</tr>
<tr>
<td>(~20-30 units of insulin to add to 100 ml of 0.9% saline)</td>
</tr>
</tbody>
</table>

7. Connect the bag containing saline, insulin and blood to the relevant infusion line and place in the pump.

8. Cannulate right wrist or hand vein retrogradely for blood sampling (if not possible then the cephalic vein near wrist will suffice). **Ensure hand is kept warm throughout study with electric heating pad.**

9. Withdraw at least 3 arterialised blood samples from wrist cannula for baseline mean plasma glucose measurement and note on CRF.
**Determination of clamp level**

If mean fasting plasma glucose value is 4.5-5.5 mMol/l then this will be the target clamp glucose level.

If fasting glucose is > 5.5 mMol/l, clamp level will be 5.5 mMol/l (100mg/dl).

If fasting glucose < 4.5 mMol/l, clamp level will be 4.5 mMol/l (80 mg/dl).

10. Attach glucose and insulin infusion apparatus to a line in the left antecubital vein using a 3-way stopcock.

11. Set clock to start timing clamp at time 0 (*deuterated tracer was started at minus 120 minutes*).

12. Start insulin infusion:

   (constant infusion rate = 15 ml per hour):- (40mU.min⁻¹.m⁻²)
   i) 4 x constant infusion (i.e. 60 ml/h) time 0-4 min
   ii) 2 x constant infusion (i.e. 30 ml/h) time 4-7 min
   iii) constant infusion (15 ml/h) time 7-120 min

13. Measure arterialised plasma glucose every 5 min in order to adjust glucose infusion (*write all values on CRF*) (start measuring plasma glucose at 2.5 minutes).

14. Start glucose infusion:

   • If fasting glucose < 5.5 mMol/l start glucose infusion at *time 5min*
   • If fasting glucose > 5.5 mMol/l (100 mg/dl), await glucose reduction to 5.5 mMol/l before starting glucose infusion
15. Convert mg of glucose per kg body weight per min to corresponding pump infusion rate (considering 20% glucose solution = 200 mg/ml) - keep continuous record of blood glucose determinations and changes in glucose infusion using the Excel spreadsheet.

<table>
<thead>
<tr>
<th>Example</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 kg subject = 55 mg/min = 3,300 mg/h</td>
<td>divide by mg of glucose per ml to give hourly infusion rate</td>
</tr>
<tr>
<td>(e.g. for 20% solution, divide by 200 = 16.5 ml/h)</td>
<td>$\rightarrow$ 2 mg/kg/min = 33 ml/h</td>
</tr>
<tr>
<td>$\rightarrow$ 3 mg/kg/min = 49.5 ml/h</td>
<td></td>
</tr>
</tbody>
</table>

16. The steady-state period (for calculations of insulin sensitivity) is measured from 80 to 120 minutes. (Mean plasma glucose should remain within ± 15% of target clamp glucose level).

17. The clamp study finishes at 120 minutes - stop the clock. Maintain glucose and insulin infusions at constant rates until after modified IVGTT completed.

**Modified IVGTT:**

18. Prepare to give i.v. bolus of 50% glucose solution (0.3 g per kg of body weight) over one minute into the right antecubital vein. The subject may experience a sensation of 1) warmth; 2) a metallic taste in mouth; 3) flushing or 4) nausea during or immediately after the injection the glucose bolus. Patient must be advised of this before giving the bolus.
19. Re-start clock precisely at the beginning of the injection. The bolus should be administered in less than 20 seconds, followed by a 10 ml saline flush.

20. Take blood samples for glucose, insulin and C-peptide **precisely** at +2 min, +4 min, +6 min and +8 min (after the *start* of the glucose bolus).

21. When all samples are taken, stop the insulin infusion but continue with the glucose infusion. Reduce the glucose infusion rate gradually over 30 minutes during which time the participant has something to eat.

22. Blood glucose concentrations must be monitored carefully until the participant is well enough to go home. Glucose levels should be >5.0 mMol/l before discontinuing the glucose infusion.

**What can go wrong during clamp:**

**Hypoglycaemia**
- *In case of severe hypoglycaemia* - **stop study**
- *In case of mild hypoglycaemia* - stop insulin, restore plasma glucose to baseline value and after 30 minutes of stable glucose levels, restart the study.

**Difficult cannulation**
- Two veins can be used instead of three. "Piggyback" glucose and insulin infusions with the tracer infusion using an additional three way tap.
Summary of Samples for Hyperinsulinaemic- Euglycaemic Clamp:

<table>
<thead>
<tr>
<th>Time</th>
<th>Samples</th>
<th>Bottles</th>
<th>Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>-120</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep [6-6^2H_2] glucose</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K2 EDTA K2</td>
<td>1 vacuette</td>
</tr>
<tr>
<td>0</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep [6-6^2H_2] glucose</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K2 EDTA K2</td>
<td>1 vacuette</td>
</tr>
<tr>
<td>80</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep [6-6^2H_2] glucose</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K2 EDTA K2</td>
<td>1 vacuette</td>
</tr>
<tr>
<td>100</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep [6-6^2H_2] glucose</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K2 EDTA K2</td>
<td>1 vacuette</td>
</tr>
<tr>
<td>120</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep [6-6^2H_2] glucose</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K2 EDTA K2</td>
<td>1 vacuette</td>
</tr>
<tr>
<td>+2</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td>+4</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td>+6</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
<tr>
<td>+8</td>
<td>Glucose</td>
<td>Fluoride / EDTA</td>
<td>1 vacuette</td>
</tr>
<tr>
<td></td>
<td>Insulin / C-Pep</td>
<td>Lithium heparin</td>
<td>2 vacuettes</td>
</tr>
</tbody>
</table>
## Appendix 2: Baseline Differences between Control and Young Type 2 Diabetes Subjects, Adjusted for Age, Gender and Fat-Free Mass.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β- Coefficient</th>
<th>Standard Error</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg m⁻²)</td>
<td>-1.82</td>
<td>1.84</td>
<td>0.33</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>-7.61</td>
<td>5.31</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (m)</td>
<td>-0.017</td>
<td>0.019</td>
<td>0.39</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>-4.5</td>
<td>2.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>-12.5</td>
<td>4.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>-2.1</td>
<td>4</td>
<td>0.58</td>
</tr>
<tr>
<td>Waist: Hip Ratio</td>
<td>-0.091</td>
<td>0.03</td>
<td>0.0058</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-1.7</td>
<td>4.1</td>
<td><strong>0.68</strong></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>-4.4</td>
<td>3.9</td>
<td>0.26</td>
</tr>
<tr>
<td>VO₂max (ml min⁻¹kg⁻¹)</td>
<td>0.66</td>
<td>0.18</td>
<td><strong>0.0012</strong></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>-0.27</td>
<td>0.34</td>
<td>0.43</td>
</tr>
<tr>
<td>HDL Chol (mmol/l)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL Chol (mmol/l)</td>
<td>-0.34</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>-1.16</td>
<td>0.4</td>
<td>0.0069</td>
</tr>
<tr>
<td>β- Cell Glucose Sens.</td>
<td>178.6</td>
<td>61.4</td>
<td>0.0073</td>
</tr>
<tr>
<td>Potentiation Factor Ratio</td>
<td>-0.16</td>
<td>0.089</td>
<td><strong>0.079</strong></td>
</tr>
<tr>
<td>Insulin Secretion Time 0</td>
<td>-149.5</td>
<td>76.9</td>
<td><strong>0.063</strong></td>
</tr>
<tr>
<td>Integral of Tot Ins Secr.</td>
<td>14.9</td>
<td>10.2</td>
<td><strong>0.15</strong></td>
</tr>
<tr>
<td>OGIS (ml min⁻¹ m⁻²)</td>
<td>149.8</td>
<td>20.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Basal HGO (mg kg⁻¹min⁻¹)</td>
<td>-0.64</td>
<td>0.26</td>
<td>0.0099</td>
</tr>
<tr>
<td>Steady State HGO</td>
<td>-0.3</td>
<td>0.16</td>
<td>0.074</td>
</tr>
<tr>
<td>Gluc Disp (mg kg⁻¹min⁻¹)</td>
<td>3.22</td>
<td>0.94</td>
<td>0.0028</td>
</tr>
<tr>
<td>AIR Insulin (pmol/l)</td>
<td>453</td>
<td>129</td>
<td>0.0027</td>
</tr>
<tr>
<td>AIR C-Peptide (pmol/l)</td>
<td>1060</td>
<td>218</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inc. area Insulin Secretion</td>
<td>420.2</td>
<td>105.1</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

* Adjusted for age, gender and fat free mass. Highlighted results are different compared to unadjusted analysis described in chapter 4.
**Appendix 3:** Adjusted Differences in Response to Exercise between Control and Young Type 2 Diabetes Subjects.

<table>
<thead>
<tr>
<th>Variable *</th>
<th>β- Coefficient</th>
<th>Standard Error</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg m^2)</td>
<td>-1.4</td>
<td>0.61</td>
<td>0.046</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>-3.84</td>
<td>1.81</td>
<td>0.05</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>-0.6</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>-2.7</td>
<td>4</td>
<td>0.52</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>-4.4</td>
<td>1.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Waist: Hip Ratio</td>
<td>-0.026</td>
<td>0.028</td>
<td>0.35</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-6.3</td>
<td>5.4</td>
<td>0.27</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>-8.1</td>
<td>3.2</td>
<td>0.025</td>
</tr>
<tr>
<td>VO2max (ml min^-1kg^-1)</td>
<td>0.13</td>
<td>0.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>-0.16</td>
<td>0.2</td>
<td>0.42</td>
</tr>
<tr>
<td>HDL Chol (mmol/l)</td>
<td>0.08</td>
<td>0.08</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL Chol (mmol/l)</td>
<td>-0.07</td>
<td>0.34</td>
<td>0.83</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>-0.64</td>
<td>0.28</td>
<td>0.035</td>
</tr>
<tr>
<td>β- Cell Glucose Sens.</td>
<td>97.4</td>
<td>54.7</td>
<td>0.097</td>
</tr>
<tr>
<td>Potentiation Factor Ratio</td>
<td>-0.15</td>
<td>0.11</td>
<td>0.22</td>
</tr>
<tr>
<td>Insulin Secretion T=0</td>
<td>-12.2</td>
<td>12.8</td>
<td>0.36</td>
</tr>
<tr>
<td>Integral of Tot Ins Secr.</td>
<td>6.5</td>
<td>6.8</td>
<td>0.36</td>
</tr>
<tr>
<td>OGIS (ml min^-1 m^-2)</td>
<td>60.5</td>
<td>39.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Basal HGO (mg kg^-1min^-1)</td>
<td>-0.034</td>
<td>0.82</td>
<td>0.97</td>
</tr>
<tr>
<td>Steady State HGO</td>
<td>0.7</td>
<td>0.72</td>
<td>0.38</td>
</tr>
<tr>
<td>Gluc Disp (mg kg^-1min^-1)</td>
<td>1.52</td>
<td>2.04</td>
<td>0.48</td>
</tr>
<tr>
<td>AIR Insulin (pmol/l)</td>
<td>466.9</td>
<td>306.7</td>
<td>0.18</td>
</tr>
<tr>
<td>AIR C-Peptide (pmol/l)</td>
<td>591.7</td>
<td>268</td>
<td>0.058</td>
</tr>
<tr>
<td>Inc. area Insulin Secretion</td>
<td>335.6</td>
<td>106</td>
<td>0.0089</td>
</tr>
</tbody>
</table>

* Adjusted for age, gender, fat free mass, baseline measurement of variable (pre-exercise) and time interval between exercise and clamp. Highlighted results are different compared to unadjusted analysis described in chapter 5.
# Appendix 4:

## Raw tracer: tracee ratios (TTR) at three time points during steady state for each clamp study.

<table>
<thead>
<tr>
<th>Clamp Number</th>
<th>TTR at 80 min</th>
<th>TTR at 100 min</th>
<th>TTR at 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>1.85%</td>
<td>1.86%</td>
<td>1.89%</td>
</tr>
<tr>
<td>Two</td>
<td>1.81%</td>
<td>1.76%</td>
<td>1.79%</td>
</tr>
<tr>
<td>Three</td>
<td>0.61%</td>
<td>0.73%</td>
<td>0.77%</td>
</tr>
<tr>
<td>Four</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
</tr>
<tr>
<td>Five</td>
<td>1.30%</td>
<td>1.28%</td>
<td>1.27%</td>
</tr>
<tr>
<td>Six</td>
<td>1.54%</td>
<td>1.61%</td>
<td>1.74%</td>
</tr>
<tr>
<td>Seven</td>
<td>3.05%</td>
<td>3.10%</td>
<td>2.98%</td>
</tr>
<tr>
<td>Eight</td>
<td>1.67%</td>
<td>1.70%</td>
<td>1.75%</td>
</tr>
<tr>
<td>Nine</td>
<td>1.41%</td>
<td>1.43%</td>
<td>1.44%</td>
</tr>
<tr>
<td>Ten</td>
<td>1.41%</td>
<td>1.56%</td>
<td>1.55%</td>
</tr>
<tr>
<td>Eleven</td>
<td>0.73%</td>
<td>0.69%</td>
<td>0.57%</td>
</tr>
<tr>
<td>Twelve</td>
<td>1.07%</td>
<td>1.09%</td>
<td>1.10%</td>
</tr>
<tr>
<td>Thirteen</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
</tr>
<tr>
<td>Fourteen</td>
<td>0.36%</td>
<td>0.35%</td>
<td>0.33%</td>
</tr>
<tr>
<td>Fifteen</td>
<td>1.52%</td>
<td>1.55%</td>
<td>1.63%</td>
</tr>
<tr>
<td>Sixteen</td>
<td>2.19%</td>
<td>2.16%</td>
<td>2.17%</td>
</tr>
<tr>
<td>Seventeen</td>
<td>Sample error</td>
<td>Sample error</td>
<td>1.46%</td>
</tr>
<tr>
<td>Eighteen</td>
<td>1.91%</td>
<td>2.13%</td>
<td>2.24%</td>
</tr>
<tr>
<td>Nineteen</td>
<td>1.80%</td>
<td>1.71%</td>
<td>1.78%</td>
</tr>
<tr>
<td>Twenty</td>
<td>2.11%</td>
<td>1.88%</td>
<td>1.86%</td>
</tr>
<tr>
<td>Twenty-one</td>
<td>0.73%</td>
<td>0.84%</td>
<td>0.88%</td>
</tr>
<tr>
<td>Twenty-two</td>
<td>1.65%</td>
<td>1.65%</td>
<td>1.54%</td>
</tr>
<tr>
<td>Twenty-three</td>
<td>1.59%</td>
<td>1.65%</td>
<td>1.66%</td>
</tr>
<tr>
<td>Twenty-four</td>
<td>1.86%</td>
<td>1.85%</td>
<td>1.91%</td>
</tr>
<tr>
<td>Twenty-five</td>
<td>1.12%</td>
<td>1.20%</td>
<td>1.14%</td>
</tr>
<tr>
<td>Twenty-six</td>
<td>1.10%</td>
<td>1.15%</td>
<td>1.22%</td>
</tr>
<tr>
<td>Twenty-seven</td>
<td>0.57%</td>
<td>0.59%</td>
<td>0.67%</td>
</tr>
<tr>
<td>Twenty-eight</td>
<td>2.04%</td>
<td>2.01%</td>
<td>2.03%</td>
</tr>
<tr>
<td>Twenty-nine</td>
<td>2.28%</td>
<td>2.23%</td>
<td>2.27%</td>
</tr>
<tr>
<td>Thirty</td>
<td>1.50%</td>
<td>1.50%</td>
<td>1.54%</td>
</tr>
<tr>
<td>Thirty-one</td>
<td>0.99%</td>
<td>1.18%</td>
<td>1.10%</td>
</tr>
<tr>
<td>Thirty-two</td>
<td>1.35%</td>
<td>1.37%</td>
<td>1.36%</td>
</tr>
<tr>
<td>Thirty-three</td>
<td>0.82%</td>
<td>0.86%</td>
<td>0.79%</td>
</tr>
<tr>
<td>Thirty-four</td>
<td>3.11%</td>
<td>3.02%</td>
<td>Sample error</td>
</tr>
<tr>
<td>Thirty-five</td>
<td>1.82%</td>
<td>1.55%</td>
<td>1.79%</td>
</tr>
<tr>
<td>Thirty-six</td>
<td>1.07%</td>
<td>1.04%</td>
<td>1.10%</td>
</tr>
<tr>
<td>Thirty-seven</td>
<td>0.63%</td>
<td>0.59%</td>
<td>0.70%</td>
</tr>
<tr>
<td>Thirty-eight</td>
<td>1.32%</td>
<td>1.27%</td>
<td>1.45%</td>
</tr>
<tr>
<td>Forty-nine</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
</tr>
<tr>
<td>Forty</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
</tr>
<tr>
<td>Forty-one</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
<td>No tracer infused</td>
</tr>
</tbody>
</table>
**Appendix 5:** Clamp measurements relative to whole body weight and corrected for fat-free mass. Figures represent the mean (±SD).

<table>
<thead>
<tr>
<th>Clamp Variable:</th>
<th>Control Pre</th>
<th>Control Post</th>
<th>P Value</th>
<th>YT2 Pre</th>
<th>YT2 Post</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state glucose infusion (clamp) mg min⁻¹ kg⁻¹</td>
<td>4.22 (0.6)</td>
<td>4.8 (0.58)</td>
<td>0.51</td>
<td>1.39 (0.3)</td>
<td>0.92 (0.38)</td>
<td>0.625</td>
</tr>
<tr>
<td>Steady state glucose infusion (clamp) mg min⁻¹ kg⁻¹ (FFM)⁻¹</td>
<td>6.62 (0.78)</td>
<td>7.4 (0.81)</td>
<td>0.492</td>
<td>2.36 (0.56)</td>
<td>1.56 (0.65)</td>
<td>0.25</td>
</tr>
<tr>
<td>Steady state glucose disappearance (clamp) mg min⁻¹ kg⁻¹</td>
<td>4.31 (0.71)</td>
<td>5.33 (0.7)</td>
<td>0.322</td>
<td>2.5 (0.3)</td>
<td>2.57 (0.42)</td>
<td>0.75</td>
</tr>
<tr>
<td>Steady state glucose disappearance (clamp) mg min⁻¹ kg⁻¹ (FFM)⁻¹</td>
<td>6.7 (0.94)</td>
<td>8.16 (0.98)</td>
<td>0.375</td>
<td>4.29 (0.71)</td>
<td>4.51 (1.05)</td>
<td>1.0</td>
</tr>
<tr>
<td>Basal glucose production (clamp) mg min⁻¹ kg⁻¹</td>
<td>1.65 (0.07)</td>
<td>1.52 (0.18)</td>
<td>1.0</td>
<td>2.09 (0.14)</td>
<td>1.92 (0.14)</td>
<td>0.625</td>
</tr>
<tr>
<td>Basal glucose production (clamp) mg min⁻¹ kg⁻¹ (FFM)⁻¹</td>
<td>2.68 (0.13)</td>
<td>2.64 (1.14)</td>
<td>0.846</td>
<td>3.57 (0.34)</td>
<td>3.26 (0.4)</td>
<td>0.313</td>
</tr>
<tr>
<td>Steady state glucose production (clamp) mg min⁻¹ kg⁻¹</td>
<td>0.284 (0.044)</td>
<td>0.383 (0.105)</td>
<td>0.426</td>
<td>0.8 (0.19)</td>
<td>1.03 (0.44)</td>
<td>1.0</td>
</tr>
<tr>
<td>Steady state glucose production (clamp) mg min⁻¹ kg⁻¹ (FFM)⁻¹</td>
<td>0.48 (0.08)</td>
<td>0.58 (0.14)</td>
<td>0.426</td>
<td>1.41 (0.39)</td>
<td>1.9 (0.86)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
References

(2005). Obesity - The policy challenges. The report of the National Taskforce on Obesity, Department of Health and Children, Dublin, Ireland.

(2007). "Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls." Nature 447(7145): 661-78.


NHLS (2003). National Health and Lifestyle Surveys. Dublin, Health Promotion Unit

Department of Health and Children.


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degree in both groups during the clamp. Both groups were insulin-resistant; however glucose disposal was markedly reduced in the type 2 diabetic subjects (2.15±0.42 mg kg⁻¹ min⁻¹) compared with obese control subjects (4.09±0.58 mg kg⁻¹ min⁻¹; p<0.03).

OGTT and IVGTT Insulin sensitivity was also calculated from the OGTT, using oral glucose insulin sensitivity (OGIS). The type 2 diabetic subjects were much more insulin-resistant (OGIS 265±10 ml min⁻¹ m⁻²) than obese control subjects (OGIS 407±13 ml min⁻¹ m⁻²; p<0.0001). Beta cell function, as assessed by OGTT modelling, was markedly impaired in type 2 diabetic subjects compared with the control group (Table 1). Glucose sensitivity, rate sensitivity and the potentiation factor ratio were all significantly reduced in type 2 diabetic subjects. Glucose sensitivity was strongly correlated with mean glucose levels during the OGTT in the whole group (r=0.95 p<0.0001, after log-transformation). Similar results were obtained with 2-h glucose.

In the IVGTT, the type 2 diabetic subjects subjects had markedly reduced beta cell response to glucose. Both acute insulin response (389±62 vs 29±71 pmol/l) and the equivalent C-peptide measurements (997±126 vs 45±80 pmol/l) were reduced in the type 2 diabetic subjects subjects, as well as the incremental area of insulin secreted after the glucose bolus (435±62 vs 19±49 pmol min⁻¹ m⁻²).

Effects of exercise intervention

The exercise intervention was completed by 14 of the obese control subjects and seven of the diabetic subjects.

Clinical and laboratory measurements Table 2 shows that there was no change in body weight, BMI, blood pressure or percent body fat in either group at the end of the 3-month exercise programme. Waist circumference was reduced in the type 2 diabetic subjects (115.2±5.5 vs 110.7±5.1 cm, p=0.031), with a similar but not significant trend in control subjects (100.8±3.3 vs 96.7±2.9 cm, p=0.075). VO₂max increased more than 20% in the obese control subjects (from 2.77±0.24 to 3.36±0.41 ml min⁻¹ kg⁻¹ p<0.0001), but did not improve significantly in the type 2 diabetic subjects (2.48±0.31 to 2.72±0.35 ml min⁻¹ kg⁻¹, p=0.078) (Fig. 1a). Change in VO₂max in the obese group was positively correlated with change in glucose disposal (r=0.55, p=0.05). Mean glucose levels during the OGTT did not change in either group after exercise. There were no significant changes in fasting lipids. Fasting concentrations of NEFA tended to be higher at baseline in the type 2 diabetic subjects, although this did not reach significance. However, after completion of the exercise, NEFA were significantly lower in the control group than in the type 2 diabetic group, in whom exercise induced no change (Fig. 1b).

Hyperinsulinaemic–euglycaemic clamp studies Basal hepatic glucose output was not changed in either group at the end of the exercise intervention (Fig. 1c). Suppression of hepatic glucose output during the clamp remained similar in both groups and was unchanged after the exercise intervention (Fig. 1d). Exercise intervention led to no significant change in whole-body glucose disposal in either group (4.31±0.71 vs 5.33±0.7 mg kg⁻¹ min⁻¹ in the control, 2.5±0.3 vs 2.57±0.42 mg kg⁻¹ min⁻¹ in the type 2 diabetic subjects, p=NS) (Fig. 1e).

OGTT and IVGTT Insulin sensitivity calculated from OGIS did not improve in either group after exercise intervention (417±14 vs 420±16 in the control group, p=NS; 281±16 vs 260±21 in the type 2 diabetic group, p=0.05) (Fig. 1f). All measures of insulin secretion remained unchanged in both groups after exercise.

Discussion

Marked visceral obesity and extreme insulin resistance are associated with early-onset type 2 diabetes. This form of diabetes is a growing public health problem. While visceral obesity is an important risk factor, the natural history of this condition in younger people is not known. Young patients with type 2 diabetes have well established loss of beta cell insulin secretion at the time of clinical presentation [7]. Two possibilities arise: (1) early-onset type 2 diabetes is identical in pathogenesis and clinical course to type 2 diabetes of later onset; or (2) type 2 diabetes of early onset is a more extreme phenotype with even greater cardiovascular and lifelong risk of complications than in older subjects. We have previously shown that patients in our clinic with onset of diabetes under 40 years are significantly more obese at an earlier age, have worse cardiovascular risk profiles than their older counterparts and have worse initial and ongoing glycaemic control, despite exactly similar care [6]. We have also reported that young type 2 subjects have a markedly adverse cardiovascular risk profile, as already established in a cohort with mean age of 22 years [7]. The clinical and research evidence to date support the hypothesis that early-onset type 2 diabetes is an extreme phenotype, distinct from the phenotype of older subjects with typical type 2 diabetes and from that of young obese subjects with normal glucose tolerance.

In the current study, we conducted baseline studies of whole-body and hepatic glucose turnover using the clamp.
Table 2  Clinical data, lipid profiles and metabolic data derived from the OGTT: comparison between obese non-diabetic control and type 2 diabetes subjects pre and post exercise

<table>
<thead>
<tr>
<th>Fixed data</th>
<th>Control subjects</th>
<th>Type 2 diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Male : female ratio</td>
<td>4:10</td>
<td>3:4</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>25.6 (0.9)</td>
<td>26.1 (0.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Per exercise status</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>97.47 (4.65)</td>
<td>95.6 (4.54)</td>
<td>108.3 (8.7)</td>
<td>109.4 (8.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.25 (1.44)</td>
<td>33.58 (1.34)</td>
<td>35.63 (1.23)</td>
<td>36.02 (1.17)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>116.1 (4)</td>
<td>112.8 (4)</td>
<td>119 (4.7)</td>
<td>121 (4.2)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.3 (2.4)</td>
<td>69.6 (2.3)</td>
<td>77 (2.5)</td>
<td>81 (2.3)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>100.8 (3.3)</td>
<td>96.7 (2.9)</td>
<td>115.2 (5.5)</td>
<td>110.7 (5.1) *</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>113.3 (3.4)</td>
<td>107.1 (3) ***</td>
<td>116.6 (4.3)</td>
<td>115.3 (3.7)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.89 (0.02)</td>
<td>0.9 (0.02)</td>
<td>0.99 (0.05)</td>
<td>0.96 (0.03)</td>
</tr>
<tr>
<td>Body fat %</td>
<td>39 (2.4)</td>
<td>38.4 (2.3)</td>
<td>39.9 (3.1)</td>
<td>39.7 (3.1)</td>
</tr>
<tr>
<td>VO2max (ml min⁻¹ kg⁻¹)</td>
<td>2.77 (0.24)</td>
<td>3.36 (0.41) ***</td>
<td>2.48 (0.31)</td>
<td>2.72 (0.35)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.97 (0.11)</td>
<td>4.92 (0.11)</td>
<td>9.39 (1.06)</td>
<td>10.64 (1.55)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.47 (0.26)</td>
<td>4.25 (0.26)</td>
<td>4.47 (0.3)</td>
<td>4.52 (0.24)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.12 (0.09)</td>
<td>1.11 (0.06)</td>
<td>0.96 (0.05)</td>
<td>0.95 (0.05)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.46 (0.22)</td>
<td>2.83 (0.25)</td>
<td>2.6 (0.26)</td>
<td>2.88 (0.18)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.37 (0.2)</td>
<td>1.19 (0.13)</td>
<td>2.63 (0.39)</td>
<td>2.26 (0.29)</td>
</tr>
<tr>
<td>Fasting plasma NEFA (mmol/l)</td>
<td>0.635 (0.053)</td>
<td>0.561 (0.036)</td>
<td>0.820 (0.104)</td>
<td>0.826 (0.043)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.43 (0.11)</td>
<td>5.32 (0.16)</td>
<td>8.46 (0.6)</td>
<td>8.41 (0.73)</td>
</tr>
<tr>
<td>Beta cell glucose sensitivity a</td>
<td>145.19 (24.68)</td>
<td>128.81 (17.39)</td>
<td>13.29 (2.92)</td>
<td>21.01 (7.44)</td>
</tr>
<tr>
<td>Rate sensitivity (pmol m⁻² mmol⁻¹ l⁻¹)</td>
<td>795.55 (195.44)</td>
<td>1095.93 (267.62)</td>
<td>241.38 (108.95)</td>
<td>196.45 (56.87)</td>
</tr>
<tr>
<td>Potentiation factor ratio</td>
<td>1.44 (0.14)</td>
<td>1.57 (0.19)</td>
<td>1.29 (0.18)</td>
<td>1.1 (0.12)</td>
</tr>
<tr>
<td>Basal insulin secretion (pmol min⁻¹ m⁻²)</td>
<td>100.05 (12.58)</td>
<td>102.1 (9.53)</td>
<td>146.43 (15.15)</td>
<td>140.56 (12.42)</td>
</tr>
<tr>
<td>Integral of total insulin secretion OGTT b</td>
<td>43.41 (2.12)</td>
<td>44.13 (3.08)</td>
<td>29.32 (2.61)</td>
<td>34.31 (6.48)</td>
</tr>
</tbody>
</table>

Figures in brackets: SEM

a pmol min⁻¹ m⁻² mmol⁻¹ l⁻¹

b nmol/m²

* p<0.05; *** p<0.001

technique with deuterated glucose tracer. We used whole-body VO2max as an index of physical fitness. Our subjects were two matched groups of severely obese young subjects, one with type 2 diabetes, the other non-diabetic. The latter non-diabetic group was nonetheless severely insulin-resistant. However, the young type 2 diabetic subjects were even more insulin-resistant at baseline. Whole-body glucose disposal was dramatically reduced and hepatic glucose production was elevated under fasting conditions. During the clamp studies, suppression of hepatic glucose production was similar between the two groups, in contrast to whole-body glucose disposal, which was significantly reduced in the diabetes group. Insulin secretion was markedly reduced in the type 2 diabetic group. The characteristics of beta cell dysfunction in young type 2 diabetic subjects are similar to those we have observed in older diabetic patients [26]. In particular, young type 2 diabetic subjects show the classical marked decrease in glucose sensitivity, rate sensitivity and potentiation factor ratio. In addition, the typical associations between mean or 2-h glucose and both beta cell glucose sensitivity and insulin sensitivity were also observed in this group. However, the association with glucose tolerance was stronger for beta cell glucose sensitivity than for insulin sensitivity, suggesting that in this insulin-resistant population the major cause of glucose intolerance is beta cell dysfunction.

Recent studies from a group in Ohio, USA, used OGTT and IVGTT to show that adolescents with type 2 diabetes have significant insulin resistance, even compared with non-diabetic subjects of similar obesity and body fatness, and impaired insulin secretion relative to their degree of insulin resistance [28]. In contrast to our findings, the diabetic subjects in those studies retained a first-phase insulin response to glucose that was comparable with lean
control subjects. Another group conducted hyperinsulinaemic-euglycaemic clamp studies in six French subjects with type 2 diabetes and a median age of 15.4 years [29]. Similar to our study, they demonstrated marked insulin resistance and beta cell failure although these subjects had very good chronic glycaemic control.

Aerobic exercise has been shown to be effective in improving insulin sensitivity in patients with obesity, pre-diabetes and type 2 diabetes [13, 14, 16]. The landmark diabetes prevention studies have proven the efficacy of exercise intervention in preventing progression from IGT to diabetes [9, 11, 12]. We have recently shown that just 7 days of exercise training led to increased glucose disposal and muscle GLUT4 protein content in middle-aged, obese subjects with type 2 diabetes (mean age 45 years, BMI 36 kg/m²) [15]. Since early-onset type 2 diabetes is characterised by obesity and severe insulin resistance, we chose to investigate the effect of exercise intervention in younger subjects with type 2 diabetes. We hypothesised that exercise alone, while maintaining a stable diet, should improve insulin sensitivity. The advantage of the study design was that all exercise sessions took place at our research unit and each session was supervised by either the exercise physiologist or study physician. The intensity of the exercise was constantly scrutinised using continuous heart rate monitoring. We only compared measurements in subjects who actually completed the study (14 control and seven type 2 diabetic subjects). To our surprise, there were essentially no metabolic improvements in either study group at the end of the 3-month programme. However, the obese control group had a 20% increase in $VO_{2\text{max}}$, which was positively correlated with glucose disposal, and a reduction in fasting NEFA when compared with the diabetic group, despite identical compliance with the training programme. This raises interesting new questions about the pathogenesis and treatment of early-onset type 2 diabetes in
obese young people. The duration (3 months) of exercise at the frequency and intensity chosen for the current study may simply have been insufficient for the improvements that were expected. However, previous studies including our own in equally obese but older subjects with obesity and type 2 diabetes, have shown improvements in insulin sensitivity and glycaemic control with similar or even less rigorous exercise regimes [13, 14]. Another question is whether it would have been preferable to combine the exercise regime with a weight-losing low-energy diet. However, subjects in the current study were instructed to maintain a stable diet during the 3-month exercise programme, and compliance with this diet regime was satisfactory. We chose to study the effect of exercise alone, not least because it was possible to accurately document exercise compliance, but also because we have found that exercise is an attractive behavioural approach to improving overall lifestyle in this kind of subject group. In addition, an alteration of diet, in conjunction with exercise, would have made it more difficult to interpret any changes in physiological measurements.

In 2005 a group in Canada reported the effects of exercise training on glucose homeostasis in almost 600 subjects, using a standard IVGTT [30]. While they observed a mean increase of 10% in insulin sensitivity after 20 weeks of aerobic training, between-subject variations in metabolic response to exercise were large, not unlike our study. In fact, most subjects showed a deterioration in the acute insulin response to glucose. It has been suggested that progressive resistance training confers greater metabolic benefits than aerobic training [31, 32]. While the exercise modality may also be relevant, most studies to date, including our own, have examined the effects of aerobic exercise training.

In this previously sedentary cohort it is also possible that our subjects were less active than previously outside the training times. We did not make any specific measurements of overall physical activity in the current study and we think it unlikely that this could have been reduced. While devices are being developed to measure physical activity continuously over several days [33], we did not use these in our study.

More basic physiological and biochemical mechanisms might explain the non-response to exercise in these patients. For example, certain diabetes susceptibility genotypes might predispose individuals to respond or not to respond to exercise. It is possible that subjects with early-onset type 2 diabetes (and severe obesity) have either genetic or acquired factors that confer resistance at the level of skeletal muscle to the expected benefits of aerobic exercise training. It is now widely recognised that mitochondrial dysfunction contributes significantly to insulin resistance and loss of insulin secretion in type 2 diabetes. The failure to increase VO2max in the diabetes subjects is consistent with mitochondrial dysfunction, which could result from a number of causes, including persistent lipotoxicity, which did not improve in this group after exercise. Further studies will be required to address these questions, which are so important for the development of effective treatments for these high-risk patients.

Acknowledgements This study was funded by the Diabetes Education and Research Fund and in part by unrestricted educational grants from Pfizer and Novo Nordisk. The authors are especially grateful to the study participants.

Duality of interest None of the authors have any duality of interest to declare.

References


Vascular inflammatory markers in early-onset obese and type 2 diabetes subjects before and after three months’ aerobic exercise training

MENSUD HATUNIC, FRANCIS FINUCANE, NICOLE BURNS, DECLAN GASPARRO, JOHN J NOLAN

Abstract

Early-onset type 2 diabetes (T2DM) may lead to very early vascular complications. Cardiovascular mortality is two to five times higher in adults with diabetes than in people without diabetes. The cardiovascular risk of young people with T2DM is unknown. T2DM in young people is associated with marked visceral obesity, insulin resistance and microalbuminuria. We recently showed that these subjects did not improve in either fitness (maximum volume of oxygen consumption, \(V_{\text{O}_2}\) max) or glucose disposal after exercise training.

Seven subjects with early-onset T2DM (aged 26.1±0.8 years, body mass index [BMI] 35.6±1.2 kg/m\(^2\)) and 14 age-matched obese subjects with normal glucose tolerance (aged 25.6±0.9 years, BMI 34.3±1.4 kg/m\(^2\)) underwent aerobic training for 12 weeks. Serum vascular inflammatory markers (high-sensitivity C-reactive protein [hsCRP], soluble intercellular adhesion molecule [sICAM-1], soluble vascular cell adhesion molecule [sVCAM-1], E-Selectin and P-Selectin) were measured before and after the training programme.

At baseline, plasma concentrations of vascular inflammatory markers were significantly elevated in both groups. They did not improve after exercise.

doi:10.3132/dvdr.2007.045

Key words: cardiovascular risk, C-reactive protein, E-Selectin, exercise, intercellular adhesion molecule, obesity, P-Selectin, type 2 diabetes, vascular inflammatory markers, vascular cell adhesion molecule.

Introduction

Patients with diabetes mellitus are at increased risk of developing cardiovascular disease. Previous studies have shown that the risk of cardiovascular mortality is two to three times higher in men with diabetes and three to five times higher in women with diabetes than in people without diabetes. The worldwide prevalence of diabetes in adults is expected to increase from 5% to 6% from 2003 to 2025. Until recently, type 2 diabetes was regarded as a disease that typically affected the middle-aged and elderly. Evidence is accumulating that the onset of type 2 diabetes in younger adults is increasing. Even children and adolescents are now part of the diabetes epidemic.

Obesity has increased by 70% in adults aged 30-39 years over the last 30 years, and type 2 diabetes has increased in parallel by 70% in adults aged 30-39 years over the last decade, making young adults the fastest-growing adult group for both obesity and type 2 diabetes.

Early-onset type 2 diabetes appears to be a more aggressive disease. We have recently reported that much younger subjects with type 2 diabetes are obese and severely insulin-resistant with markedly abnormal cardiovascular risk markers, similar to the findings in patients 30 years older. In fact, the population of young adults with type 2 diabetes has a more adverse risk profile for cardiovascular disease when compared with age-matched subjects without diabetes, but also relative to older patients with type 2 diabetes. If these findings are confirmed in other larger populations, they will have profound social and economic implications.

Individuals who report regular physical activity are less likely than sedentary individuals to die from coronary heart disease and stroke. Several studies have assessed the independent and combined effect of obesity and physical fitness on mortality. There is an abundance of evidence that exercise delays or prevents the development of type 2 diabetes in at-risk populations. Smaller studies have shown that exercise improves insulin sensitivity and glucose metabolism. We have demonstrated that short-term exercise training increases insulin sensitivity by more than 50% in obese middle-aged patients (mean age 45 years) with type 2 diabetes.

However, in contrast to the middle-aged patients, we have recently shown that patients with early-onset (before age 25) obesity and type 2 diabetes are resistant to the expected metabolic benefits of aerobic exercise training.
Neither maximum volume of oxygen consumption (VO₂max) nor insulin sensitivity improved in the young diabetes subjects despite full compliance with three months of exercise training. VO₂max did improve (20% increase) in the non-diabetic obese subjects, but glucose disposal did not.²²

Plasma levels of soluble adhesion molecules have been studied in various inflammatory and pro-thrombotic disorders. An increase in soluble vascular cell adhesion molecule (VCAM) and soluble intercellular adhesion molecule (ICAM) has been reported in coronary artery disease (CAD). An increase of soluble P-Selectin (sP-Selectin) has also been reported in arteriosclerosis.²³ Soluble E-Selectin (sE-Selectin) is increased in patients with early atherosclerosis or those with manifest atherosclerotic disease.²⁴ Evidence is accumulating that high-sensitivity C-reactive protein (hsCRP) is predictive of future coronary events.²⁵

The aim of this study was to compare circulating levels of cardiovascular inflammatory markers (hsCRP, VCAM, ICAM, sP-Selectin, sE-Selectin) in the previously described young obese and young T2DM subjects at baseline and after 12 weeks of supervised aerobic exercise intervention. We hypothesised that, even without whole body changes in VO₂max and glucose uptake, which might require a more prolonged or varied intervention, exercise would lead to an improvement in a variety of the surrogate markers of cardiovascular risk in these subjects.

Methods
Patients aged between 15 and 30 years with obesity or with type 2 diabetes were recruited from the outpatient clinics at St James’s Hospital, as previously described.²¹ Subjects with co-existing illnesses or secondary forms of diabetes were excluded from the study. The local Research Ethics Committee approved the protocol and written informed consent was obtained.

Subjects attended the Metabolic Research Unit for testing at 8 am for initial investigations. A full history and routine fasting blood samples were taken. Diabetes was excluded in the obese group by a standard 75 g oral glucose tolerance test. Each subject with diabetes was confirmed negative for glutamic acid decarboxylase antibodies. Waist:hip ratio, weight, height and body mass index (BMI) were measured. Blood pressure was measured using the left arm after the subject had been sitting comfortably for five minutes, using an oscillometric device (Omron® 705 CP). Three readings were taken and the lowest one recorded. Body composition was assessed using an electrical impedance device (Tanita® Body Composition Analyser). Urinalysis and 12-lead ECG were also performed.

VO₂max was measured in an exercise laboratory, under the supervision of an exercise physiologist. The test involved progressively increasing the workload on a bicycle ergometer to the subject’s maximal ability, in a stepwise fashion. Heart rate and blood pressure were monitored during the test.

Following successful screening, the training protocol consisted of 60 minutes of aerobic exercise four days a week for 12 weeks at 70% of each subject’s maximum oxygen uptake on a bicycle ergometer or treadmill.²² Subjects had their blood pressure and heart rate monitored at each training session throughout the period of the study. The subjects maintained a stable diet and treatment for diabetes during the period of the exercise programme. All measurements were repeated after 12 weeks of the programme.

The vascular inflammatory markers were measured in frozen fasting serum collected and stored at -80°C. Serum concentration of hsCRP was determined by immunonephelometric assay (Immulite® 2000): normal values were < 5 mg/L. sCAM-1, sVCAM-1, s-ESelectin and sP-Selectin levels were assessed with an enzyme-linked immunosorbent assay using monoclonal antibodies specific for each of those adhesion molecules (R&D Systems, Abingdon, Oxfordshire, UK).
This study has shown that concentrations of a range of cardiovascular inflammatory markers (hsCRP, sP-Selectin, sE-Selectin) in young obese and young T2DM subjects are elevated at baseline and do not change significantly after 12 weeks of supervised aerobic exercise. The values of sVCAM and sICAM were in the high normal range and were not significantly different after the exercise programme.

It is known that diabetes mellitus and obesity confer higher risk of cardiovascular disease and represent an important global public health problem. In our previous studies we confirmed that our young patients with T2DM are obese, severely insulin-resistant and have a more adverse cardiovascular risk profile than older patients. Because early-onset type 2 diabetes is a relatively new clinical phenomenon, it is not clear which factors (such as severe obesity, insulin resistance or low-grade inflammation) are responsible for this adverse risk profile. There are as yet no long-term studies that describe the natural history of diabetes and its complications in these subjects. Early-onset visceral obesity and severe insulin resistance are probable contributors to the inflammatory cardiovascular risk milieu and to vascular stiffness, but this remains to be confirmed.

We hypothesised that aerobic exercise training would improve insulin sensitivity, physical fitness and a range of the associated phenotypic abnormalities in this very insulin-resistant population. Although VO2max increased by 20% in the obese control group, there was no significant improvement in the group with T2DM. Nor was there any improvement in whole body glucose uptake in either group, as recently reported. These patients have a markedly adverse cardiovascular risk profile that did not improve with short-term exercise training. Surprisingly, the surrogate markers of cardiovascular risk were not improved by this three-month intervention. Thus, it is possible that the underlying cellular abnormalities conferring severe insulin resistance and exercise resistance in these patients may overlap with those contributing to risk of early cardiovascular disease. One likely shared mechanism could be the effects of lipotoxicity in both the target tissues for insulin action, particularly skeletal muscle and liver, and in the cardiovascular system. These patients are clearly dyslipidaemic at baseline, and remain so after aerobic exercise intervention. Lipotoxicity may ultimately be exerted through impairment of mitochondrial function, another cellular mechanism potentially linking...

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**Table 2. Comparison markers of vascular inflammation in young obese controls and T2DM subjects pre- and post-exercise**

<table>
<thead>
<tr>
<th></th>
<th>YO pre-exercise</th>
<th>YO post-exercise</th>
<th>p value</th>
<th>T2DM pre-exercise</th>
<th>T2DM post-exercise</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>6.3±1.7</td>
<td>7.1±2.3</td>
<td>0.3</td>
<td>5.9±2.6</td>
<td>7.2±2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>E-Selectin (ng/ml)</td>
<td>72 (40-85)</td>
<td>61 (37-85)</td>
<td>0.3</td>
<td>63 (43-89)</td>
<td>52 (43-64)</td>
<td>0.2</td>
</tr>
<tr>
<td>P-Selectin (ng/ml)</td>
<td>115 (91-143)</td>
<td>122 (100-133)</td>
<td>0.7</td>
<td>127 (98-147)</td>
<td>130 (80-148)</td>
<td>0.9</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>526 (440-655)</td>
<td>541 (429-685)</td>
<td>0.9</td>
<td>667 (528-789)</td>
<td>668 (560-1026)</td>
<td>0.8</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
<td>252 (205-297)</td>
<td>256 (213-334)</td>
<td>0.9</td>
<td>316 (285-377)</td>
<td>292 (270-375)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard error of mean or the median (interquartile ranges) in parentheses.

Key: YO = young obese; T2DM = type 2 diabetes; hsCRP = high-sensitivity C-reactive protein; VCAM = vascular cell adhesion molecule; ICAM = intercellular adhesion molecule.
the resistance to exercise in skeletal muscle and the effects on the cardiovascular system. Low-grade inflammation is another potential mechanism integrating these abnormalities. Whether inflammation per se represents a modifiable risk factor in obesity is currently uncertain, although recent studies have suggested that some common preventive therapies, such as the use of statins, may reduce inflammatory markers. None of the patients in the current study were receiving statin therapy, nor is it current practice to administer statins to patients in this age group with type 2 diabetes.

While the current results are disappointing, additional studies will clearly be needed to investigate the role of dose, intensity and duration of exercise and other concurrent interventions in the modification of both insulin resistance and adverse cardiovascular risk in high-risk young patients with type 2 diabetes and severe insulin resistance.

Acknowledgements
This project was supported in part by an unrestricted educational grant from Pfizer. We thank the study volunteers and their families for their participation.

Conflicts of interest statement
None declared.

References
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Plasma Visfatin is Reduced after Aerobic Exercise in Early Onset Type 2 Diabetes Mellitus.

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<th><em>Diabetes, Obesity and Metabolism</em></th>
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<td>Brema, Imad; St James's Hospital, Metabolic Research Unit Hatunic, Mensud; St James's Hospital, Metabolic Research Unit Finucane, Francis; St James's Hospital, Metabolic Research Unit Burns, Nicole; St James's Hospital, Metabolic Research Unit Haider, Dominik; Medical University of Vienna, Clinical Pharmacology Wolzt, Michael; Medical University of Vienna, Clinical Pharmacology ludvik, bernhard; Medizinische Universität Wien, Universitätsklinik für Innere Medizin 3 Nolan, John; Trinity College Dublin, Metabolic Research Unit</td>
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<tr>
<td>Key Words:</td>
<td>diabetes mellitus, obesity, aerobic exercise, visfatin</td>
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</table>
Plasma Visfatin is Reduced after Aerobic Exercise in Early Onset Type 2 Diabetes Mellitus.

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Dublin 8, Ireland

Running Title: I. Brema et al: Visfatin and exercise in young subjects with type 2 diabetes
Total Word Count 781 words
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>YOb</td>
<td>Young Obese(Control subject)</td>
</tr>
<tr>
<td>YT2DM</td>
<td>Young Type 2 Diabetes Subject</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist: Hip Ratio</td>
</tr>
<tr>
<td>OGIS</td>
<td>Oral Glucose Insulin Sensitivity Index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
</tbody>
</table>
Introduction:

Visfatin is an adipokine (1) that has been shown to be significantly elevated in people with type 2 diabetes mellitus and independently associated with waist-hip ratio (2) and BMI (3). However, no correlations have been found between visfatin and insulin sensitivity (3-5). We showed that short-term exercise training in obese middle-aged subjects with type 2 diabetes resulted in a 50% increase in glucose disposal (6). We recently reported, however, that in young subjects with type 2 diabetes, neither glucose disposal nor VO$_2$$_{max}$ were improved after 3 months aerobic training (7). We measured plasma visfatin in this cohort of young patients at baseline and after three months aerobic exercise.

Methods:

Patients aged between fifteen and thirty years with type 2 diabetes or obesity were recruited, as previously described (7). Full history and physical examination was performed and patients had standard 75g OGTT, and fasting laboratory profiles.

VO$_2$ Max testing

All subjects exercised for four-one hour weekly sessions at 75% VO$_2$ Max under supervision of an exercise physiologist. The test involved progressively increasing the workload on a bicycle ergometer to the subject’s maximal ability, in a stepwise fashion. Dietary intake remained unchanged during the exercise intervention programme.

Visfatin assay:

Visfatin was measured using a commercially available ELISA Kit with an intra- and interassay coefficient of variation of <6% (Phoenix Peptides, Karlsruhe, Germany).
**Insulin sensitivity:**

Insulin sensitivity was calculated using both the hyperinsulinaemic euglycaemic clamp and oral glucose insulin sensitivity Index OGIS (8)

**Statistical Analysis:**

All statistical analysis was performed using SPSS 11.5 version for windows. Baseline characteristics were compared using Mann-Whitney U Test. Wilcoxon signed rank test was used for post exercise comparisons. Spearman's correlations were used to examine correlation between plasma visfatin and other biomarkers. All statistical analyses were two sided and P value of < 0.05 was considered significant.

**Results:**

Characteristics of the study subjects are shown in Table 1. Apart from a small reduction in waist circumference (P <0.027), no significant changes were observed in any of the biomarkers listed in Table 1 after completion of the exercise programme, as previously reported (7). At baseline, and prior to any exercise intervention, plasma visfatin was similar between the young type 2 diabetes and young obese subjects (55.8±17.2 ng/ml vs.64.7±10.7ng/ml, respectively, P=0.169). Plasma Visfatin was substantially reduced in both groups post exercise (11.6±4.4 ng/ml in young type 2 diabetes and 29.5±7.1 ng/ml in the young obese, P=0.02 and 0.002 for diabetes and obese, respectively). However, after exercise, visfatin was reduced to a much greater extent in diabetes patients (P< 0.019). In the young type 2 diabetes patients, Visfatin correlated with percent body fat (r=0.933, P= 0.003) and HDL Cholesterol (r=0.893, P= 0.007) post exercise. All other correlations were negative, both pre and post exercise in this group and no correlations were found between visfatin and any of the biomarkers listed in Table 1 in the young obese subjects.
Discussion:

Plasma visfatin concentration was significantly reduced by approximately 80% and 50% after 12 weeks aerobic exercise training in severely obese young subjects with Type 2 diabetes or normal glucose tolerance, respectively. There was no difference in plasma visfatin concentrations between these two groups of subjects at baseline. To our knowledge, this is the first report to describe the effect of aerobic exercise on circulating plasma visfatin concentrations in insulin resistant subjects. The mechanism through which exercise is associated with this reduction in circulating visfatin in young type 2 diabetes patients remains unclear, since, apart from a small reduction in waist circumference, no significant physiological changes were found post exercise in either group. Moreover, whole body insulin sensitivity, hepatic insulin sensitivity, insulin secretion and OGIS were unchanged post exercise in either group (7). We previously demonstrated a reduction of visfatin following exercise in subjects with type 1 diabetes mellitus (9). We also demonstrated a significant reduction in plasma visfatin in morbidly obese patients following gastric banding. However, weight, BMI and waist circumference were significantly reduced in that study (10).

In our study, visfatin did not correlate with insulin sensitivity quantified by either the clamp method or OGIS, which is comparable to previous studies, which showed lack of correlation between visfatin and insulin sensitivity quantified by the hyperinsulinaemic euglycaemic clamp or HOMA (3-5).

The current study is limited by the sample size and lack of measurement of both visceral and subcutaneous visfatin mRNA expression. The duration of the aerobic exercise may have been too short to result in changes in body weight, BMI and insulin
sensitivity. Therefore, the interpretation of our results should be with caution. Further studies on larger samples will be needed to clarify the role of visfatin in the pathogenesis of type 2 diabetes, obesity and insulin resistance.
Table 1. Characteristics of study population:

<table>
<thead>
<tr>
<th></th>
<th>YT2DM N=8</th>
<th>YOb N=14</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>25.6±0.8</td>
<td>24.6±0.9</td>
<td>0.361</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>108.1±7.5</td>
<td>97.45±4.7</td>
<td>0.413</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>115.2±5.5</td>
<td>100.8±3.3</td>
<td>&lt;0.022</td>
</tr>
<tr>
<td>WHR</td>
<td>1.0±0.0</td>
<td>0.9±0.0</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.8±1.3</td>
<td>34.2±1.4</td>
<td>0.609</td>
</tr>
<tr>
<td>Systolic BP, (mmHg)</td>
<td>120.6±4.3</td>
<td>116.1±4.0</td>
<td>0.916</td>
</tr>
<tr>
<td>Diastolic BP, (mmHg)</td>
<td>74.9±3.2</td>
<td>71.3±2.4</td>
<td>0.339</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>10.0±0.9</td>
<td>5.0±0.1</td>
<td>&lt;0.0001</td>
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<tr>
<td>Fasting insulin (pmol/L)</td>
<td>84.6±30.6</td>
<td>63.2±9.0</td>
<td>&lt;0.048</td>
</tr>
<tr>
<td>HBA1C (%)</td>
<td>8.4±0.1</td>
<td>5.4±0.1</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Triglyceride, (mmol/L)</td>
<td>2.7±0.3</td>
<td>1.4±1.0</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>HDL-cholesterol, (mmol/L)</td>
<td>1.0±0.0</td>
<td>1.1±0.1</td>
<td>0.207</td>
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<tr>
<td>LDL-cholesterol, (mmol/l)</td>
<td>2.7±0.2</td>
<td>2.5±0.2</td>
<td>0.48</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>38.2±3.2</td>
<td>39.0±2.4</td>
<td>0.89</td>
</tr>
<tr>
<td>OGIS (ml.min⁻¹.m⁻²)</td>
<td>281.4±15.9</td>
<td>417.1±21.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VO₂ Max (ml/min/kg)</td>
<td>2.5±0.2</td>
<td>2.8±0.2</td>
<td>0.78</td>
</tr>
<tr>
<td>Visfatin Pre-exercise (ng/ml)</td>
<td>55.8±17.2</td>
<td>64.7±10.7</td>
<td>0.169</td>
</tr>
<tr>
<td>Visfatin Post-Exercise (ng/ml)</td>
<td>11.6±4.4</td>
<td>29.5±7.1</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Data are presented as Mean ±SEM
*P-values obtained by Mann-Whitney U test. **(Wilcoxon signed rank test)
References:


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Secretion, Severe Insulin Resistance and a lack of Response to Aerobic Exercise Training. Diabetologia. 50:1500-1508

