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TRINITY COLLEGE DUBLIN

TYPE 2 DIABETES, INSULIN RESISTANCE AND EFFECTS OF EXERCISE
IN YOUNG ADULTS

By

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BA (Kinesiology), MSc (Exercise Physiology)

Thesis submitted for the degree of Doctor of Philosophy in Clinical Medicine at
the University of Dublin, Trinity College

Submitted

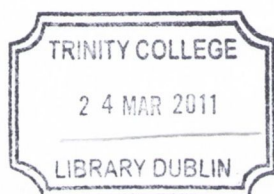
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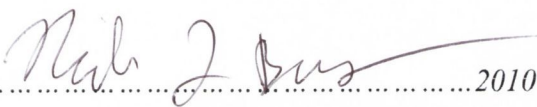
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Summary

Study 1- Characteristics of the physically active but insulin resistant phenotype

Methods: Data from the European Group on Insulin Resistance- Relationship between Insulin Sensitivity and Cardiovascular Risk study was used in this analysis. Participants underwent a euglycaemic-hyperinsulinaemic clamp with a continuous insulin infusion rate of $240 \text{ pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ and a variable dextrose infusion that was adjusted to maintain a plasma glucose concentration between 4.5-5.5 $\text{mmol}\cdot\text{l}^{-1}$. Physical activity was measured over 7 days using an accelerometer. Phenotypic and biochemical differences between subjects in the upper tertile of physical activity and lower tertile of insulin sensitivity (AIR) were compared with those in the lower tertile of physical activity and upper tertile of insulin sensitivity (IIS).

Results: The AIR group had significantly higher body mass index, waist circumference and waist: hip ratio (all $P<0.01$). The 2 hr post oral glucose tolerance test (OGTT) plasma glucose concentrations and fasting plasma insulin concentrations in the AIR group were significantly elevated compared to the IIS group (both $P<0.005$). The AIR group had lower plasma adiponectin concentrations and high-density lipoprotein (HDL) cholesterol concentrations (both $P<0.005$) than the IIS group, but significantly higher free fatty acid concentrations ($P<0.05$).

Study 2- Clinical comparison of younger and older patients with Type 2 diabetes.

Methods: This was a retrospective study of two distinct clinical populations with type 2 diabetes mellitus (T2DM). Early-onset patients (<40 years) identified from our clinical database were compared to randomly identified later-onset patients (>50 years). Each patient's first and last clinical visit and lab episode was examined.

Results: The early-onset patients were significantly more obese as measured by body mass index and weight (both $P<0.05$). High density lipoprotein (HDL) cholesterol was significantly lower while total cholesterol: HDL ratio and triglyceride concentrations were higher in the early-onset cohort (all $P<0.05$). Mean glycated haemoglobin (HbA1c) at diagnosis was higher in the early-onset patients and continued to be higher at follow-up (both $P>0.05$), despite active titration of treatment.

Study 3- Characterization of the phenotype of early-onset T2DM.

Methods: Two sedentary groups of young subjects (<30 years), one with type 2 diabetes (YT2) and one without (control), were closely matched for age and body mass index. All participants underwent a full clinical assessment, including a 2 hour oral glucose tolerance

test and a euglycaemic-hyperinsulinaemic clamp with tracer. Exercise capacity (VO_2peak) was determined using a graded exercise test to exhaustion.

Results: YT2 subjects had a higher waist circumference, waist: hip ratio, systolic blood pressure and triglyceride concentration when compared to controls (all $P<0.05$). The YT2 subjects were much more insulin-resistant both during the OGTT ($P<0.0001$) and during the euglycaemic-hyperinsulinaemic clamp (increased basal hepatic glucose output ($P<0.001$) and reduced glucose disposal ($P<0.05$)). Marked defects in insulin secretion were also displayed. Concentrations of vascular inflammatory markers were markedly elevated when compared to healthy control values, in both groups of subjects.

Study 4- *Aerobic exercise intervention*

Methods: Subjects from the previous study (characterization of the phenotype of early-onset T2DM) were enrolled in a 12 week supervised exercise intervention. Each participant exercised at 70% VO_2peak for 1 hour a day, 4 times per week. Baseline tests were then repeated.

Results: VO_2peak in the control group increased significantly ($P<0.001$) by 20%, whereas no change was seen in the YT2 group. Exercise training had no effect on whole-body or hepatic insulin sensitivity or on insulin secretion. There were no changes in lipids, anthropometric parameters or inflammatory markers in either group.

Study 5- *Markers of mitochondrial function in response to acute and chronic exercise*

Methods: Muscle biopsies were taken from participants during two separate exercise studies. Fasting samples were taken at baseline and following the 12 weeks of aerobic exercise described in Study 4 (chronic study). Additionally samples were taken at baseline, post 1hr and post 7 days exercise (acute study).

Results: At baseline YT2 subjects showed a reduced expression of mitofusion-2 (Mfn2) ($P<0.01$) and the alpha subunit of ATP synthase ($P<0.05$), when compared to controls. Acute exercise caused a substantial induction in PCG-1 α gene expression in the skeletal muscle from control subjects ($P<0.05$), whereas no change was seen in the YT2 subjects. Chronic exercise training led to an increase in Mfn2 ($P<0.05$), with no change in the YT2 group.

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List of Abbreviations

Abbreviation	Definition
ADA	American Diabetes Association
ACSM	American College of Sports Medicine
AIR	Active insulin resistant subjects
ATP	Adenosine triphosphate
ATP5a1	Alpha subunit of ATP synthase
B	Beta
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CHD	Coronary heart disease
COXIII	Mitochondrial gene chromosome oxidase III
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DPP	Diabetes Prevention Program Research Group
DPS	Diabetes Prevention Study
ECG	Electrocardiogram
EGIR	European Group on Insulin Resistance
ERR α	Estrogen-related receptor-alpha
FFA	Free fatty acid
FHL1	Four-and-a-half LIM domains 1
FTO	Fat Mass and Obesity Associated Gene
GINF	6-6 ² H ₂ 1.6% glucose/glucose in 500mL bag of 20% glucose
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein cholesterol

hr	Hour
hsCRP	High-sensitivity C-reactive protein
IGT	Impaired glucose tolerance
IHD	Ischaemic heart disease
IIS	Inactive insulin sensitive subjects
I.V.	Intravenous
IVGTT	Intravenous glucose tolerance test
kg	Kilogram
LDL	Low-density lipoprotein cholesterol
M	Insulin sensitivity determined from euglycaemic-hyperinsulinaemic clamp
mmHg	Millimeters of mercury
mmol	millimole
MC4R	Melanocortin 4 receptor
Mfn2	Mitofusin 2
MI	Myocardial Infarction
M/I	Insulin sensitivity as a ratio to mean plasma insulin
min	Minute
MODY	Maturity onset diabetes of the young
mRNA	Messenger ribonucleic acid
NaCl	Sodium Chloride
Ndufa9	p37 subunit of Complex I of the respiratory chain
NGT	Normal glucose tolerance
NINF	6- $^6\text{H}_2$ glucose in 50mL of 0.9% NaCl
OGIS	Oral Glucose Insulin Sensitivity

OGTT	Oral Glucose Tolerance Test
OXPHOS	Oxidative phosphorylation pathways
PCR	Polymerase chain reaction
PGC-1 α	Proliferator-activated receptor-coactivator alpha
PGC-1 β	Proliferator-activated receptor-coactivator beta
POMC	Proopiomelanocortin gene
RER	Respiratory exchange ratio
RISC	Relationship between Insulin Sensitivity and Cardiovascular Risk
rpm	Revolutions per minute
SBP	Systolic blood pressure
SD	Standard Deviation
SEM	Standard Error of the Mean
sICAM	Soluble intercellular adhesion molecule
sVCAM	Soluble vascular adhesion molecule
T2DM	Type 2 diabetes mellitus
U.S.	United States
VO ₂ peak	Peak Oxygen Uptake
WHR	Waist: Hip Ratio
yrs	Years
YT2	Young person with type 2 diabetes

Chapter One:

Introduction

Physical activity contributes to a healthy lifestyle. Regular physical activity has been shown to prevent both cardiovascular and metabolic diseases (1-3). Physically active adults tend to develop and maintain a higher level of physical fitness. This increased fitness allows for a higher level of exertion which may be required to affect risk factors for cardiovascular and metabolic diseases; further emphasizing the inter-dependence of fitness and exercise training on exercise response (4). The American College of Sports Medicine (ACSM) recommends that adults should include as part of their daily activities at least 30min of moderate-intensity physical activity on at least five days a week, or 20 min of vigorous-intensity physical activity on at least three days a week, or an equivalent combination, in order to promote and maintain health (5, 6). Exceeding these minimum recommendations may lead to improvements in fitness, reductions in risk for chronic diseases and may prevent weight gain (6, 7)

Recent population studies have shown a decline in leisure-time physical activity (8, 9). Physical inactivity increases the risk for obesity, hypertension, heart disease, osteoarthritis, type 2 diabetes and certain malignancies (10-12). As westernized societies are becoming more sedentary, obesity rates have increased rapidly (13-16). The worldwide obesity epidemic is simultaneously triggering higher rates of diabetes. In the United States, the incidence of diabetes rose from 4.9% to 6.5% from 1990 to 1998 (17). A year later, the same author reported that 7.3% of Americans had diabetes (8). This trend of increasing diabetes prevalence is not unique to the United States (U.S.) (18, 19). In the year 2000, approximately 171 million adults worldwide had diabetes; the prevalence is predicted to more than double to 366 million adults by 2030 (20).

The global prevalence of overweight children and adolescents has increased (21-26) as they have become more sedentary (27-29). Inactivity in these age groups may also contribute to long term health consequences (30, 31). There is accumulating evidence that early-onset type 2 diabetes is linked to the current obesity epidemic (32-36). Over the past few decades the estimated incidence of type 2 diabetes in adolescents has increased almost

10-fold in North America (36, 37); similar trends have been seen in other westernized countries (32, 38, 39). The new epidemics of obesity and type 2 diabetes in adolescents and young adults will have profound social and economic implications (34, 40).

A key objective of this thesis is to study the clinical and metabolic characteristics of type 2 diabetes in an early-onset population compared to a range of control populations. The core of this thesis focuses on the effects of an aerobic exercise intervention on insulin sensitivity, cardiovascular risk markers, and mitochondrial function.

Literature Review

1.1 Diabetes Mellitus

Diabetes remains the leading preventable cause of renal failure, amputations and blindness. It is characterised by hyperglycaemia resulting from a deficiency in insulin secretion or action, or both. Hyperglycaemia leads to glycosylation of tissues which is associated with long-term damage to various organs including the eyes, kidneys and blood vessels (41, 42). There are many distinct clinical forms of diabetes. Type 1 diabetes mellitus is an autoimmune disease with near loss of β -cell insulin secretion and comprises almost 10% of all diabetes cases worldwide. Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder. It is characterized by insulin resistance, gradual loss of β -cell insulin secretion, dyslipidaemia, hypertension, obesity and cardiovascular disease and comprises approximately 90% of all diabetes cases worldwide. Many other less common phenotypes of diabetes have been documented (43). This thesis is focused exclusively on type 2 diabetes of early-onset (individuals aged <30 yrs).

1.2 Type 2 Diabetes

Type 2 diabetes mellitus was previously referred to as non-insulin-dependent diabetes or adult-onset diabetes because it occurred almost exclusively in the adult population and was primarily managed with oral hypoglycaemic agents. The pathophysiology of T2DM is still

not fully understood; however, it is known to be a progressive disease with onset from years to decades. Overt T2DM develops when pancreatic β -cells fail to compensate for the insulin resistance in peripheral tissues. Clinically, patients present with symptoms of hyperglycaemia (with concurrent hyperinsulinaemia) or with vascular complications of the disease. Principal contributors to the disease pathology are: genetic and environmental factors, insulin resistance, and impaired β -cell function, Figure 1.1.

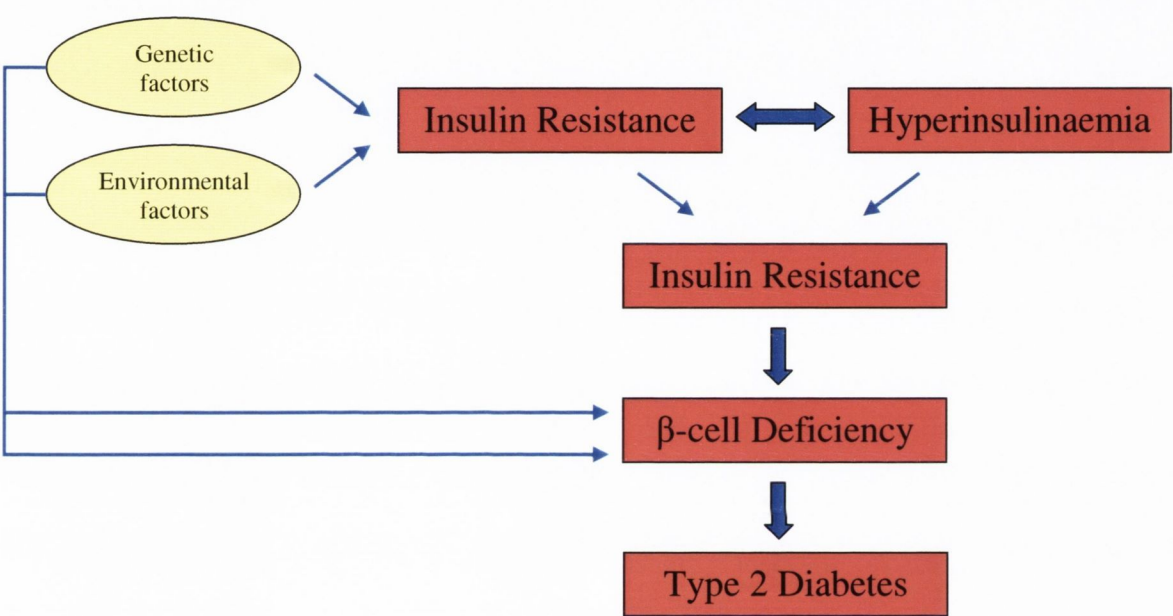


Figure 1.1 Insulin resistance and β -cell dysfunction. Insulin resistance is caused by a combination of genetic and environmental factors. Insulin resistance leads to additional insulin requirements. Over time overt type 2 diabetes develops when the compensatory insulin secretion does not meet the requirements (β -cell dysfunction) and hyperglycaemia arises.

T2DM is a condition with multiple risk factors. Although it is known that age, ethnicity and family history may all contribute to the pathogenesis of T2DM, obesity remains the most prominent risk factor. Approximately 80% of those diagnosed with T2DM are obese (44, 45).

These same risk factors also contribute strongly to the pathogenesis of T2DM in younger populations. In early-onset T2DM there is commonly a significant family history of the disease with 45-80% of patients reporting having at least one parent with T2DM (32, 37). In addition, ethnic minorities represent 94% of the children presenting with T2DM in the

U.S. (36). There is also a slight increase in the female: male prevalence rate; this pattern is consistent with the ratio of female to male adults diagnosed. Obesity appears to be a continuous risk rather than a threshold risk for the onset of T2DM (35), as early-onset T2DM is more closely associated with obesity than with any other clinical condition (46-48).

1.3 Pathogenesis of Type 2 Diabetes

1.3.1 Insulin Resistance

Insulin resistance manifests itself clinically as an impairment of the effect of insulin on glucose uptake in skeletal muscle, adipose tissue, and the liver as well as a failure of insulin to inhibit hepatic glucose production (49-52). In vivo, higher concentrations of insulin are needed for normal glucose disposal, Figure 1.2.

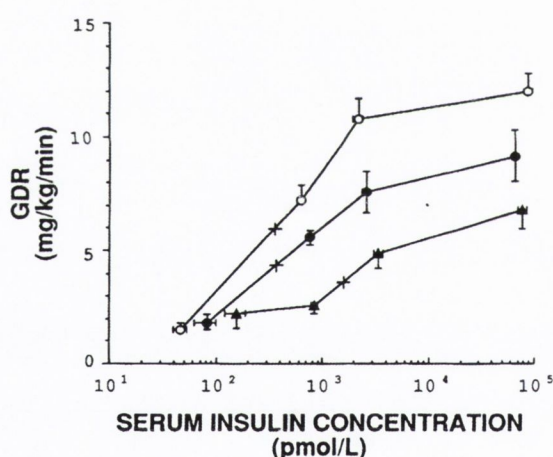


Figure 1.2 Insulin dose-response. Insulin resistance requires a higher insulin concentration to elicit the same glucose disposal rate. In vivo glucose disposal was measured in lean nondiabetic subjects (○), obese nondiabetic subjects (●), and subjects with T2DM (▲). Glucose disposal was lower in T2DM vs. lean controls ($P<0.0005$), T2DM vs. obese controls ($P<0.05$), and obese vs. lean controls ($P<0.05$). *Role of human skeletal muscle insulin receptor kinase in the in vivo insulin resistance of noninsulin-dependent diabetes mellitus and obesity.* Nolan JJ et al., Copyright 1994, The Endocrine Society (53).

Insulin binds to insulin receptors on target tissues i.e. muscle, liver, and adipose tissue. This binding then leads to a series of events that promote intracellular protein phosphorylation; which triggers glucose uptake, fatty acid and protein metabolism and mitogenesis, Figure 1.3. The protein structure of the insulin molecule and the insulin

receptor in T2DM patients are almost always normal. However, molecular defects have been demonstrated in intracellular signalling pathways and this is what ultimately leads to the inability of normal insulin levels to produce appropriate biological responses (49, 54). Insulin resistance does not always result in the development of T2DM.

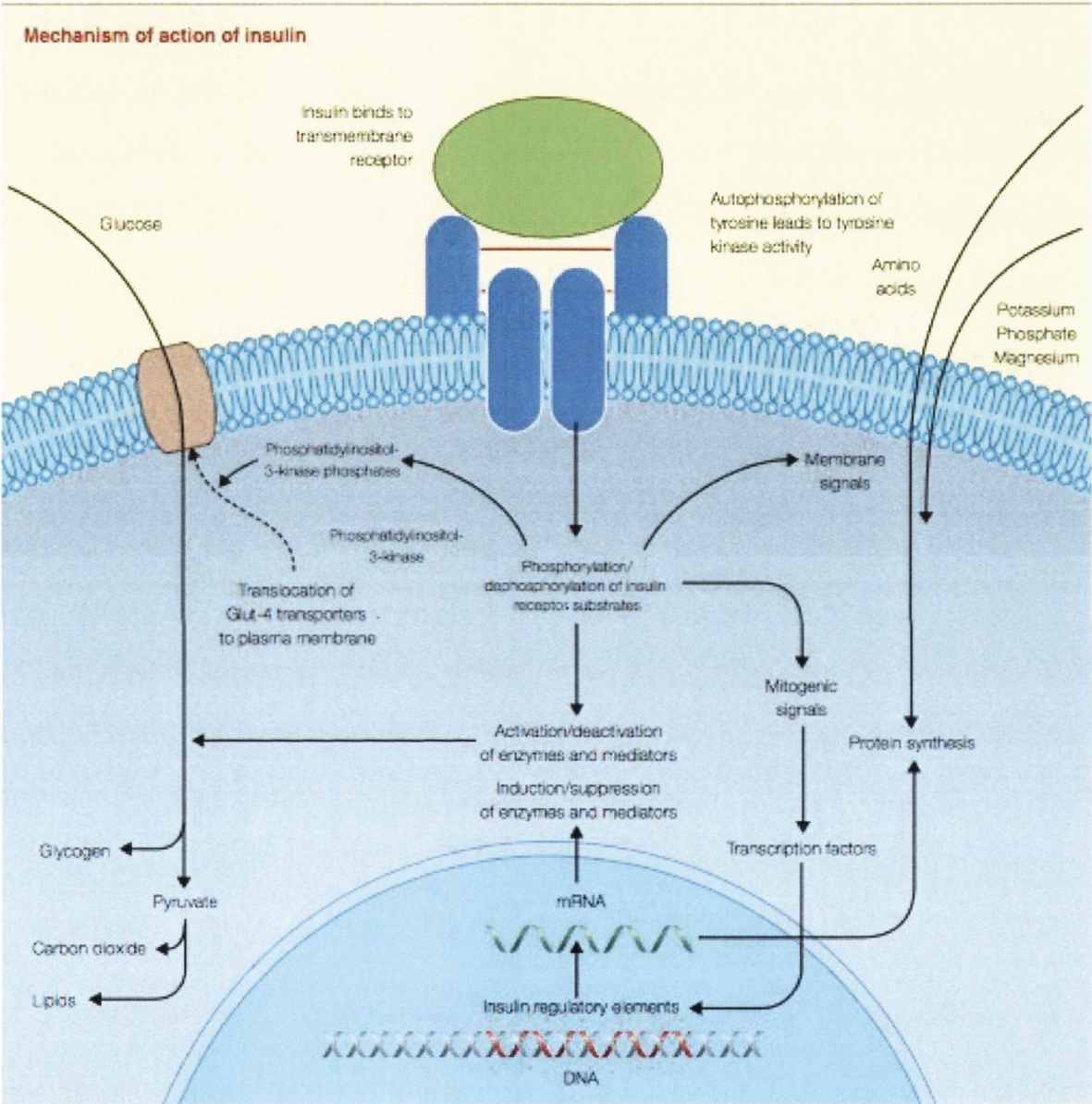


Figure 1.3 Mechanisms of insulin action. Schematic of insulin receptor and intercellular signalling pathways. Insulin binds to a transmembrane receptor which triggers the phosphorylation of insulin receptor substrates. Which leads to glucose uptake, fatty acid and protein metabolism and mitogenesis. *What is Type 2 Diabetes?* Nolan J, Copyright 2006, Elsevier Ltd. (54)

Insulin resistance in skeletal muscle has a major impact on whole-body glucose disposal. Skeletal muscle glucose transport accounts for approximately 75% of whole-body insulin-stimulated glucose uptake (55, 56), and is the rate-limiting step in whole-body glucose

disposal (57, 58). GLUT4 is mainly responsible for insulin-mediated glucose uptake in skeletal muscle and adipose tissue (57, 59), Figure 1.3. GLUT4 expression in skeletal muscle has been shown to be unchanged in T2DM patients; however, cell surface GLUT4 content is reduced under insulin stimulated conditions (60, 61). Impairment in GLUT4 translocation contributes to the decreased glucose uptake in T2DM and results in abnormalities in glucose homeostasis (62). It has been speculated that, in response to persistent hyperglycaemia, the glucose transport system in skeletal muscle may become desensitized to insulin as a protective mechanism (63). Mitochondrial dysfunction has also been shown to contribute to insulin resistance and to the pathophysiology of T2DM (64-66).

Insulin resistance is associated with the accumulation of visceral body fat (67, 68). Adipose tissue modulates metabolism by releasing free fatty acids (FFA), as well as other hormones such as adiponectin and leptin, and proinflammatory cytokines (69-71). Visceral adipose tissue is metabolically more active than subcutaneous adipose tissue (68). FFA in visceral deposits contribute significantly to increased hepatic glucose production (68, 72). Elevations of plasma FFA concentrations inhibit insulin-stimulated glucose transport as well as phosphorylation activity in skeletal muscle, contributing to insulin resistance (52, 73-75).

1.3.2 Impaired β -cell Function

Pancreatic islet β -cells regulate insulin release dependent on the nature, quantity and route of a stimulus and the circulating plasma glucose concentrations (72). In healthy individuals, a feedback loop between insulin sensitive tissues and the β -cells exists, which serves to increase insulin supply in response to demand from the liver, muscles and adipose tissue. The β -cells help to maintain a balance between hepatic glucose production and the rate of insulin-dependent utilization of glucose by the muscles and adipose tissue.

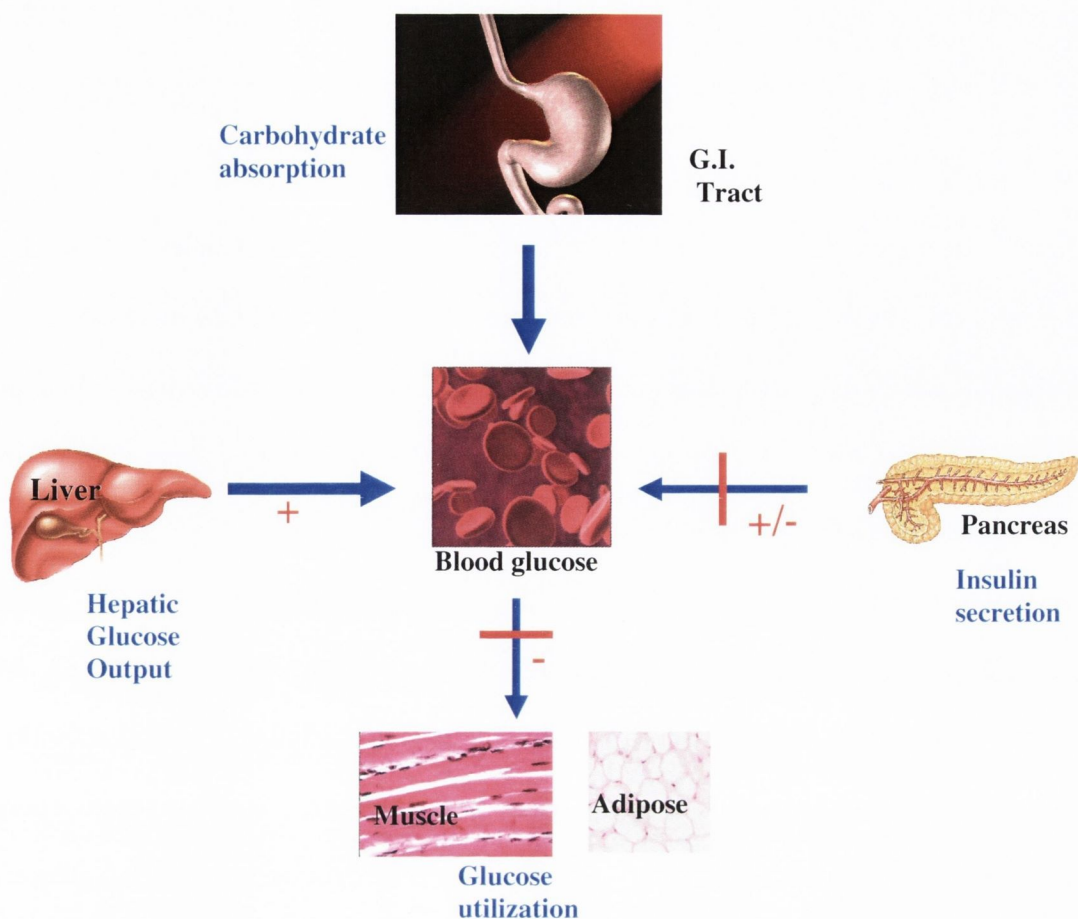


Figure 1.4 Pathogenesis of hyperglycaemia in type 2 diabetes. Carbohydrates are absorbed through the gastrointestinal tract (GI tract) and enter the blood stream as glucose. The pancreas secretes insulin to stimulate glucose uptake in muscle and adipose tissue, and suppress hepatic glucose production. Hyperglycaemia ultimately results from the β -cells' failure to secrete enough insulin to compensate for insulin resistance in the liver, muscle and adipose tissue.

As insulin resistance develops the β -cells secrete more insulin to stimulate glucose uptake. However, over time, in some individuals, the β -cells fail to produce enough insulin to adequately decrease blood glucose concentrations. Thus, insulin resistance and lack of adequate insulin secretion leads to an overproduction of glucose by the liver and a underutilization of glucose by peripheral tissues (68), Figure 1.4. As progressive β -cell failure occurs, subjects with normal glucose tolerance (NGT) progress to having overt T2DM (51, 76-78). In addition, in patients with T2DM glycaemic control continues to deteriorate over time due to increasing insulin resistance and β -cell failure (79-81).

1.3.3 Environmental Factors- Diet/Physical Activity

Environmental factors significantly contribute to the progression to T2DM in those who are genetically susceptible. Excessive food intake, inactivity and obesity all increase insulin resistance and thus are key triggers for progression of T2DM. Studies from the U.S. have shown that for every 1 kg increase in body weight, the risk of T2DM increased by 9% (8). Other studies have shown that preventing weight gain, by 1 body mass index unit (BMI), resulted in a 12.5% reduction in the incidence of T2DM in patients with normal glucose tolerance (82).

Obesity is strongly associated with sedentary behaviour (i.e. high television viewing time and low physical activity time (9)) and consumption of energy dense foods (83, 84). Balancing energy intake (food consumption) with energy expenditure (physical activity) is necessary for weight maintenance. Weight gain occurs if energy intake exceeds energy expenditure. A study from the U.S. has recently shown that the increasing prevalence of obesity has occurred in parallel with an increase in the energy density of food consumed (85). Changes in social behaviour (i.e. decreased physical activity) have also occurred during the same period which have also contributed to weight gain (13). A recent study from the United Kingdom has shown that average recorded energy intake has declined as obesity rates have continued to increase which implies that energy expenditure has decreased even more (86).

In Pima Indians, an ethnic group that is genetically very susceptible to T2DM, habitual physical activity has been shown to prevent obesity and the development of T2DM (6.9% vs. 38% prevalence rate, active vs. inactive respectively) (87). Other studies have indicated that the majority of patients with diabetes or those at high risk for developing diabetes do not engage in regular physical activity (88). Furthermore, the likelihood of developing diabetes and diabetes-related cardiovascular co-morbidities tends to increase with physical inactivity regardless of BMI (12). Thus, there is a clear relationship between physical inactivity and progression to diabetes in those predisposed.

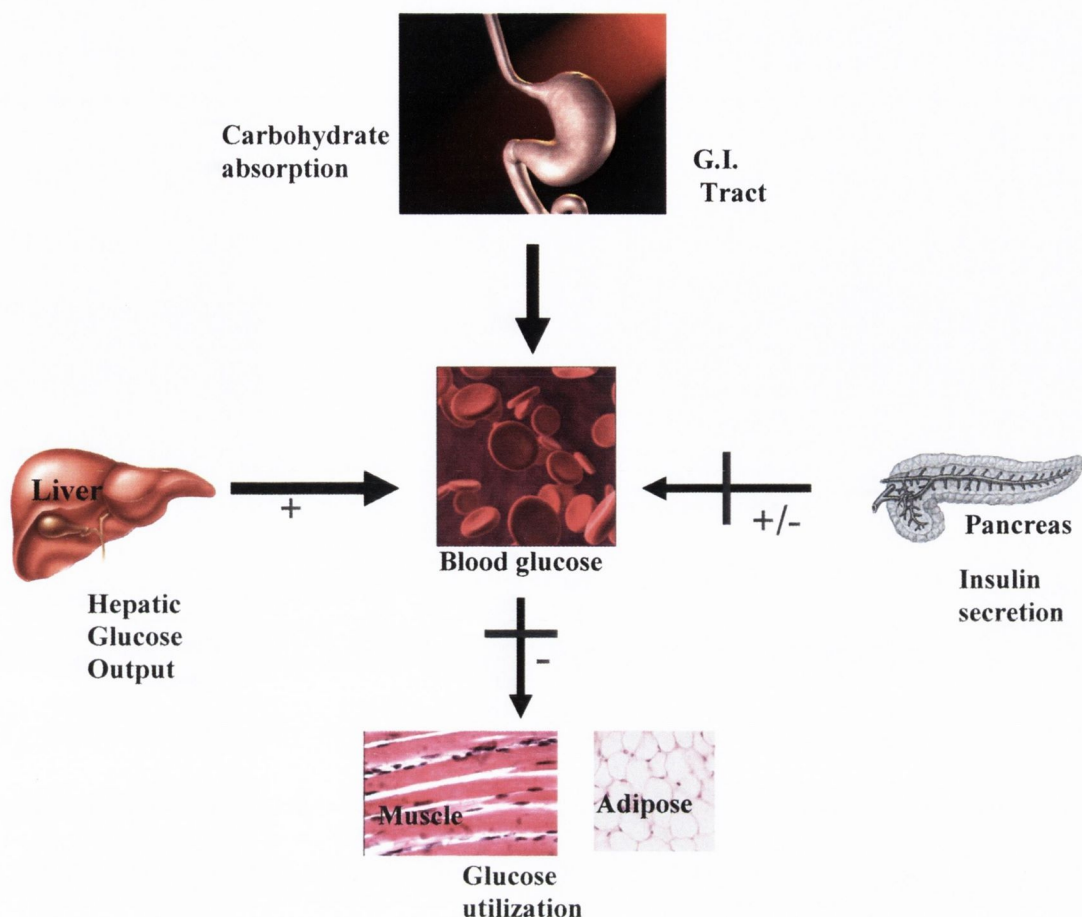


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1.3.4 Genetic Predisposition

1.3.4.1 Obesity-related genes

There are two distinct monogenic forms of obesity: the leptin/leptin receptor deficiency (89-91) and the melanocortin 4 receptor deficiency (92-95). Melanocortin 4 receptor (MC4R) deficiency is the most common monogenic cause of human obesity (92); in obese populations the prevalence of carriers ranges from 2.4-5.8% (95-98). A mutation in the gene that codes for MC4R has been shown to cause severe obesity, increases in lean body mass, increases in linear growth, hyperphagia, and severe insulin resistance (95, 98). Patients with a deficiency in leptin (or the receptor) are severely obese from a young age, hyperinsulinaemic, hyperphagic, and have a high energy intake (89, 90).

Genome-wide association studies have led to the discovery of the Fat Mass and Obesity Associated Gene (FTO) (99-102) and the proopiomelanocortin gene (POMC) (89, 103, 104). Variants in the FTO gene have shown a strong and highly significant association with obesity and obesity-related traits (99-101, 105). In addition, variations in FTO can contribute to extreme early-onset obesity (102). The POMC region on chromosome 2 (2p22) has been associated with plasma leptin concentrations (106-108). Variations in POMC expression may be influential in determining body weight (103) and regulating metabolism (89, 104).

1.3.4.2 Diabetes-related genes

Population studies have previously shown that a family history of T2DM contributes strongly to future risk of developing the disease (109-111). Although many genes have been implicated in the pathogenesis of T2DM, the influence of genetic susceptibility remains poorly understood. T2DM is acknowledged to be a polygenic disease. Thus each partial contributor adds a very small increment of risk. The majority of genes identified to date have been associated not with insulin resistance but with insulin secretion. Increased

susceptibility to T2DM has been implicated with variations and mutations in the genes listed in Table 1.1.

Table 1.1 Genes associated with type 2 diabetes.

TCF7L2	Transcription factor 7-like-2 (112-121)
PPARg	Peroxisome Proliferator Activator Receptor Gamma (122-125)
KCNJ11	Kir6.2 (126-130)
FTO	Fat Mass and Obesity Associated Gene (109, 131)
CAPN10	Calpain 10 (132, 133)
IRS-1	Insulin Receptor Substrate-1 (134, 135)
CDKAL1	CDK5 regulatory subunit associated protein 1 like-1 (136-138)
HHEX	Hematopoietically expressed homeobox (125, 139-141)
SCL30A8	Solute carrier family 30 (Zinc Transporter), member 8 (139, 140, 142)
CDKN2A/B	Cyclin- Dependent Kinase Inhibitor 2A/B (139-141)
IGF2BP2	Insulin-like Growth Factor 2 mRNA Binding Protein 2 (125, 140, 141)

Maturity onset diabetes of the young (MODY) represents a number of monogenetic forms of type 2 diabetes, Table 1.2. MODY patients present with a phenotype quite distinct from that to be investigated in the current thesis. All forms of MODY involve genetically determined symptoms with the loss of insulin secretion as the primary defect (143, 144). MODY subjects are not typically insulin resistant in contrast to subjects with early-onset T2DM (143, 144).

Table 1.2 Genes associated with MODY.

MODY 1	HNF-4 α	Hepatocyte nuclear factor- 1 alpha (145-147)
MODY 2	GCK	Glucokinase (145-148)
MODY 3	HNF-1 α	Hepatocyte nuclear factor- 4 alpha (145-147)
MODY 4	IPF-1	Insulin Promoter factor- 1 (145-147)
MODY 5	HNF-1 β	Hepatocyte nuclear factor- 1 beta (145-147)
MODY 6	NeuroD1	Neurogenic differentiation 1 (145, 146)

1.4 Cardiovascular Disease Associated with Type 2 Diabetes

Type 2 diabetes has long been associated with adverse patterns of cardiovascular risk factors including hypertension, central obesity, and dyslipidaemia (149). T2DM, in itself,

has been proven to be an independent predictor of cardiovascular disease (CVD) (150-152), which accounts for nearly 70% of all deaths in people with diabetes (153). The risk of cardiovascular mortality is two to three times higher in men and three to five times higher in women with diabetes than in those without diabetes (149, 154-158), Table 1.3.

Table 1.3 Cardiovascular disease risk and diabetes. Various landmark studies relating death from cardiovascular disease to type 2 diabetes status.

	N =, gender	Age (yrs)	Follow-up	Main outcomes
Framingham study	5209, male & female	30-62 yrs	20yrs initially	2 to 3-fold ↑ risk in atherosclerotic disease in people with diabetes (154)
Nurses Health study	116,117 Female	30-55 yrs, Free of known CHD, stroke, & cancer	8yrs	↑ nonfatal and fatal cardiovascular events in women with T2DM (157)
Multiple Risk Factor Intervention Trial (MRFIT)	347,978 Male	35-57 yrs, 5163 had diabetes upon entry	12yrs	3x higher absolute risk from CVD death in men with T2DM (156)
Coronary heart disease equivalent	2,432 male & female	1373 had diabetes upon entry	18yrs	Diabetes without prior MI and prior MI without diabetes have similar risk of CHD death (159)

Abbreviations: CHD= coronary heart disease, CVD= cardiovascular disease, T2DM= type 2 diabetes, MI= myocardial infarction

One of the main findings from the Framingham study was a 2 to 3 fold increase in atherosclerotic disease in subjects with T2DM (154). Patients who have T2DM but who have not had a prior myocardial infarction (MI) carry the same risk of coronary heart disease (CHD) mortality as nondiabetic patients who have had a previous MI (159-161). The Nurses Health study helped to establish that both nonfatal and fatal cardiovascular events were substantially increased among woman with T2DM (157). Diabetes status proved to be a major independent determinant of cardiovascular risk. Only 2% of the total study population had T2DM; however, an estimated 13.8% of coronary events, 12.2% of cerebrovascular accidents, and 14.8% of cardiovascular events occurred in these women (157). The Multiple Risk Factor Intervention Trial (MRFIT) confirmed the above findings

in men; 603 deaths were reported in men with T2DM (11.7%) compared to 8965 deaths (2.6%) among men without T2DM (156).

Vascular complications represent the leading cause of death among people with diabetes. While the degree of hyperglycaemia and duration of diabetes have been associated with the risk of microvascular complications, the same relationship has not been demonstrated with macrovascular complications (162). The increased risk of macrovascular complications may start before the clinical diagnosis of T2DM due to the metabolic disturbances during the period before it presents clinically (162, 163). Often, once diabetes is diagnosed vascular complications are already present.

Early-onset T2DM appears to be a more aggressive form of the disease in terms of elevated cardiovascular risk. Young adults diagnosed with T2DM have a more adverse cardiovascular risk profile with markedly abnormal cardiovascular risk markers (35, 47, 164). They also carry a higher relative risk of MI and CVD mortality due to the longer duration of the disease, although the absolute rate is higher in older adults (35, 165).

1.4.1 Impaired Glucose Metabolism and Cardiovascular Disease

Disturbances in glucose metabolism play an important role in the pathogenesis of coronary heart disease. Hyperglycaemia in otherwise healthy populations has been associated with an elevation in cardiovascular risk (166, 167). Men with stable angina but no previous history of diabetes were screened with oral glucose tolerance tests (OGTT). Over 50% of the patients had disturbances in glucose metabolism, and of those almost 50% were reported to have T2DM (168). The disruption in glucose metabolism was observed more in men with pronounced atherosclerotic lesions and with two- and three-vessel disease, indicating that the prevalence of glucose abnormalities increased with the progression of CHD (168). The LURIC study demonstrated similar findings: 32% of patients who underwent coronary angiography had T2DM, 17% of which were previously undiagnosed (169). The EURO Heart survey has shown that nearly 70% of patients that presented with

an acute MI had previously undiagnosed impairments in glucose metabolism, either impaired glucose tolerance (IGT) or T2DM (170). Data from the same study showed that survival following an initial MI was worse for people diagnosed with IGT or T2DM compared to those that had normal glucose tolerance (NGT) (171). The Whitehall study has confirmed that the risk of mortality from CHD and stroke increases significantly for glucose intolerant subjects (155). There was a 2-fold increase in CHD mortality in participants without T2DM who had a raised 2 hr plasma glucose concentration following a 50 g OGTT ($5.3-11.0 \text{ mol.l}^{-1}$) (155, 172).

1.4.2 Hyperinsulinaemia and Cardiovascular Disease

Hyperinsulinaemia is closely associated with hyperlipidaemia and hypertension (50, 173). Recently, insulin sensitivity in addition to fasting insulin has been shown to be strongly correlated to elements of the metabolic syndrome (173). A number of population studies have shown that hyperinsulinaemia predicts the development of CVD, more specifically ischaemic heart disease (IHD) in men (174-176). In a prospective study conducted in men without T2DM, a high fasting plasma insulin concentration was associated with increased incidence of IHD (177). At baseline, the men who developed IHD had an 18% higher plasma insulin concentration than those who did not develop IHD. In addition, the progression to T2DM increased among men in whom IHD developed (177). In abnormal glucose tolerance, insulin has been shown to be a predictor of death independent of total cholesterol, smoking status, and hypertension (149). Other population studies have shown that individuals with NGT who are hyperinsulinaemic have elevated cardiovascular risk (162, 178). The San Antonio Heart Study found that subjects with pre-diabetes were more hyperinsulinaemic and had a more atherogenic pattern of cardiovascular risk when compared to a healthy population (162).

1.4.3 Dyslipidaemia in Diabetes

Dyslipidaemia is present before the onset of T2DM in many subjects (162, 163). It is a major contributor to CVD risk and mortality in individuals with diabetes (152, 179), and is characterised by elevated plasma triglycerides and decreased high-density lipoprotein (HDL) cholesterol concentrations (162, 180). Insulin resistance and increased FFA transport may contribute to the lipid abnormalities observed (181, 182). Dyslipidaemia tends to be more severe in patients with T2DM, and in women when compared to men (181). Women with T2DM and decreased HDL cholesterol concentrations have an increased probability of CHD mortality compared to men and women without diabetes (183). Elevations in triglyceride concentrations are strongly associated with insulin resistance (181) and endothelial dysfunction (184). Triglycerides are atherogenic and strongly predict increased cardiovascular mortality (151).

1.4.4 Endothelial Dysfunction and Type 2 Diabetes

Endothelial dysfunction is implicated in the pathogenesis of CVD and precedes the development of atherosclerosis (185-189). Insulin resistance is an important factor in the early stages of endothelial dysfunction (50).

Independent of glycaemic control, endothelial function is poorer in patients with T2DM when compared to patients with type 1 diabetes due to insulin resistance (190). The concentrations of many inflammatory markers are increased in T2DM patients, especially in those with macrovascular complications (191-193). Elevation of several inflammatory markers has been associated with insulin resistance (193-195), and has been shown to be predictive of future cardiovascular events (196-201). The endothelium of the microvascular, macrovascular, and retinal systems are all insulin-sensitive. Insulin-induced vasodilatation has been shown to be impaired in nondiabetic obese individuals who display insulin resistance (184, 202-204). This may be due, in part, to a decrease in the vascular action of insulin (202, 205). Normally insulin stimulates basal nitric oxide production,

relaxing vascular smooth muscle cells, and decreasing the release of FFA (184). In insulin resistance there is a decreased anti-lipolytic activity of insulin resulting in increased circulating FFA concentrations, which decreases endothelium-dependent vasodilatation (184, 202, 206). The effects of FFA on the endothelium may be strongly dependent on the ability of endothelium lipase to hydrolyse triglycerides and phospholipids.

Hyperglycaemia may cause changes in endothelial function due to oxidative shear stress and the interaction of advanced glycosylation end-products with their specific receptors on the endothelium (184). In addition, increases in plasma soluble adhesion molecules are directly related to hyperglycaemia (184, 192-194). Although improvement of endothelial function is seen with the control of blood glucose levels, the degree of improvement depends on the initial level of hyperglycaemia (184).

Obesity also appears to contribute to endothelial activation in patients with T2DM (194, 207-209). Adipose tissue, as an endocrine organ, is thought to contribute to systemic and vascular inflammation, by releasing certain adipokines that contribute to the development of insulin resistance, endothelial dysfunction, hypertension and atherosclerosis (210).

1.5 Exercise

Daily physical activity is a major determinant of insulin sensitivity (211). A recent review has verified that adherence to physical activity recommendations can substantially reduce the risk of T2DM (212).

1.5.1 Exercise in the Prevention of Type 2 Diabetes in at Risk Populations

Lifestyle interventions have been shown to prevent or delay T2DM in persons at high risk for the disease (1, 2, 213-215), Table 1.4. One of the first intervention studies to look at the effects of lifestyle on T2DM risk was conducted in Malmö, Sweden (214). In this study, men with IGT or T2DM underwent 6 months of exercise training and 6 months of diet counselling in a cross-over design, and were evaluated annually for 5 yrs. Men who

participated in the intervention lost weight, improved their fitness and had a greater reversal of glucose intolerance when compared to those who received standard care (214), Table 1.4. After 12 yrs, subjects with IGT who participated in the intervention had a similar mortality rate to those who were NGT and half the mortality rate of subjects with IGT who did not participate in the intervention (214).

Table 1.4 Diabetes prevention studies. Landmark studies that have prevented T2DM in at risk populations.

	n =, age, status	Intervention time	Intervention	Outcomes in intervention groups
Malmö	n = 114 NGT n = 181 IGT n = 41 T2DM 47-49 yrs	5 yrs	▪ control ▪ lifestyle intervention, 6 months supervised physical training and 6 months diet treatment in cross over design, with annual check-ups	▪ 2.3-3.7% weight loss ▪ 10-14% ↑ VO₂max ▪ NGT in >50% of IGT patients ▪ >50% of diabetes patients in remission after 6 yrs (214)
Da Qing	n = 577 IGT	6 yrs	▪ control ▪ diet ▪ exercise ▪ diet + exercise	▪ 31% ↓ T2DM by diet ▪ 46% ↓ T2DM by exercise ▪ 42% ↓ T2DM by diet + exercise (213)
Finnish Diabetes Prevention Study (DPS)	n = 522 IGT 40-64 yrs	Mean duration 3.2 yrs	▪ control ▪ individualized counselling for weight reduction of ≥5% by diet (↓ total and sat fat intake, ↑ fibre) and ↑ exercise	▪ weight loss after 3 yrs 3.5 ± 5.5 kg ▪ Risk of diabetes reduced by 58% in intervention group (2)
Diabetes Prevention Program (DPP)	n = 3234 IGT Mean age 51yrs Mean BMI 34	Mean follow-up 2.8 yrs	▪ placebo ▪ metformin ▪ lifestyle modification 7% weight loss, 150 min physical activity weekly	▪ lifestyle intervention ↓ incidence T2DM by 58% ▪ metformin ↓ 31% (1)
Indian Diabetes Prevention Programme (IDPP-1)	n = 531 IGT age = 45.9 ± 3.5 yrs BMI 25.8± 3.5	Mean follow-up 30 months	▪ control ▪ lifestyle modification ▪ metformin ▪ lifestyle and metformin	▪ 28.5% ↓ T2DM with lifestyle ▪ 26.4% ↓ T2DM with metformin ▪ 28.2% ↓ T2DM with lifestyle + metformin (215)

Abbreviations: NGT= normal glucose tolerant, IGT= impaired glucose tolerant, T2DM= type 2 diabetes mellitus, BMI= body mass index

In the Da Qing study, a dietary and exercise intervention led to a significant decrease in the incidence of T2DM in individuals at risk (213). Participants in the dietary intervention

were given individual and group counselling throughout the study, while participants in the exercise intervention were taught and encouraged to increase their leisure time physical activity. The diet, exercise, and diet plus exercise intervention groups were associated with a 31%, 46%, and 42% reduction in the risk of developing T2DM respectively, when compared to the control group (213).

The Finnish Diabetes Prevention study (DPS), which had a similar intervention to the Malmö study, also showed similar results (2). The goal was 5% weight loss. Subjects in the intervention group were given dietary advice to consume less fat (total fat <30% energy consumed, saturated fat <10% energy consumed) and to increase their fibre intake (15 g per 1000 kcal). They were also encouraged to increase their physical activity by at least 30 min of moderate intensity activity per day. The intervention group experienced greater weight loss when compared to the control group (3.5 vs. 0.9 kg, $P<0.0001$), and had substantial improvements in measures of glycaemic control and dyslipidaemia after 3 yrs (216). Additionally, insulin secretion and sensitivity were examined in a subgroup of 87 male participants. After 4 yrs, insulin sensitivity tended to be higher in the intervention group ($P=0.067$), and was strongly correlated with weight loss (217).

More recently, intervention studies have been performed utilising antihyperglycaemic agents. The Diabetes Prevention Program (DPP), for example, studied 3234 participants who had IGT (1). Participants were randomly assigned to three groups: lifestyle intervention, metformin, and placebo. The lifestyle intervention consisted of at least 150 min of physical activity per week. The incidence of diabetes was decreased by 58% by lifestyle intervention alone, and by 31% in the metformin-treated group when compared to the placebo group (1). These results were similar in men and women, and in all ethnicities and age groups studied. The lifestyle intervention group showed greater decreases in Glycated haemoglobin (HbA1c) and increased rates of glucose disposal compared to those in the metformin-treated group (1). The Indian Diabetes Prevention Programme replicated

the DPP study in younger, leaner, more insulin resistant native Asian Indian subjects who had IGT, with similar results (215), Table 1.4.

Even more recently, intervention studies have been conducted in populations with normal glucose tolerance. In the ProActive trial, changes in physical activity, energy expenditure, and aerobic fitness in 365 subjects with a family history of T2DM were examined (218). Following 1 yr of participation, increases in activity ($P=0.004$) and fitness ($P=0.003$) were associated with reduced metabolic risk (218). In the HERITAGE family study, increased physical activity, defined as 50 min per day 3 times per week, for 20 weeks, led to an increase in mean insulin sensitivity ($P<0.001$) and glucose disappearance ($P=0.02$) among previously healthy but sedentary men and women (219). Alternations in physical activity and/or weight loss appear to be the key contributors to changes in insulin sensitivity and glucose tolerance independent of initial glucose metabolism.

1.5.2 Exercise and Improved Insulin Sensitivity

Physically active adults with T2DM have been shown to have a more favourable metabolic and cardiovascular profile when compared to sedentary adults with T2DM (220). In a meta-analysis of exercise trials looking at glycaemic control independent of changes in body weight, exercise training was associated with a reduced HbA1c (221).

Acute exercise improves insulin sensitivity in the liver and muscle (44, 222). In skeletal muscle, exercise has been shown to increase glucose uptake which can lead to decreased plasma glucose levels (44, 223-225). Exercise that depletes muscle glycogen stores leads to increased insulin sensitivity as the body “super compensates” for the utilised glycogen (226). A single bout of aerobic exercise at 85% VO_2max for 2 min, alternating with 3 min rest periods until exhaustion, has been shown to increase peripheral and splanchnic insulin sensitivity in men with T2DM (227).

Adaptive responses to chronic exercise are mediated primarily through skeletal muscle (222, 228). Chronic exercise elicits enhancements in insulin stimulated skeletal muscle

glucose transport, reduced hepatic glucose production, and improved blood flow to skeletal muscle (44, 222). Habitual physical activity results in increased insulin sensitivity across all levels of glucose tolerance, from NGT to T2DM (229). During exercise, GLUT4 translocation is increased, thus increasing the rate of glucose transport into skeletal muscle. The increases in glucose uptake can persist for several hours, resulting in a fall in glycaemia (44). This results in a lower basal and glucose-stimulated insulin concentration, which contributes to a decreased insulin response to glucose challenges (223). One week of aerobic exercise training has been shown to increase whole-body insulin mediated glucose disposal in obese subjects with T2DM. This increase was associated with an increase in muscle GLUT4 protein content independent of changes in insulin signalling proteins (230). In addition, increased glucose tolerance has been associated with increased mitochondrial fatty acid oxidation in obese subjects with and without T2DM after exercise training (231, 232). Improvements in insulin sensitivity have also been associated with increases in mitochondrial density and mitochondrial oxidative enzymes (233, 234).

1.5.3 Exercise and Improvements in Cardiovascular Disease Risk Markers

Regular physical activity, in leisure time or at work, can reduce the risk of mortality from CVD (3, 235, 236). A dose-response relationship exists between the amount and intensity of exercise and improvements in the risk parameters of cardiovascular health (228). Acute bouts of endurance exercise lead to acute reductions in triglycerides and low-density lipoprotein (LDL) cholesterol and increases in HDL cholesterol (4, 237-239). Chronic exercise training has been associated with more permanent increases in HDL cholesterol (238, 240-243) and decreases in LDL cholesterol (238). Triglyceride concentrations have not been shown to be consistently lowered by exercise interventions in individuals with T2DM (244); however, studies have shown significant decreases among obese controls (240, 245). Exercise can lead to significant reductions in blood pressure and these reductions appear to increase with energy expenditure (4, 246-248). It has been speculated

that exercise has a protective effect on the endothelium, and may contribute to a reduced CVD risk (249). This is reinforced by the improved concentrations of endothelial risk markers after exercise (249-254). Weight reduction in obese individuals with and without T2DM has been associated with an improvement in endothelial inflammatory markers (255-257).

More recently the idea of fitness, as opposed to physical activity, has been examined in relation to mortality. The Aerobics Centre Longitudinal study revealed that low cardiorespiratory fitness was a strong independent predictor of CVD and all-cause mortality (258). In agreement with the above study, the Lipid Research Clinics study further concluded that higher fitness levels did not completely reverse the increased risk associated with excess adiposity (259). However, in subjects with T2DM the relationship between fitness and mortality has been shown to be independent of BMI; the mortality rate is higher among less fit subjects (260).

1.5.4 Exercise Guidelines for Type 2 Diabetes

Exercise is beneficial in the prevention and treatment of T2DM and in addition to diet and pharmacotherapy forms the basis of diabetes treatment. In children and adolescents, exercise and diet are recommended as the primary treatment for T2DM (48). The goals of exercise training for obese subjects with T2DM are to improve glycaemic control, assist with weight maintenance, and reduce the risk of CVD (261). The most recent statement from the American Diabetes Association (ADA) recommended 150 min/week of moderate activity (40-60% VO_2max) or 90 min/week of vigorous activity (>60% VO_2max) distributed over at least 3 days with no more than two days without physical activity (261). Both moderate and vigorous intensity exercise, with equivalent caloric expenditure, has been shown to significantly reduce insulin resistance (262). These exercise recommendations are the same as those proposed for all adults to facilitate the maintenance of health. More recently, studies have shown that adding resistance training to an aerobic

exercise programme may help improve glucose metabolism (263-265). The addition of resistance training has also recently been adopted by the ADA (261). The American College of Sports Medicine has recommended that people with T2DM should aim to achieve 1000 kcal of physical activity a week through aerobic exercise in addition to participating in a well-rounded resistance training programme (266, 267). Many recent reviews have confirmed the benefits of aerobic training, resistance training and aerobic combined with resistance training on improving insulin sensitivity in both young and older subjects with T2DM (268, 269). To date, there remain no standard clinical guidelines for effective exercise interventions in the treatment of T2DM.

1.6 Summary:

The phenotype of young adults with T2DM is incompletely understood. These patients have elevated cardiovascular risk markers and are at increased risk of future cardiovascular events. Traditional intensive diabetes treatment may not suffice in lowering the risk of diabetes-related complications. There are serious economic implications with the decreasing age of T2DM diagnosis: given the cost of long-term treatment, insulin therapy, diabetes-related devices, and further medications for diabetes-related complications. This is only exacerbated by the longevity of the disease. A focused lifestyle treatment programme will be necessary to address diabetes and cardiovascular disease in young adults.

Chapter Two:

Characteristics of the Physically Active but Insulin Resistant Phenotype

This study was completed with the help of Prof. JJ. Nolan, Dr. H. Thabit, and Dr. S. Shah on behalf of the EGIR-RISC Study Group

2.1 INTRODUCTION

Habitual physical activity has been shown to independently contribute to insulin sensitivity (211, 229). Increasing physical activity can lead to enhancements in insulin action in insulin-resistant tissues (222, 226). Exercise has been shown to improve various cardiometabolic risk factors, such as insulin resistance and elevated cholesterol concentrations (217-219, 248). Even at moderate intensity, exercise can have a positive effect on body fat distribution by decreasing the proportion of visceral fat (270).

Although most studies have shown an improvement in insulin sensitivity with exercise, the insulin sensitivity response to exercise training differs among individuals. The gene-environment interaction which contributes strongly to insulin resistance may also be a factor in exercise training outcomes. The Heritage study has recently identified genes that contribute to exercise responsiveness (271, 272). The four-and-a-half LIM domains 1 (FHL1) gene was found to be over-expressed by 50-470% of those who had the highest insulin sensitivity improvement. The ability to identify genotype and phenotype variances in response to exercise may eventually play a role in the management of T2DM and obesity (273-275). This would allow clinicians to prescribe complete management protocols (including exercise and medication) for their patients that will promote the best cardiometabolic outcomes.

2.1.1 Aim

The aim of this study was to examine the phenotypic and biochemical differences between two well-defined cohorts: subjects who are physically active but insulin resistant (AIR), and subjects who are physically inactive and insulin sensitive (IIS).

2.2 METHODS

2.2.1 General Experimental Design

The European Group on Insulin Resistance -Relationship between Insulin Sensitivity and Cardiovascular Risk (EGIR- RISC) study was a pan-European, prospective observational cohort study in which 18 clinical centres from 13 European countries participated (276). The main aims of the protocol were to study insulin sensitivity and CVD risk in a population of healthy European subjects. Initially, subjects attended for baseline visits. Following successful entry into the study they were followed for several years. This study is still ongoing, see full methodology in Hills *et al.*, 2004 (276). This report contains baseline characteristics only.

Subjects attended for a clinical examination, oral glucose tolerance test and were fitted with an accelerometer. One week later they returned for a euglycaemic-hyperinsulinaemic clamp with tracer.

2.2.2 Subjects

1,500 subjects between the ages of 30 and 60 participated in the EGIR-RISC Study between 2002 and 2004. The subjects were not included for screening if: they were on treatment for obesity, hypertension, dyslipidaemia, chronic systemic illnesses, cardiovascular disease, were diagnosed with cancer in the last 5 yrs, on steroid therapy, pregnant, or had weight change > 5 kg in the last month. Following the screening visit and a 75 g oral glucose tolerance test, subjects were excluded if they had: high blood pressure (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg), plasma cholesterol ≥ 7.8 mmol.l⁻¹, triglycerides ≥ 4.6 mmol.l⁻¹, fasting or post-prandial plasma glucose ≥ 7.0 mmol.l⁻¹ or 11.1 mmol.l⁻¹ respectively, or electrocardiogram (ECG) abnormalities. Not all subjects completed the physical activity protocol (accelerometer recordings).

Data on physical activity and insulin sensitivity are available for 343 males and 455 females.

2.2.3 Anthropometric Measurements

All centres performed measurements according to a common protocol (276). Measurements were taken from subjects wearing minimal clothing without shoes. Height was measured with the subject standing upright using a clinical stadiometer in centimetres to the nearest 0.5 cm. Weight, BMI, total percentage body fat, body fat mass and lean body mass were measured using a bipodal bio-electrical impedance device (Tanita TBF-300 Body Composition Analyser, TANITA corp., Tokyo, Japan). Waist, hip and thigh measurements were measured using a tape measure. An automated 12-lead ECG was performed. After sitting for at least 10 min, blood pressure and heart rate were measured three times using an automated oscillometric blood pressure cuff (Omron 705 CP, Matsusaka Co., Japan).

2.2.4 Physical Activity Measurements

Physical activity for each participant was measured objectively using a CSA Actigraph (MTI: Manufacturing Technology, Fort Walton Beach, Florida, USA). The Actigraph has been shown in various studies to provide a validated measure of physical activity (277). The device reports physical activity in counts. It measures changes in acceleration 30 times per second during an epoch period (1min). At the end of 1 epoch, 1,800 measurements are summed. The total physical activity was determined for each subject, as the total number of counts per day

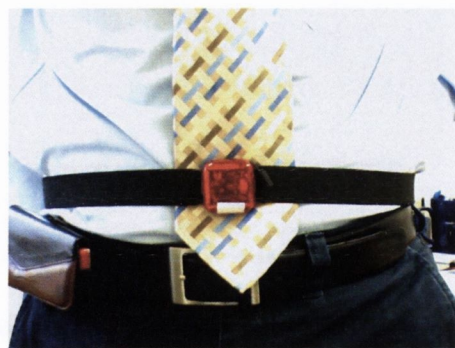


Figure 2.1

An Actigraph

averaged over the days worn. The device was secured around the subject's waist using an elasticated belt, Figure 2.1. Subjects were asked to wear it upon waking until going to bed, and were asked only to remove it when bathing or during water-based physical activities.

Data was used only from days in which the Actigraph was worn for more than 10 hrs. Data from participants who wore the Actigraph for less than three days were excluded.

2.2.5 Insulin Sensitivity Measurements

Insulin sensitivity was measured using a 2 hr euglycaemic-hyperinsulinaemic clamp, the procedures of which were also standardized. A primed-continuous infusion rate of $240 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ of insulin was used and a variable dextrose infusion was adjusted every 5-10 min to maintain the target plasma glucose concentration between 4.5-5.5 mmol.l⁻¹ ($\pm 15\%$). The steady state period for calculation of the insulin sensitivity (M) was calculated over the final 40 min of the clamp (from 80-120 min), and is expressed as the ratio of the M value to the mean plasma insulin (M/I, in $\mu\text{mol min}^{-1} \text{kg}_{\text{ffm}}^{-1} \text{nM}^{-1}$).

2.2.6 Laboratory Analysis

Plasma and serum collected during the studies were centrifuged, aliquoted and stored at -20°C for glucose, insulin and C-peptide, and -80°C for lipids, FFA and adiponectin. Biological samples were assayed at the central laboratory in Odense, Denmark.

2.2.7 AIR vs. IIS Subanalysis

Data collected from individuals with both physical activity counts and insulin sensitivity values were divided into tertiles.

Subjects classified in the upper physical activity tertile (active) were defined as those with the highest average number of counts per minute from the Actigraph. Those in the lowest physical activity tertile (inactive) had the lowest average number of counts per minute. Subjects in the upper tertile of insulin sensitivity were found to have the highest M/I values (insulin sensitive= IS). Those in the lowest tertile of insulin sensitivity had the lowest M/I values (insulin resistant= IR). Each tertile consisted of an equal number of subjects ($n = 266$ subjects).

Subjects in the upper tertile of physical activity who were in the lowest tertile of insulin sensitivity were then collated to produce a subgroup of subjects who were physically *active* but remained *insulin resistant* (AIR). Similarly, a subgroup of subjects who were physically *inactive* but *insulin sensitive* (IIS) was formed, Figure 2.2. In total, 65 AIR and 61 IIS subjects were subdivided and analysed.

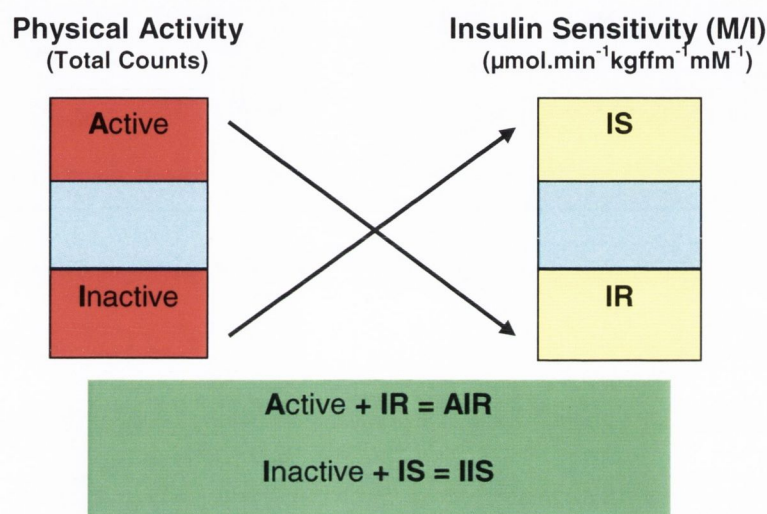


Figure 2.2 Creation of AIR vs. IIS subgroups. AIR $n = 65$, IIS $n = 61$.

2.2.8 Statistical Analysis

All statistical analyses were performed using SPSS version 13 (SAS). Data are presented as Mean $\pm$ standard deviation (SD) or percent, where appropriate. Comparisons between AIR and IIS were performed using independent-sample t-tests for normally distributed variables. Logarithmic transformations were performed for non-normally distributed variables, but data are presented transformed back to their original units. $P < 0.05$ was considered statistically significant.

2.3 RESULTS

2.3.1 Baseline Characteristics

The baseline characteristics from the study cohort that had both activity counts and insulin sensitivity measurements are shown in Table 2.1. The mean waist circumference, waist: hip ratio (WHR), systolic and diastolic blood pressures were within normal limits. BMI was on the higher side of normal. Fasting plasma glucose, lipids and 2 hr post OGTT plasma glucose were all within normal limits.

Table 2.1 Baseline characteristics of the study cohort. A total of 798 subjects were included.

	Mean ± SD
Percent male	43%
Age (yrs)	44.3 ± 8.4
Weight (kg)	73.1 ± 14.3
BMI (kg.m ⁻²)	25.1 ± 3.8
Fat mass (kg)	20.2 ± 8.8
Waist (cm)	85.9 ± 12.2
WHR	0.86 ± 0.1
Systolic blood pressure (mmHg)	117.5 ± 12.6
Diastolic blood pressure (mmHg)	74.2 ± 7.9
Total cholesterol (mmol.l ⁻¹)	4.8 ± 0.9
High-density lipoprotien cholesterol (mmol.l ⁻¹)	1.5 ± 0.4
Low-density lipoprotien cholesterol (mmol.l ⁻¹)	2.9 ± 0.8
Triglycerides (mmol.l ⁻¹)	1.1 ± 0.7
Fasting plasma glucose (mmol.l ⁻¹)	5.1 ± 0.5
2hr post OGTT glucose (mmol.l ⁻¹)	5.7 ± 1.5
Fasting insulin (pmol.l ⁻¹)	33.4 ± 18.1
Adiponectin (mg.l ⁻¹)	8.8 ± 3.8
Fasting FFA (mmol.l ⁻¹)	0.5 ± 0.2
M/I (μmol.min ⁻¹ .kg _{ffm} ⁻¹ .nM ⁻¹)	149.8 ± 73.2

Data are presented as Mean ± SD. Abbreviations: BMI= body mass index, WHR= waist: hip ratio, OGTT= oral glucose tolerance test, FFA= free fatty acids, M/I= insulin sensitivity as a ratio to mean plasma insulin

2.3.2 Comparison between AIR and IIS subjects

The comparison between the AIR and IIS subjects are shown in Table 2.2. There were a higher percentage of males in the AIR group than in the IIS group (57% vs. 27%). There were no differences in age between the two groups. On average, subjects in the AIR group did twice as much physical activity per day as measured by the actigraph ($P<0.01$). The AIR group had significantly higher BMI ($P<0.01$), waist circumference ($P<0.005$) and WHR ($P<0.005$) than the IIS group; however, there was no significant difference in fat mass between the two groups.

Table 2.2 Comparison between AIR vs. IIS subjects.

	AIR (n = 65)	IIS (n = 61)	P-value (<0.05)
Percent male	57%	23%	
Age (yrs)	43.9 ± 7.2	44.6 ± 8.8	NS
BMI (kg.m ⁻²)	25.9 ± 3.8	23.6 ± 3.0	0.01
Waist (cm)	88.5 ± 9.9	80.5 ± 9.9	<0.005
WHR	0.88 ± 0.07	0.82 ± 0.1	<0.005
Fat mass (kg)	20.7 ± 7.7	19.3 ± 6.5	NS
Total cholesterol (mmol.l ⁻¹)	4.64 ± 0.87	4.71 ± 0.82	NS
HDL cholesterol (mmol.l ⁻¹)	1.35 ± 0.36	1.61 ± 0.4	<0.005
LDL cholesterol (mmol.l ⁻¹)	2.75 ± 0.77	2.69 ± 0.78	NS
Triglycerides (mmol.l ⁻¹)	1.2 ± 0.83	0.87 ± 0.38	<0.01
Fasting glucose (mmol.l ⁻¹)	5.2 ± 0.5	5.0 ± 0.4	NS
2hr post OGTT glucose (mmol.l ⁻¹)	6.2 ± 1.3	5.2 ± 1.2	<0.005
Fasting insulin (pmol.l ⁻¹)	38.7 ± 18.4	25.5 ± 9.8	<0.005
Adiponectin (mg.l ⁻¹)	6.9 ± 3.1	10.1 ± 4.2	<0.005
Actigraph counts	570.9 ± 235.7	232.1 ± 45.7	<0.005
M/I (μmol.min ⁻¹ .kg _{ffm} ⁻¹ .nM ⁻¹)	86.7 ± 21.7	232.0 ± 104.5	<0.005

Data are expressed as Mean ± SD. Abbreviations: BMI= body mass index, WHR= waist: hip ratio, HDL= high-density lipoprotein, LDL= low-density lipoprotein, OGTT= oral glucose tolerance test, M/I= Insulin sensitivity as a ratio to mean plasma insulin.

There were no differences in fasting plasma glucose; however, the 2 hr post OGTT plasma glucose was significantly elevated, as was fasting plasma insulin in the AIR group (both $P<0.005$). The AIR group was significantly more insulin resistant (M/I) when compared to the IIS group ($P<0.005$). The AIR group had significantly higher FFA values (0.60 ± 0.22 vs. 0.51 ± 0.22 mmol.l⁻¹, $P<0.05$), Figure 2.3, but significantly lower plasma adiponectin concentrations ($P<0.005$). Triglycerides were elevated ($P<0.01$) and HDL cholesterol concentrations ($P<0.005$) were lower than those measured in the IIS group. There were no differences in total or LDL cholesterol concentrations, Table 2.2.

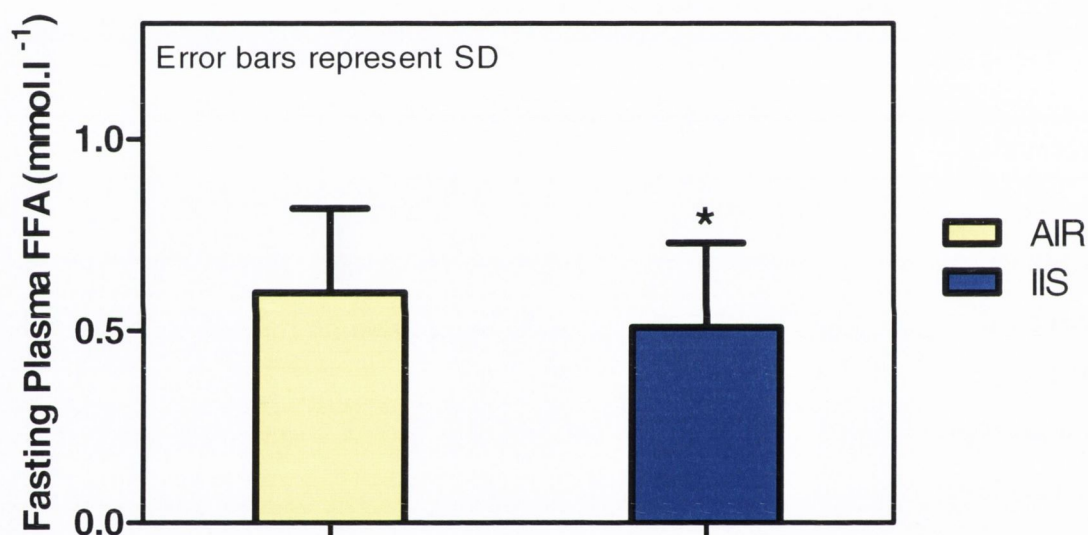


Figure 2.3 Comparison of plasma FFA concentrations in the AIR and IIS groups. Data are presented as Mean $\pm$ SD. *Significantly different from AIR group ($P<0.05$)

2.3.3 AIR vs. IIS gender comparison

The AIR and IIS groups were further subdivided according to gender, see Table 2.3 for characteristics. In comparing AIR and IIS males, there were no significant differences in age, BMI, fat mass, waist circumference, or WHR between the two groups. All plasma cholesterol concentrations were similar between the two male groups.

Despite having no difference in age, the AIR female group had a significantly higher BMI ($P<0.05$), fat mass ($P<0.05$) and waist circumference ($P<0.05$) when compared to the

female IIS group, Table 2.3. There were no differences between any of the previously mentioned cholesterol concentrations.

Table 2.3 AIR vs. IIS gender comparison

	Males		Females	
	AIR n = 37	IIS n = 14	AIR n = 28	IIS n = 47
Age (yrs)	43.6 ± 7.6	40.2 ± 9.6	44.4 ± 6.9	45.9 ± 8.2
Weight (kg)	82.4 ± 10.9	80.8 ± 9.4	68.9 ± 11.5	63.8 ± 8.5
BMI (kg.m ⁻²)	26.0 ± 2.9	25.1 ± 2.6	25.9 ± 4.7	23.2 ± 2.9*
Fat mass (kg)	17.9 ± 6.4	16.0 ± 5.8	24.4 ± 7.8	20.3 ± 6.4*
Waist (cm)	92.3 ± 7.8	88.9 ± 10.1	83.6 ± 10.3	78.0 ± 8.5*
Waist: hip ratio	0.91 ± 0.06	0.90 ± 0.07	0.83 ± 0.06	0.80 ± 0.1
Total cholesterol (mmol.l ⁻¹)	4.62 ± 0.85	4.27 ± 0.91	4.67 ± 0.91	4.84 ± 0.76
HDL cholesterol (mmol.l ⁻¹)	1.22 ± 0.27	1.32 ± 0.27	1.52 ± 0.41	1.69 ± 0.39
LDL cholesterol (mmol.l ⁻¹)	2.80 ± 0.69	2.48 ± 0.71	2.69 ± 0.86	2.76 ± 0.79
Triglycerides (mmol.l ⁻¹)	1.34 ± 1.0	1.0 ± 0.35	1.02 ± 0.41	0.84 ± 0.39

Data are presented as Mean ± SD. Abbreviations: BMI = Body mass index, WHR = waist: hip ratio, HDL = High density lipoprotein, LDL = low-density lipoprotein. *Significantly different from AIR subjects (*P*<0.05).

There were no differences in fasting plasma glucose in either the males (5.36 ± 0.51 vs. 5.16 ± 0.36 mmol.l⁻¹) or females (4.99 ± 0.46 vs. 4.99 ± 0.4 mmol.l⁻¹) in either group (AIR vs. IIS respectively); however, post OGTT glucose concentrations were significantly elevated in both the AIR males (6.32 ± 1.34 vs. 4.87 ± 1.27 mmol.l⁻¹, *P*<0.01) and AIR females (6.19 ± 1.23 vs. 5.3 ± 1.21 mmol.l⁻¹, AIR vs. IIS respectively *P*<0.01).

Adiponectin remained significantly lower in the AIR group when compared to the IIS group in both males (5.7 ± 2.6 vs. 6.9 ± 1.7 mg.l⁻¹, *P*<0.05) and females (8.6 ± 2.8 vs. 11.1 ± 4.3 mg.l⁻¹, *P*<0.01), Figure 2.4. In contrast, FFA remained significantly elevated in both AIR males and females when compared to the IIS groups (0.56 ± 0.24 vs. 0.38 ± 0.23

and 0.64 ± 0.18 vs. $0.54 \pm 0.21\text{mmol.l}^{-1}$, males and females respectively, both $P<0.05$),
Figure 2.5.

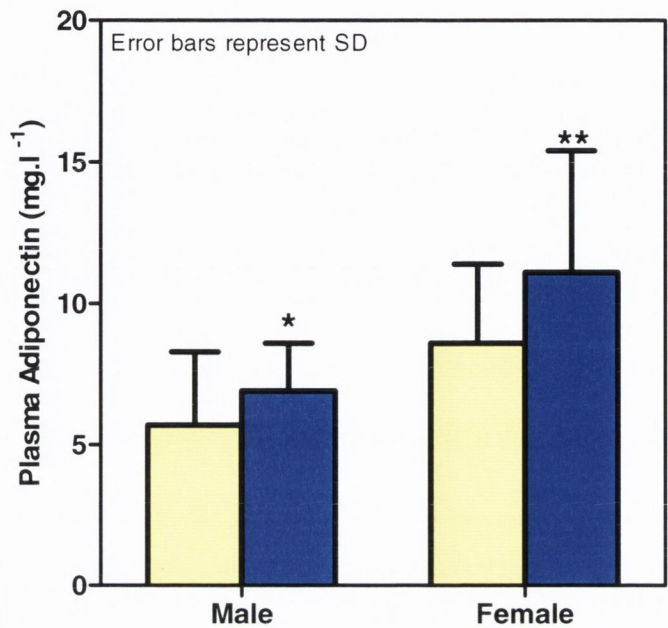


Figure 2.4
Comparison of adiponectin concentrations by gender in AIR and IIS subjects.
Values are presented as Mean $\pm$ SD. *Significantly different from AIR group ($P<0.05$) **Significantly different from AIR group ($P<0.01$).

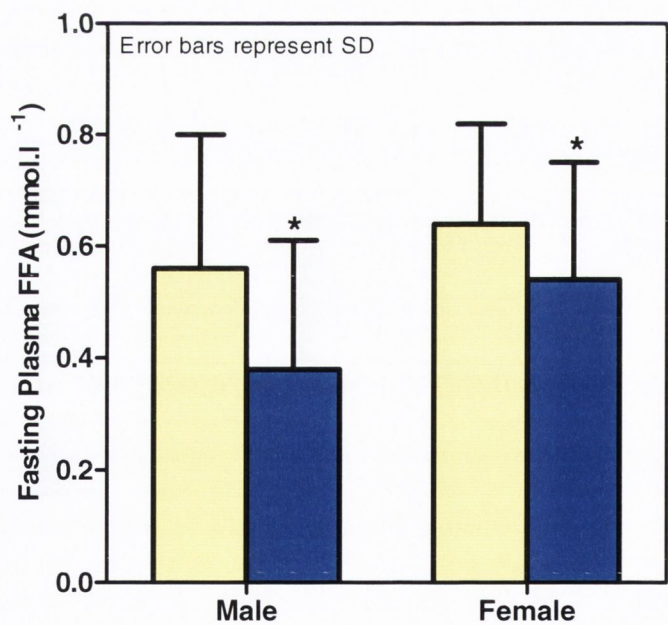


Figure 2.5
Comparison of FFA concentrations by gender in AIR and IIS subjects.
Values are presented as Mean $\pm$ SD. *Significantly different from AIR group ($P<0.05$).

2.4 DISCUSSION

The cohorts described in this chapter represent two subgroups (AIR and IIS) from a normal, healthy, middle-aged population. As can be seen in Table 2.1 and 2.2, subjects were non-obese with a normal BMI. They had a normal waist circumference, were normotensive and had lipid and glucose values within normal ranges. The subjects we have deemed as “physically active” represent the most active participants in this very “average” adult cohort. It is important to note that they may, however, still represent a relatively inactive group, as many studies have shown that the majority of adults do not meet current physical activity recommendations (278, 279). Nonetheless, when the cohort was divided by activity level and insulin sensitivity, significant differences were found in a number of variables.

The insulin resistance present in the AIR group when compared to the IIS group is the opposite of what is expected in physically active adults (211). Physical activity of moderate to vigorous intensity has been associated with reductions in insulin resistance and reduced risk of T2DM (212, 218, 280). In addition, time spent in light or moderate activity has been shown to be positively associated with 2 hr plasma glucose values (281, 282), contrary to our findings, Table 2.2. However, in spite of several studies demonstrating that physical activity improves insulin sensitivity, others have shown a stronger association for BMI with insulin sensitivity (283, 284). Various studies have described the relationship between insulin resistance and obesity (285-288). Although, participants in the current study were not obese, the AIR group had a significantly higher BMI ($P<0.01$), waist circumference ($P<0.005$), and WHR ($P<0.05$) when compared to the IIS group. Consistent with this, data from the Insulin Resistance Arteriosclerosis study has shown that abdominal obesity predicts declining insulin sensitivity and future development of metabolic syndrome, a precursor to T2DM and CVD (287, 289).

Abnormal fatty acid metabolism may lead to the accumulation of its metabolites in the liver and muscle contributing towards insulin resistance in these tissues (290, 291). An

important finding in this study is the elevated FFA concentrations in the AIR group, Figure 2.3. When the groups were further divided by gender, AIR subjects continued to have an elevated FFA concentration when compared to the IIS subjects, Figure 2.5. Consistent with this study, the Longitudinal Study of Aging has also shown that subjects with elevated post-OGTT glucose concentrations also have elevated fasting FFA concentrations (292). In the Longitudinal Study of Aging, the rate of suppression of lipolysis was a determinant of insulin sensitivity (292). Impaired FFA metabolism may contribute to the process of glucose intolerance in susceptible individuals (293). However, exercise has been shown to induce alterations in fatty acid partitioning and upregulate mitochondrial oxidative phosphorylation within the skeletal muscle to improve insulin sensitivity (231-234, 294, 295).

Reductions in plasma adiponectin concentrations have consistently been associated with insulin resistance and risk of T2DM (296-298); however, the pathophysiology of how adiponectin affects insulin resistance is not yet fully understood (299). Another key biochemical finding was the significantly lower adiponectin concentration observed in the AIR group when compared to the IIS group ($P<0.005$), a finding that was again present when the groups were divided by gender ($P<0.05$ vs. $P<0.01$, male vs. female respectively), Figure 2.4. Consistent with previous studies, males had a lower plasma adiponectin concentration when compared to females in both the AIR (5.7 ± 2.7 vs. 8.6 ± 2.6 mg.l⁻¹, male vs. female respectively, $P<0.005$) and IIS groups (6.9 ± 1.7 vs. 11.1 ± 4.3 mg.l⁻¹, male vs. female respectively, $P<0.005$) (299, 300). Although exercise has been shown to improve insulin sensitivity, there have been no consistent findings regarding physical activity and the effect it has on increasing adiponectin levels (301-305).

2.4.1 Conclusions

This is the first study to clinically compare two groups of subjects with opposing features of insulin sensitivity and physical activity in a healthy population. Strengths of the current

study included the objective recording of physical activity, using a validated accelerometer, and use of the euglycaemic-hyperinsulinaemic clamp, the “gold standard” in assessment of insulin sensitivity.

We have shown that the active group, who were insulin resistant, had higher concentrations of FFA and triglycerides as well as decreases in adiponectin and HDL cholesterol concentrations. Although both groups were of normal weight, the female AIR group was heavier and had a higher fat mass than the female IIS group; a finding not seen in the males.

Exercise has always been one of the primary treatments in the clinical management of obese patients and those with T2DM. However, evidence is emerging that there may be subgroups of patients that do not respond metabolically to exercise training (306, 307). The current study has shown that it may be possible to identify individuals who have a blunted response to exercise; and a slight alteration in lipid profiles may contribute to this lack of metabolic response. Interestingly we have also identified individuals who remained insulin sensitive despite being less active. This group may have inherently more efficient hepatic or intramuscular glucose disposal systems, therefore protecting them against insulin resistance. Further studies are needed to investigate factors that impair the metabolic response to physical activity.

Chapter Three:

Clinical Comparison of Younger Patients and Older Patients with Type 2 Diabetes

3.1 INTRODUCTION

Little is known about the clinical and cardiovascular risk status of younger onset type 2 diabetes patients compared to those that present later in life. Adolescents with T2DM are severely insulin resistant, even when compared to subjects of similar obesity and body fatness, with impaired insulin secretion relative to their degree of obesity (308-310). At follow-up, glucose control is often poor, and diabetes-related complications can occur earlier (34, 36). Progression of both microvascular and macrovascular complications have been documented in young individuals diagnosed with diabetes (31, 34). For example, neuropathy has been shown to be twice as prevalent in individuals with type 2 diabetes when compared to type 1 diabetes, with similar age of onset (311). Early-onset T2DM appears to be a more aggressive form of diabetes from a cardiovascular standpoint (165). Children and adolescents who have T2DM show early signs of cardiovascular risk in the form of increased abdominal obesity, insulin resistance, abnormal lipid profiles and elevated blood pressure (30). Data from our research group has shown early-onset T2DM patients have markedly abnormal cardiovascular risk markers, similar to those seen in patients thirty years older (47). Younger adults with T2DM appear to have a much higher risk of cardiovascular disease in comparison to age-matched subjects without diabetes (35, 165).

3.1.1 Aim

The aim of this study was to compare, in our clinic population, the clinical and metabolic characteristics of younger and older patients with T2DM.

3.2 METHODS

3.2.1 Study Design

This was a retrospective study of two distinct populations from the same outpatient clinic. Subjects were identified from our computerized clinical database. Each patient's first clinic visit and lab episode was examined to determine lipid and diabetes parameters at diagnosis, including HbA1c, C-peptide, total, HDL and LDL cholesterol, total cholesterol/HDL ratio, and triglycerides. Blood pressure, weight, and BMI were established from the same entry in the database. The most recent entry was used to identify the most up-to-date HbA1c value.

3.2.2 Subjects

The cohort of young T2DM subjects included all patients diagnosed under the age of 40, who had been attending our service for at least 1 year. A total of 149 younger subjects were included in the study with a mean age of 35.2 ± 0.4 yrs. An additional 217 older patients with T2DM diagnosed between the ages of 50-70 yrs (mean age 61.6 ± 0.4 yrs) were randomly identified from the same database. Both groups were controlled for the duration of diabetes. Type 1 diabetes was excluded on clinical grounds and on the biochemical basis of elevated fasting C-peptide levels (C-peptide $>0.7\mu\text{g.l}^{-1}$ in all subjects at diagnosis). All subjects gave their written informed consent for inclusion into the clinical database.

3.2.3 Data Collection

Subjects were weighed, without shoes, using electronic scales (Seca, Ltd. Germany), in kilograms to the nearest 0.1 kg. Height was measured with the subjects standing upright, without shoes, using a stadiometer (Seca Ltd., Germany) in centimeters to the nearest 0.5 cm.

Body mass index was calculated using the following equation from Mc Ardle *et al.*, (1994):

$$\text{BMI (kg.m}^{-2}\text{)} = \text{Body mass (kg)} / \text{Height}^2 \text{ (m}^2\text{)}$$

Blood Pressure was measured on the left arm with the subjects seated comfortably using a sphygmomanometer (Riester Big Ben Round Sphyg, Germany), cuff (Bainbridge, Trimline Medical Products, Branchburg, NJ, USA) and a stethoscope (3M Littmann). Routine bloods tests were taken one week prior to clinic visits, see Appendix for laboratory analysis.

3.2.4 Statistical Analysis

Data were expressed as Mean $\pm$ standard error of the mean (SEM). Comparisons between the young and older group were made using student unpaired t-tests. A two-tailed model was used and statistical significance was set at $P < 0.05$. JMP statistical software (version 5.1, SAS Institute, Cary, NC, USA) was used in statistical analysis. Data pertaining to treatment modality were expressed as percentages.

3.3 RESULTS

3.3.1 Clinical Data

Patient characteristics are outlined in Table 3.1. The majority of patients in both cohorts were male. The younger patients were significantly heavier at diagnosis than the older patients (95.5 vs. 87.0 kg, $P<0.05$). Both cohorts were obese, but the early-onset patients were significantly more obese ($P<0.05$) as measured by BMI (33.3 vs. 30.7 kg.m⁻²).

Table 3.1 Mean clinical data from early- and later-onset patients with T2DM.

	Younger T2DM n = 149	Older T2DM n = 217	P value (<0.05)
Male (%)	67.00	63.57	
Age (yrs)	35.2 ± 0.4	61.6 ± 0.4	
BMI (kg.m ⁻²)	33.3 ± 1.7	30.7 ± 0.5	<0.05
Weight (kg)	95.5 ± 1.9	87.0 ± 1.2	<0.05
HbA1c at diagnosis (%)	9.38 ± 0.21	8.57 ± 0.14	<0.05
HbA1c current (%)	8.02 ± 0.19	7.35 ± 0.09	<0.05
Total cholesterol (mmol.l ⁻¹)	4.98 ± 0.12	4.83 ± 0.07	NS
LDL cholesterol (mmol.l ⁻¹)	2.83 ± 0.07	2.79 ± 0.06	NS
HDL cholesterol (mmol.l ⁻¹)	1.07 ± 0.03	1.17 ± 0.02	<0.05
Total cholesterol/HDL ratio	4.83 ± 0.11	4.23 ± 0.06	<0.05
Triglycerides (mmol.l ⁻¹)	2.60 ± 0.22	2.09 ± 0.13	<0.05
C-peptide at diagnosis (µg.l ⁻¹)	3.27 ± 0.34	3.48 ± 0.25	NS
Systolic BP (mmHg)	124 ± 1	136 ± 2	NS
Diastolic BP (mmHg)	80 ± 1	78 ± 1	NS

Values are presented as Mean ± SEM. Abbreviations: BMI= body mass index, HbA1c= glycated haemoglobin, LDL= low-density lipoprotein, HDL= high-density lipoprotein, BP= blood pressure, NS= not significant.

There were no significant differences in the total cholesterol concentration or the mean LDL concentration between the two cohorts at diagnosis, Table 3.1. However, HDL cholesterol was significantly lower in the younger patients when compared to the older patients (1.07 vs. 1.17 mmol.l⁻¹, $P<0.05$). In addition, the mean ratio of total cholesterol/HDL was higher (4.83 vs. 4.23, $P<0.05$) in the cohort with early-onset T2DM. The mean triglyceride concentration was significantly higher in the younger patients when compared

to the older patients (2.60 vs. 2.09 mmol.l⁻¹, $P<0.05$). There were no significant differences in C-peptide concentrations at diagnosis. No differences were seen in mean systolic or diastolic blood pressure, Table 3.1.

Mean HbA1c at diagnosis was higher in the younger patients when compared to the older patients and continued to be worse at follow-up (both $P>0.05$), Figure 3.1.

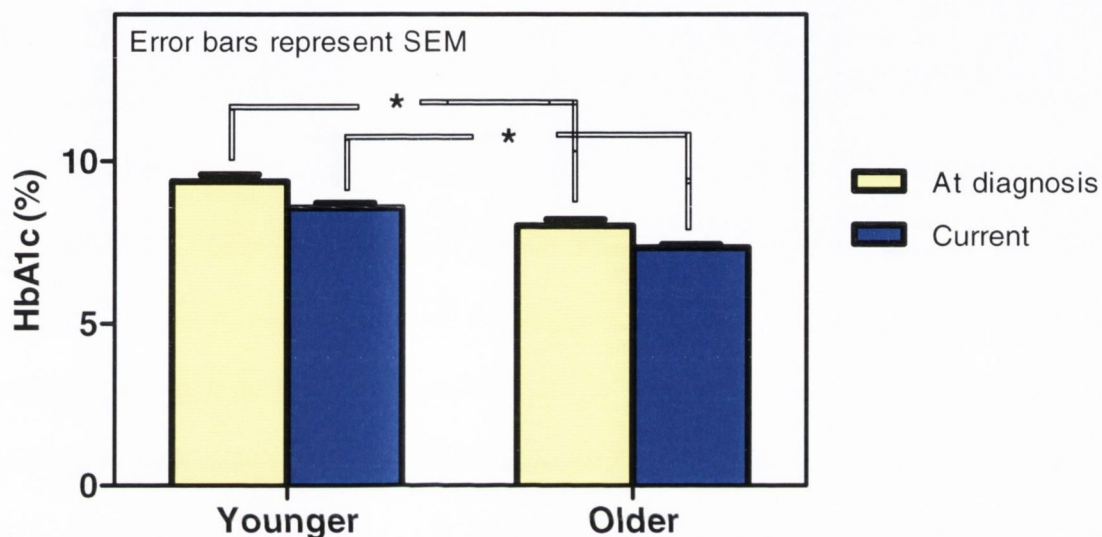


Figure 3.1 HbA1c at diagnosis and at follow-up in early- and later-onset patients with T2DM. Measurements are Mean $\pm$ SEM. *Significantly different from young group ($P<0.05$)

The proportion of patients who required insulin therapy was higher in the younger cohort (21.5%) when compared to the older cohort (13.4%), Table 3.2.

Table 3.2 Diabetes treatment in early- and later-onset patients with T2DM.

	OAD	Insulin/OAD	Diet only
Younger T2DM	80 (53.7%)	32 (21.5%)	37 (24.8%)
Older T2DM	133 (61.3%)	29 (13.4%)	55 (25.3%)

Values are presented as number of patients (percentages in parenthesis). Abbreviations: T2DM= type 2 diabetes mellitus, OAD= oral antidiabetes drugs

3.4 DISCUSSION

Early-onset T2DM is associated with visceral adiposity and dyslipidaemia, which constitute classical components of insulin resistance and T2DM. In the current comparative study, the clinical phenotype of subjects with early- and later-onset T2DM has been compared. Early-onset patients were more obese (on average 8.5 kg heavier), and had a more adverse lipid profile (lower HDL, higher total cholesterol/HDL ratio, and higher triglycerides) at diagnosis than patients diagnosed later in life (all $P<0.05$), Table 3.1. This is consistent with several previous studies (36, 46, 47, 310). Dyslipidaemia in diabetes has been associated with increased CVD risk and mortality (151, 152, 179). Thus, due to the more abnormal lipid profile and significant increase in obesity it can be assumed that these early-onset patients are at higher risk of future CVD complications.

The younger patients have proven to be more difficult to stabilize at follow-up, despite active management protocols, drug-dose titration and similar duration of the disease, Figure 3.1. Consistent with a previous report, more have progressed to insulin therapy compared to the older patients (21.5% vs. 13.4%) (35). Increased insulin resistance and diminished insulin secretion may contribute to the progression of T2DM in younger subjects. Fasting C-peptide levels in both cohorts were similarly elevated, Table 3.1; however, the younger group had worse initial and ongoing glycaemic control. Therefore, it can be postulated that a more severe form of β -cell dysfunction may contribute to the pathogenesis of T2DM in younger patients. The characteristic insulin resistance found in younger patients may also contribute towards a more adverse glucose metabolism in comparison to older subjects. Other studies completed in even younger cohorts (adolescents) have shown impairments in β -cell function in the setting of worsening insulin resistance (308-310, 312). The persistent hyperglycaemia in the younger group also imposes additional cardiovascular risk (152, 155).

3.4.1 Conclusions

The characteristics of younger and older (more typical) patients with T2DM from our clinic were compared. The younger group were more obese and had a more adverse lipid profile. Younger patients presented with worse initial glycaemic control, and continued to have worse on-going glycaemic control when compared to older patients from the same clinics despite similar treatment protocols. Early-onset patients will have a longer duration of diabetes, probably with more severe hyperglycaemia. Patients who are diagnosed with diabetes at even younger ages than those in the current study are likely to experience even greater cardiovascular risk.

Strengths of the current study included the relatively large number of early-onset subjects. The random identification of older subjects (aged >50 yrs) meant the early-onset subjects were compared to a representative sample of our typical clinic patients.

The continuing trend of T2DM presenting at an earlier age represents a serious challenge for diabetes management. Early-onset T2DM patients present with a more adverse phenotype from a metabolic and cardiovascular standpoint (35, 165, 313). More prospective studies are still needed in order to fully understand the pathophysiology of T2DM in the young, in order that prevention and treatment can be made more appropriate for this group.

Chapter Four:

Characterization of the Phenotype of Early-Onset Type 2 Diabetes Mellitus

Burns *et al.*, 2007 (306)

4.1 INTRODUCTION

The clinical phenotype of younger adults with diabetes is gradually being revealed; early-onset adults (<40 yrs) are more obese, have worse cardiovascular risk profiles and have worse initial and on-going glycaemic control than their older counterparts, Chapter 3. However, the exact events leading to the development of T2DM in adolescents and young adults is still not fully understood. Obesity, puberty and inactivity are known to promote insulin resistance and possibly the conversion to T2DM. During normal puberty, insulin resistance is increased and as a compensatory mechanism insulin secretion increases, resulting in mild to moderate hyperinsulinaemia (314, 315). In susceptible adolescents, this insulin resistance can lead to β -cell dysfunction as the pancreatic β -cells fail produce enough insulin to meet the requirements of glucose storage. This process can ultimately result in the progression to T2DM. At the time of clinical presentation, young patients with T2DM have a well-established loss of β -cell insulin secretion, coupled with extreme insulin resistance (47, 308-310). Our group has also reported that younger adults with T2DM have elevated CVD risk markers and elevated markers of inflammation (47, 164), implying a disproportionate cardiovascular risk burden associated with T2DM in younger patients (35, 165).

The clinical and research evidence to date is beginning to demonstrate that early-onset T2DM is an extreme phenotype, noticeably different from the phenotype of older subjects with typical T2DM and from that of young obese insulin resistant but normal glucose tolerant subjects.

4.1.1 Aim

The aim of this study was to fully characterise the clinical and metabolic phenotype of young adults with type 2 diabetes.

4.2 METHODS

4.2.1 General Experimental Design

Day one: Consent signed. Physical examination and medical history, including diet and exercise history. Baseline laboratory measurements. Oral glucose tolerance test (2 hrs).

Day two: Euglycaemic-hyperinsulinaemic clamp with tracer. Modified intravenous glucose tolerance test (IVGTT).

Day three: graded exercise test.

4.2.2 Subjects

Subjects who had previously been diagnosed with type 2 diabetes (YT2) were recruited from a young person's diabetes clinic, see Table 4.1 for patient characteristics. Subjects without diabetes served as controls and were matched for age and BMI to the YT2 cohort. Control subjects were recruited from an endocrine clinic and from the community.

Table 4.1 Patient Characteristics

	Control (n= 18)	YT2 (n= 13)
Male: female ratio	4:14	8:5
Age (yr)	24 ± 1	26 ± 1
Weight (kg)	99.6 ± 4.4	109.0 ± 5.0
BMI (kg.m ⁻²)	35.1 ± 1.4	34.9 ± 1.4

Values are presented as Mean ± SEM. Abbreviations: BMI= body mass index

All participants had normal renal, cardiac and hepatic function and were not treated with any medication which could affect carbohydrate or lipid metabolism (other than those for diabetes). Subjects with co-existing illnesses or secondary forms of diabetes were excluded. No participant had been involved in regular exercise, defined as at least once weekly, for three months prior to the start of the study. Before carrying out this study, ethics approval was obtained from the local research ethics committee.

4.2.3 Anthropometric Measurements

Waist circumference was measured around the umbilicus. Hip girth was measured half way between the ilium and the greater trochanter. Height was measured with the subject standing upright without shoes, using a stadiometer (Seca Ltd., Germany). All measurements were taken in centimetres to the nearest 0.5 cm.

The subject was weighed in light indoor clothing without socks or shoes, using electronic scales (Seca Ltd., Germany). Total percentage body fat, body fat mass and lean body mass were measured using the Tanita TBF-300 Body Composition Analyser (TANITA corp., Tokyo, Japan), which measures impedance, Figure 4.1. Body mass index was calculated (kg.m^{-2}).



Figure 4.1
Tanita TBF-300
Body Composition Analyzer

A standard automated 12-lead electrocardiogram (ECG) was performed (Burdick Eclipse Plus, USA). Blood pressure was measured in a semi-recumbent position, using an automated oscillometric blood pressure cuff (Omron 705 CP, Matsusaka Co., Japan). Three readings were taken and the lowest one was recorded.

To rule out the possibility of pregnancy in females an hCG+ pregnancy test (Abbott Laboratories, USA) was performed.

4.2.4 Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) was performed after a 12 hr fast. The subject was resting comfortably in a semi-recumbent position in a bed. Fasting baseline blood samples were taken. These included samples for glucose, insulin and C-peptide. Samples were also taken for lipid profiles and HbA1c. See Appendix II for details on processing of blood samples.

Additional blood samples were taken at baseline and further processed for free fatty acids and endothelial risk markers. These samples were spun in a centrifuge (Clinispin 2000,

Woodley Equipment Company, New York) at 2000 (rpm) for 15 min, and then the serum or plasma was separated into 1 ml aliquots. All samples were labelled and frozen at -80°C within 30 min. Once all the subjects completed the testing protocol, these samples were processed together. See Appendix II for processing of these samples.

The glucose-loading dose was a standardised 75 grams. The participant was given 200 ml of a glucose/water solution (Polycal®, Nutricia Zoetermeer, The Netherlands), which was consumed over 2 min, and followed by 100 ml of water. The stopwatch started when the patient began to drink the Polycal® solution. Further blood samples were taken for glucose, insulin, and C-peptide at 30, 60, 90 and 120 min.

4.2.5 Euglycaemic-Hyperinsulinaemic Clamp

For two days preceding the euglycaemic-hyperinsulinaemic clamp the participants did not take part in heavy or unusual physical activity. On the morning of the study all participants arrived at the hospital before 9:00am after a 12 hr fast. A urine sample was obtained prior to the start of the test and was tested with urinalysis and in the case of females, for pregnancy. After passing urine, the subject remained in a supine position on a bed throughout the duration of the test.

The clamp procedure was identical to that followed by the EGIR-RISC group (276). An antecubital vein was cannulated for infusion of [6-6²H₂]-glucose; this deuterated stable isotope of glucose acts as a tracer. On the opposite arm an antecubital vein was cannulated for infusion of labeled glucose and insulin until the end of the clamp. A heating pad was used to warm the hand on the same side as the tracer (for the duration of the study), this acted to ‘arterialize’ venous blood, Figure 4.2. Once warm, the wrist or hand dorsal vein was cannulated retrogradely for blood sampling.

Pre-clamp phase

At time -120 min a [6-6²H₂]-glucose bolus of 4 mg.kg⁻¹ lean body mass was given through the cannula. From time -120 until time +15 min (see timeline in Appendix I) a constant

infusion of [6-6²H₂]-glucose in 50 ml 0.9% NaCl at a rate of 0.04 mg.min⁻¹.kg⁻¹ lean body mass was infused. This 0.9% NaCl based solution is termed NINF and acts as the tracer.

Euglycaemic-hyperinsulinaemic clamp phase

1.6 g of [6-6²H₂]-glucose was added to a bag of 500 ml of 20% glucose solution (Baxter Healthcare Ltd., England) to make up a final concentration of [6-6²H₂] 1.6% glucose/glucose. This enriched glucose solution is called GINF.

Insulin (Actrapid, Novo Nordisk A/S., Denmark) was added to a 100ml bag of 0.9% NaCl, according to the subject's body surface area (40 mU.min⁻¹.m⁻²).

The formula used to calculate the body surface area (BSA) in m² was derived from Dubois and Dubois (316):

$$BSA = W^{0.425} \times H^{0.725} \times 71.84 \times 10^{-4} \text{ (H= height in cm, W= weight in kg).}$$

The formula used to calculate the amount of insulin to be added to solution was:

$$BSA \times 2.4 \div \text{constant infusion rate (15 ml.hr}^{-1}\text{)} \times \text{volume (100 ml).}$$

Figure 4.2
A subject undergoing the euglycaemic-hyperinsulinaemic clamp study. Tracer, insulin and glucose infusion apparatus attached to patient. The heating pad ensures the hand is kept warm.



Once the insulin and glucose infusions were prepared, 3 arterialized blood samples were withdrawn at 5 min intervals for baseline plasma glucose measurements. The clamp level was determined from the average of these three samples:

- If fasting plasma glucose was 4.5-5.5 mmol.l⁻¹ (80-100 mg.dl⁻¹) then the clamp level was 4.5-5.5 mmol.l⁻¹ (i.e. isoglycaemic clamp)
- If fasting glucose was >5.5 mmol.l⁻¹ (100 mg.dl⁻¹), the clamp level was 5.5 mmol.l⁻¹
- If fasting glucose was < 4.5 mmol.l⁻¹ (80 mg.dl⁻¹), the clamp level 4.5 mmol.l⁻¹

The insulin infusion was started at time 0 at a rate of 60 ml.hr⁻¹. At time 4 min, it changed to an infusion rate of 30 ml.hr⁻¹. From time 7 to 120 min the insulin was maintained at a constant infusion rate of 15 ml.hr⁻¹ (40 mU.min⁻¹.m⁻²).

Plasma glucose was measured from blood samples taken from the arterialized vein every 5 min and the glucose infusion was adjusted accordingly. If fasting plasma glucose was <5.5 mmol.l⁻¹ (100 mg.dl⁻¹) glucose infusion commenced at time 5 min. If fasting plasma glucose was >5.5 mmol.l⁻¹ (100mg.dl⁻¹), plasma glucose was reduced to 5.5 mmol.l⁻¹ (100 mg.dl⁻¹) before the glucose infusion was started.

The steady state period used for the calculation of insulin sensitivity was between times 80-120 min. Mean plasma glucose was maintained within $\pm 15\%$ of the target. At 120 min the euglycaemic-hyperinsulinaemic clamp concluded and the clock was stopped. Glucose and insulin infusions were maintained at the same constant rate as at 120 min until the modified intravenous glucose tolerance test was completed.

During the clamp a YSI blood glucose analyzer (YSI Inc., USA) was used for measuring blood glucose values. At various times throughout the clamp, blood samples were sent to the laboratory for measurement of glucose, insulin and C-peptide (-120, 80, 100, and 120 mins). In addition, at times -120, 0, 80, 100, and 120 mins samples were taken to trace the amount of endogenous glucose production and compared with the [6-6²H₂] glucose (tracer) given between times -120 and +15 min. These samples were spun in the centrifuge

at 2000 rpm for 15 min, and then a serum sample was taken in a 1ml aliquot and frozen at -80°C within 30 min for later processing.

4.2.5.1 Modified Intravenous Glucose Tolerance Test (IVGTT)

An i.v. bolus of 50% glucose solution (B.Braun Medical Ltd., Ireland), 0.3 g per kg of body weight, was prepared. The clock was re-started precisely at the beginning of the injection, which was immediately following the conclusion of the clamp, see timeline in Appendix I. Blood samples for glucose, insulin and C-peptide were taken precisely at +2, +4, +6, and +8 min after the start of the glucose bolus. The insulin infusion was stopped at time = +8 min. The glucose infusion rate was slowly reduced, while the participant had something to eat. They were monitored carefully after the discontinuation of the glucose infusion before being discharged and were advised of hypoglycaemic symptoms and the actions to take if necessary.

4.2.6 Graded Exercise Test

Oxygen and carbon dioxide consumption, as well as heart rate were measured during a graded cycle ergometer exercise test.

The subject was fitted with a mouth-breathing silicone face mask (Cortex Biophysik GmbH, Hans Rudolph Inc., USA), which was connected to a metalyzer (Cortex Biophysik GmbH, Hans Rudolph Inc., USA). Heart rate was monitored using radiotelemetry (Cardiosport Ltd. Taiwan), Figure 4.3. A small amount of electrophoresis gel was applied to the heart rate monitor for more accurate and consistent readings. Blood pressure was taken manually using a blood pressure cuff (Accoson, England) and a stethoscope (Fannin Medicare Ltd., Ireland). An electronically braked isokentic cycle ergometer was pre-



Figure 4.3
Cardiosport heart rate monitor
and radiotelemetry watches

programmed to change workload intensities (Excalibur Sport: LODE Ltd., Groningen, The Netherlands), Figure 4.4. Saddle height, handlebar height and “reach” were adjusted to best fit each subject.

The subject sat stationary on the bicycle for the first 3 min ($t=0$ to $t=3$) while baseline measurements were taken, see timeline in Appendix I. Heart rate was recorded at 2, 2.5, and 3 min. VO_2 , ventilation and respiratory exchange ratio (RER) were averaged over the last minute. Blood pressure was taken at rest. Following the 3 minute rest period, the test commenced with an initial load of 25 watts. The load was then increased by 25 watts every

3 min for the initial 3 loads. During each of the first three intervals the pattern of data collection was the same as at baseline (heart rate, VO_2 , ventilation, and RER). Starting at the fourth interval, the load was increased by 10 watts every

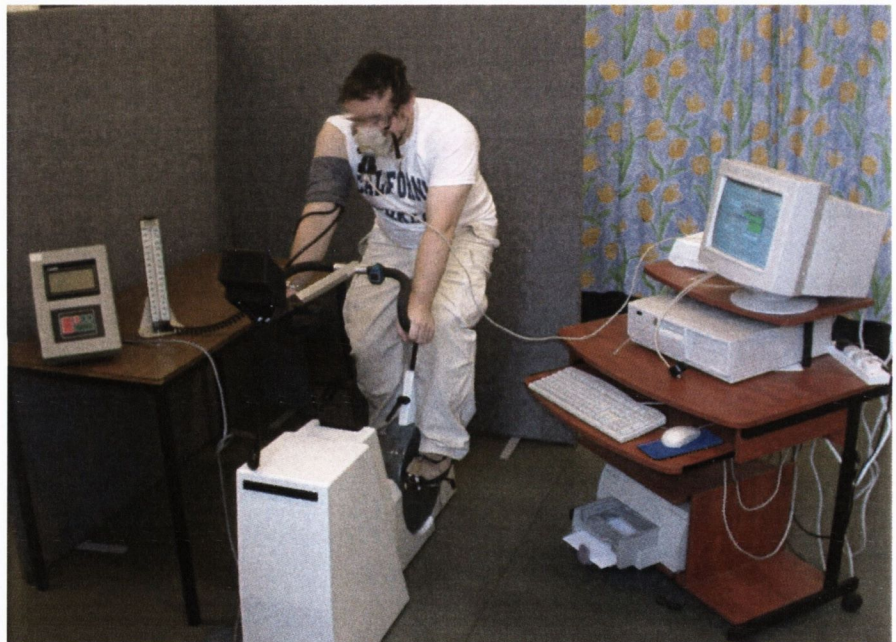


Figure 4.4
A subject undergoing a graded cycle ergometer exercise test. The heart rate monitor is worn across the thorax under the clothing. The facemask is connected via the nafion tubing to the metalyzer. The blood pressure cuff is worn on the right arm.

minute until exhaustion. Heart rate was recorded at 40, 50 and 60 seconds. VO_2 , ventilation, and RER were averaged over the last 20 seconds in each interval. The subject was encouraged to maintain a pedalling speed between 60-70 revolutions per minute (rpm) throughout the duration of the test. The test terminated when the subject failed to maintain a cadence above 60 rpm for 3 seconds.

4.2.7 Statistical Analysis

Data are presented as Mean $\pm$ SEM. Due to the small sample size of the population and inter-subject variability, nonparametric statistical tests were used. Comparisons between the control and young type 2 subjects used an unpaired Mann-Whitney *U* test. Statistical significance was set at $P < 0.05$. InStat 2.03 statistical software, version 4.0 (Graphpad Software Inc., California, USA) was used in statistical analysis.

4.3 RESULTS

4.3.1 Clinical Data

13 subjects with type 2 diabetes (YT2) and 18 non-diabetic subjects were recruited. Clinical characteristics of the subjects are shown in Table 4.2. The two groups were closely matched for age, body mass index, and body fat. 7 of the 13 YT2 subjects and 11 of the 18 obese subjects had a documented family history of type 2 diabetes. All of the subjects had completed puberty. 8 of the 13 YT2 subjects were receiving metformin, 2 were receiving sulphonylureas, 3 were receiving insulin (alone or in combination with oral agents) and 2 were on no hypoglycaemic medications. There were 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, Table 4.1. None of these differences were significant when genders were analysed separately (not shown).

Table 4.2 Mean clinical data; comparison between control and YT2 subjects

	Control (n= 18)	YT2 (n= 13)
SBP (mmHg)	115 ± 3	125 ± 3*
DBP (mmHg)	73 ± 2	78 ± 3
Waist circumference (cm)	102.1 ± 2.7	117.2 ± 3.8**
Hip girth (cm)	116.4 ± 3.3	115.3 ± 2.8
Waist: hip ratio	0.88 ± 0.02	1.02 ± 0.03**
Body fat (%)	41.2 ± 2.2	38.3 ± 3.0
VO ₂ peak (ml.kg ⁻¹ .min ⁻¹)	27.0 ± 1.7	22.9 ± 1.1

Values are presented as Mean ± SEM. Abbreviations: SBP= systolic blood pressure, DBP= diastolic blood pressure, VO₂peak= peak oxygen uptake. *Significantly different from controls ($P<0.05$). ** Significantly different from controls ($P<0.01$).

The YT2 group had significantly a higher systolic blood pressure ($P<0.05$), waist circumference ($P<0.01$), and WHR ($P<0.01$), Table 4.2. Both groups had similar levels of fitness ($P=NS$).

4.3.2 Metabolic Data and Lipid Profiles

The YT2 cohort had significantly higher fasting concentrations of glucose ($P<0.001$), and C-peptide ($P<0.05$) as well as significantly elevated 2 hr post-prandial glucose values ($P<0.001$), Table 4.3. HbA1c levels among the YT2 cohort were significantly elevated. Plasma FFA concentrations tended to be higher in the YT2 cohort, although this did not reach significance, Table 4.3.

Table 4.3 Lipid profiles and metabolic data; comparison between control and YT2 subjects

	Control (n= 18)	YT2 (n= 13)	Reference ranges
HbA1c (%)	5.4 ± 0.2	8.8 ± 0.3***	4.8-6.9
Fasting glucose (mmol.l ⁻¹)	4.9 ± 0.3	10.3 ± 0.4***	4.0-6.0
Fasting insulin (pmol.l ⁻¹)	76.1 ± 9.0	212.5 ± 114.8	0.00-83.3
Fasting C-peptide (µg.l ⁻¹)	2.5 ± 0.3	3.7 ± 0.5*	0.2-3.2
2-hr PP glucose (mmol.l ⁻¹)	6.0 ± 0.3	17.3 ± 0.8***	<7.8
Fasting plasma FFA (mmol.l ⁻¹)	0.642 ± 0.041	0.748 ±0.236	
Total cholesterol (mmol.l ⁻¹)	4.42 ± 0.20	4.69 ± 0.24	3.00-5.20
HDL cholesterol (mmol.l ⁻¹)	1.09 ± 0.07	0.94 ± 0.04	1.00-2.10
LDL cholesterol (mmol.l ⁻¹)	2.44 ± 0.17	2.67 ± 0.20	2.00-3.36
Triglycerides (mmol.l ⁻¹)	1.49 ± 0.19	2.90 ± 0.37***	0.50-2.00

Data are presented as Mean ± SEM. Abbreviations: HbA1c= glycated haemoglobin, PP= post-prandial, FFA = free fatty acids, HDL= high-density lipoprotein, LDL= low-density lipoprotein. Reference ranges are those for the central pathology laboratory at St. James’ Hospital, Dublin, Ireland. *Significantly different from controls $P<0.05$, ***Significantly different from controls $P<0.001$

The total and LDL cholesterol values between the groups did not differ. Triglycerides were elevated in the YT2 cohort when compared to controls ($P<0.001$). Although there was a tendency for HDL cholesterol to be lower in the YT2 cohort, this reduction did not reach statistical significance, Table 4.3.

Oral Glucose Insulin Sensitivity Index (OGIS), a mathematical model for oral glucose sensitivity, was calculated from the OGTT. The YT2 subjects were much more insulin-resistant than the obese control subjects (OGIS 265 ± 10 vs. 407 ± 13 ml.min⁻¹.m⁻², YT2

vs. control, respectively, $P<0.0001$). Beta cell function, as assessed by OGTT modelling, was markedly impaired in YT2 subjects, Table 4.4. Glucose sensitivity, rate sensitivity and the potentiation factor ratio were all significantly reduced in the YT2 subjects, Table 4.4. 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 hr glucose.

Table 4.4 Metabolic data derived from OGTT; comparison between control and YT2 subjects

	Control (n= 18)	YT2 (n= 13)
OGIS ($\text{ml} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$)	407 ± 13	$265 \pm 10^{***}$
Beta Cell Glucose Sensitivity ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$)	187.9 ± 44.8	$16.6 \pm 3.3^{***}$
Rate Sensitivity ($\text{pmol} \cdot \text{m}^2 \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$)	758.9 ± 165.0	$175.3 \pm 60.7^*$
Potentiation factor ratio	1.892 ± 0.440	$1.117 \pm 0.111^*$
Basal insulin secretion ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$)	107.1 ± 10.5	$141.7 \pm 13.4^*$
Integral of total insulin secretion OGTT ($\text{nmol} \cdot \text{m}^{-2}$)	52.7 ± 6.9	$30.9 \pm 3.6^{***}$

Data are presented as Mean $\pm$ SEM. Abbreviations: OGIS= oral glucose insulin sensitivity, OGTT= oral glucose tolerance test. *Significantly different from controls $P<0.05$, ***Significantly different from controls $P<0.001$

Basal insulin secretion was markedly elevated in the YT2 cohort ($P<0.05$), Table 4.4. During the OGTT, the integral of total insulin secretion was significantly reduced in the YT2 subjects ($P<0.001$).

During the euglycaemic-hyperinsulinaemic clamp studies basal hepatic glucose output was increased in the YT2 subjects ($2.6 \pm 0.25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) compared with the controls ($1.72 \pm 0.14 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($P<0.001$), Figure 4.5. Glucose production was suppressed in both groups to a similar degree during the clamp (0.89 ± 0.23 vs. $0.30 \pm 0.04 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, YT2 vs. control, respectively, $P=\text{NS}$), Figure 4.6. Both groups were insulin resistant; however, glucose disposal was markedly reduced in the YT2 cohort when compared to controls (2.15 ± 0.42 vs. $4.09 \pm 0.58 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($P<0.05$), Figure 4.7.

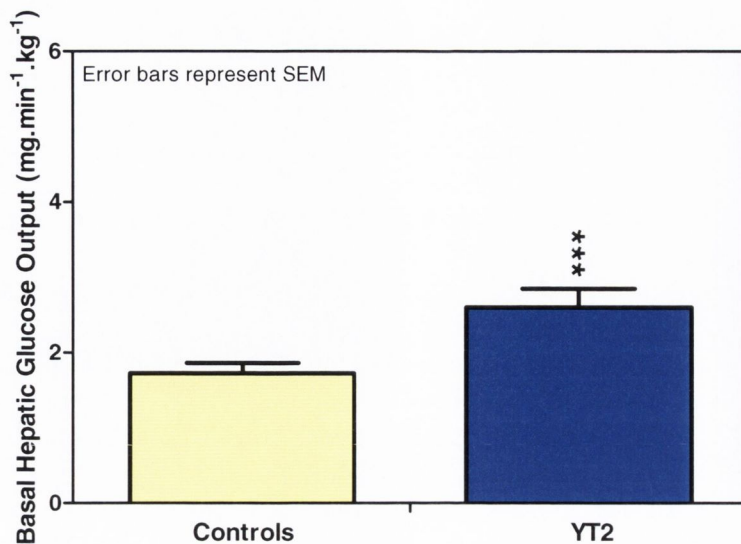


Figure 4.5

Basal hepatic glucose output measurements for control and YT2 subjects. Measurements are presented as Mean $\pm$ SEM. ***Significantly different from controls ($P<0.001$)

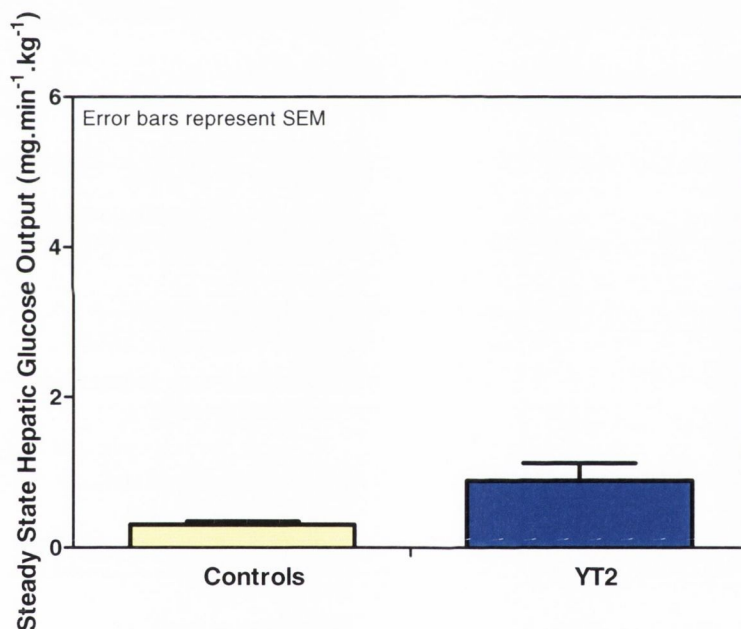


Figure 4.6

Hepatic glucose output measurements for control and YT2 subjects calculated during the steady-state of the clamp (80-120min). Measurements are presented as Mean $\pm$ SEM.

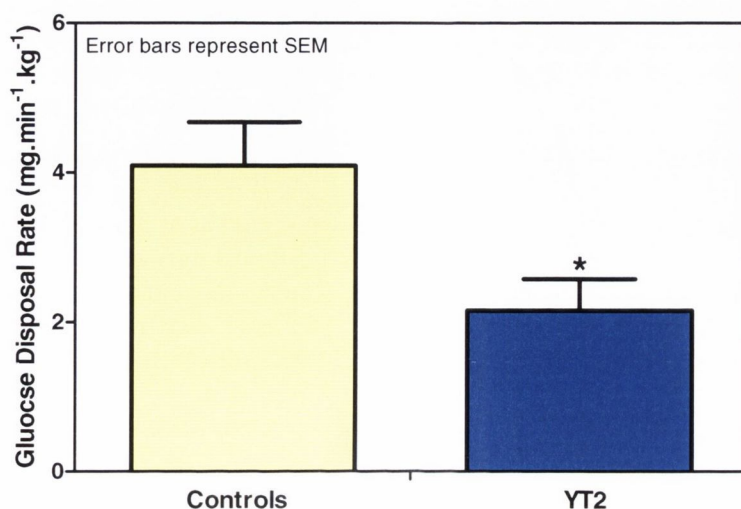


Figure 4.7

Glucose disposal rate measurements for control and YT2 subjects calculated during the steady-state of the clamp (80-120min). Measurements are presented as Mean $\pm$ SEM. *Significantly different from controls ($P<0.05$)

During the intravenous glucose tolerance test (IVGTT), the YT2 subjects had a markedly reduced first phase insulin response to the glucose bolus. Insulin and C-peptide concentrations in the YT2 cohort were significantly reduced at 2, 4, 6, and 8 min ($P<0.01$), Figure 4.8 (insulin not shown). Additionally, YT2 subjects had significantly higher serum concentrations of glucose during each of the time points 0, 2, 4, 6, and 8 min, Figure 4.9. The acute insulin response (29 ± 71 vs. 389 ± 62 pmol.l⁻¹) and the equivalent C-peptide measurements (45 ± 80 vs. 997 ± 126 pmol.l⁻¹) were reduced in the YT2 subjects (both $P<0.001$). The incremental area of insulin secreted after the glucose bolus (19 ± 49 vs. 435 ± 62 pmol.min⁻¹.m⁻²) was decreased in the YT2 cohort when compared to the controls ($P<0.001$)

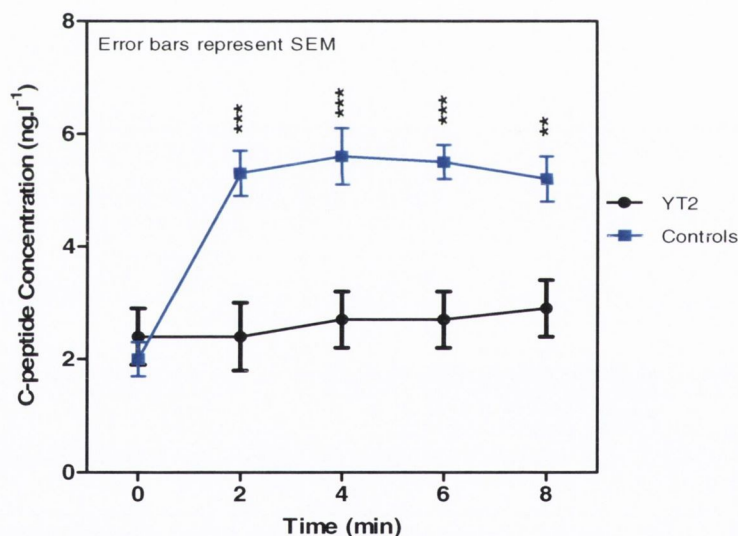


Figure 4.8
Serum C-peptide concentration during the IVGTT.
 Concentrations are presented as Mean ± SEM. **Significantly elevated when compared to controls ($P<0.01$). ***Significantly elevated when compared to controls ($P<0.001$)

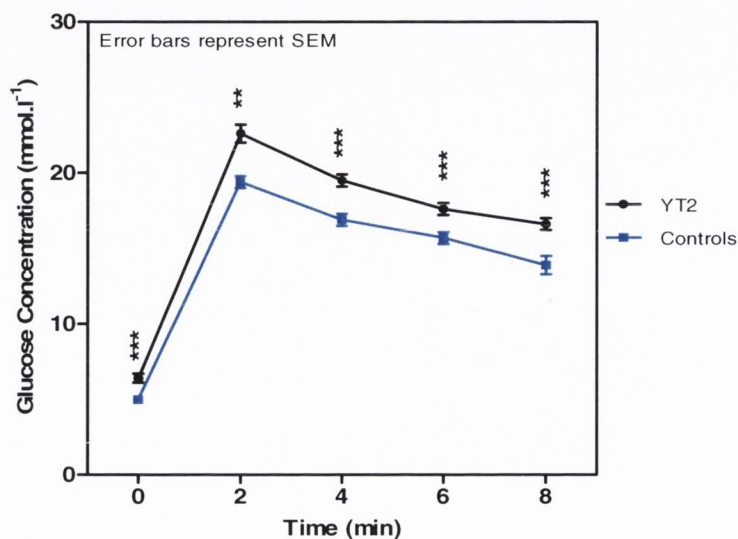


Figure 4.9
Serum glucose concentration during the IVGTT.
 Concentrations are presented as Mean ± SEM. **Significantly elevated when compared to controls ($P<0.01$) ***Significantly elevated when compared to controls ($P<0.001$)

4.3.3 Inflammatory Markers

The only inflammatory marker that was significantly elevated in the YT2 group when compared to controls was soluble intercellular adhesion molecule (sICAM) ($P<0.05$), Table 4.5. Other inflammatory markers tended to be slightly elevated in the YT2 cohort, but did not reach significance (E-selectin, P-selectin, soluble vascular adhesion molecule (sVCAM)). High sensitivity C-reactive protein (HsCRP) tended to be higher in the control group, although this was not significant.

Table 4.5 Vascular inflammatory markers

	Control (n= 18)	YT2 (n= 13)
hsCRP (mg.l ⁻¹)	6.58 ± 1.55	5.41 ± 1.08
E-selectin (ng.ml ⁻¹)	59.91 ± 7.66	70.33 ± 11.71
P-selectin (ng.ml ⁻¹)	108.9 ± 8.90	123.1 ± 9.17
sVCAM (ng.ml ⁻¹)	585.2 ± 36.01	684.0 ± 39.57
sICAM (ng.ml ⁻¹)	275.8 ± 17.59	336.8 ± 15.66*

Data are presented as Mean ± SEM. Abbreviations: hsCRP= high-sensitivity C-reactive protein, sVCAM= soluble vascular adhesion molecule, sICAM= soluble intercellular adhesion molecule.
*Significantly different from controls $P<0.05$

4.4 DISCUSSION

In the current study, whole-body and hepatic glucose turnover were investigated using the euglycaemic-hyperinsulinaemic clamp technique with deuterated glucose tracer. Whole body peak oxygen consumption (VO_{2peak}) was used as an index of fitness. Two age- and BMI-matched groups of severely obese young subjects, one with T2DM and one without, were recruited. The two groups had similar fitness levels, and were sedentary at baseline. The control group was insulin resistant (317); however, the YT2 subjects were much more insulin resistant. Under fasting conditions, hepatic glucose output was elevated and glucose disposal was decreased in the YT2 group. During the clamp, suppression of hepatic glucose production was similar between the two groups; however, whole-body glucose disposal remained significantly reduced in the YT2 group.

Insulin secretion was markedly reduced in the YT2 group. During the IVGTT, the YT2 subjects showed no additional glucose-induced insulin secretion in contrast to the obese controls that had a 3- to 4-fold increase over baseline values. β -cell glucose sensitivity, assessed by OGTT modeling, was markedly impaired in the YT2 group when compared to the control group ($P < 0.001$). YT2 subjects show the classic marked decrease in glucose sensitivity, rate sensitivity and potentiation factor ratio seen in older subjects with T2DM (78). In addition, the typical associations between mean and 2 hr glucose with both β -cell glucose sensitivity and insulin sensitivity were observed. However, the association with glucose tolerance was stronger for β -cell glucose sensitivity than for insulin sensitivity, suggesting that in this young population the major cause of glucose intolerance is β -cell dysfunction (318).

Consistent with our study, a group in France conducted euglycaemic-hyperinsulinaemic clamps in adolescents (mean age 15.4 yrs) with T2DM. Decreased peripheral glucose uptake and insulin resistance was seen in all patients (308). The French patients showed variable responses to IV glucose stimulation; however, overall the data stressed that β -cell dysfunction was a major-component in adolescent T2DM (308). Consistent with these

findings, a study from the U.S. has demonstrated that adolescents with T2DM (mean age 17.9 yrs) had severe insulin resistance and impaired insulin secretion when compared to subjects of similar obesity and body fatness without diabetes (309). However, in contrast to our findings, the subjects in that study retained a first phase insulin secretory response which was comparable with lean control subjects (309).

Both groups of participants in the current study have an increased future risk of CVD. It is well established that T2DM and obesity confer a higher risk of cardiovascular disease when compared to normal weight healthy populations (154, 156, 161). Both groups of subjects were extremely overweight, with an average BMI of 35 kg.m^{-2} . The YT2 subjects had a greater degree of abdominal obesity, with mean waist circumferences and WHR above that seen in the obese control group and above what is considered within healthy ranges (319-321). Both groups had elevated FFA concentrations (322, 323). In addition, the YT2 group was more lipotoxic (higher triglycerides, and lower HDL). Both groups of subjects had elevated concentrations of inflammatory markers when compared to other at-risk populations (196, 198, 207, 255). These elevations are also consistent with other studies conducted in insulin resistant populations (192-195, 207, 209, 324).

It can be postulated that the YT2 cohort has an increased risk of CVD when compared to the BMI-matched control group. Many studies have shown elevations in inflammatory markers to be predictive of future cardiovascular disease (189, 196, 198-200, 325). Significant elevations in waist circumference, WHR, and triglyceride concentrations in combination with the elevations in inflammatory markers impose an increased future risk of CVD in the YT2 group (179, 180, 326). In addition, a low fitness level is clinically associated with increased mortality in healthy individuals, individuals with a primary risk factor for CVD and individuals with T2DM (327, 328). Especially troubling is the young age of these participants, which allows additional time for continued damage to occur to the cardiovascular system.

Interestingly, the YT2 group did not display a strong family history of diabetes (only 7 out of 13) this is in direct contrast to other studies that have shown that early-onset T2DM is strongly associated with a family history of the disease (36, 48, 329, 330). A limitation to this study could have been in the method for obtaining family history. Family history was ascertained via interview during the medical examination. It is possible that the young patients were not completely aware of their family histories and thus reported negatively. Care should be taken in the interpretation of these results, in the context of the current literature.

4.4.1 Conclusions

Early-onset T2DM is a relatively new clinical phenomenon. The clinical and research evidence to date support the hypothesis that early-onset T2DM is an extreme phenotype, distinct from the phenotype of older subjects with typical T2DM and from that of young obese subjects with normal glucose tolerance. Visceral obesity is an important risk factor. Using the euglycaemic-hyperinsulinaemic clamp technique, severe insulin resistance has been demonstrated in these early-onset subjects. Oral and IV glucose challenges have also confirmed β -cell dysfunction. The role that lipotoxicity may have contributed to the increased insulin resistance remains unclear (52, 73, 75, 293, 323).

From a cardiovascular standpoint, subjects with early-onset T2DM appear to have even greater lifelong risk of complications and CVD events when compared to older subjects. It is not clear which factors (severe obesity, insulin resistance, or low-grade inflammation) are responsible. To date, there are no long term studies that describe the natural history of T2DM and its complications in younger subjects. Early-onset obesity and severe insulin resistance are probably contributors to the inflammatory cardiovascular risk profile seen, but this remains to be confirmed.

Chapter Five:

Aerobic Exercise Intervention

Burns *et al.*, 2007 (306)
Hatunic *et al.*, 2007 (331)
Brema *et al.*, 2008 (332)

5.1 INTRODUCTION

The phenotype of early-onset T2DM patients was described in the previous chapter. YT2 subjects are viscerally obese, extremely insulin resistant and have elevated cardiovascular risk. Physical exercise is a proven and effective modality for the treatment and prevention of diabetes (1, 2, 213, 214, 221, 223, 333). Most of the previous studies relate to treatment and prevention in middle-aged or older adult populations. Physically active T2DM subjects have a more favourable metabolic (lower plasma glucose, HbA1c, percentage body fat) and cardiovascular profile (lower blood pressure, higher VO₂max) when compared to sedentary subjects (220), and are less likely to suffer from cardiovascular events (3, 235).

Regular exercise has been associated with decreases in weight, blood pressure, serum cholesterol and triglycerides, and increases in HDL cholesterol (237, 243, 246). Chronic exercise can potentially improve metabolic control, by consistently decreasing insulin resistance, increasing insulin sensitivity, and improving glucose uptake (44, 223, 229, 334), all of which have been shown to occur transiently.

Studies from our own group have recently shown that short-term exercise training (7 days) increased whole-body insulin mediated glucose disposal and muscle GLUT4 protein content in obese middle-aged patients with T2DM (mean age 45 yrs, BMI 36 kg.m⁻²) (230). Studies from another group have shown that in men with insulin resistance both moderate and high intensity exercise training, of equal energy cost, led to similar improvements in insulin sensitivity after 24 weeks duration (262). The efficacy of exercise intervention programs in improving insulin sensitivity and in the prevention and treatment of T2DM is well established (2, 335-337). However, the optimum exercise regime in much younger subjects is not yet known.

5.1.1 Aim

The aim of this study was to quantify changes in clinical and metabolic parameters in severely insulin resistant young subjects after a 12 week aerobic exercise intervention.

5.2 METHODS

5.2.1 General Experimental Design

- I. *Baseline*: Examinations were carried out as part of the baseline study, see Chapter 4.
- II. *Exercise*: The subjects exercised at 70% of their peak oxygen uptake (VO₂peak) for 60 min, 4 times a week, for 12 weeks.

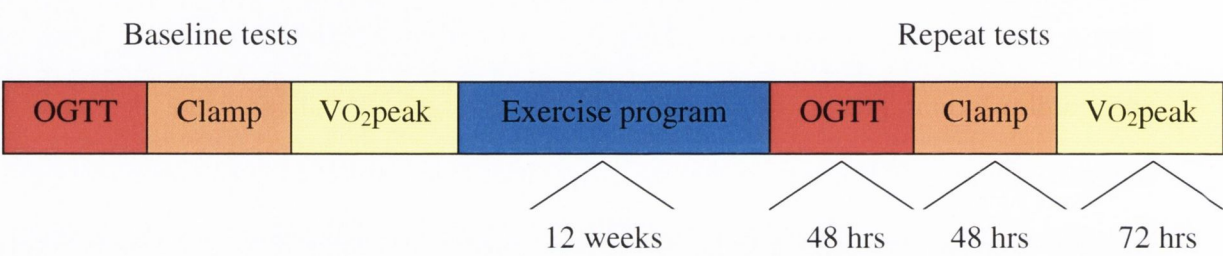


Figure 5.1
Timeline of exercise study

III. *End of study measurements*: Subjects were asked to continue with exercise between tests so that no more than 48 hrs passed prior to testing (72 hrs in the case of the graded exercise test), Figure 5.1. The protocols for all examinations were identical to the baseline testing procedures, see Chapter 4 for details.

The study protocol was approved by the local research ethics committee and written informed consent was obtained from participants prior to any testing.

5.2.2 Subjects

Subjects were recruited from the baseline study. Participants were between the ages of 15 and 30 yrs old at the time of enrolment into the study and had a BMI >28 kg.m⁻², Table 5.1. Participants were sedentary; none reported engaging in physical activity, more than once weekly, before the start of the study.

Table 5.1 Baseline characteristics of the subgroup enrolled in the exercise study.

	Control (n= 14)	YT2 (n= 7)
Male: Female ratio	4:10	3:4
Age (yr)	25 ± 1	26 ± 1
Weight (kg)	97.5 ± 4.7	108.3 ± 8.7
BMI (kg.m ⁻²)	34.3 ± 1.4	35.6 ± 1.2

Values are presented as Mean ± SEM. Abbreviations: BMI = body mass index

5.2.3 Exercise Protocol

The exercise intervention involved 1 hr of exercise training, 4 times per week for 12 weeks. Cycle ergometers and treadmills as well as outdoor walking/jogging were all valid modes of exercise. Each exercise session was fully supervised. There were between 1 and 3 participants at any one session. Sessions consisted of a 5 min warm- up, 60 min of aerobic exercise at 70% VO₂peak (with continuous heart rate monitoring) and a 5 min cool down. Exercise sessions were offered 7 days a week. Any participant that missed more than two consecutive sessions was telephoned to make sure they would attend the next session.



Figure 5.2
Cycle ergometer

During the first 3 weeks of exercise, participants had to use the cycle ergometers provided, Figure 5.2. After the introductory 3 week period they were then allowed to walk/jog as well as cycle and could use outdoor training facilities.

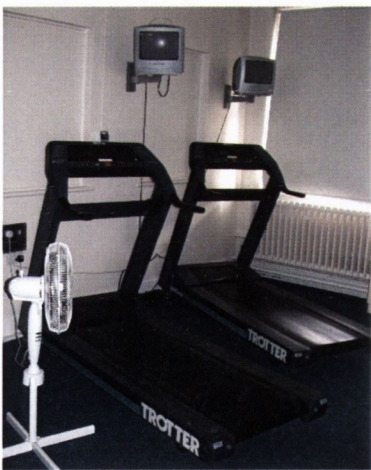


Figure 5.3
Treadmill

During each exercise session heart rate was monitored using a Cardiosport heart rate monitor (Cardiosport Ltd., Taiwan); a small amount of water or electrophoresis gel (Bluescan ultrasound gel, Lina Medical ApS, Denmark) was applied to the heart rate

monitor for more accurate and consistent readings. During the first 3 weeks, participants exercised at heart rates reflective of 50-65% $\text{VO}_{2\text{peak}}$. From weeks 4 to 12 participants exercised at heart rates reflective of 65-75% $\text{VO}_{2\text{peak}}$. Compliance with the exercise programme was 95% throughout the study.

5.2.4 Dietary Protocol

A dietician met with each subject at baseline and weekly thereafter to ensure a stable energy intake during the study. The participants completed a three day diet diary prior to beginning the study. This was used to ensure caloric intake was similar each week.

5.2.5 Statistical Analysis

Data are presented as Mean $\pm$ SEM. Due to the small sample size of the population and skewed distribution, nonparametric statistical tests were used. A Wilcoxon Signed Ranked Test was performed to examine differences before and after exercise within the groups. Unpaired Mann-Whitney *U*-tests were used to compare the two groups to each other pre-exercise and post-exercise. Spearman's correlations were used to examine correlations between variables. Statistical significance was set at $P < 0.05$. Prism 5 statistical software (version 5.01, Graphpad Software Inc., California, USA) was used in statistical analysis.

5.3 RESULTS

5.3.1 *Baseline Data*

Participants in the exercise study were recruited from the baseline study, Chapter 4. Upon completion of baseline testing, each subject was offered the opportunity to participate in the exercise intervention. In the subgroups that completed the exercise study, both groups remained matched for age and BMI. At baseline, there were no differences in systolic or diastolic blood pressure, percentage body fat or WHR. Before the start of the exercise regime the YT2 subjects had a significantly higher waist circumference ($P<0.05$), Table 5.2. The VO_2 peak of the control group was significantly higher than that of the YT2 cohort ($P<0.05$). Physical activity patterns did not differ between the two groups and both were sedentary.

5.3.2 *Changes with Exercise*

5.3.2.1 Clinical Data

Table 5.2 shows that despite good compliance, there were no mean changes in body weight, BMI, blood pressure or percentage body fat in either group following the 3-month exercise regime. Although there was some inter-subject variability with respect to weight change (+3.4 to -11.3 kg), overall there was no significant difference. Neither mean fat free mass nor mean fat mass changed significantly, as seen in the consistent percentage body fat.

Mean fasting glucose concentrations did not change in either group after exercise. Nor were there any significant differences observed in the percent HbA1c.

There were no statistically significant changes seen within the groups for systolic or diastolic blood pressure, Table 5.2. The difference in DBP between groups was significant only after exercise ($P<0.01$). This variance was due to a slight non-significant decrease in DBP of the control group and slight non-significant elevation in the YT2 group.

Table 5.2 Mean clinical data; comparison between control and YT2 subjects pre- and post-exercise.

	Control subjects		YT2 subjects	
Male: Female ratio	4:10		3:4	
Age (yr)	25 ± 1		26 ± 1	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Weight (kg)	97.5 ± 4.7	95.6 ± 4.5	108.3 ± 8.7	109.4 ± 8.4
BMI (kg.m ⁻²)	34.3 ± 1.4	33.6 ± 1.3	35.6 ± 1.2	36.0 ± 1.2
SBP (mmHg)	116 ± 4	113 ± 4	119 ± 5	121 ± 4
DBP (mmHg)	71 ± 2	70 ± 2	77 ± 3	81 ± 2 ^{##}
Fasting Glucose (mmol.l ⁻¹)	5.0 ± 0.1	5.0 ± 0.1	9.4 ± 1.1 ^{###}	10.6 ± 1.6 ^{###}
HbA1c (%)	5.4 ± 0.1	5.3 ± 0.2	8.5 ± 0.6 ^{##}	8.4 ± 0.7 ^{###}
Waist circumference (cm)	100.8 ± 3.3	96.7 ± 2.9	115.2 ± 5.5 [#]	110.7 ± 5.1 ^{*#}
Hip girth (cm)	113.3 ± 3.4	107.1 ± 3.0 ^{**}	116.6 ± 4.3	115.3 ± 3.7
Waist: Hip ratio	0.89 ± 0.02	0.90 ± 0.02	0.99 ± 0.05	0.96 ± 0.03
Body Fat (%)	39.01 ± 2.39	38.38 ± 2.29	39.90 ± 3.11	39.73 ± 3.05
VO ₂ peak (ml.kg ⁻¹ .min ⁻¹)	28.61 ± 1.94	35.15 ± 2.95 ^{***}	22.67 ± 1.57 [#]	24.40 ± 1.50 ^{##}

Values are presented as Mean ± SEM. Abbreviations: BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, HbA1c= glycated haemoglobin, VO₂peak= peak oxygen uptake. *Post-exercise is significantly different from pre-exercise ($P<0.05$) **Post-exercise is significantly different from pre-exercise ($P<0.01$). *** Post-exercise is significantly different from pre-exercise ($P<0.001$). [#]Significantly different from controls pre-exercise ($P<0.05$) ^{###}Significantly different from controls pre-exercise ($P<0.001$) [#]Significantly different from controls post-exercise ($P<0.05$) ^{##}Significantly different from controls post-exercise ($P<0.01$) ^{###}Significantly different from controls post-exercise ($P<0.001$).

Waist circumference was reduced in the YT2 subjects (115.2 ± 5.5 vs. 110.7 ± 5.1 cm, $P<0.05$), with a similar but not significant trend seen in control subjects. Waist circumference in the YT2 group remained significantly elevated above that of the control group after exercise ($P<0.05$). Hip girth in the control group decreased significantly (113.3 ± 3.4 to 107.1 ± 3.0 cm, $P<0.01$) by the end of exercise programme. Although there were significant changes seen in hip and waist measurements, no significant change in WHR was seen within or between groups, Table 5.2.

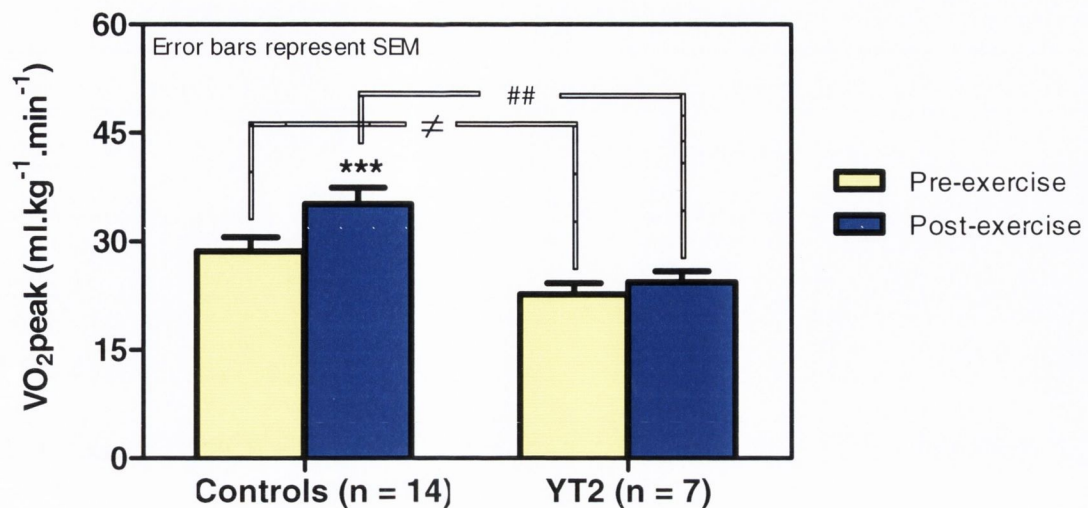


Figure 5.4 Peak oxygen consumption measurements for control and YT2 subjects pre- and post-exercise. Measurements are presented as Mean $\pm$ SEM. ***Significantly different from pre-exercise measurement ($P<0.001$) *Significantly different from controls pre-exercise ($P<0.05$) ##Significantly different from controls post-exercise ($P<0.01$).

VO₂peak increased more than 20% in the obese control subjects (from 28.61 ± 1.94 to 35.15 ± 2.95 ml.kg⁻¹.min⁻¹, $P<0.001$), but did not change in the YT2 cohort, Figure 5.4. The change in VO₂peak in the control group was positively correlated with change in glucose disposal ($r = 0.55$, $P=0.05$). Following the exercise regime, there remained a significant ($P<0.01$) difference in VO₂peak between the two groups.

5.3.2.2 Lipid Profiles and Free Fatty Acids

There was no difference in the response to exercise within or between groups in relation to lipid parameters, Table 5.3. There were no significant changes in any of the fasting lipid concentrations in either group. Mean total cholesterol and triglycerides tended to decrease slightly, while LDL showed a slight but non-significant increase.

Table 5.3 Lipid profiles and free fatty acids; comparison between control and YT2 subjects pre- and post-exercise.

	Control subjects (n = 14)		YT2 subjects (n = 7)	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Total Cholesterol (mmol.l ⁻¹)	4.47 ± 0.26	4.25 ± 0.26	4.47 ± 0.30	4.52 ± 0.24
HDL cholesterol (mmol.l ⁻¹)	1.12 ± 0.09	1.11 ± 0.06	0.96 ± 0.05	0.95 ± 0.05
LDL cholesterol (mmol.l ⁻¹)	2.46 ± 0.22	2.83 ± 0.25	2.60 ± 0.26	2.88 ± 0.18
Triglycerides (mmol.l ⁻¹)	1.37 ± 0.20	1.19 ± 0.13	2.63 ± 0.39**	2.26 ± 0.29**
Fasting Plasma FFA (mmol.l ⁻¹)	0.635 ± 0.053	0.561 ± 0.036	0.820 ± 0.104	0.826 ± 0.043###

Data are presented as Mean ± SEM. Abbreviations: HDL= high-density lipoprotein, LDL= low-density lipoprotein. FFA= free fatty acids. **Significantly different from controls pre-exercise ($P<0.01$) ###Significantly different from controls post-exercise ($P<0.01$) ###Significantly different from controls post-exercise ($P<0.001$).

Fasting FFA concentrations tended to be higher at baseline in the YT2 subjects, although this trend did not reach significance, Table 5.3. However, after completion of the exercise programme, FFA concentrations were significantly lower in the control group than in the YT2 cohort, in whom exercise induced no change, Figure 5.5.

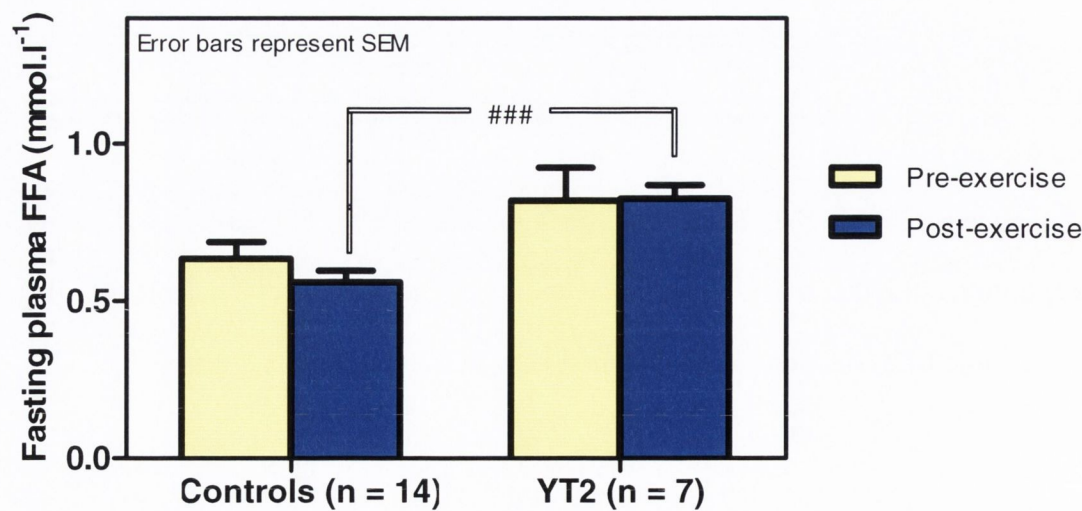


Figure 5.5 Fasting plasma FFA concentrations for control and YT2 subjects pre- and post-exercise. Measurements are presented as Mean ± SEM. ###Significantly different from controls post-exercise ($P<0.001$).

5.3.2.3 Metabolic Data

There was no difference in the response to exercise within or between groups in relation to metabolic parameters, Table 5.4. Overall, both groups demonstrated no change in glucose tolerance nor insulin sensitivity.

Table 5.4 Metabolic data derived from the OGTT; comparison between control and YT2 subjects pre- and post-exercise.

	Control subjects (n = 14)		YT2 subjects (n = 7)	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Beta Cell Glucose Sensitivity (pmol.min ⁻¹ .m ⁻² .mmol ⁻¹ .l ⁻¹)	145.19 ± 24.68	128.81 ± 17.39	13.29 ± 2.92 ^{***}	21.01 ± 7.44 ^{###}
Rate Sensitivity (pmol.m ⁻² .mmol ⁻¹ .l ⁻¹)	759.55 ± 195.44	1095.93 ± 267.62	241.38 ± 108.95	196.45 ± 56.87 [#]
Potential factor ratio	1.44 ± 0.14	1.57 ± 0.19	1.29 ± 0.18	1.10 ± 0.12
Basal insulin secretion (pmol.min ⁻¹ .m ⁻²)	100.05 ± 12.58	102.1 ± 9.53	146.43 ± 15.15 [*]	140.56 ± 12.42
Integral of total insulin secretion during OGTT (nmol.m ⁻²)	43.41 ± 2.12	44.13 ± 3.08	29.31 ± 2.61 [*]	34.31 ± 6.48

Data are presented as Mean ± SEM. Abbreviations: ^{*}Significantly different from controls pre-exercise (*P*<0.05) ^{**}Significantly different from controls pre-exercise (*P*<0.01) ^{***}Significantly different from controls pre-exercise (*P*<0.001) [#]Significantly different from controls post-exercise (*P*<0.05) ^{###}Significantly different from controls post-exercise (*P*<0.001).

Data derived from the OGTT showed that there were no improvements in glucose tolerance or β-cell function following the exercise intervention. All measures of insulin secretion (β-cell glucose sensitivity, rate sensitivity and potentiation factor ratio) remained unchanged in both groups, Table 5.4. There were no significant differences between the responses of the two groups to the IVGTT after exercise (data not shown).

Insulin sensitivity measured by OGIS was significantly reduced in the YT2 group at baseline and did not improve following the exercise programme (278 ± 16 vs. 293 ± 35ml.min⁻¹.m⁻²), YT2 pre- and post-exercise, respectively, *P*=NS). The differences between groups remained significant after the intervention (*P*<0.001), Figure 5.6.

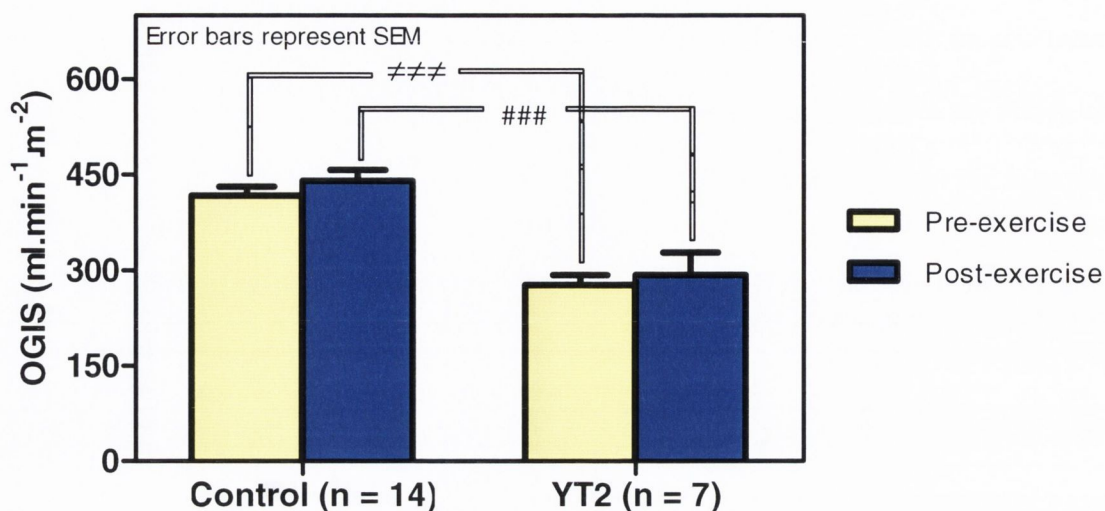


Figure 5.6 Oral Glucose Insulin Sensitivity measurements for control and YT2 subjects pre- and post-exercise. Measurements are presented as Mean $\pm$ SEM. ###Significantly different from controls pre-exercise ($P<0.001$) ###Significantly different from controls post-exercise ($P<0.001$).

There was no change in insulin sensitivity measured by the euglycaemic-hyperinsulinaemic clamp study in either group following the exercise intervention. Basal hepatic glucose output was unchanged in either group, Figure 5.7. Mean basal hepatic glucose output was higher in the YT2 subjects at baseline ($P<0.05$); however, differences between the groups post-exercise no longer remained significant. Hepatic glucose output during the clamp was similarly suppressed in both groups and unchanged after the exercise intervention, Figure 5.8. The intervention led to no significant change in whole-body glucose disposal in either group, and differences between groups remained significant post-exercise ($P<0.001$), Figure 5.9.

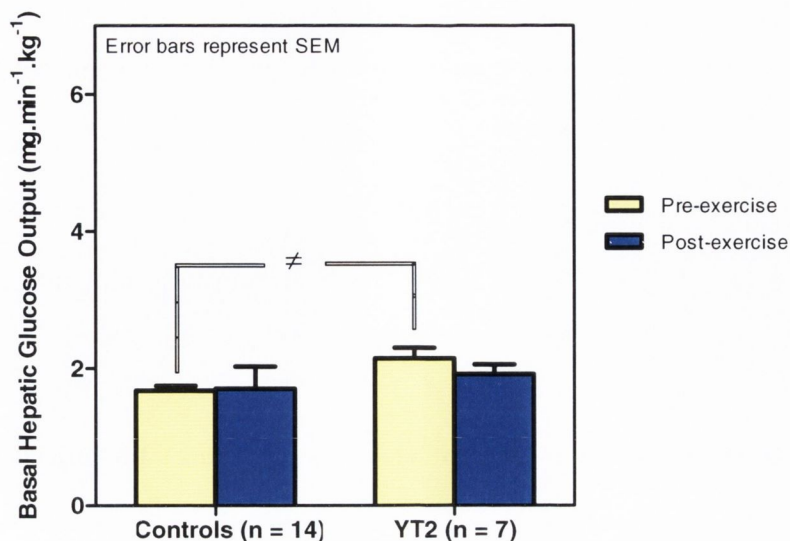


Figure 5.7

Basal hepatic glucose output measurements for control and YT2 subjects pre- and post-exercise.

Measurements are presented as Means $\pm$ SEM.

*Significantly different from controls pre-exercise ($P < 0.05$)

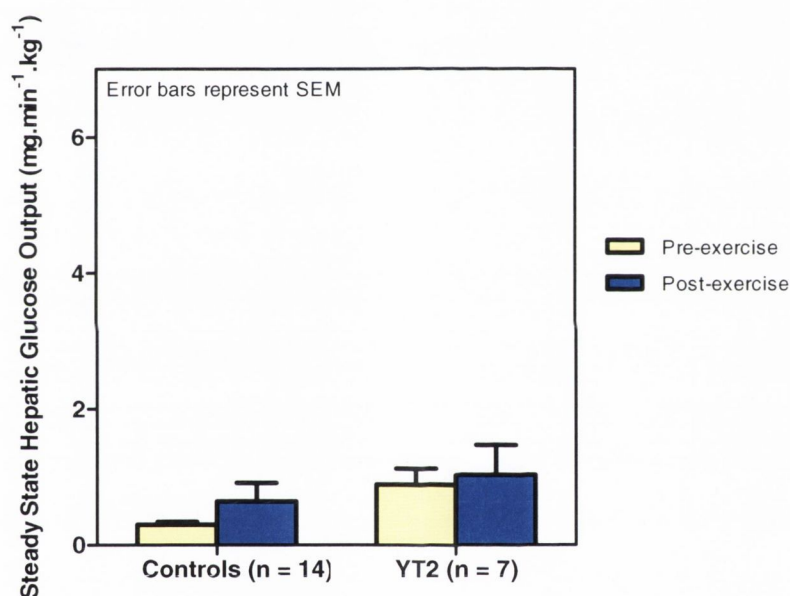


Figure 5.8

Steady state hepatic glucose output measurements for control and YT2 subjects calculated during the steady-state of the clamp (80-120 min) pre- and post-exercise.

Measurements are presented as means $\pm$ SEM.

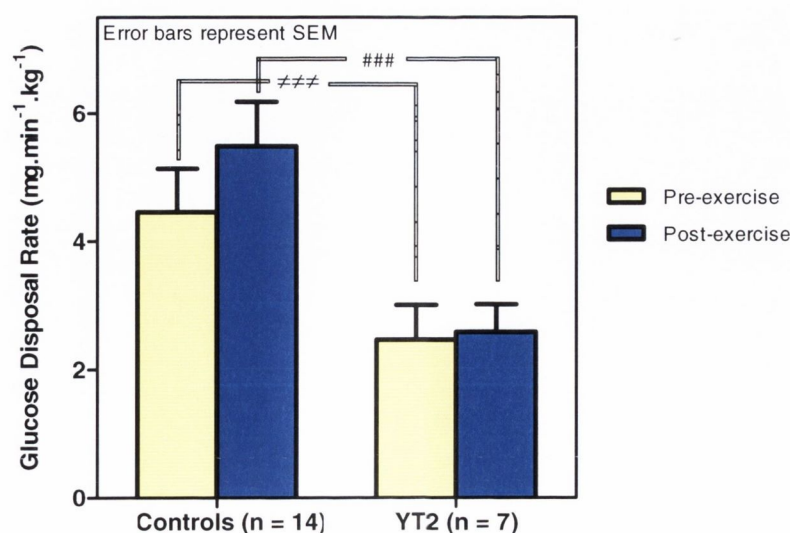


Figure 5.9

Glucose disposal rate measurements for control and YT2 subjects calculated during the steady-state of the clamp (80-120 min) pre- and post-exercise.

Measurements are presented as Mean $\pm$ SEM.

***Significantly different from controls pre-exercise ($P < 0.001$). ###Significantly different from controls post-exercise ($P < 0.001$)

5.3.2.4 Vascular Inflammatory Markers

Measurements of vascular inflammatory markers and visfatin are outlined in Table 5.5. In both groups, the concentrations of hsCRP, sE-selectin and sP-selectin were markedly elevated at baseline, and did not change after the three months of supervised aerobic exercise. The concentrations of sVCAM and sICAM were in the high normal range at baseline and did not change after exercise.

Table 5.5 Vascular inflammatory markers; comparison between control and YT2 subjects pre- and post-exercise

	Control subjects (n = 14)		YT2 subjects (n = 7)	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
hsCRP (mg.l ⁻¹)	6.3 ± 1.7	7.1 ± 2.3	5.9 ± 2.6	7.2 ± 2.6
E-Selectin (ng.ml ⁻¹)	61 ± 9	59 ± 7	69 ± 10	56 ± 7
P-Selectin (ng.ml ⁻¹)	117 ± 10	118 ± 8	120 ± 13	120 ± 13
sVCAM (ng.ml ⁻¹)	560 ± 32	563 ± 38	681 ± 56	735 ± 90
sICAM (ng.ml ⁻¹)	260 ± 21	269 ± 20	323 ± 19	315 ± 25

Values are presented as Mean ± SEM. Abbreviations: hsCRP= high sensitivity C-reactive protein, sVCAM= soluble vascular adhesion molecule, sICAM= soluble intercellular adhesion molecule.

5.3.2.5 Visfatin

Plasma visfatin concentration was similar between the control and YT2 group before exercise (64.7 ± 10.7 vs. 40.0 ± 7.8 ng.ml⁻¹, control and YT2, respectively, $P=NS$), Figure 5.10.

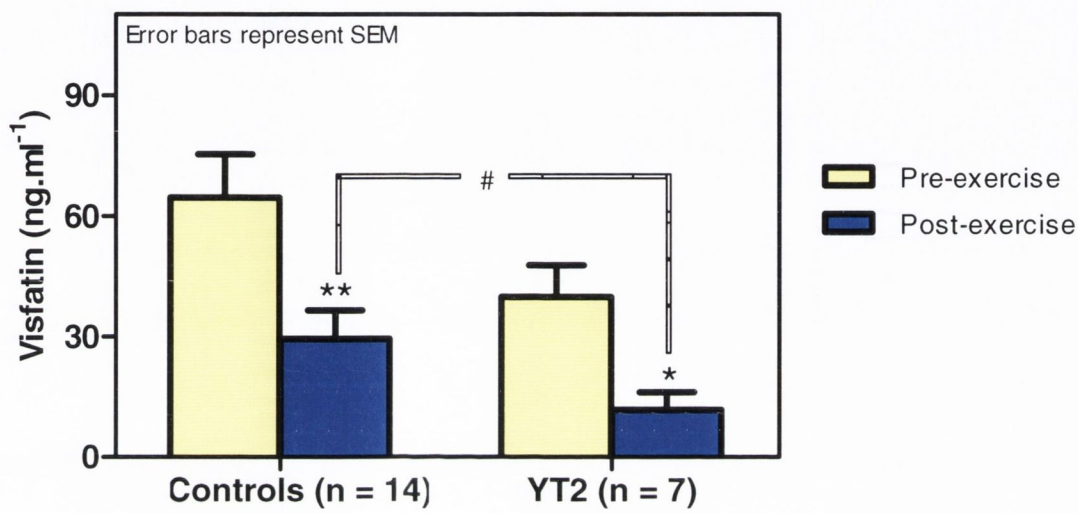


Figure 5.10 Fasting plasma visfatin concentrations for control and YT2 subjects pre- and post-exercise. Measurements are presented as Mean $\pm$ SEM. * Significantly different from pre-exercise measurement ($P<0.05$) **Significantly different from pre-exercise measurement ($P<0.01$) #Significantly different from controls post-exercise ($P<0.05$)

Following the exercise intervention, visfatin was substantially reduced in both groups (29.8 ± 6.6 in controls subjects and 11.8 ± 4.4 ng.ml⁻¹ in YT2 subjects, $P<0.01$ and $P<0.05$ for control and YT2, respectively). However, plasma visfatin concentration was reduced to a much greater extent in YT2 subjects post-exercise ($P<0.05$).

5.4 DISCUSSION

Participants in the current study exercised at 70% of their VO_2peak , four times a week for twelve weeks. As expected, there was a 20% increase in the VO_2peak of the control group; however, there was surprisingly no change in the VO_2peak of the YT2 group, Figure 5.4. In contrast to this study, many studies have shown significant changes in VO_2peak after similar periods and intensities of exercise training in individuals with T2DM (238, 243, 338-340). Exercise training that leads to increased cardiorespiratory fitness (VO_2peak), has also been shown to improve glucose metabolism (264). Consistent with this, the control group that experienced a significant increase in VO_2peak also experienced increases in glucose disposal during the clamp that were positively correlated with the change in VO_2peak ($r = 0.55$, $P=0.05$).

Aerobic exercise has been proven to be effective in improving glucose control, insulin sensitivity, and ameliorating insulin resistance in patients with obesity, pre-diabetes, and T2DM (4, 221, 335-337). In an acute exercise study, lower fasting plasma glucose concentrations, significant increases in total glucose utilization, and normalized hepatic sensitivity to insulin were shown 12 hrs after glycogen depleting cycle exercise in subjects with T2DM (227). Similar results were found after 8 days of single leg exercise in a group of young NGT men (25 ± 1 yr), with no further improvement seen at Day 14 or 30 (341). Data from a chronic exercise study of 12 weeks duration has shown a significant improvement in oral glucose tolerance and an 11% increase in glucose disposal at higher insulin concentrations during a clamp (336). A 31-week residential lifestyle modification programme reported improved glycaemic control (HbA1c), blood pressure, BMI, VO_2peak (all $P<0.0001$), and cholesterol concentrations ($P<0.05$) in T2DM participants (243).

However, despite twelve weeks of aerobic exercise, in the current study, there were no significant changes in insulin sensitivity measured by OGIS or the euglycaemic-hyperinsulinaemic clamp in either group of subjects. In addition, there was no change in glucose control as indicated by the stable HbA1c. Although not expected, these results are

consistent with other short and long-term studies. A similar study (12 weeks of aerobic exercise) reported a decrease in hepatic glucose production and no improvement in insulin sensitivity measured by a euglycaemic-hyperinsulinaemic clamp in men with T2DM. These men also experienced no change in body weight (338). Another study of 6 weeks duration showed a significant decrease in HbA1c in T2DM subjects with only minimal improvement insulin sensitivity during the OGTT. Similar to our study body weight and adiposity remained constant (334).

There were no significant changes to insulin secretion during the IVGTT following the exercise protocol among either group of subjects. Data from the Heritage study in 2005 reported the effects of endurance training on glucose homeostasis in almost 600 subjects, using a standard IVGTT (219). 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, not unlike in our study. The acute insulin response to an I.V. glucose challenge increased 7% in the quartile with the lowest baseline glucose tolerance and actually decreased 14% in the quartile with the highest baseline glucose tolerance. Improvements in fasting insulin were transitory, disappearing 72 hr after the last exercise bout (219).

In our cohort, there was no change to fasting FFA concentrations within the groups following the exercise programme. However, at the end of the 12-week programme the FFA concentration in the YT2 group was significantly elevated ($P < 0.001$) when compared to the control group (0.826 ± 0.043 vs. 0.561 ± 0.036 mmol.l⁻¹), a trend that was not seen pre-exercise, Figure 5.5. Similar to our study, data from the SLIM study has shown that following 3 yrs of exercise, 30 min a day for 5 days a week, there were no changes in fasting FFA concentrations. However, they found a significant decrease in FFA concentrations 2 hrs after a glucose load (342). Another study has shown that FFA oxidation measured during exercise (after a year-long exercise intervention), was

significantly increased in individuals with T2DM (343). However, unlike these studies, FFA were not measured during exercise or postprandially in the current study.

In Chapter 4, it was shown that both groups of subjects had increases in a number of cardiovascular risk markers. Following the intervention there was no change in markers of obesity (weight, BMI, or percentage body fat) in either group. Although, the control group experienced a modest decrease in hip girth and the YT2 group experienced a modest decrease in waist circumference ($P<0.05$), consistent with other studies of similar duration (344), there was no change in WHR. In the current study, no significant changes to blood lipid profiles were seen in either group, Table 5.3. Aerobic exercise training is believed to stimulate improvements in lipid profiles (240-243, 245). However, data from other studies have shown little or no change in lipid parameters with increases in physical activity (244, 248, 344), consistent with findings from this study.

Following the 12-week exercise protocol there were no differences observed in the concentrations of any of the inflammatory markers, Table 5.5. The lack of change in hsCRP concentration is in agreement with other studies (345, 346), although others have shown a decrease in hsCRP concentration with weight loss in middle aged or older people (257, 347, 348). In contrast to the current study, decreases in both P-selectin and E-selectin have been shown following exercise interventions both with and without weight loss (253, 255, 348-350). Changes in sICAM and sVCAM have not been consistently documented. (253, 255, 256, 348, 349). However, similar to our study, 12 weeks of aerobic exercise in overweight female adolescents ($n=19$, 13 ± 1.8 yrs, 26.8 ± 3.9 kg.m⁻²) did not change circulating levels of sICAM or sVCAM (346).

Visfatin is an adipokine that has been shown to be significantly elevated in people with obesity, T2DM and CVD (351, 352). It is independently associated with WHR (353) and with BMI (354). However, no correlations have been found between visfatin and insulin sensitivity quantified by the euglycaemic-hyperinsulinaemic clamp or HOMA (354-356). In the current study, visfatin did not correlate with insulin sensitivity quantified by OGIS.

The plasma visfatin concentration was significantly elevated in the control group compared to the YT2 group following the exercise intervention ($P<0.05$), Figure 5.10. Visfatin concentrations were significantly reduced by approximately 70% and 50% in the YT2 and obese control group respectively ($P<0.05$, $P<0.01$), consistent with other studies (357, 358). The mechanism through which exercise is associated with reductions in circulating visfatin remains unclear.

5.4.1 Conclusions

Prior to the exercise intervention, both groups of subjects were severely obese, extremely insulin resistant and had markedly adverse cardiovascular risk profiles, Chapter 4. Unexpectedly, there were essentially no metabolic improvements in either study group at the end of the three-month intervention. However, the obese control group had a 20% increase in VO_{2peak} , that was positively correlated with improved glucose disposal, and a reduction in fasting FFA, in contrast to the YT2 group. Both groups showed no improvement in their cardiovascular risk profiles.

The advantage of the current study was that all exercise sessions were supervised, and compliance was documented. Measurements were only compared in subjects who completed the study (14 controls and 7 YT2). These findings have raised interesting new questions concerning the pathogenesis and treatment of early-onset T2DM in obese young patients. Are there exercise responders and non-responders? Are three months of exercise at the frequency and intensity chosen for the current study sufficient to elicit the improvements that were expected? Would it have been preferable to combine the exercise regime with a hypocaloric diet for weight loss?

Previous studies, including some from our research group, in equally obese but older subjects have shown improvements in insulin sensitivity and glycaemic control with similar or even lesser aerobic exercise regimes (230, 335, 336). Recently, it has been suggested that progressive resistance training confers greater metabolic benefits when

compared to aerobic training alone (263, 359). The most recent guidelines for exercise in subjects with T2DM suggests a combination of aerobic and resistance exercises, focusing on increasing energy expenditure and losing weight (261). Subjects in the current study increased energy expenditure; however, contrary to expectations, they lost no weight. Participants were instructed to maintain a stable diet during the three-month exercise programme, and self-reported compliance with this diet regime was satisfactory.

More basic physiologic and biochemical mechanisms might explain the non-response to exercise in these participants. It is possible that obese, insulin resistant young adults, with and without early-onset T2DM, have either genetic or acquired factors that confer resistance at the level of skeletal muscle to the expected benefits of aerobic exercise training. Mitochondrial dysfunction contributes significantly to both insulin resistance and loss of insulin secretion in T2DM (64-66). The failure to increase VO_{2peak} in the subjects with diabetes is consistent with mitochondrial dysfunction which could result from a number of causes, including the persistent lipotoxicity which did not improve in the YT2 group after exercise.

The underlying cellular abnormalities conferring severe insulin resistance and a lack of response to exercise in both groups of subjects may overlap with those contributing to the risk of early cardiovascular disease. Whether inflammation is a modifiable risk factor in dealing with obesity is uncertain.

Chapter Six:

Mitochondrial Dysfunction in Type 2 Diabetes of the Young

6.1 INTRODUCTION

Insulin resistance has been shown to be associated with reduced oxidative capacity in skeletal muscle and mitochondrial dysfunction (66, 361-366). Multiple factors contribute to mitochondrial dysfunction in insulin resistant states. These include a reduction in the activity of enzymes in the oxidative phosphorylation (OXPHOS) pathways (367) as well as an impaired mitochondrial capacity for fat oxidation during fasting (361, 368). Reduced mitochondrial activity results in accumulation of intracellular triglyceride and lipid intermediates which contribute to insulin resistance (65, 369, 370).

Peroxisome proliferator-activated receptor-coactivator alpha (PGC-1 α) is a transcriptional co-activator of nuclear receptors which regulates a number of mitochondrial genes involved in mitochondrial biogenesis and oxidative phosphorylation. Independent muscle biopsy studies have shown a reduction in a number of oxidative genes under the control of PGC-1 α which encode for key enzymes in oxidative metabolism and mitochondrial function in obesity and T2DM (362, 371-373). Reduction in the expression of these genes could contribute to reduced fat oxidative capacity (370).

Mitofusion 2 (Mfn2) is a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the regulation of mitochondrial metabolism (374). Mfn2 expression causes energization of the mitochondria altering the regulation of OXPHOS subunits in muscle cells (374). Mfn2 gene expression has been shown to be down-regulated in obesity and T2DM (375-377). PGC-1 α up-regulates Mfn2 mRNA and protein expression in muscle cells through interaction with the transcription factor estrogen-related receptor-alpha (ERR α) (378).

Exercise training is known to improve mitochondrial function and induce mitochondrial biogenesis in insulin resistant subjects and those with T2DM (233, 379). With exercise, PGC-1 α expression increases (380). This increase has been associated with increased transcription of different nuclear genes encoding mitochondrial proteins (381, 382).

In the previous chapter it was reported that patients with early-onset obesity and T2DM had no improvement in whole-body or hepatic insulin sensitivity or in VO_2peak despite aerobic exercise training for 12 weeks. This suggested the possibility that in these exercise ‘non-responders’ alterations may exist in their mitochondrial function in skeletal muscle. It is hypothesised that deregulation of the OXPHOS system, via a diminished response from PGC-1 α , could contribute to mitochondrial dysfunction in early-onset T2DM.

6.1.1 Aim

The aims of this study were to examine components of mitochondrial function at baseline and after aerobic exercise training of various frequencies, and to correlate these parameters with indices of whole body glucose metabolism and fitness.

6.2 METHODS

6.2.1 General Experimental Design

Muscle biopsies were taken from participants during two separate exercise studies.

I. *Study 1*: 12-week aerobic exercise study, Chapter 5. Fasting muscle biopsies were taken at baseline and 48 hrs following the final exercise bout.

II. *Study 2*: Acute exercise protocol, Figure 6.1.

Day 1 (Screening): Physical examination, medical history, ECG, graded exercise test and anthropometric measurements.

Day 2 (Baseline): Fasting blood samples and fasting muscle biopsy.

Day 3 (Acute Exercise): 1 hr of exercise at 70% VO₂peak on cycle ergometer.
Following exercise – blood samples and muscle biopsy.

Days 4-10: 7 consecutive days of exercise at 70% VO₂peak for 1 hr per session.

On Day 10, following exercise- blood samples and muscle biopsy

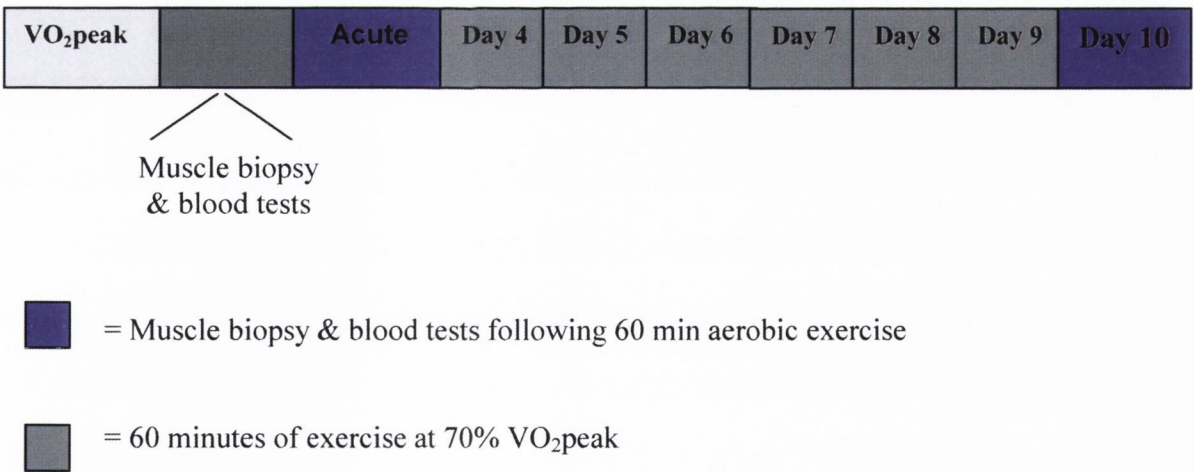


Figure 6.1
Timeline of Muscle Biopsy Study.

Subjects did not have to attend on consecutive days for Days 1, 2, and 3 of the study. Days 4-10 were consecutive.

6.2.2 Subjects

Subjects with early-onset T2DM (diagnosed before the age of 25) were recruited from the young person's diabetes clinic. Age- and BMI-matched control subjects were recruited from endocrine clinics and from the community. Participant characteristics are detailed in Tables 6.1 (study 1) and 6.2 (study 2).

All participants had normal renal, cardiac and hepatic function and were not treated with any medication which could affect carbohydrate or lipid metabolism (other than those for diabetes). Subjects with co-existing illnesses or secondary forms of diabetes were excluded. No participant had been involved in regular exercise, defined as at least once weekly, for three months prior to the start of the study. All subjects received an information leaflet and signed a consent form prior to participation.

6.2.3 Study 2

6.2.3.1 Screening- Day 1

Participants arrived on the first morning prior to 9:00am. Identical to the baseline measurements taken in Chapter 4, a doctor performed a physical exam and documented each subject's full history including: medical history, family history, dietary and exercise habits. Anthropometric measurements were taken, see Section 4.2.3 for procedures. The subjects then completed a VO_2 peak test, see Section 4.2.6.

Prior to the baseline visit (Day 2), control subjects attended on one additional day for an Oral Glucose Tolerance test to confirm that they did not have diabetes, see Section 4.2.4.

6.2.3.2 Baseline Visit- Day 2

Baseline fasting blood samples were taken. These included samples for glucose, insulin, C-peptide, HbA1c, and lipids, see Appendix II for details on processing of blood samples.

Following blood sampling a fasting vastus lateralis muscle biopsy was performed. The subjects lay supine on a bed for the duration of this procedure. Aseptic techniques were

followed. The vastus lateralis muscle on the left leg was identified and cleaned with videne 10% w/w iodinated povidone antiseptic solution (Adams healthcare, England). The biopsy area was anaesthetised with 6-9 ml of 1% w/v lidocaine HCl injection BP (B/Braun®, USA). A small incision was made with a #11 sterile disposable scalpel (Swann-Morton®, England). Pressure was then applied to the wound to prevent excess blood flow. A muscle biopsy needle, Figure 6.2, was inserted into the incision about a centimetre into the muscle fascia. A sample was obtained using a cutting and rotating technique. The needle with the sample inside was then removed from the biopsy site. The sample was removed from the needle and placed into liquid nitrogen. It was then placed into a sterile aliquot, labelled and frozen at -80°C.



Figure 6.2
Muscle Biopsy Needle

Once the biopsy needle was removed from the biopsy site, pressure was applied to the wound for 5-10 min to ensure homeostasis. Once bleeding ceased a ¼" x 3" Steri-Strip™ (3M Health Care, USA) was used to seal the wound. It was then bandaged with sterile gauze (Rociale Medical Ltd., Cambridge, England) and a 7.5 cm x 4.5 m elastic crepe bandage (NOVOLAST, Midland bandages, Ltd., Ireland). The subject was given advice on wound care and released after a short time.

6.2.3.3 One Hour Exercise- Day 3

Participants attended before 9:00am, fasting for this visit. 70 min of exercise was performed, comprised of a 5 min warm-up, 60 min of exercise on a cycle ergometer at heart rates corresponding to 70% VO₂peak, and a 5 min cool-down. During exercise, heart rate was monitored.

Immediately following the exercise session, fasting blood samples were taken and processed as described previously, including those for FFA. A fasting muscle biopsy was performed from the right vastus lateralis.

6.2.3.4 Seven Consecutive Days of Exercise - Days 4-10

On Days 4-9, participants were allowed to exercise between 7:30am and 7:30pm. On Day 10, participants had to begin exercise before 8:30am. Each exercise session lasted 70 min and was fully supervised and monitored as detailed previously. On Day 10, immediately following exercise, fasting blood samples were taken and a final muscle biopsy was performed on the left leg at least one centimetre in distance from the initial biopsy site.

6.2.4 Processing of Muscle Biopsy Samples

Upon completion of all participants in study 1, the muscle biopsy samples were processed for protein extraction (pre- and 12 weeks post-exercise) see Appendix II for details. Following completion of all subjects in study 2, the samples were subsequently processed for both RNA and protein extraction (pre-, 1 hr and 7 days post-exercise), see Appendix II for details.

Briefly: RNA from approximately 25 mg of skeletal muscle was extracted using RNeasy kit (Qiagen, GmbH). A real-time polymerase chain reaction (PCR) was used for measurement of specific mRNAs (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems, Foster City, CA). All PCR runs were performed in duplicate.

A portion of biopsied skeletal muscle tissue from both groups was homogenized and the expression of mitochondrial proteins was studied. Protein homogenate was extracted from approximately 25 mg of skeletal muscle and the protein concentration was then determined using a Micro BCA protein assay (Pierce, Rockford, IL, USA). Western blot assays were used to identify concentrations of Mfn2 (Bach et al. 2003), two subunits of the OXPHOS system complex I (anti-NDUFA9) and complex V (anti-subunit a from H₉-F1-ATP synthase) from molecular probes and porin (Calbiochem, La Jolla, CA, USA).

6.2.5 Statistical Analysis

Data are presented as Mean $\pm$ SEM. Unpaired t-tests were performed to compare muscle protein expression between controls and subjects with diabetes. Paired t-tests were performed to compare the effects of chronic and acute exercise on the expression of a range of proteins and RNAs. In all cases, significance levels for the t-tests was set at $P < 0.05$.

6.3 RESULTS

6.3.1 Baseline Clinical and Metabolic Measurements

Study 1

The baseline clinical and metabolic characteristics of study 1 participants (12 week exercise intervention) were described in Chapter 4. The subgroup that had muscle biopsies had similar characteristics to the whole cohort, as detailed in Table 6.1 below. At baseline, the groups were matched for BMI and fitness (VO_{2peak}). The YT2 group had higher triglycerides and lower HDL levels ($P<0.05$) when compared to controls.

Table 6.1 Mean clinical data; comparison between control and YT2 subjects from Study 1

	Control (n= 6)	YT2 (n= 7)
Male: Female ratio	0:6	5:2
Age (yr)	22 ± 1	27 ± 1*
Weight (kg)	108.7 ± 10.6	108.1 ± 6.7
BMI (kg.m ⁻²)	37.78 ± 3.43	33.23 ± 1.81
SBP (mmHg)	104 ± 3	122 ± 5*
DBP (mmHg)	71 ± 3	72 ± 3
Waist circumference (cm)	113.2 ± 4.6	117.4 ± 3.2
WHR	0.91 ± 0.03	1.00 ± 0.04
VO _{2peak} (ml.kg ⁻¹ .min ⁻¹)	24.35 ± 1.39	21.96 ± 2.16
HbA1c (%)	5.5 ± 0.2	8.2 ± 0.6**
Fasting glucose (mmol.l ⁻¹)	5.2 ± 0.2	9.1 ± 0.8**
Fasting insulin (pmol.l ⁻¹)	105.5 ± 10.9	89.2 ± 14.3
Fasting C-peptide (µg.l ⁻¹)	3.4 ± 0.3	3.1 ± 0.4
Total cholesterol (mmol.l ⁻¹)	4.33 ± 0.21	4.50 ± 0.42
HDL cholesterol (mmol.l ⁻¹)	1.11 ± 0.08	0.88 ± 0.03*
LDL cholesterol (mmol.l ⁻¹)	2.57 ± 0.23	2.34 ± 0.35
Triglycerides (mmol.l ⁻¹)	1.41 ± 0.27	3.05 ± 0.64*

Values are presented as Mean ± SEM. Abbreviations: BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, WHR= waist: hip ratio, VO_{2peak} = peak oxygen uptake, HDL= high-density lipoprotein, LDL= low-density lipoprotein. *Significantly different from control group ($P<0.05$) **Significantly different from control group ($P<0.01$).

Study 2

The participants in study 2 (acute exercise intervention) had a similar clinical and metabolic phenotype (Table 6.2) to those reported from our research group previously (47, 306). The control group was matched for age and BMI with the YT2 group, with no significant differences in body weight, waist circumference or WHR. Both groups had similar fitness levels (VO_{2peak}). The $HbA1c$ was significantly higher ($P<0.01$), as was fasting glucose in the YT2 group. Insulin was similar between groups. Both groups had similar cholesterol profiles, however HDL was significantly lower in the YT2 group ($P<0.05$).

Table 6.2 Mean clinical data; comparison between control and YT2 subjects from Study 2

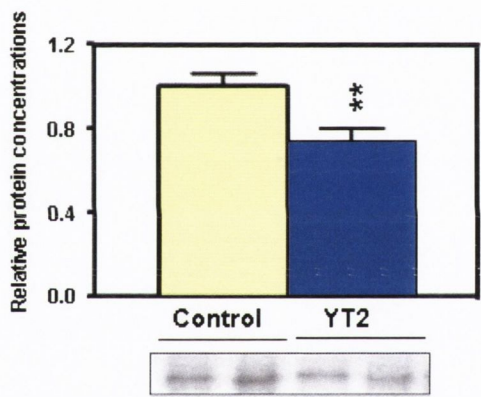
	Control (n= 7)	YT2 (n= 12)
Male: female ratio	2:5	10:2
Age (yr)	26 ± 2	27 ± 1
Weight (kg)	113.9 ± 11.2	114.5 ± 7.3
BMI (kg.m ⁻²)	39.13 ± 2.54	36.13 ± 1.78
SBP (mmHg)	117 ± 3	127 ± 4
DBP (mmHg)	75 ± 4	81 ± 3
Waist circumference (cm)	120.2 ± 6.1	111.4 ± 4.8
WHR	0.98 ± 0.03	0.99 ± 0.02
VO_{2peak} (ml.kg ⁻¹ .min ⁻¹)	22.85 ± 2.71	23.79 ± 1.79
$HbA1c$ (%)	5.6 ± 0.1	7.5 ± 0.5**
Fasting glucose (mmol.l ⁻¹)	5.2 ± 0.1	8.0 ± 0.7**
Fasting insulin (pmol.l ⁻¹)	161.7 ± 69.3	196.4 ± 71.4
Fasting C-peptide (µg.l ⁻¹)	5.3 ± 1.2	3.4 ± 0.4*
Total cholesterol (mmol.l ⁻¹)	4.13 ± 0.32	4.29 ± 0.26
HDL cholesterol (mmol.l ⁻¹)	1.30 ± 0.17	0.95 ± 0.05*
LDL cholesterol (mmol.l ⁻¹)	2.21 ± 0.29	2.63 ± 0.28
Triglycerides (mmol.l ⁻¹)	1.35 ± 0.20	1.76 ± 0.26

Values are presented as Mean ± SEM. Abbreviations: BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, WHR= waist: hip ratio, VO_{2peak} = peak oxygen uptake, $HbA1c$ = glycated haemoglobin, HDL= high-density lipoprotein, LDL= low-density lipoprotein. *Significantly different from controls ($P<0.05$), **Significantly different from controls ($P<0.01$).

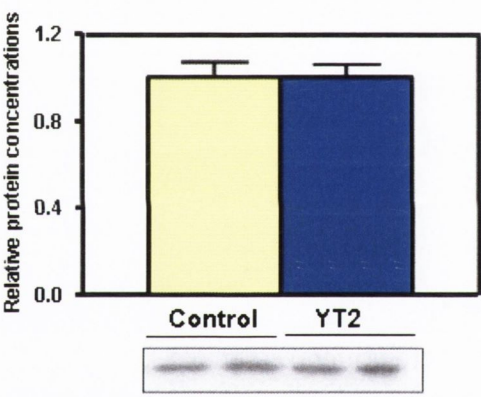
6.3.2 Expression of Skeletal Muscle Mitochondrial Proteins at Baseline

The two study groups were combined for baseline mitochondrial protein analysis. When analysed separately, similar results were found (data not shown). The total protein concentration was similar in the homogenate samples from both control and YT2 subjects (54.1 ± 2.6 vs. 54.8 ± 3.1 mg.g⁻¹, control vs. YT2 respectively, $P=NS$). At baseline, subjects with T2DM showed a reduced expression of Mfn2 (26% reduction in the YT2 group) and the alpha subunit of ATP synthase (ATP5a1) (39% reduction in YT2 group), Figures 6.3A&C. The abundance of porin (a marker of mitochondrial mass) and of the p37 subunit of Complex I of the respiratory chain (Ndufa9) was unaltered, Figures 6.3B&D.

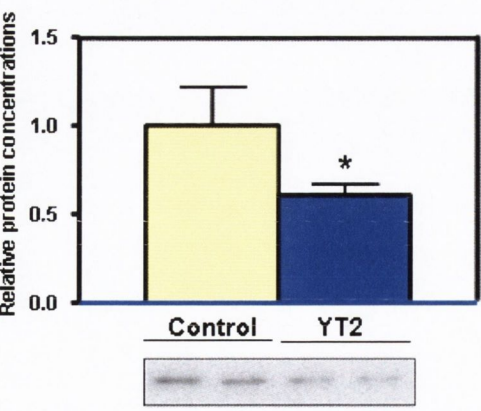
6.3A Mfn2



6.3B Porin



6.3C Atp5a1



6.3D Ndufa9

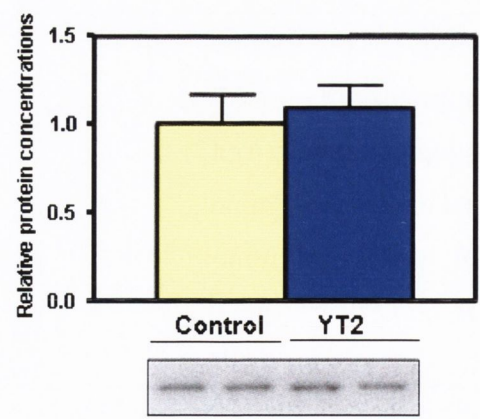


Figure 6.3A-D. Basal key mitochondrial protein concentrations from skeletal muscle from both control and YT2 subjects. Values are expressed as YT2 concentrations relative to the control group (control = 1), Mean $\pm$ SEM. Control n=11, YT2 n=16. *Significantly different from control group ($P<0.05$). **Significantly different from control group ($P<0.01$). Representative autoradiograms are also shown.

6.3.3 Expression of Skeletal Muscle Mitochondrial Proteins After 12 Weeks of Exercise

Exercise training was associated with the selective induction of Mfn2 (2.8-fold increase), porin (1.6-fold increase) and p37 subunit of Complex I (Ndufa9) (1.7-fold increase) in skeletal muscle from controls, Figures 6.4A, B&C. In YT2 patients, the expression of Mfn2 was unchanged, while there was a significant induction of Ndufa9 (2.0-fold increase) Figures 6.4A&C. Porin also tended to increase (1.6-fold increase), although this was not significant, Figure 6.4B.

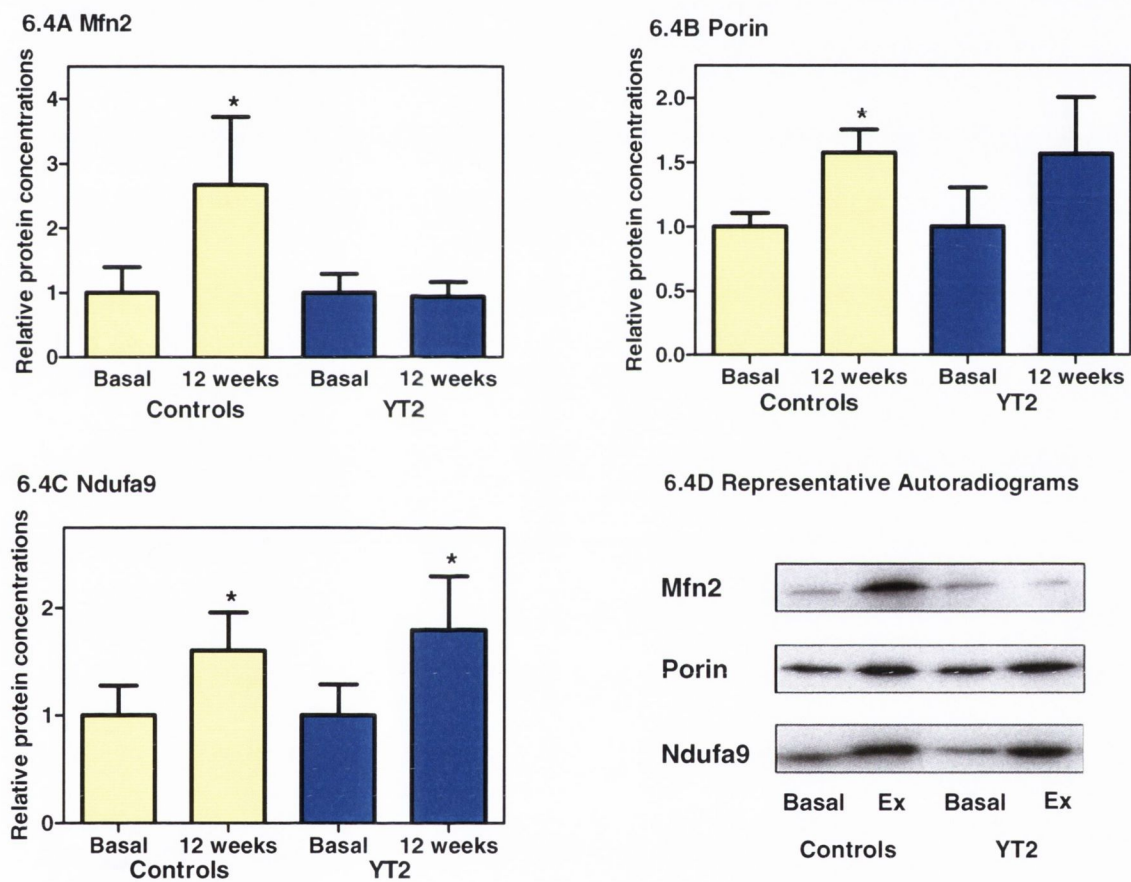


Figure 6.4A-D. Expression of key mitochondrial protein concentrations before and after 12-weeks of aerobic exercise. Chronic exercise caused a poorer induction of skeletal muscle mitochondrial proteins in YT2 subjects when compared to controls. Values are expressed as post-exercise concentrations relative to baseline, where baseline = 1, Mean $\pm$ SEM. Control n=3, YT2 n=3. *Significantly different from baseline values ($P<0.05$). Representative audoradiograms are also shown.

6.3.4 Skeletal Muscle Mitochondrial Gene Expression in Response to Acute Exercise.

Muscle biopsies taken after 1hr of acute exercise caused a substantial induction in PGC-1 α gene expression in control subjects (4-fold increase), Figure 6.5A. However, following seven days of exercise these changes had reverted back to baseline concentrations. Control subjects also showed an induction of ERR α gene expression (2.2-fold increase), although this trend did not reach statistical significance, Figure 6.5C. There were no changes in the expression of PGC-1 β , porin, Mfn2, or the mitochondrial gene COXIII in control subjects, Figures 6.5B, D, E&F.

In contrast, acute exercise led to no change in gene expression of PGC-1 α in the skeletal muscle from YT2 subjects, Figure 6.5A. In addition, a significant reduction was seen in the gene expression of ERR α (23% reduction), porin (23% reduction) and Mfn2 (30% reduction) (all $P<0.05$), Figures 6.5C, D&E. However, these reductions did not remain significant after seven days of exercise. No changes in gene expression of PGC-1 β or COXIII were detected in the YT2 group, Figures 6.5B&F.

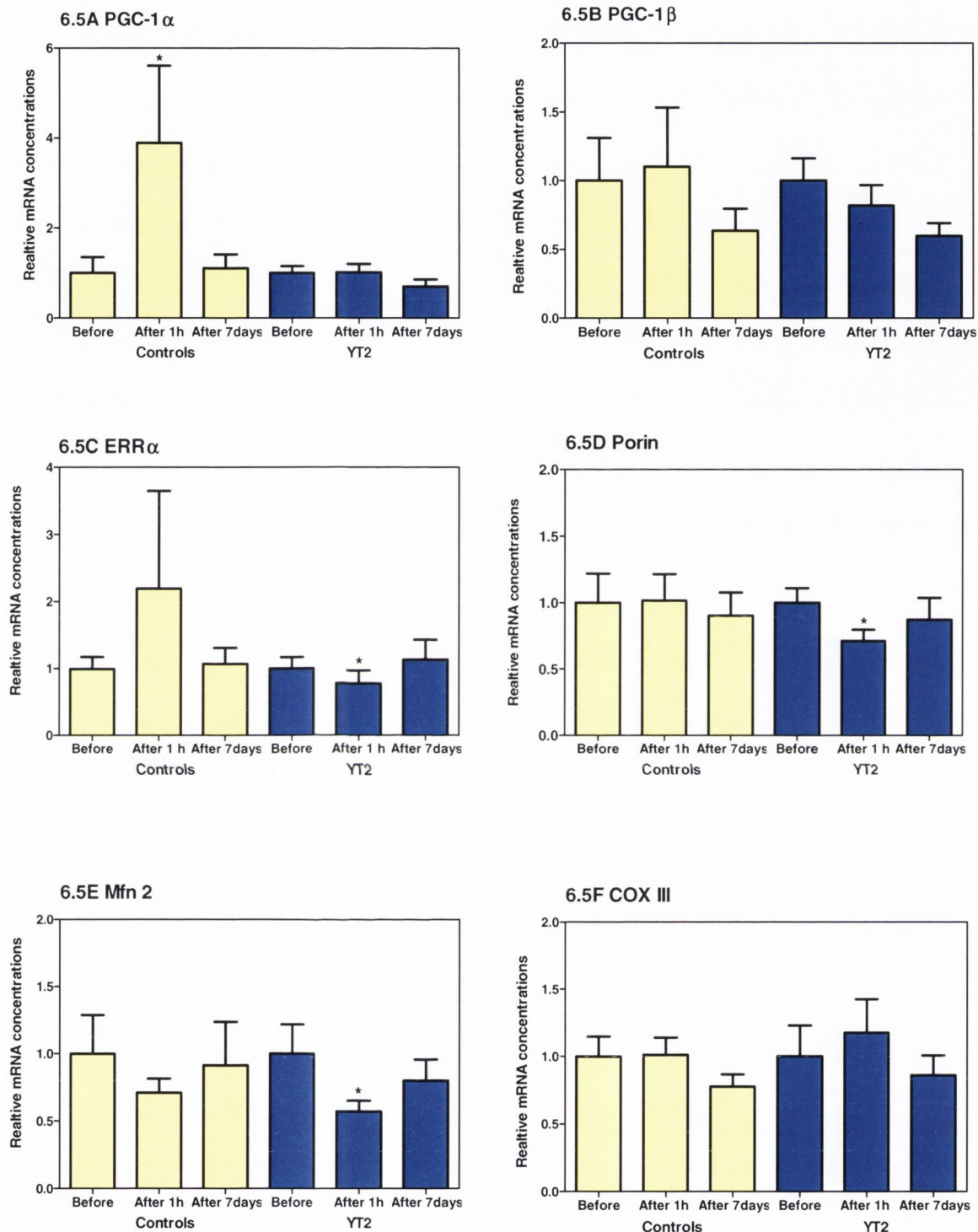


Figure 6.5A-F Mitochondrial gene expression before and after acute exercise (1hr and 7days). Acute exercise induces skeletal muscle PGC-1 α gene expression in control but not in YT2 subjects. Values are expressed as post-exercise concentrations relative to baseline, where baseline = 1, Mean $\pm$ SEM. Controls n=9, YT2 n=6. *Significantly different from baseline values ($P < 0.05$).

6.4 DISCUSSION

The YT2 participants in the acute study (study 2) had a similar clinical and metabolic phenotype to those reported in previous studies from our research group (47, 306). As such, in this chapter direct comparisons between the two studies (acute and chronic) will be made. In the acute study, VO_2peak was not re-measured after one week, as this was not expected to change.

As detailed in Chapter 5, 12 weeks of aerobic exercise (chronic exercise) led to no significant change in whole-body or hepatic insulin sensitivity in either the controls or YT2 subjects. VO_2peak increased by 20% in the control group, but was unchanged in the YT2 group. Fasting plasma FFA concentrations tended to be lower in the control group at baseline (0.635 vs. 0.820 mmol.l^{-1} , control vs. YT2 respectively, $P=\text{NS}$), and were significantly lower following the exercise intervention (0.561 vs. 0.826 mmol.l^{-1} , control vs. YT2, respectively, $P<0.001$), Figure 5.5.

At baseline, the YT2 subjects showed an altered pattern of mitochondrial protein expression compared with equally obese young people without diabetes. Subjects with diabetes displayed a modest decrease in the protein expression of Mfn2 and some OXPHOS subunits, Figure 6.3. Porin and Ndufa9 protein expression were similar between the two groups, which suggests that the changes in Mfn2 and ATP synthase were not secondary to changes in mitochondrial mass. Previous studies have shown that Mfn2 is a regulator of mitochondrial metabolism. In mice, a targeted null mutation in Mfn1 and Mfn2 in embryonic fibroblasts exhibited a loss of mitochondrial membrane potential, reduced endogenous respiration and an incapacity to increase respiration upon stimulation (383). Others have shown that the repression of Mfn2 reduces glucose, pyruvate and fatty acid oxidation as well as mitochondrial membrane potential (374, 376). The Mfn2 loss of function mutation causes alterations in substrate oxidation that have been shown to be unrelated to mitochondrial mass (374). In addition, previous research has shown that Mfn2 protein expression is positively correlated with insulin sensitivity (377). Consistent with

this is the lower expression of Mfn2 in the YT2 group, who are severely insulin resistant and are characterized by a higher WHR and dyslipidaemia. Thus, it is possible that lipotoxicity at baseline could either cause or result from the baseline mitochondrial abnormalities.

The mitochondrial changes observed following exercise are consistent with the whole body findings in both groups of subjects. Acute exercise (1hr) in the obese control group led to a four-fold increase in PGC-1 α gene expression (Figure 6.5A) and a later increase in both Mfn2 and porin concentrations following the 12-week exercise intervention (Figures 6.4A & B). The increase in mitochondrial proteins is consistent with the increased whole-body maximal oxygen uptake (VO_{2peak}) seen after 12 weeks. In subjects with diabetes, there was neither an early stimulation of PGC-1 α nor any change to Mfn2, which is consistent with the lack of change in VO_{2peak}.

PGC-1 α has been shown to have a stimulatory effect on Mfn2 mRNA, protein and promoter expression in muscle cells (378). Increases in FFA levels, by high fat diets coupled with lack of physical activity, have been shown to reduce PGC1 α expression (384-386). However, both acute and chronic aerobic exercise have been shown to increase the activity of PGC-1 α in combination with increases in mitochondrial enzyme activity (387-390). Increases in PGC-1 α have been associated with improved exercise performance and oxygen uptake (391, 392). In addition, ERR α has been shown to increase in parallel with increases in PGC-1 α expression (393). Based on the lack of response to exercise, it can be suggested that a defective activation of the PGC-1 α /Mfn2 regulatory pathway could alter mitochondrial metabolism in muscle from subjects with early-onset T2DM, and that this could be an inherited trait.

One interesting finding from this study is that chronic exercise increased skeletal muscle porin (Figure 6.4B, P =NS) and Ndufa9 (Figure 6.4C, P <0.05) expression in YT2 subjects although there were no changes to PGC-1 α or Mfn2 expression. This suggests that alternative mechanisms of mitochondrial biogenesis may be triggered by physical exercise.

This is consistent with findings from a PGC-1 α knockout mouse study, in which chronic exercise induced mitochondrial proteins in mouse skeletal muscle in the absence of PGC-1 α (394).

Previous studies have examined the effect of weight loss on the expression of mitochondrial proteins. These studies have shown that weight loss induced by diet and/or exercise can stimulate mitochondrial activity in skeletal muscle in obese subjects (233, 395), and are associated with improved insulin sensitivity (375, 379, 387, 396). However, the effects of weight loss on mitochondrial activity in subjects with T2DM are less consistent (233, 375). Although weight loss leads to improved insulin sensitivity, in the absence of physical activity it does not increase muscle oxidative capacity (375, 397). More recently, studies have shown a delayed and reduced response in PGC-1 α expression in muscle from obese insulin resistant subjects after exercise (307, 372). Furthermore, the expression of genes downstream of PGC-1 α have also shown a reduced response after exercise (307). Therefore, data from that and the current study taken together imply that there could be an association between insulin resistance and a defective regulation of PGC-1 α ; so that in severe insulin resistance the induction of PGC-1 α in response to exercise is nearly abolished. Future studies should investigate whether the lack of PGC-1 α induction with exercise causes or results from insulin resistance.

6.4.1 Conclusion

These results indicated that early-onset T2DM is associated with a reduced expression of Mfn2. It can be speculated that the extreme insulin resistance seen in the YT2 group has a negative impact on Mfn2 expression which in turn down regulates certain subunits of the OXPHOS system. It is also possible that the lipotoxicity in the YT2 group could either cause or result from the mitochondrial abnormalities. It is unclear which of these defects is the primary one.

Following 12 weeks of aerobic exercise training, the young obese control group experienced significant increases in the protein expression of Mfn2, which was associated with improvements in skeletal muscle oxidative capacity, VO_{2peak} . The upregulation in Mfn2 is consistent with the increase in PGC-1 α expression after the acute exercise bout. The YT2 group experienced a different pattern of muscle changes. It has been shown that the upregulation of the OXPHOS system via PGC1 α is abnormal in the YT2 group following exercise. The variation in Mfn2 expression and the failure to stimulate PGC-1 α may be related to the inability of the YT2 group to improve VO_{2peak} in response to the exercise training. These alterations may contribute to the mitochondrial dysfunction seen in the pathogenesis of early-onset T2DM.

This is the first study to investigate mitochondrial function in young obese people with type 2 diabetes. However, this study is limited by the small sample size. With the small subject number it was impossible to match the groups for gender. In the chronic study (which was completed first), the skeletal muscle investigations were limited by the biopsy amount and it was not possible to measure PGC-1 α or other gene expression. Future studies should focus on examining a more complete mitochondrial function profile. A full understanding of these mechanisms and how they affect the pathophysiology of T2DM in adolescents and young adults will be crucial to designing lifestyle interventions for prevention and treatment.

Chapter Seven:

Summary and Conclusions

7.1 Phenotype of Early-Onset Type 2 Diabetes

Subjects with early-onset T2DM (YT2) exhibit an extreme phenotype. Early-onset obesity coupled with physical inactivity are likely to accelerate the pathogenesis of T2DM in those who are susceptible due to genetic or other environmental factors (30, 31, 34), Figure 7.1. Similar to adult-onset diabetes, YT2 is characterised by insulin resistance with subsequent loss of β -cell insulin secretion.

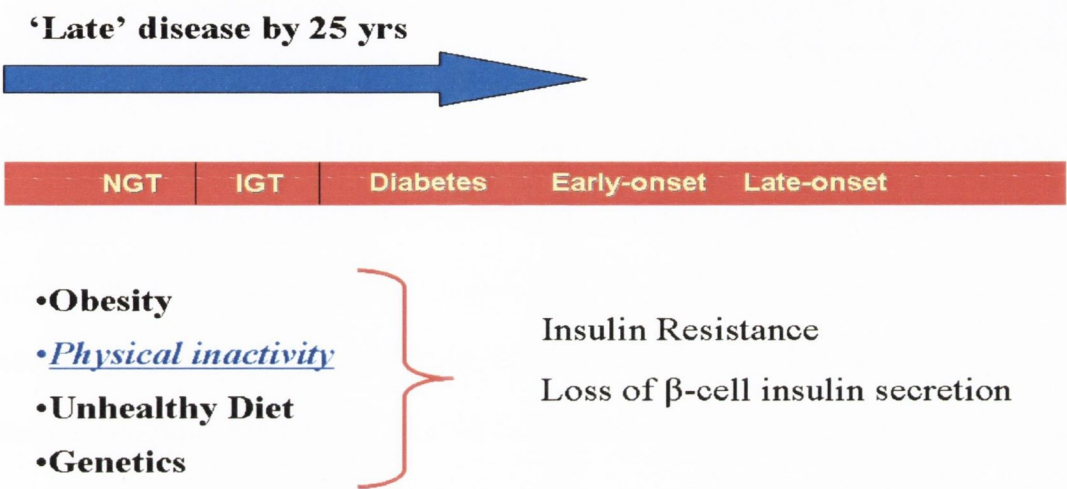


Figure 7.1 Pathogenesis of early-onset T2DM. The classical T2DM phenotype occurs 20-30 yrs earlier in early-onset patients. Abbreviations: NGT= normal glucose tolerance, IGT= impaired glucose tolerance

In chapter 4, young subjects with and without T2DM who were age- and BMI-matched were studied. Although they were of similar weight, those with diabetes were more viscerally obese (greater waist circumference and WHR, both $P<0.01$). YT2 subjects were also much more insulin resistant, as determined from the euglycaemic-hyperinsulinaemic clamp. Furthermore, they displayed marked β -cell dysfunction. These findings are in accordance with previous research from our group, and others (47, 308-310). In agreement with the literature, the YT2 group also tended to be more dyslipidaemic when compared to the control group (as indicated by higher triglycerides ($P<0.001$), and a trend towards higher FFA and lower HDL cholesterol concentrations) (73, 293, 323).

Early-onset T2DM is also associated with a similar, but more severe clinical and metabolic phenotype when compared to later-onset T2DM, Chapter 3. Subjects with early-onset

T2DM have been shown to have an elevation in cardiovascular risk markers when compared to older adults (47, 164, 306). This is manifested by higher levels of classical risk markers such as BMI, waist circumference, WHR, hyperglycaemia, as well as elevations in inflammatory markers. Due to this, early-onset T2DM patients have proven to be a greater challenge to manage with currently available conventional therapies. Coupled with elevated cardiovascular disease risk factors, young T2DM subjects have an enhanced risk of future morbidity and mortality (35, 165).

In Table 7.1 the early-onset cohort from Chapter 3 (i.e. those diagnosed <40 yrs) has been compared to the YT2 cohort (i.e. those diagnosed <30 yrs). The YT2 cohort has a more adverse lipid profile, presenting with lower HDL cholesterol concentrations and a trend towards higher triglycerides when compared to the older cohort (<40 yrs). Their ongoing glycaemic control was again poorer (manifested by the higher HbA1c), whereas baseline C-peptide was similar and a similar percentage of patients in each group progressed onto insulin therapy (23% in the YT2 cohort vs. 21% in the cohort <40 yrs).

Table 7.1 Mean clinical data comparison between subjects diagnosed <40 yrs and <30 yrs.

	< 40 yrs (n = 149)	<30 yrs (YT2) (n = 13)	P value (<0.05)
Male (%)	67.00	61.00	
Age (yrs)	35.2 ± 0.4	26 ± 1	<0.0001
BMI (kg.m ⁻²)	33.3 ± 1.7	34.9 ± 1.4	NS
Weight (kg)	95.5 ± 1.9	109.0 ± 5.0	0.02
HbA1c (%)	8.02 ± 0.19	8.8 ± 0.3	0.04
Total cholesterol (mmol.l ⁻¹)	4.98 ± 0.12	4.69 ± 0.24	NS
LDL cholesterol (mmol.l ⁻¹)	2.83 ± 0.07	2.67 ± 0.20	NS
HDL cholesterol (mmol.l ⁻¹)	1.07 ± 0.03	0.94 ± 0.04	0.01
Triglycerides (mmol.l ⁻¹)	2.60 ± 0.22	2.90 ± 0.37	NS
C-peptide (µg.l ⁻¹)	3.27 ± 0.34	3.7 ± 0.5	NS

Values are presented as Mean ± SEM. Abbreviations: YT2= young patients with type 2 diabetes, BMI= body mass index, HBA1c= glycated haemoglobin, LDL= low-density lipoprotein, HDL = high-density lipoprotein.

Interestingly, the YT2 group was heavier (by 14 kg) (*P*<0.02) when compared to the group approximately 10 yrs their senior. In fact, obesity proves to be a key determinant for the

development of diabetes among younger patients (37, 398). Figure 7.2 illustrates the relationship between BMI and age of onset of T2DM in patients from our clinic. The greater the BMI, the earlier the age of onset. In an earlier study from our research group in which younger and older subjects were BMI matched, the younger subjects were significantly heavier (average 20 kg heavier, $P<0.005$) and taller ($P<0.002$) when compared to older subjects (47).

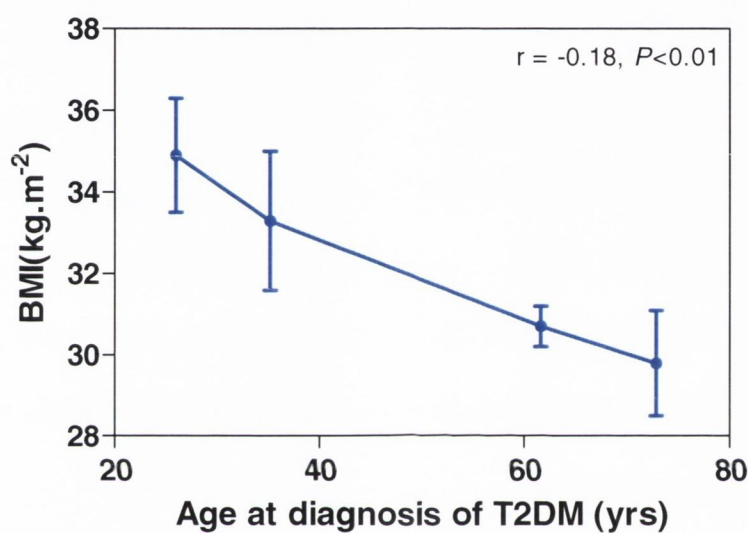


Figure 7.2 Influence of BMI on the progression to T2DM in the young. Data from our clinical database, presented as Mean $\pm$ SEM.

In chapter 2, an active but insulin resistant (AIR) phenotype among healthy subjects was described. The AIR cohort was markedly more obese, more insulin resistant and had elevations in FFA concentrations when compared to the inactive insulin sensitive cohort (IIS), Table 2.2 and Figure 2.3. The AIR group seem to be exercise “non-responders”. Although they were almost twice as active, physical activity did not provide this cohort with protection against insulin resistance as they still demonstrated impaired peripheral glucose disposal as measured by the euglycaemic-hyperinsulinaemic clamp. As described previously, the YT2 cohort did not respond to a 12 week aerobic exercise regime, Chapter 5. There were no changes in insulin sensitivity or fitness, as measured by the graded exercise test. The same metabolic features seen in this young cohort were also

observed in the AIR vs. IIS study. Similar to the AIR cohort, the YT2 cohort was significantly more insulin resistant and dyslipidaemic in comparison to the young obese control group who experienced an increase in fitness. Baseline studies of skeletal muscle showed that the YT2 group had an altered pattern of mitochondrial protein expression, more specifically, a decreased expression of Mfn2 and some OXPHOS subunits, Figure 6.3. In addition, the inability to increase their whole-body maximal oxygen uptake is consistent with the underlying alterations in mitochondrial protein and gene expression (specifically PGC-1 α and Mfn2), Figures 6.4 and 6.5. These findings are consistent with previous studies in obese insulin resistant subjects (307, 372). This study further strengthens the hypothesis that the association between insulin resistance, lipotoxicity and the lack of metabolic response observed in this unique cohort may be related at least in part to impaired mitochondrial function. This raises the question whether a similar defect in mitochondrial function may be present in healthy subjects (such as the AIR cohort), who are active, lipotoxic and insulin resistant.

Aerobic exercise is associated with improvements in insulin sensitivity (222, 229, 230). The landmark diabetes prevention studies have shown that diet and exercise can lead to improved insulin sensitivity, which in turn reduces the demand on failing β -cell function in those who are already predisposed to diabetes (1, 2). However, more recent studies have shown that not all subjects respond to the same degree to physical activity (271, 272). This raises the question as to whether certain genetic determinants contribute to the lack of response to aerobic exercise training (272).

7.2 Future directions

These studies outline a lack of response among young adults with T2DM to exercise. Early-onset T2DM is a more extreme phenotype than older-onset (traditional) T2DM. BMI- and age-matched controls have been compared to a group of young adults with diabetes and the YT2 cohort displayed greater insulin resistance and lipotoxicity, relative

to their degree of obesity. These subjects demonstrated an altered mitochondrial protein expression at baseline and following both acute and chronic exercise. We have also described a sub-phenotype among healthy middle-aged individuals who are insulin resistant despite adequate levels of physical activity.

It is likely that some forms of insulin resistance are genetically acquired. Studies have shown that exercise can prevent progression to T2DM in at risk individuals and improve diabetic control in others, through its effect on improving insulin sensitivity. Although the current studies have shown a lack of response to aerobic exercise training at 70% $\text{VO}_{2\text{peak}}$, for 1 hr per day, 4 days per week, this does not mean that aerobic exercise is not beneficial. Questions remain in relation to the optimal intensity, frequency, and duration of exercise that may be beneficial to this particular group of patients.

To improve diabetes prevention and management in younger subjects, future studies could focus on training programs which include a hypocaloric diet to initiate and/or increase weight loss in obese adolescents, similar to the approach taken in many previous intervention programs (2, 213, 214, 399-401). More in depth inquiries can be made into identifying genetic contributors to insulin resistance and exercise response, such as the FHL1 gene. In addition, the contribution of mitochondrial dysfunction to insulin resistance and the lack of response to exercise training requires much more detailed study. Furthermore, the effect of lipotoxicity on the progression and management of T2DM in the young will require much further study.

Early-onset type 2 diabetes presents a major challenge to all components of diabetes care, but particularly to the design of lifestyle interventions, which must still form the mainstay of treatment, even in light of the current research findings.

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Appendix I

**Timelines for euglycaemic-hyperinsulinaemic clamp study
and graded exercise test**

Timeline- euglycaemic-hyperinsulinaemic clamp

Time (minutes)

-120min	[6-6²H₂] glucose bolus of 4mg/kg lean body mass given constant infusion of [6-6²H₂] glucose in 50ml 0.9% NaCl at a rate of 0.04mg/min/kg body mass started Blood samples taken for glucose, insulin, C-peptide and tracer
-20min	three arterialized blood samples drawn for glucose measurements a) If fasting plasma glucose value was 4.5-5.5mmol/l (80-100mg/dl) then the fasting glucose concentration was the clamp level (i.e. isoglycaemic clamp) b) If fasting glucose was > 5.5 mmol/l, the clamp level was 5.5 mmol/l (100mg/dl) c) If fasting glucose was < 4.5 mmol/l, the clamp level 4.5 mmol/l (80mg/dl).
0min	insulin solution started at rate of 60ml/hr Blood sample taken for glucose and tracer
4min	insulin infusion rate changed to 30ml/hr unlabelled glucose infusion started per guidelines above glucose infusion checked and changed accordingly to keep patient within clamp level- this continues every 5min throughout duration of clamp
7min	insulin infusion rate changed to 15ml/hr (remains at this rate until end of intravenous glucose tolerance test)
15min	[6-6²H₂] glucose infusion stopped
80min	blood samples taken for glucose, insulin C-peptide and tracer
80-120min	steady state period used to calculate insulin sensitivity
100min	blood samples taken for glucose, insulin, C-peptide and tracer
120, 0min	insulin and unlabelled glucose infusion are maintained at same constant rate clock re-started to t=0 an I.V. bolus of 50% glucose solution (0.3mg/kg body weight) administered over one minute into antecubital vein
+2,+4,+6, and +8min	blood samples taken for glucose, insulin and C-peptide
+8min	insulin infusion stopped, unlabelled glucose infusion slowly reduced, patient given something to eat

Timeline- Graded exercise test

Time (minutes)

-15min	weight and height measured, BMI calculated heart rate monitor put on face mask fitted bike adjustments made
0-3min	rest period on bike VO ₂ , respiratory exchange ratio (RER) and heart rate (HR) recorded over 1min (from minute 2 to minute 3), BP measured
3-6min	subject begins to cycle at 25W HR, RER and VO ₂ recorded during last minute
6-9min	intensity increased to 50W HR, RER and VO ₂ recorded during last minute
9-12min	intensity increased to 75W HR, RER and VO ₂ recorded during last minute
12-13min	intensity increased to 85W HR, RER and VO ₂ recorded over last 20 seconds
13- 14min	intensity increased to 95W HR, RER and VO ₂ recorded over last 20 seconds
14 min- failure	intensity increase by 10W each minute until failure HR, RER and VO ₂ recorded during last 20 seconds of each interval

Appendix II

**Laboratory analysis procedures for processing of blood samples and
muscle biopsies**

Routine blood sample laboratory analysis:

- Plasma glucose was measured using a glucose oxidase method (bioMérieux kit; Hitachi Modular, USA).
- Serum insulin was measured using an AutoDELFIA™ Insulin time-resolved fluoroimmunoassay kit (Auto-Delfa, Wallac Oy, Finland).
- Serum C-peptide was assayed using an AutoDELFIA® C-peptide time-resolved fluoroimmunoassay kit (PerkinElmer Life and Analytical Sciences, Wallac Oy, Finland).
- HbA_{1c} was determined enzymatically by an Arkay HbA_{1c} model HA 8140 kit (Chapter 3) or HA 1060 kit (Chapters 4, 5 and 6) (Menarini Group, Florence, Italy).
- Plasma total cholesterol was measured using human liquicolor CHOP PAP enzymatic methods (Chapter 3: Hitachi Modular, Roche Diagnostics, Basel Switzerland) and (Chapters 4, 5, and 6: Omega Diagnostics Ltd., UK).
- Plasma triglycerides were measured using human liquicolor GPO PAP enzymatic methods (Chapter 3: Hitachi Modular, Roche Diagnostics, Basel Switzerland) and (Chapters 4, 5, and 6: Omega Diagnostics Ltd., UK).
- Plasma high-density lipoprotein (HDL) cholesterol was measured directly by enzymatic methods (Randox direct kits; Hitachi Modular, Roche Diagnostics, Basel Switzerland)
- Plasma low density lipoprotein (LDL) cholesterol was measured directly by enzymatic methods (Randox direct kits; Hitachi Modular, Roche Diagnostics, Basel Switzerland).
- Antibodies to GAD were determined using a direct radioligand assay (Randox Laboratories, Antrim, UK).
- Serum free fatty acids (FFA) were determined using a spectrophotometric assay (Randox Laboratories, Antrim, UK).

Markers of endothelial inflammation and visfatin laboratory analysis:

- sICAM was assayed using the quantitative sandwich enzyme immunoassay technique (R&D Systems Europe Ltd., Abingdon, Oxfordshire, UK); normal values 115-306ng.ml⁻¹.
- sVCAM was immunoassayed using a 2-hour solid-phase ELISA designed to measure human sVCAM-1 in serum or plasma (R&D Systems Europe Ltd., Abingdon, Oxfordshire, UK); normal values 379-991ng.ml⁻¹.

- E-selectin was assayed using the quantitative sandwich enzyme immunoassay technique (R&D Systems Europe Ltd., Abingdon, Oxfordshire, UK); normal values 29.1-63.4ng.ml⁻¹.
- P-selectin immunoassay is a 1.25 hour solid-phase ELISA that measures P-selectin in serum and plasma (R&D Systems Europe Ltd., Abingdon, Oxfordshire, UK); normal values 51-113ng.ml⁻¹.
- hsCRP was assayed by means of a particle enhanced immunonephelometric assay (Immulite® 2000, Siemens Medical Solutions Diagnostics, USA); normal values were <5mg.l⁻¹.
- Visfatin was measured using a commercially available ELISA kit (Phoenix Peptides, Karlsruhe, Germany); with an intra- and inter-assay coefficient of variation of <6%

Analysis methods for OGTT and IVGTT data:

Beta cell function was assessed using a model described previously (1, 2). The model parameters were estimated from glucose and C-peptide concentrations by regularised least-squares, as previously described (1). 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. The following terms from the model are used to describe beta cell function:

- “Beta cell glucose sensitivity” represents the mean slope of the insulin secretion vs. glucose concentration graph. It is a dose-response function.
- The “potentiation factor” modifies the dose-response and encompasses several potentiating factors: prolonged exposure to hyperglycaemia, non-glucose substrates, gastrointestinal hormones and neurotransmitters. The potentiation factor is a positive function of time and averages one. It expresses a relative increase in insulin secretion as a response to plasma glucose concentration and increases during the OGTT (1-3). This increase has been quantified as the ratio between the potentiation factor value at 2hrs post OGTT and that at time zero.

- “Rate sensitivity” represents the dependence of insulin secretion on the rate of change of glucose concentration. It is related to early insulin release (1-3).
- The “acute insulin response” was calculated as the mean incremental insulin concentration during the eight-minute IVGTT (4).

Processing of muscle biopsy samples

RNA extraction and real time PCR

- RNA was extracted from approximately 25mg of skeletal muscle using a RNeasy kit (Qiagen, GmbH).
- 40 ng of RNA was reverse transcribed to cDNA using Sensiscript enzyme (Invitrogen, USA).
- Real-time PCR was used for measurement of specific mRNAs (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems, Foster City, CA).

Determination of mRNA levels was done by measuring fluorescence from the progressive binding of SYBR green I dye to double-stranded DNA. All PCR runs were performed in duplicate.

- The primers and probe for PGC-1 α , PGC-1 β , and ATP5A1 (Co V) were supplied as a TaqMan Reagents kit from Applied Biosystems [Hs01016721_m1, Hs00991677_m1, and Hs00900735_m1, respectively].
- mRNA expression was calculated using the Δ CT method (5).
- The oligonucleotide sequences for the primer pairs are detailed below:

Mfn2: 5-CCCCCTTGCTCTTTATGCTGATGTT-3

and 5-TTTTGGGAGAGGTGTTGCTTATTTC-3

Cytochrome C Oxidase subunit III (COX III):

5-CGCCTGATACTGGCATTTC-3

and 5-TTTTTTTTTTTTTTTTTTTTTTTTAAGACC-3

porin: 5-ACGTGGACTGAAGCTGACCT-3
and 5-CCAAACTCTGTCCCGTCATT-3

Homogenate extracts from muscle biopsies.

- Protein homogenate was extracted from approximately 25 mg of skeletal muscle.
- The muscle was immersed in ice-cold medium buffer A (0.1 m KCl, 5 mm MgCl₂, 5mm EGTA, 5mm sodium pyrophosphate, pH adjusted to 7.4, 2 µm leupeptin, 2 µm pepstatin, 0.5mm phenylmethylsulphonylfluoride (PMSF)) for 2 ×10 min.
- Following this, the muscle was finely minced in 1/10 (w/v) buffer B (0.25 m sacharose, 50mmKCl, 5 mm EDTA, 1mm sodium pyrophosphate, 5mm MgCl₂, pH adjusted to 6.8, 2 µm leupeptin, 2 µm pepstatin, 0.5mm PMSF) and then disrupted with a motor driven Teflon/glass homogenizer.
- The entire procedure was performed at 0–4°C.
- The protein concentration was determined using a Micro BCA protein assay (Pierce, Rockford, IL, USA).

Western blot assays.

- 35µg of skeletal muscle protein homogenate were subjected to a 10% acrylamide/bisacrylamide SDS–PAGE. It was then electroblotted onto nylon filters.
- These nylon filters were probed with specific antibodies raised against Mfn2 (6), two subunits of the OXPHOS system (complex I (anti-NDUFA9) and complex V (anti-subunit a from Hp-F1-ATP synthase) (from Molecular Probes)) and porin (Calbiochem, La Jolla, CA, USA).
- The specific proteins were detected by the ECL western blotting detection analysis system (Amersham, GE healthcare, UK). The signal obtained with each antibody was quantified on autoradiography films using a laser densitometer.

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Appendix III

**Contrasting clinical and cardiovascular risk status between early
and later onset type 2 diabetes.**

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Contrasting clinical and cardiovascular risk status between early and later onset type 2 diabetes

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Abstract

The prevalence of type 2 diabetes (T2DM) is increasing rapidly and the age of presentation is falling. These changes are likely to be linked to the current obesity epidemic. Our objective was to compare the characteristics of younger patients with T2DM (diagnosed at age < 40 years) with those of older patients (diagnosed at age 50–70 years).

We identified 149 younger patients with T2DM, from our diabetes clinic database, and compared them with 217 older T2DM patients randomly identified from the same database.

Younger patients with T2DM were more obese, more hypertriglyceridaemic, with lower high-density lipoprotein (HDL) cholesterol, higher total cholesterol/HDL ratio and worse initial and ongoing glycaemic control than older patients from the same clinic. Additional cardiovascular risk factors are associated with T2DM in the young. Treatment should be aimed at early modification of lifestyle and other forms of therapy to avoid long-term complications.

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Key words: early onset type 2 diabetes, obesity, dyslipidaemia, cardiovascular risk.

Introduction

We are witnessing an escalating epidemic of type 2 diabetes, in conjunction with increasing obesity and sedentary lifestyle.^{1,2} The worldwide prevalence of diabetes in adults is expected to increase from 5% to 6.2% from 2003 to 2025. This will mean that in 2025 some 328 million people will have diabetes.³ Until recently, type 2 diabetes (T2DM) was typically regarded as a disease of the middle-aged and elderly. Evidence is accumulating, however, that onset in younger

adults is increasing; even children and adolescents are now part of the diabetes epidemic.⁴ Obesity has increased by 70% in adults aged 18–29 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 development of both obesity and type 2 diabetes.^{2,5}

Early onset type 2 diabetes appears to be a more aggressive disease from a cardiovascular standpoint. We have recently reported that much younger subjects with type 2 diabetes (average age of onset 19 years) are obese and severely insulin-resistant, with markedly abnormal cardiac risk markers, similar to those seen in patients thirty years older.⁶ Young adults with type 2 diabetes appear to have a much higher risk of cardiovascular disease in comparison to age-matched subjects without diabetes.^{6,7} This pattern of disease has profound social and economic implications.⁴

The prevalence of type 2 diabetes has been increasing rapidly and the age of presentation is falling. These factors are likely to be linked to the current obesity epidemic.^{8,9} Despite the rapid change in age of onset of type 2 diabetes, little is known about the differences in clinical and cardiovascular risk status between early and later onset type 2 diabetes.

The aim of this study was to compare, in our clinic population, the characteristics of younger patients with T2DM (diagnosed at age < 40 years) with those from older patients (diagnosed at age 50–70 years).

Research design and methods

We retrospectively identified 149 younger patients with T2DM (diagnosed at age < 40 years), from our Diamond® computerised diabetes clinic database, and compared them with 217 older T2DM patients (diagnosed at age 50–70 years) randomly identified from the same database. Serum insulin and C-peptide were measured by commercially available fluoroimmunoassays (Auto-Delfia). Plasma total cholesterol and triglycerides were measured using enzymatic methods (Human liquicolor kits/Hitachi Modular). Plasma high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (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) and glycosylated haemoglobin (HbA_{1c}) was measured using a Hi-Auto A1c analyser (Menarini HA 8140). All subjects gave their written informed consent for inclusion in the clinical database.

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Table 1. Baseline characteristics of patients in the two groups

	Younger T2DM	Older T2DM	p value
Number	149	217	
Male (%)	67.00	63.59	
Age (years)	35.16 (0.39)	61.61 (0.38)	
BMI (kg/m ²)	33.28 (1.65)	30.66 (0.5)	<0.05
Weight (kg)	95.5 (1.9)	87.0 (1.2)	<0.05
HbA _{1c} at diagnosis (%)	9.38 (0.21)	8.57 (0.14)	<0.05
HbA _{1c} current (%)	8.02 (0.19)	7.35 (0.09)	<0.05
Total cholesterol (mmol/L)	4.98 (0.12)	4.83 (0.07)	NS
LDL (mmol/L)	2.83 (0.07)	2.79 (0.06)	NS
HDL (mmol/L)	1.07 (0.03)	1.17 (0.02)	<0.05
Total cholesterol/HDL ratio	4.83 (0.11)	4.23 (0.06)	<0.05
Triglycerides (mmol/L)	2.60 (0.22)	2.09 (0.13)	<0.05
C-peptide at diagnosis (µg/L)	3.27 (0.34)	3.48 (0.25)	NS
Systolic BP (mmHg)	124 (1.23)	136 (1.5)	NS
Diastolic BP (mmHg)	80 (0.91)	78 (0.81)	NS

Values are presented as mean, with standard error of the mean in parentheses.

Key: T2DM = type 2 diabetes; BMI =body mass index; BP = blood pressure; NS = not significant; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol

Statistical analysis

Data were expressed as means ± standard error of mean. The significance of the difference of means was tested by t-test for independent samples. A two-tailed model was used and a p value < 0.05 was considered to be statistically significant. Data pertaining to treatment modality were expressed as percentages.

Results

Patient characteristics are outlined in table 1. We included in the younger group all patients attending our service for at least one year with type 2 diabetes diagnosed at age < 40 years. Type 1 diabetes was excluded on clinical grounds and on the basis of fasting C-peptide levels (C-peptide > 0.7 µg/L in all subjects at diagnosis). The total number was 149, with a mean age 35.2±0.4 years.

We compared these with 217 older patients with type 2 diabetes diagnosed at age 50–70 years, randomly identified from the same database, with a similar duration (range 1–6 years) of clinical follow-up. The mean age in the older group was 61.6±0.4 years. Most of the patients were male in both groups, younger and older (67.0% vs. 63.6%). Both groups of patients were obese, but the younger-onset group was significantly more obese as measured by body mass index (BMI) than the older group (BMI 33.3 vs. 30.7 kg/m², p<0.05) and weight (95.5 vs. 87.0 kg, p<0.05).

The mean HbA_{1c}, at diagnosis was higher in young adults with type 2 diabetes than in older adults (9.38% vs. 8.57%, p<0.05), and HbA_{1c} when stabilised at follow-up continued to be worse in the younger group than in the older group (8.02% vs. 7.35%, p<0.05).

There was no statistically significant difference in the mean total cholesterol level (4.98 vs. 4.83 mmol/L) or the mean LDL level (2.83 vs. 2.79 mmol/L) between the two groups.

HDL cholesterol was significantly lower in the younger group compared to the older group (1.07 vs. 1.17 mmol/L, p<0.05), and the mean ratio of total cholesterol/HDL was higher (4.83 vs. 4.23, p<0.05) in the group with younger onset T2DM. The mean triglyceride concentration was significantly higher in younger than in older patients (2.60 vs. 2.09 mmol/L, p<0.05).

There was no significant difference between the two groups in mean levels of C-peptide (3.27 vs. 3.48 µg/L), systolic blood pressure (124 vs. 136 mmHg) or diastolic blood pressure (80 vs. 78 mmHg).

The proportion of the patients requiring some form of insulin treatment was higher in the younger onset group than in the later onset (21.5% vs. 13.4%), table 2.

Discussion

We compared the clinical phenotype and cardiovascular risk status between patients with early and later onset T2DM. We have shown that patients with early onset T2DM have worse glycaemic control and dyslipidaemia than their older counterparts from the same clinic despite identical clinical treatment protocols and follow-up.

We have recently confirmed that patients with type 2 diabetes of teenage onset are obese and severely insulin-resistant, much more insulin-resistant than equally obese and age-matched young people with normal glucose tolerance.⁶ These young subjects with type 2 diabetes were

Table 2. Diabetes treatment in the two groups

	OAD	Insulin/OAD	Diet
Younger T2DM	80 (53.7%)	32 (21.5%)	37 (24.8%)
Older T2DM	133 (61.3%)	29 (13.4%)	55 (25.3%)

Values are presented as numbers of patients, with percentages in parentheses

Key: T2DM = type 2 diabetes; OAD = oral antidiabetes drug

dyslipidaemic, with higher triglycerides (1.9 mmol/L vs. 1.26 mmol/L) and lower HDL (0.96 vs. 1.06 mmol/L) compared with equally obese non-diabetic teenagers.⁶

Our current study, in a cohort of average age 35 years, confirms that type 2 diabetes of earlier onset is strongly associated with obesity and the classical dyslipidaemia of insulin resistance and type 2 diabetes. In the current study, the younger group, aged 35 years at diagnosis, have classical type 2 diabetes with onset on average 27 years earlier than the older group.

It is not clear why the younger subjects have developed diabetes at this early stage. It is likely that early onset visceral obesity and associated insulin resistance are the main risk factors for early progression and loss of beta cell insulin secretion. Consistent with this, the younger patients in the current study were on average 8.5 kg heavier at diagnosis than the older patients with later onset T2DM (95.5 kg vs. 87.0 kg, $p < 0.05$). Fasting C-peptide levels in both younger and older groups were similarly elevated, despite the worse initial glycaemic control in the younger group. This is consistent with the hypothesis that the younger group have progressed more rapidly because of their insulin resistance.

An important implication of the current study is the long-term risk of diabetes complications in patients with earlier onset of disease. Previous studies have demonstrated that hyperglycaemia is an independent predictor of cardiovascular disease in type 2 diabetes.^{10,11} It is known that metabolic control of type 2 diabetes generally deteriorates with increasing duration of the disease, irrespective of the mode of treatment.¹² Younger patients in the current study presented with a worse degree of initial hyperglycaemia and have proven more difficult to stabilise despite uniform protocols for treatment titration. More have progressed to insulin therapy compared to those in the older group, and even though their glycaemic control improved (HbA_{1c} fell from 9.38% to 8.02%), the younger group on average continued to be more hyperglycaemic than the older group.

Another major contributor to cardiovascular mortality is diabetic dyslipidaemia.¹³ The dyslipidaemia in type 2 diabetes patients is characterised by elevated triglycerides and decreased HDL cholesterol levels.¹⁴ Previous studies have demonstrated that the ratio of total to HDL cholesterol is the strongest lipid predictor for cardiovascular disease.^{15,16} Based on both of these observations, the younger group with type

2 diabetes in the current study is at significantly higher long-term risk of cardiovascular complications.

Conclusion

Younger patients with T2DM are more obese than older patients. They are more hypertriglyceridaemic, and have lower HDL and higher total cholesterol/HDL cholesterol ratios. They also present with worse initial hyperglycaemia and continue to have poorer ongoing glycaemic control than older patients from the same clinic, in spite of identical treatment and follow-up protocols.

This phenomenon is a new and serious challenge for diabetes management. Younger patients with T2DM present with a higher cardiovascular risk profile than older patients, and will also face a longer disease duration and possibly worse severity of hyperglycaemia. Treatment of younger patients with T2DM is likely to require early aggressive lifestyle modification and intensive multifactorial therapy in order to prevent long-term complications. This will require further prospective study.

Conflict of interest disclosure

There are no relevant conflicts of interest to be disclosed.

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Appendix IV

Early-onset type 2 diabetes in obese white subjects is characterised by a marked defect in beta cell insulin secretion, severe insulin resistance and a lack of response to aerobic exercise training.

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Early-onset type 2 diabetes in obese white subjects is characterised by a marked defect in beta cell insulin secretion, severe insulin resistance and a lack of response to aerobic exercise training

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Abstract

Aims/hypothesis Early-onset type 2 diabetes is associated with marked visceral obesity and extreme insulin resistance, but its pathogenesis and response to treatment are not completely understood. We studied physical fitness, whole-body and hepatic glucose turnover, and insulin secretion in young obese Irish subjects before and after 3 months of aerobic exercise training. We hypothesised that exercise alone, with stable diet, should improve insulin sensitivity. **Materials and methods** Anthropometric parameters and maximum volume of oxygen utilisation ($\dot{V}O_{2\max}$) were measured in 13 subjects with type 2 diabetes and 18 non-diabetic control subjects, matched for age and BMI. Insulin sensitivity and hepatic glucose turnover were measured using the hyperinsulinaemic–euglycaemic clamp. Insulin secretion was assessed from an OGTT and a modified

intravenous glucose tolerance test. Some subjects (seven type 2 diabetic, 14 non-diabetic control subjects) then completed a 12-week supervised aerobic exercise programme. All measurements were repeated on completion of the exercise programme.

Results Type 2 diabetic subjects had higher WHR, systolic blood pressure and triacylglycerols than non-diabetic control subjects. They were significantly more insulin-resistant as measured both by the clamp and oral glucose insulin sensitivity. They also displayed marked defects in insulin secretion in response to oral and intravenous glucose challenges. Exercise intervention had no significant effect on whole-body or hepatic insulin sensitivity or insulin secretion. $\dot{V}O_{2\max}$ increased significantly in the non-diabetic control subjects, but not in the type 2 diabetic subjects after exercise training.

Conclusions/interpretation Young obese subjects with type 2 diabetes are severely insulin-resistant with marked loss of beta cell function compared with control subjects matched for age and obesity. Neither group responded metabolically to aerobic exercise intervention.

Keywords Early onset · Beta cell function · Exercise · Glucose sensitivity · Hyperinsulinaemic clamp · Insulin resistance · Insulin secretion · Type 2 diabetes · Visceral Obesity · Young

N. Burns and F. M. Finucane contributed equally to this work.

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Abbreviations

GLUT4 glucose transporter 4
IVGTT intravenous glucose tolerance test
OGIS oral glucose insulin sensitivity
 $\dot{V}O_{2\max}$ maximum volume of oxygen utilisation

Introduction

Marked visceral obesity and extreme insulin resistance are associated with early-onset type 2 diabetes. The prevalence of diabetes is predicted to more than double to 366 million by 2030 [1]. A more recent trend has been the shift of type 2 diabetes to middle-aged and younger populations. The new epidemic of obesity and type 2 diabetes in young people is becoming a clinical and health economic priority [2].

In Ireland, in the last decade, the prevalence of obesity has increased by 67% [3]. Irish adolescent boys and girls ranked third and second highest respectively in an international study of obesity prevalence rates [4]. This has been associated with significant changes in physical activity and diet in this age group [5]. However, the exact sequence of events leading to type 2 diabetes in youth is not fully understood. We have reported that younger Irish adults (aged less than 40 years) with type 2 diabetes 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 [6]. In a separate study of younger white Irish subjects with type 2 diabetes (mean age 22 years), we used an insulin-modified intravenous glucose tolerance test (IVGTT) to demonstrate severe insulin resistance and loss of first-phase insulin secretion [7]. This cohort had evidence of high risk for cardiovascular complications, with elevated markers of endothelial dysfunction. This suggests that a disproportionate cardiovascular risk burden is associated with type 2 diabetes in younger patients. To date there have been no studies of insulin sensitivity using the hyperinsulinaemic–euglycaemic clamp technique in this population.

There is considerable evidence that exercise delays or prevents the development of type 2 diabetes in at-risk populations [8–12]. Smaller studies have shown that exercise improves insulin sensitivity and glucose metabolism [13, 14]. We have recently shown that short-term exercise training increased insulin sensitivity by more than 50% in obese middle-aged patients (mean age 45 years) with type 2 diabetes [15]. In these patients the main biochemical change was an increase in glucose transporter 4 (GLUT4) protein content in skeletal muscle. These improvements, while impressive, are likely to be proportional to the duration and intensity of the exercise regime [16]. The metabolic benefits of exercise are known to be short-lived [17–20]. This has important implications for the design and optimisation of exercise interventions.

The aim of the current study was to examine the effects of a 3-month aerobic exercise training programme in young obese insulin-resistant subjects with and without type 2 diabetes. We hypothesised that exercise would lead to an improvement in insulin sensitivity in these severely insulin-resistant subjects. We used the glucose clamp and tracer

techniques to measure the effects of exercise on whole-body and hepatic insulin sensitivity and insulin secretion.

Subjects and methods

Study population

Patients aged between 15 and 30 years, who were attending either the endocrinology or diabetes services at St James' Hospital, Dublin and had obesity or type 2 diabetes, were invited to take part in the study. Subjects with co-existing illnesses or secondary forms of diabetes were excluded. The study protocol was approved by the local Research Ethics Committee and written informed consent was obtained.

Screening

Subjects attended the Metabolic Research Unit on one morning at 08.00 h for documentation of their full history and a physical examination. Routine blood samples were taken for blood count, renal, liver, bone profile, thyroid function tests and fasting lipids. Each subject with diabetes was confirmed negative for GAD antibodies.

Anthropometric measurements

Waist-to-hip ratio, weight, height and BMI were measured. Blood pressure was measured using the left arm after the subject had been sitting comfortably for 5 min, using an oscillometric device (Omron 705 CP; Omron, Matsusaka, Japan). Three readings were taken and the lowest one recorded. Body composition was assessed using an electrical impedance device (Tanita TBF-300 Body Composition Analyser; Tanita, Tokyo, Japan). Urinalysis and 12-lead ECG were performed.

Testing of maximum volume of oxygen utilisation

Maximum volume of oxygen utilisation ($\dot{V}O_{2\max}$) was measured using a bicycle ergometer (Excalibur, Groningen, the Netherlands) in a stepwise fashion. Heart rate and oxygen consumption were recorded. Blood pressure was monitored.

OGTT

An OGTT (75 g glucose load) was performed. Insulin and C-peptide levels were measured in addition to blood glucose, at 30-min intervals over 2 h.

Hyperinsulinaemic–euglycaemic clamp study

On a separate morning (within 7 days of the OGTT), subjects attended the unit for a 4-h hyperinsulinaemic–

euglycaemic clamp study. Subjects fasted for 12 h prior to the clamp. Diabetic subjects taking insulin omitted the basal dose on the night before the clamp as well as the dose on the morning of the clamp study. Subjects taking metformin stopped this 1 week before the study, while subjects taking sulfonylureas stopped these 3 days before the clamp. During a 2-h basal phase, $[6,6\text{-}^2\text{H}_2]$ glucose tracer (Cambridge Isotopes, Cambridge, MA, USA) was infused through an intravenous cannula in the right antecubital fossa. Subsequently a 2-h insulin infusion (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was commenced through an intravenous cannula in the left antecubital fossa at a rate of $40 \text{ mU m}^{-2} \text{ min}^{-1}$. Simultaneously a $[6,6\text{-}^2\text{H}_2]$ glucose solution was infused in order to maintain euglycaemia. To monitor blood glucose levels during the clamp study, arterialised venous blood was taken at 5-min intervals from a retrograde cannula on the dorsum of the right hand, which was warmed. The mean glucose infusion rate between 80 and 120 min after commencement of the insulin infusion was calculated. The glucose disposal rate was calculated after correction for residual hepatic glucose production using the $[6,6\text{-}^2\text{H}_2]$ glucose tracer [21–23].

Modified intravenous glucose tolerance test

At the end of the clamp study (at 240 min), a rapid bolus of intravenous glucose was administered (0.3 g/kg) over 20 s and serial blood samples taken every 2 min for 8 min to measure insulin, C-peptide and glucose. The IVGTT has been used in similar cohorts to assess beta cell function in the past [7].

Analysis methods for OGTT and IVGTT data

Beta cell function was assessed using a model that describes the relationship between insulin secretion and glucose concentration and that has been illustrated in detail previously [24, 25]. 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 potentiating factors (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 1 during the experiment. It thus expresses a relative potentiation of the secretory response to glucose. Previous studies [24–26] 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 h 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 [24–26].

The model parameters were estimated from glucose and C-peptide concentrations by regularised least-squares, as previously described [24, 25]. 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 ($\sim 1\%$ for glucose and $\sim 4\%$ 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 $\text{pmol min}^{-1} \text{ m}^{-2}$ of body surface area.

The acute insulin response was calculated as the mean incremental insulin concentration during the 8-min IVGTT, where the mean was determined by trapezoidal integration. Analogous indices were obtained using C-peptide concentration and insulin secretion, which was calculated by deconvolution from C-peptide concentration [27].

Laboratory analysis

Serum insulin and C-peptide were measured using commercially available fluoroimmunoassays (Auto-Delfia, Wallac-Oy, Finland). Plasma total cholesterol and triacylglycerols were measured using enzymatic methods (Human liquicolor kits; Hitachi Modular; Roche Diagnostics, Basel Switzerland). 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) and HbA_{1c} was measured using an analyser (Hi-Auto A1c HA 8140; Menarini, Florence Italy). Antibodies to GAD were measured using a direct radioligand assay. Serum NEFA were determined using a spectrophotometric assay (Randox Laboratories, Antrim, UK).

Exercise protocol

The exercise programme was conducted entirely at the Metabolic Research Unit gym and involved 1 h of exercise training, four times per week for 12 weeks. Each exercise session was supervised by an exercise physiologist or one of the study physicians. Each subject completed a 5-min

warm-up, followed by 60 min of aerobic exercise at 70% $\dot{V}O_{2max}$ (with continuous heart rate monitoring) and a 5-min cool down either on a cycle ergometer or treadmill. Compliance with the exercise was excellent with 95% attendance throughout the study.

Diet

A dietitian met with each subject weekly to ensure a stable energy intake during the study.

Statistical analysis

Data are presented as means (SEM). Comparisons between control and young 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. Statistical significance was set at $p<0.05$. JMP statistical software (version 5.1; SAS Institute, Cary, NC, USA) was used in statistical analysis.

Results

Baseline studies

Clinical and laboratory measurements We recruited 13 subjects with type 2 diabetes and 18 non-diabetic control

subjects for the baseline study. Clinical characteristics of the subjects are shown in Table 1. The two groups were matched for age, BMI, body fat and physical fitness. In seven of the 13 subjects with type 2 diabetes and 11 of the 18 obese subjects there was a documented family history of type 2 diabetes. All of the subjects had completed puberty. Of the 13 type 2 diabetic subjects, six were receiving metformin alone, two were receiving metformin with sulfonylureas, three were receiving insulin (alone or in combination with oral agents) and two were on no hypoglycaemic medications. No differences were subsequently noted in the responses between these treatment sub-groups. There was a higher proportion of men in the type 2 diabetic subjects 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 the sexes were analysed separately (not shown). The type 2 diabetic subjects had higher waist circumference, WHR, fasting triacylglycerol and slightly higher systolic blood pressure than the obese control subjects. In the subgroup (14 obese control, seven type 2 diabetic subjects) who completed the exercise intervention, the baseline trends were similar.

Hyperinsulinaemic–euglycaemic clamp studies Basal hepatic glucose output was increased in the type 2 diabetic subjects (2.6 ± 0.25 mg kg⁻¹ min⁻¹) compared with the obese control subjects (1.72 ± 0.14 mg kg⁻¹ min⁻¹; $p=0.0015$). Glucose production was suppressed to a similar

Table 1 Clinical data, lipid profiles and metabolic data derived from the OGTT prior to exercise intervention: comparison between obese control and obese type 2 diabetic subjects

	Control (n=18)	Type 2 diabetic subjects (n=13)
Male:female ratio	4:14	8:5
Mean age (years)	23.7 (0.9)	25.8 (1.2)
Weight (kg)	99.6 (4.4)	109 (5)
BMI (kg/m ²)	35.2 (1.4)	34.9 (1.4)
Blood pressure (mmHg)		
Systolic	114.9 (3.3)	124.9 (3.4) *
Diastolic	72.9 (2.1)	77.5 (2.5)
Waist circumference (cm)	102.1 (2.72)	117.2 (3.8) **
Hip circumference (cm)	116.4 (3.3)	115.3 (2.8)
WHR	0.88 (0.02)	1.02 (0.03) **
Body fat %	41.2 (2.15)	38.3 (3.02)
$\dot{V}O_{2max}$ (ml min ⁻¹ kg ⁻¹)	2.66 (0.19)	2.55 (0.2)
Fasting plasma glucose (mmol/l)	4.94 (0.34)	10.31 (0.42) ***
Total cholesterol (mmol/l)	4.42 (0.2)	4.69 (0.24)
HDL-cholesterol (mmol/l)	1.09 (0.07)	0.94 (0.04)
LDL-cholesterol (mmol/l)	2.44 (0.17)	2.67 (0.2)
Triacylglycerol (mmol/l)	1.49 (0.19)	2.9 (0.37) ***
Fasting plasma NEFA (mmol/l)	0.642 (0.041)	0.748 (0.236)
HbA _{1c} (%)	5.39 (0.23)	8.78 (0.28) ***
Beta cell glucose sensitivity ^a	187.9 (44.8)	16.6 (3.3) ***
Rate sensitivity ^b	758.9 (165)	175.3 (60.7) *
Potential factor ratio	1.892 (0.44)	1.117 (0.111) *
Basal insulin secretion ^c	107.1 (10.5)	141.7 (13.4) *
Integral of total insulin secretion OGTT ^d	52.7 (6.9)	30.9 (3.6) ***

Values in brackets: SEM.
^a pmol min⁻¹ m⁻² mmol⁻¹ l⁻¹
^b pmol m⁻² mmol⁻¹ l⁻¹
^c pmol min⁻¹ m⁻²
^d nmol/m²
* $p<0.05$; ** $p<0.01$;
*** $p<0.001$

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

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 $265 \pm 10 \text{ ml min}^{-1} \text{ m}^{-2}$) than obese control subjects (OGIS $407 \pm 13 \text{ ml min}^{-1} \text{ m}^{-2}$; $p < 0.0001$). 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 had markedly reduced beta cell response to glucose. Both acute insulin response (389 ± 62 vs $29 \pm 71 \text{ pmol/l}$) and the equivalent C-peptide measurements (997 ± 126 vs $45 \pm 80 \text{ pmol/l}$) were reduced in the type 2 diabetic subjects, as well as the incremental area of insulin secreted after the glucose bolus (435 ± 62 vs $19 \pm 49 \text{ pmol min}^{-1} \text{ m}^{-2}$).

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 $110.7 \pm 5.1 \text{ cm}$, $p = 0.031$), with a similar but not significant trend in control subjects (100.8 ± 3.3 vs $96.7 \pm 2.9 \text{ cm}$, $p = 0.075$). $\dot{V}\text{O}_{2\text{max}}$ increased more than 20% in the obese control subjects (from 2.77 ± 0.24 to $3.36 \pm 0.41 \text{ ml min}^{-1} \text{ kg}^{-1}$ $p < 0.0001$), but did not improve significantly in the type 2 diabetic subjects (2.48 ± 0.31 to $2.72 \pm 0.35 \text{ ml min}^{-1} \text{ kg}^{-1}$, $p = 0.078$) (Fig. 1a). Change in $\dot{V}\text{O}_{2\text{max}}$ 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 $5.33 \pm 0.7 \text{ mg kg}^{-1} \text{ min}^{-1}$ in the control, 2.5 ± 0.3 vs $2.57 \pm 0.42 \text{ mg kg}^{-1} \text{ min}^{-1}$ in the type 2 diabetic subjects, $p = \text{NS}$) (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, $p = \text{NS}$; 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

	Control subjects		Type 2 diabetic subjects	
Fixed data				
<i>n</i>	14		7	
Male : female ratio	4:10		3:4	
Mean age (years)	25.6 (0.9)		26.1 (0.9)	
Per exercise status	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Weight (kg)	97.47 (4.65)	95.6 (4.54)	108.3 (8.7)	109.4 (8.4)
BMI (kg/m ²)	34.25 (1.44)	33.58 (1.34)	35.63 (1.23)	36.02 (1.17)
Systolic BP (mmHg)	116.1 (4)	112.8 (4)	119 (4.7)	121 (4.2)
Diastolic BP (mmHg)	71.3 (2.4)	69.6 (2.3)	77 (2.5)	81 (2.3)
Waist circumference (cm)	100.8 (3.3)	96.7 (2.9)	115.2 (5.5)	110.7 (5.1) *
Hip circumference (cm)	113.3 (3.4)	107.1 (3) ***	116.6 (4.3)	115.3 (3.7)
WHR	0.89 (0.02)	0.9 (0.02)	0.99 (0.05)	0.96 (0.03)
Body fat %	39 (2.4)	38.4 (2.3)	39.9 (3.1)	39.7 (3.1)
$\dot{V}O_{2max}$ (ml min ⁻¹ kg ⁻¹)	2.77 (0.24)	3.36 (0.41) ***	2.48 (0.31)	2.72 (0.35)
Fasting plasma glucose (mmol/l)	4.97 (0.11)	4.92 (0.11)	9.39 (1.06)	10.64 (1.55)
Total cholesterol (mmol/l)	4.47 (0.26)	4.25 (0.26)	4.47 (0.3)	4.52 (0.24)
HDL-cholesterol (mmol/l)	1.12 (0.09)	1.11 (0.06)	0.96 (0.05)	0.95 (0.05)
LDL-cholesterol (mmol/l)	2.46 (0.22)	2.83 (0.25)	2.6 (0.26)	2.88 (0.18)
Triacylglycerols (mmol/l)	1.37 (0.2)	1.19 (0.13)	2.63 (0.39)	2.26 (0.29)
Fasting plasma NEFA (mmol/l)	0.635 (0.053)	0.561 (0.036)	0.820 (0.104)	0.826 (0.043)
HbA _{1c} (%)	5.43 (0.11)	5.32 (0.16)	8.46 (0.6)	8.41 (0.73)
Beta cell glucose sensitivity ^a	145.19 (24.68)	128.81 (17.39)	13.29 (2.92)	21.01 (7.44)
Rate sensitivity (pmol m ⁻² mmol ⁻¹ l ⁻¹)	795.55 (195.44)	1095.93 (267.62)	241.38 (108.95)	196.45 (56.87)
Potentiation factor ratio	1.44 (0.14)	1.57 (0.19)	1.29 (0.18)	1.1 (0.12)
Basal insulin secretion (pmol min ⁻¹ m ⁻²)	100.05 (12.58)	102.1 (9.53)	146.43 (15.15)	140.56 (12.42)
Integral of total insulin secretion OGTT ^b	43.41 (2.12)	44.13 (3.08)	29.32 (2.61)	34.31 (6.48)

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 $\dot{V}O_{2max}$ 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

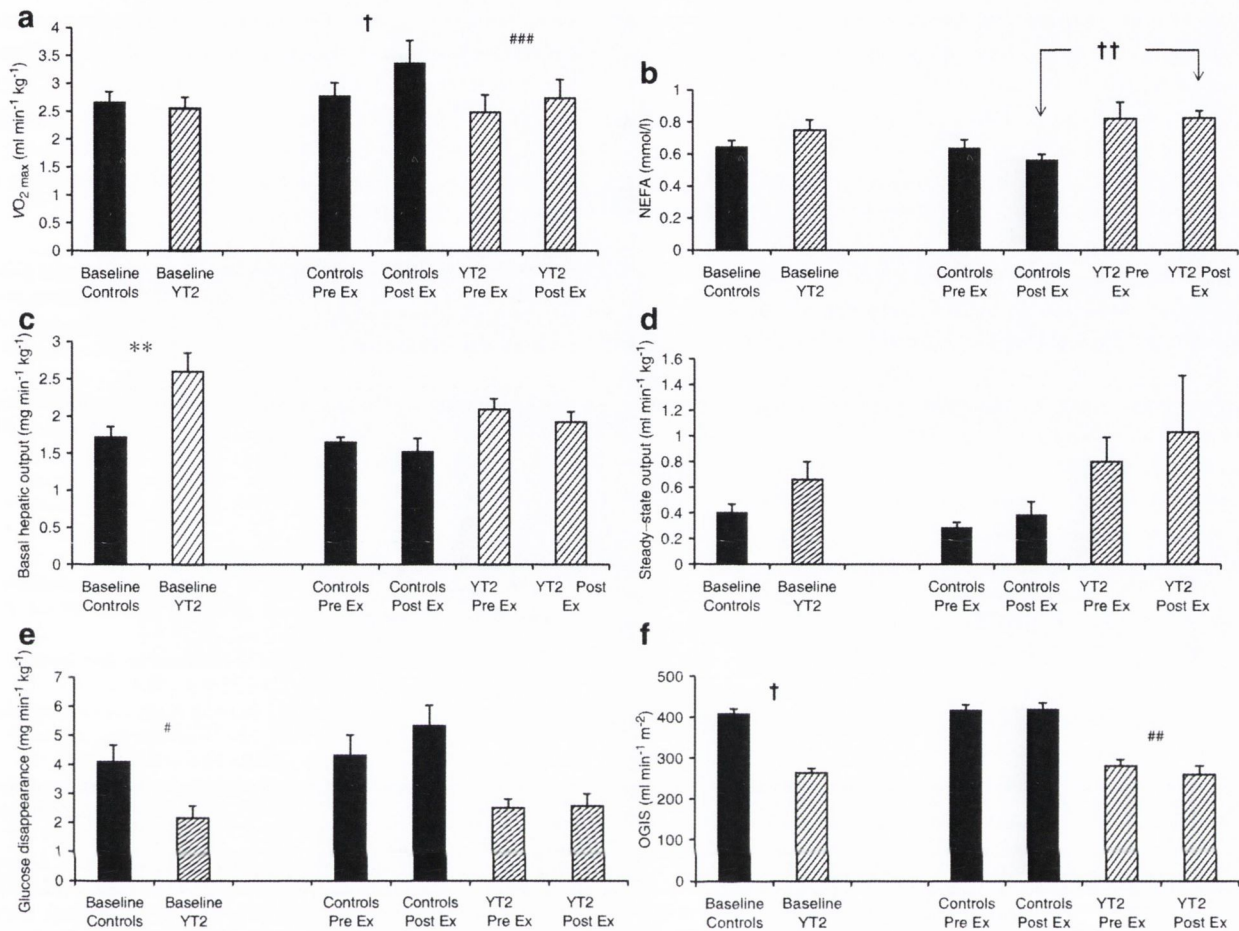


Fig. 1 Comparison of measurements between obese non-diabetic control and type 2 diabetes subjects at baseline and then pre-exercise (Pre Ex) and post-exercise (Post Ex) in those who completed the exercise programme. **a** $\dot{V}O_{2\max}$; **b** fasting NEFA; **c** basal hepatic

glucose output (clamp); **d** steady-state hepatic glucose output (clamp); **e** glucose disappearance during clamp; and **f** OGIS. Error bars: SEM. YT2, young type 2 diabetic subjects. ^{**} $p=0.001$, [#] $p=0.03$; ^{###} $p=0.047$; ^{††} $p=0.0001$; [†] $p=0.0003$

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 $\dot{V}O_{2\max}$, 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 $\dot{V}O_{2\max}$ 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.

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

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Appendix V

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

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

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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, $\text{VO}_{2\text{max}}$) or glucose disposal after exercise training.

Seven subjects with early-onset T2DM (aged 26.1 ± 0.9 years, body mass index [BMI] $35.6 \pm 1.2 \text{ kg/m}^2$) and 14 age-matched obese subjects with normal glucose tolerance (aged 25.6 ± 0.9 years, BMI $34.3 \pm 1.4 \text{ kg/m}^2$) 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.

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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.^{1,4} The worldwide prevalence of diabetes in adults is expected to increase from 5% to 6.2% from 2003 to 2025.⁵ 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.⁶ Obesity has increased by 70% in adults aged 18–29 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.^{7,8}

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.⁹ 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.^{9–11} 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.^{12–14} There is an abundance of evidence that exercise delays or prevents the development of type 2 diabetes in at-risk populations.^{15–18} Smaller studies have shown that exercise improves insulin sensitivity and glucose metabolism.^{19,20} 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.²¹

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.²²

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Table 1. Clinical and anthropometric characteristics in young obese and diabetic subjects pre- and post-exercise						
	Controls pre	Controls post	p value	YT2 pre	YT2 post	p value
N	14			7		
M:F ratio	4:10			3:4		
Mean age (years)	25.6±0.9			26.1±0.9		
Height (m)	1.69±0.03			1.73±0.05		
Weight (kg)	97.47±4.65	95.6±4.54	0.07	108.3±8.7	109.4±8.4	NS
BMI (kg/m ²)	34.25±1.44	33.58±1.34	NS	35.63±1.23	36.02±1.17	NS
Systolic BP (mmHg)	116.1±4	112.8±4	NS	119±4.69	121±4.15	NS
Diastolic BP (mmHg)	71.3±2.4	69.6±2.3	NS	77±2.5	81±2.3	NS
Waist:hip ratio	0.89±0.02	0.9±0.02	NS	0.99±0.05	0.96±0.03	NS
HbA _{1C} (%)	5.43±0.11	5.32±0.16	NS	8.46±0.6	8.41±0.73	NS
VO ₂ max (ml min ⁻¹ kg ⁻¹)	2.77±0.24	3.36±0.41	<0.0001	2.48±0.31	2.72±0.35	0.078

Values are presented as mean ± standard error of the mean in parentheses

Key: YT2 = young type 2 diabetes; BMI = body mass index; BP = blood pressure; NS = not significant; HbA_{1C} = glycosylated haemoglobin; M = male; F = female; VO₂max = maximum volume of oxygen consumption

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).²³ An increase of soluble P-Selectin (sP-Selectin) has also been reported in arteriosclerosis.²⁴ Soluble E-Selectin (sE-Selectin) is increased in patients with early atherosclerosis or those with manifest atherosclerotic disease.²⁵ Evidence is accumulating that high-sensitivity C-reactive protein (hsCRP) is predictive of future coronary events.²⁶

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.²² 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 test-

ing 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. sICAM-1, sVCAM-1, sE-Selectin 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,

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

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

Values are presented as mean $\pm$ 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

UK). Normal reference range values: for sICAM 115–306 ng/ml, sVCAM 379–991 ng/ml, sE-Selectin 29.1–63.4 ng/ml, sP-Selectin, 51–113 ng/ml.

Statistical analysis

Data were expressed as mean $\pm$ standard error of mean or the median (interquartile ranges 25–75%). Differences between groups were analysed by a Student's paired *t*-test or unpaired *t*-test. Because of the skewed distribution of soluble adhesion molecules, differences in concentration were evaluated by non-parametric statistical procedures (Mann-Whitney *U*). Wilcoxon Signed Rank Test was performed to examine differences before and after exercise. A *p*-value < 0.05 was considered to be statistically significant. SPSS for Windows 12.0 was used for statistical analysis.

Results

Patient characteristics are outlined in table 1. Both groups were matched for age and BMI. There were no significant differences between the groups at baseline in VO_2max , systolic or diastolic BP. The T2DM group had a significantly higher glycosylated haemoglobin ($\text{HbA}_{1\text{C}}$) level and waist to hip ratio. After completion of the exercise programme, VO_2max increased by 24% in the obese non-diabetic control group, but did not increase in the group with T2DM. Nor were there any significant improvements in anthropometrics or insulin sensitivity, measured with the hyperinsulinaemic glucose clamp technique, as we recently reported.²²

Measurements of vascular inflammatory markers are outlined in table 2. There were no significant differences between the groups for any of the markers, either at baseline or after exercise intervention.

In both groups, the concentrations of hsCRP, sE-Selectin and sP-Selectin were markedly elevated at baseline, and did not change after three months of supervised aerobic exercise intervention. In both groups, the concentrations of sVCAM and sICAM were in the high normal range at baseline and did not change after exercise.

Discussion

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.^{1–5} 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.^{9,10} 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 VO_2max increased by 20% in the obese control group, there was no significant improvement in the group with diabetes. Nor was there any improvement in whole body glucose uptake in either group, as recently reported.²² 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.²² Lipotoxicity may ultimately be exerted through impairment of mitochondrial function,²⁷ another cellular mechanism potentially linking

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.²⁸ 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

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Conflicts of interest statement

None declared.

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Appendix VI

**Plasma visfatin is reduced after aerobic exercise
in early-onset type 2 diabetes mellitus.**

**Brema I, Hatunic M, Finucane F, Burns N, Nolan JJ, Haider D,
Wolzt M, Ludvik B**

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RESEARCH LETTER

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Plasma visfatin is reduced after aerobic exercise in early onset type 2 diabetes mellitus

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 body mass index (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 $\text{VO}_{2\text{ max}}$ were improved after 3 months of aerobic training [7]. We measured plasma visfatin in this cohort of young patients at baseline and after 3 months of aerobic exercise.

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Methods

Patients aged between 15 and 30 years with type 2 diabetes or obesity were recruited as previously described [7]. Full history and physical examination was performed and patients had standard 75 g oral glucose tolerance test, and fasting laboratory profiles.

$\text{VO}_{2\text{ max}}$ Testing

All subjects exercised for four 1 h weekly sessions at 75% $\text{VO}_{2\text{ max}}$ 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 enzyme-linked immunosorbent assay kit with an intra- and interassay coefficient of variation of <6% (Phoenix Peptides, Karlsruhe, Germany).

Insulin Sensitivity

Insulin sensitivity was calculated using both the hyper-insulinaemic 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 postexercise comparisons. Spearman’s correlations were used to examine correlation between plasma visfatin and other biomarkers. All statistical analyses were two sided and *p* < 0.05 was considered significant.

Results

Characteristics of the study subjects are shown in Table 1. Apart from a small reduction in waist circumference (WC) (*p* < 0.027) in young type 2 diabetes subjects, 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 ± 10.7 ng/ml, respectively, *p* = 0.169). Plasma visfatin was substantially reduced in both groups postexercise (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 negatively correlated with percent body fat (*r* = −0.933, *p* = 0.003) and high-density lipoprotein cholesterol (*r* = −0.893, *p* = 0.007) postexercise. All other correlations were negative, both pre- and postexercise 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 of aerobic

Table 1 Characteristics of study population

	YT2DM (N = 8)	YOb (N = 14)	p value*
Age in years	25.6 ± 0.8	24.6 ± 0.9	0.361
Weight (kg)	108.1 ± 7.5	97.45 ± 4.7	0.413
Waist circumference (cm)	115.2 ± 5.5	100.8 ± 3.3	<0.022
Waist:hip ratio	1.0 ± 0.0	0.9 ± 0.0	<0.04
Body mass index (kg/m ²)	34.8 ± 1.3	34.2 ± 1.4	0.609
Systolic BP (mmHg)	120.6 ± 4.3	116.1 ± 4.0	0.916
Diastolic BP (mmHg)	74.9 ± 3.2	71.3 ± 2.4	0.339
Fasting blood glucose (mmol/l)	10.0 ± 0.9	5.0 ± 0.1	<0.0001
Fasting insulin (pmol/l)	84.6 ± 30.6	63.2 ± 9.0	<0.048
HbA1C (%)	8.4 ± 0.1	5.4 ± 0.1	<0.003
Triglyceride (mmol/l)	2.7 ± 0.3	1.4 ± 1.0	<0.003
High-density lipoprotein cholesterol (mmol/l)	1.0 ± 0.0	1.1 ± 0.1	0.207
Low-density lipoprotein cholesterol (mmol/l)	2.7 ± 0.2	2.5 ± 0.2	0.48
Percent body fat (%)	38.2 ± 3.2	39.0 ± 2.4	0.89
Oral glucose insulin sensitivity index (ml/min/m ²)	281.4 ± 15.9	417.1 ± 21.1	<0.0001
VO ₂ max (ml/min/kg)	2.5 ± 0.2	2.8 ± 0.2	0.78
Visfatin pre-exercise (ng/ml)	55.8 ± 17.2	64.7 ± 10.7	0.169
Visfatin postexercise (ng/ml)	11.6 ± 4.4	29.5 ± 7.1	0.019
p value†	0.02	0.002	

Data are presented as mean ± SEM.
BP, blood pressure; YT2DM, young type 2 diabetes subject; YOb, young obese (control subject).
**p* values obtained by Mann–Whitney *U*-test.
†Wilcoxon signed rank test.

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 because apart from a small reduction in WC, no significant physiological changes were found postexercise in either group. Moreover, whole body insulin sensitivity, hepatic insulin sensitivity, insulin secretion and OGIS were unchanged postexercise 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 WC 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.

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Appendix VII

Subjects with early-onset type 2 diabetes show defective activation of the skeletal muscle PGC-1 α /Mitofusin-2 regulatory pathway in response to physical activity.

Hernandez-Alvarez MI, Thabit H, Burns N, Shah S, Brema I, Hatunic M, Finucane F, Liesa M, Chiellini C, Noan D, Zorzano A, Nolan JJ

Subjects With Early-Onset Type 2 Diabetes Show Defective Activation of the Skeletal Muscle PGC-1 α /Mitofusin-2 Regulatory Pathway in Response to Physical Activity

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OBJECTIVE — Type 2 diabetes is associated with insulin resistance and skeletal muscle mitochondrial dysfunction. We have found that subjects with early-onset type 2 diabetes show incapacity to increase Vo_{2max} in response to chronic exercise. This suggests a defect in muscle mitochondrial response to exercise. Here, we have explored the nature of the mechanisms involved.

RESEARCH DESIGN AND METHODS — Muscle biopsies were collected from young type 2 diabetic subjects and obese control subjects before and after acute or chronic exercise protocols, and the expression of genes and/or proteins relevant to mitochondrial function was measured. In particular, the regulatory pathway peroxisome proliferator-activated receptor γ coactivator (PGC)-1 α /mitofusin-2 (Mfn2) was analyzed.

RESULTS — At baseline, subjects with diabetes showed reduced expression (by 26%) of the mitochondrial fusion protein Mfn2 and a 39% reduction of the α -subunit of ATP synthase. Porin expression was unchanged, consistent with normal mitochondrial mass. Chronic exercise led to a 2.8-fold increase in Mfn2, as well as increases in porin, and the α -subunit of ATP synthase in muscle from control subjects. However, Mfn2 was unchanged after chronic exercise in individuals with diabetes, whereas porin and α -subunit of ATP synthase were increased. Acute exercise caused a fourfold increase in PGC-1 α expression in muscle from control subjects but not in subjects with diabetes.

CONCLUSIONS — Our results demonstrate alterations in the regulatory pathway that controls PGC-1 α expression and induction of Mfn2 in muscle from patients with early-onset type 2 diabetes. Patients with early-onset type 2 diabetes display abnormalities in the exercise-dependent pathway that regulates the expression of PGC-1 α and Mfn2.

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Early-onset type 2 diabetes is increasing in prevalence, in parallel with the worldwide obesity epidemic (1), and is typically characterized by early-onset obesity and severe insulin resistance in young people with a strong family history of type 2 diabetes (1,2). Weight reduction and increased physical exercise are effective treatments to improve insulin sensitivity. We have been studying the effects of a variety of exercise and dietary regimens in these very insulin-resistant patients. We recently demonstrated that a 3-month, four times weekly, aerobic exercise intervention in subjects with early-onset type 2 diabetes failed to improve Vo_{2max} and had no significant effect on whole-body or hepatic insulin sensitivity (3). Equally obese nondiabetic control subjects had a 20% increase in Vo_{2max} following the same regime. This suggested the possibility that, in these diabetic patients, chronic exercise training failed to activate a mitochondrial oxidative response.

Defective mitochondrial function in skeletal muscle has been reported in a variety of insulin-resistant states, including type 2 diabetes (4,5). Muscle mitochondria from type 2 diabetic subjects show reduced size and reduced activity of the electron transport chain (4,6). In parallel, type 2 diabetes is associated with reduced expression of genes of oxidative metabolism as well as repression of the mitofusin-2 (Mfn2) gene, which encodes the mitochondrial fusion protein mitofusin-2 (7–9). Decreased expression of nuclear genes encoding proteins of oxidative phosphorylation has been reported in skeletal muscle of nondiabetic individuals with a family history of type 2 diabetes (8,9), along with reduced in vivo oxidative phosphorylation (5). These findings suggest that mitochondrial abnormalities in type 2 diabetes may have a heritable component.

Chronic exercise induces mitochondrial biogenesis in skeletal muscle and enhances mitochondrial function (10). Exercise is known to induce PGC-1 α (11), which in turn induces the transcription of different nuclear genes encoding mitochondrial proteins (12,13). One example is Mfn2, which is induced by PGC-1 α through interaction with the transcription factor ERR α (14). This may be particularly relevant, since it has been reported that Mfn2 regulates not just mi-

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tochondrial fusion but also mitochondrial function through changes in mitochondrial membrane potential and the expression of OXPHOS subunits (15). The stimulatory effect of exercise on mitochondrial biogenesis and function has also been reported in muscle in insulin-resistant conditions such as obesity (6) and aging (16) and in type 2 diabetes (17).

Subjects who took part in the chronic exercise protocol (3) underwent skeletal muscle biopsies at baseline and after 3 months of exercise training. To address mechanistic questions, we measured the expression of mitochondrial proteins from this initial study. We then conducted an acute (short-term) exercise intervention protocol in a similar cohort of patients with early-onset type 2 diabetes and examined the expression in muscle biopsies of a range of specific mitochondrial genes and proteins. We hypothesized that the lack of a whole-body response to exercise training in the early-onset type 2 diabetic subjects may be a consequence of alterations in the abundance or activity of relevant mitochondrial proteins in skeletal muscle.

RESEARCH DESIGN AND METHODS

Subjects— Subjects with early-onset type 2 diabetes (i.e., diagnosed before age 25 years and negative for GAD antibodies) were recruited from our clinic, along with obese and otherwise healthy subjects who were as far as possible matched for age and BMI, but with normal glucose tolerance and without family history of diabetes. All subjects were sedentary. All gave written informed consent for the study, which had been approved by the local research ethics committee.

Clinical and metabolic characteristics of both groups of subjects, in each study, are summarized in Tables 1 and 2.

Concurrent medications

None of the obese control subjects was receiving medications during the course of these studies.

Chronic exercise study. All subjects were receiving metformin. One subject was also receiving gliclazide. Doses of oral hypoglycemic agents were unchanged during the course of the study.

Acute exercise study. Three subjects were on dietary management alone. Seven of the 12 subjects were receiving oral hypoglycemic agents alone. Two subjects were on oral and insulin combination therapy (metformin plus basal bolus insulin treatment). All subjects on oral

Table 1—Baseline characteristics of subjects in the chronic exercise study

	Control subjects	Youth with type 2 diabetes
n	6	7
Male:female ratio	0:6	5:2
Age (years)	22 $\pm$ 1	27 $\pm$ 1*
Duration of diabetes (years)	NA	3.2 $\pm$ 1.5
Treatment for diabetes	NA	Met 7; Met/SU 1
Weight (kg)	108.7 $\pm$ 10.6	108.1 $\pm$ 6.7
BMI (kg/m ²)	37.78 $\pm$ 3.43	33.23 $\pm$ 1.81
Systolic blood pressure (mmHg)	104 $\pm$ 3	122 $\pm$ 5*
Diastolic blood pressure (mmHg)	71 $\pm$ 3	72 $\pm$ 3
Waist circumference (cm)	113.2 $\pm$ 4.6	117.4 $\pm$ 3.2
Waist-to-hip ratio	0.91 $\pm$ 0.03	1.00 $\pm$ 0.09
A1C (%)	5.5 $\pm$ 0.2	8.2 $\pm$ 0.6†
Fasting glucose (mmol/l)	5.2 $\pm$ 0.2	9.1 $\pm$ 0.8†
Fasting insulin (pmol/l)	91.1 $\pm$ 9.4	77.1 $\pm$ 12.3
Fasting C-peptide (μ g/l)	3.4 $\pm$ 0.3	3.1 $\pm$ 0.4
Total cholesterol (mmol/l)	4.33 $\pm$ 0.21	4.50 $\pm$ 0.42
HDL (mmol/l)	1.11 $\pm$ 0.08	0.88 $\pm$ 0.03*
LDL (mmol/l)	2.57 $\pm$ 0.23	2.34 $\pm$ 0.35
Triglycerides (mmol/l)	1.41 $\pm$ 0.27	3.05 $\pm$ 0.64*
Free fatty acids (mmol/l)	0.635 $\pm$ 0.053	0.820 $\pm$ 0.104
Glucose disposal (μ mol/min/kg/mU/l)	26.25 $\pm$ 4.66	16.13 $\pm$ 4.16
Vo _{2max} (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	28.61 $\pm$ 1.94	22.67 $\pm$ 1.57*

Data are means $\pm$ SE, unless specified otherwise. *Significantly different from control group ($P < 0.05$). †Significantly different from control group ($P < 0.01$). Met, metformin; SU, sulfonylurea.

hypoglycemic agents were on stable doses throughout the duration of the study. Subjects on the combination therapy had their insulin doses reduced by $\sim$ 20% during the course of the study.

Baseline studies

Baseline studies were identical for both the chronic and acute exercise studies (3) and included the following.

Screening. Each subject was screened with a medical history and physical examination and routine blood and urine biochemistries. Waist-to-hip ratio, weight, height, and BMI were measured. Blood pressure was measured using the left arm after the subject had been sitting comfortably for 5 min, 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 Analyzer). An exercise stress test with electrocardiogram and oxygen uptake was performed. Subjects with any abnormal stress response were excluded, as were those with clinically significant abnormalities on routine lab testing.

Aerobic capacity (Vo_{2peak}). Maximal oxygen consumption was measured by treadmill, as previously described (3).

Muscle biopsy. Muscle biopsies were taken either after an overnight fast or immediately after the most recent session of exercise, as described below.

Biopsies were obtained under local anesthesia from the vastus lateralis muscle. The muscle samples were immediately frozen in liquid nitrogen and stored for protein extraction for samples from pre- and 12 weeks post-exercise program (chronic exercise study) and subsequently for both RNA and protein extraction (acute exercise study: for samples pre- and 1-h and 7 days post-exercise).

Hyperinsulinemic-euglycemic clamp. A clamp study (with insulin infusion 40 mU $\cdot$ m⁻² $\cdot$ min⁻¹) was performed at baseline and after exercise training in the chronic exercise study (3). Subjects taking insulin omitted the basal dose on the night before the clamp as well as the dose on the morning of the clamp study. The glucose disposal rate (between 80 and 120 min after commencement of the insulin infusion) was calculated after correction for residual hepatic glucose production using the [6,6-²H₂] glucose tracer.

Table 2—Baseline characteristics of subjects in the acute exercise study

	Control subjects	Youth with type 2 diabetes
n	7	12
Male:female ratio	2:5	10:2
Age (years)	26 ± 2	27 ± 1
Duration of diabetes (years)	NA	2.5 ± 0.7
Treatment for diabetes	NA	Met 9; Met/Ins 2; Diet 3
Weight (kg)	113.9 ± 11.2	114.5 ± 7.3
BMI (kg/m ²)	39.13 ± 2.54	36.13 ± 1.78
Systolic blood pressure (mmHg)	117 ± 3	127 ± 4
Diastolic blood pressure (mmHg)	75 ± 4	81 ± 3
Waist circumference (cm)	120.2 ± 6.1	111.4 ± 4.8
Waist-to-hip ratio	0.98 ± 0.03	0.99 ± 0.02
A1C (%)	5.6 ± 0.1	7.5 ± 0.5†
Fasting glucose (mmol/l)	5.2 ± 0.1	8.0 ± 0.7†
Fasting insulin (pmol/l)	163.0 ± 65.3	169.6 ± 61.7
Fasting C-peptide (μg/l)	5.3 ± 1.2	3.4 ± 0.4*
Total cholesterol (mmol/l)	4.13 ± 0.32	4.29 ± 0.26
HDL (mmol/l)	1.30 ± 0.17	0.95 ± 0.05*
LDL (mmol/l)	2.21 ± 0.29	2.63 ± 0.28
Triglycerides (mmol/l)	1.35 ± 0.20	1.76 ± 0.26
Free fatty acids (mmol/l)	0.67 ± 0.16	0.77 ± 0.22
VO _{2max} (ml · kg ⁻¹ · min ⁻¹)	22.85 ± 2.71	23.79 ± 1.79

Data are means ± SE, unless specified otherwise. *Significantly different from control group ($P < 0.05$).

†Significantly different from control group ($P < 0.01$). Ins, insulin; Met, metformin.

Exercise training

For more information on exercise training, see supplementary Figs. 1 and 2 in the online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc09-1305/DC1>. The subjects maintained a stable diet and treatment for diabetes during both exercise programs. Subjects exercised either on a treadmill or a stationary bicycle ergometer. Each session lasted for a total of 70 min (5-min warm-up, 60-min exercise, 5-min cool down) and was fully supervised by an exercise physiologist or physician. Each exercise session was conducted at the same intensity, i.e., at 70% of the subject's VO_{2max}. This intensity of exercise has previously been shown by us and others to improve insulin sensitivity in obese middle-aged subjects with type 2 diabetes. Blood glucose was checked before exercise, and heart rate and blood pressure were monitored throughout exercise. Compliance with the exercise regimens was greater than 90%.

Chronic exercise. After completion of all baseline measurements and a baseline muscle biopsy, subjects exercised for 4 1-h sessions each week. Each exercise session was conducted at 70% of the subject's VO_{2max}. The study continued for a total of 12 weeks. Baseline measurements were repeated and a final muscle biopsy was taken immediately

after the last exercise session at the end of the study (3) (supplementary Fig. 1).

Acute exercise. After completion of all baseline measurements and a baseline muscle biopsy, subjects exercised for a single 1-h session at 70% of VO_{2max}. A second muscle biopsy was taken immediately after the first session of exercise. The subjects then exercised for 1 h daily for 7 days, followed by a final muscle biopsy immediately after the final exercise session at the end of this study (supplementary Fig. 2).

RNA extraction and real-time quantitative PCR (acute exercise study). For more information on the RNA extraction and real-time quantitative PCR, see the online appendix.

Homogenate extracts from muscle biopsies and Western blot assays (acute and chronic exercise studies). Protein homogenate was extracted from ~25 mg skeletal muscle. The muscle was immersed in ice-cold medium buffer A (0.1 mM KCL, 5 mM MgCl₂, 5 mM EGTA, 5 mM sodium pyrophosphate, pH adjusted to 7.4, 2 μM leupeptin, 2 μM pepstatin, 0.5 mM phenylmethylsulfonylfluoride for 2 × 10 min). After this, the muscle was finely minced in 1/10 (wt/vol) buffer B (0.25 mM saccharose, 50 mM KCL, 5 mM EDTA, 1 mM sodium pyrophosphate, 5

mm MgCl₂, pH adjusted to 6.8, 2 μM leupeptin, 2 μM pepstatin, 0.5 mM phenylmethylsulfonylfluoride) and then disrupted with a motor-driven Teflon/glass homogenizer. The entire procedure was performed at 0–4°C. The protein concentration was determined using a Micro BCA protein assay (Pierce, Rockford, IL). Western blot assays were performed as reported (15) (see online appendix).

Statistical analysis

Unpaired *t* tests were performed to compare muscle protein expression between control subjects and subjects with diabetes at baseline (Fig. 1A). Paired *t* tests were performed to compare the effects of chronic exercise (Fig. 1B) and acute exercise (Fig. 1C) on the expression of a range of proteins and RNAs. In all cases, significance level for the *t* tests was set at $P < 0.05$.

RESULTS

Clinical and metabolic measurements

Chronic exercise study. Baseline characteristics (Table 1) of the chronic exercise participants have been described previously (3). The nondiabetic control subjects were matched for BMI with the diabetic group, but the diabetic subjects were slightly older. The 3-month exercise training program led to no significant change in whole-body or hepatic insulin sensitivity in either the control subjects or the subjects with diabetes (3). VO_{2max} increased by 20% in the control group (from 28.61 ± 1.94 to 35.15 ± 2.95 ml · kg⁻¹ · min⁻¹, $P < 0.01$), but was unchanged in the group with diabetes (22.67 ± 1.57 vs. 24.40 ± 1.50 ml · kg⁻¹ · min⁻¹, $P = \text{NS}$) after exercise. Fasting plasma free fatty acid concentrations tended to be lower at baseline, although not reaching significance, in the control group (0.635 vs. 0.820 mmol/l, $P = \text{NS}$) and were significantly lower (0.561 vs. 0.826 mmol/l, $P = 0.003$) in the control subjects after the 3-month exercise intervention (3). No correlation was found between either age or sex and VO_{2max} (data not shown).

Acute exercise study. The acute exercise participants (Table 2) had a similar phenotype to individuals we have reported from previous studies (2,3). The control subjects were matched for age and BMI and had similar VO_{2max} at baseline to individuals with diabetes (22.85 ± 2.71 vs. 23.79 ± 1.79 ml · kg⁻¹ · min⁻¹, respectively; $P = \text{NS}$).

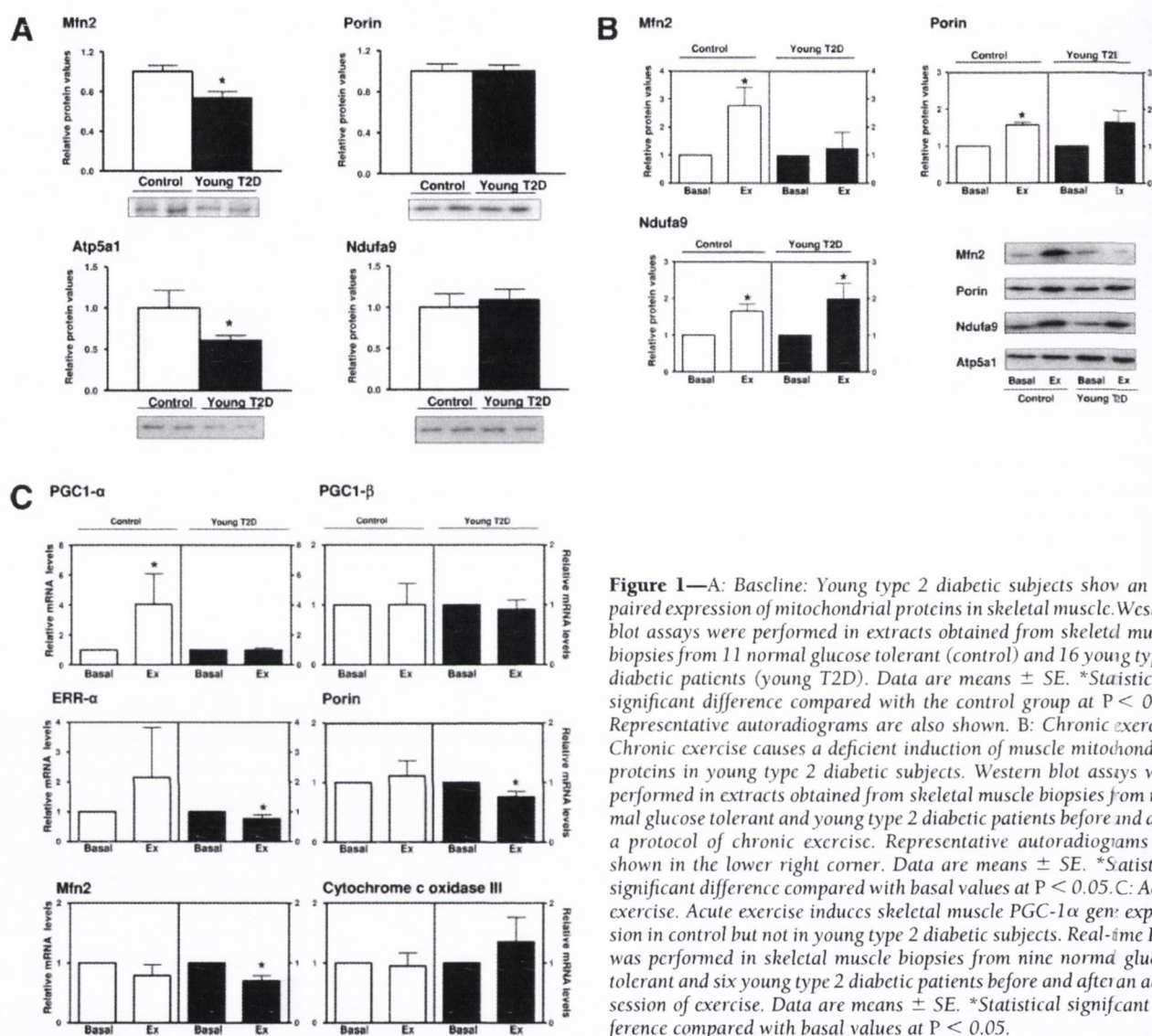


Figure 1—A: Baseline: Young type 2 diabetic subjects show an impaired expression of mitochondrial proteins in skeletal muscle. Western blot assays were performed in extracts obtained from skeletal muscle biopsies from 11 normal glucose tolerant (control) and 16 young type 2 diabetic patients (young T2D). Data are means $\pm$ SE. *Statistically significant difference compared with the control group at $P < 0.05$. Representative autoradiograms are also shown. B: Chronic exercise. Chronic exercise causes a deficient induction of muscle mitochondrial proteins in young type 2 diabetic subjects. Western blot assays were performed in extracts obtained from skeletal muscle biopsies from normal glucose tolerant and young type 2 diabetic patients before and after a protocol of chronic exercise. Representative autoradiograms are shown in the lower right corner. Data are means $\pm$ SE. *Statistical significant difference compared with basal values at $P < 0.05$. C: Acute exercise. Acute exercise induces skeletal muscle PGC-1 α gene expression in control but not in young type 2 diabetic subjects. Real-time PCR was performed in skeletal muscle biopsies from nine normal glucose tolerant and six young type 2 diabetic patients before and after an acute session of exercise. Data are means $\pm$ SE. *Statistical significant difference compared with basal values at $P < 0.05$.

Neither $\text{VO}_{2\text{max}}$ nor insulin sensitivity were re-measured at the end of this protocol, since neither of these parameters were expected to change after only 1 week of exercise training.

Expression of skeletal muscle mitochondrial proteins at baseline (combined baseline data from the chronic and acute studies)

Muscle biopsies were collected from young type 2 diabetes or control subjects, homogenates were obtained and the expression of mitochondrial proteins was studied. The yield of total proteins was similar in homogenates from control and young type 2 diabetic subjects (54.1 ± 2.6 and 54.8 ± 3.1 mg protein/g of tissue in control and young type 2 diabetic groups, respectively).

The young type 2 diabetic group showed a reduced expression of the mitochondrial fusion protein, Mfn2 (26% reduction in those with diabetes) and the alpha subunit of ATP synthase (ATP5a1) (39% reduction in those with diabetes) (Fig. 1A). Under these conditions, the abundance of porin (a marker of mitochondrial mass) and of the p37 subunit of Complex I of the respiratory chain (Ndufa9) was unaltered indicating that the changes of Mfn2 and ATP synthase were not secondary to changes in mitochondrial mass (Fig. 1A).

Expression of skeletal muscle mitochondrial proteins after 3 months exercise: chronic exercise study

Chronic exercise was associated with the induction of Mfn2 (2.8-fold increase),

porin (1.6-fold increase), and p37 subunit of Complex I (Ndufa9) (1.7-fold increase) in skeletal muscle from control subjects (Fig. 1B). In the young type 2 diabetic group, chronic exercise caused a different pattern of muscle changes. The expression of Mfn2 was unchanged, while there was induction in both Ndufa9 (2.0-fold increase) and porin (1.6-fold increase but not reaching statistical significance) (Fig. 1B).

Mitochondrial gene expression in skeletal muscle in response to acute exercise: acute exercise study

Muscle samples taken after 7 days of exercise showed no significant changes in gene expression (data not shown). However, acute exercise after 1 h caused a substantial induction in PGC-1 α gene

expression in skeletal muscle from control subjects (fourfold induction) (Fig. 1C). These changes in PGC-1 α had reverted to baseline after 7 days of exercise (data not shown). Control subjects also showed an increased ERR α gene expression (2.2-fold increase), although this did not reach statistical significance and no changes in the gene expression of PGC-1 β , porin, Mfn2, or the mitochondrial gene COXIII (Fig. 1C). In contrast, acute exercise led to no change in PGC-1 α gene expression in skeletal muscle in the young type 2 group, and the expression of ERR α (23% reduction), Mfn2 (30% reduction), or porin (23% reduction) was significantly reduced (Fig. 1C). No changes in PGC-1 β or COXIII gene expression were detected in young type 2 diabetic subjects (Fig. 1C). We analyzed whether AMPK was stimulated under these conditions; however, we detected no phosphorylation of AMPK in either the control or type 2 diabetic subjects (data not shown). No correlation was found between $\text{VO}_{2\text{max}}$ and the expression of mitochondrial genes or proteins (data not shown).

CONCLUSIONS — We have previously reported that young subjects with type 2 diabetes are severely insulin resistant, achieving a maximal glucose disposal rate of only $2.15 \pm 0.42 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, compared with $4.09 \pm 0.58 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in very obese control subjects (3). We undertook the current studies to directly analyze a range of parameters of muscle mitochondrial function in these subjects at baseline and after different durations of exercise training. The mitochondrial parameters reveal interesting new abnormalities in the subjects with diabetes both at baseline and after exercise. At baseline, subjects with diabetes display modestly ($\sim 25\%$) reduced expression of Mfn2 and some OXPHOS subunits. Thus, in the baseline state, without any intervention, subjects with diabetes showed a defective pattern of mitochondrial protein expression in muscle compared with equally obese young people without diabetes. The patients with type 2 diabetes, in comparison with matched obese control subjects, were markedly dyslipidemic. Lipotoxicity in the patients with diabetes at baseline could either cause or result from the mitochondrial abnormalities that we have demonstrated in the current study.

After exercise, the mitochondrial changes we have observed are much more pronounced and are consistent with the

whole-body findings in these subjects. Thus, in the obese nondiabetic subjects, acute exercise intervention was associated with increased muscle expression of genes encoding for PGC-1 α and ERR α under conditions in which Mfn2 or porin was unchanged. These data are consistent with prior observations indicating that AMPK activity is stimulated by acute exercise in skeletal muscle in humans (18) and that PGC-1 α gene expression and promoter activity are stimulated by AMPK (19). Moreover, and based on the observations that Mfn2 gene transcription is induced by ERR α , and coactivated by PGC-1 α (14), we propose that enhanced PGC-1 α activity will promote an increase in Mfn2 gene transcription. In contrast with this profile, acute exercise caused a reduction in the expression of genes encoding Mfn2 and porin in muscle from the subjects with diabetes under conditions in which PGC-1 α remained unchanged. These data can be explained by the incapacity of acute exercise to stimulate AMPK activity in patients with diabetes, which cancels the induction of PGC-1 α gene expression. We also propose that the reduced expression of the Mfn2 gene (and perhaps the porin gene, which also encodes for a mitochondrial protein) may be due to reduced transcriptional activities of either PGC-1 α or PGC-1 β , key regulators of Mfn2 transcription (14,20). In the obese nondiabetic subjects, chronic exercise training led to an increase in both Mfn2 (2.8-fold) and porin (1.6-fold), consistent with the increase in $\text{VO}_{2\text{max}}$ observed after 3 months. In subjects with diabetes, however, we found no increase in Mfn2, consistent with the lack of stimulation of whole-body oxygen uptake. In spite of the lack of induction of PGC-1 α or Mfn2, chronic exercise induced porin or Ndufa9 expression in the type 2 diabetic subjects. The induction of porin or Ndufa9 in the absence of PGC-1 α suggests the existence of mechanisms of mitochondrial biogenesis, alternative to PGC-1 α gene induction, that are triggered by physical exercise.

Several studies have analyzed the effect of dietary and/or exercise interventions aimed to promote weight loss in obese or type 2 diabetic subjects. These studies have detected that weight loss induced by diet/exercise stimulate mitochondrial activity in skeletal muscle both in obese subjects as well as in type 2 diabetic patients (17,21,22). In a recent study, a delayed and reduced response in

PGC-1 α expression was detected in muscle from obese subjects in response to exercise (22). Under these conditions, the expression of genes downstream of PGC-1 α such as NRF-1 or cytochrome c oxidase subunit VIc also showed a reduced response in obese subjects after exercise (22).

Interestingly, we have also noted a markedly different response to bariatric surgery in morbidly obese diabetic subjects compared with a matched nondiabetic cohort (23). Despite similar weight loss ($\sim 60 \text{ kg}$) and a marked improvement in insulin sensitivity in both groups, the patients with diabetes showed a blunted response in terms of glucose oxidation and no significant changes in the expression of Mfn2, porin, and citrate synthase (23). In all, these data together with data from our current study suggest a link between insulin resistance and a defective regulation of PGC-1 α and downstream mitochondrial proteins in response to exercise, so that under conditions of severe insulin resistance, the induction of PGC-1 α in response to exercise is abolished. Further studies should investigate whether the defective PGC-1 α expression is a primary defect or whether it is secondary to insulin resistance or reversible lipotoxicity.

There are further possible explanations to link environmental effects such as diet and physical activity with abnormalities of mitochondrial oxidative proteins in patients with type 2 diabetes. In a recent muscle biopsy study in human subjects including control subjects, subjects with impaired glucose tolerance, and subjects with type 2 diabetes, it was shown that type 2 diabetes is associated with hypermethylation of PGC-1 α , concomitant with reduced mitochondrial content (24). Epigenetic effects may also modulate the effect of diet and activity on the pathogenesis of diabetes and could explain at least some of the abnormalities observed in the current study.

We acknowledge some limitations in the current studies. The overall number of subjects included is small, and it was not possible with these numbers to match groups for sex. Early-onset type 2 diabetes remains uncommon, and it is challenging to recruit young subjects of working age for these relatively complex protocols. In the initial (chronic exercise) study, the skeletal muscle investigations were limited by the biopsy amount, and it was not possible to measure PGC-1 α or other gene expression.

In summary, our results indicate that early-onset type 2 diabetes is associated, at baseline, with reduced expression of skeletal muscle Mfn2, which is associated with concomitant reduction in activity of certain oxidative phosphorylation subunits. In addition, subjects with early-onset type 2 diabetes are characterized by a deficient capacity to induce PGC-1 α or Mfn2 in response to aerobic exercise training. These alterations in Mfn2 expression and the failure to stimulate PGC-1 α may be relevant to the observed incapacity in these patients to enhance whole-body $\text{VO}_{2\text{max}}$ in response to exercise training. Further mechanistic studies of these pathways in this patient group are clearly indicated. A more complete understanding of these mechanisms will be crucial to the design of lifestyle interventions to prevent and treat type 2 diabetes in adolescents and young adults.

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Appendix VIII

Fighting the battle of the bulge: exercise training in early-onset type 2 diabetes.

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Fighting the battle of the bulge: exercise training in early-onset type 2 diabetes

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Abbreviations

$\dot{V}O_{2\max}$ maximum volume of oxygen utilisation

As we enter the twenty-first century, alarming clinical and epidemiological statistics reflect the shift to a sedentary lifestyle, as technology increasingly replaces any remaining daily requirement to be active. The role of visceral obesity in the development of insulin resistance and type 2 diabetes, and of other diabetes-related disorders, is underscored by the rising trend of physical inactivity and over-nutrition. Obesity often lays the foundation for the development of type 2 diabetes and other linked metabolic disorders, including cardiovascular disease and cancer, and is becoming excessively common in the young. For example, the prevalence of obesity in adolescents aged 12–19 years in the USA increased from 5.0 to 17.4% between the late 1970s and 2004 [1]. Unchecked, this striking rise in childhood obesity will increase the prevalence of fatal and non-fatal diseases, and the steady rise in

life expectancy over the past two centuries may come to a halt or even be reversed.

In this issue of *Diabetologia*, Burns et al. [2] shed new light on the impact of obesity in young people with early-onset type 2 diabetes, and raise intriguing questions regarding the efficacy of exercise training for improvement of insulin sensitivity in this population. Their study determined the effect of adiposity in young, obese, insulin-resistant individuals with and without type 2 diabetes, and investigated whether exercise training improves insulin-mediated glucose metabolism. Obese individuals with diabetes exhibited increases in WHR, fasting triacylglycerol levels and systolic blood pressure compared with the obese non-diabetic individuals, and additionally showed marked reductions in glucose disposal and beta cell function, as reflected by an increase in basal hepatic glucose output. The authors considered two different explanations for these metabolic impairments: (1) that early-onset type 2 diabetes is identical to later-onset type 2 diabetes in terms of pathogenesis and clinical progression; and (2) that it is a more extreme phenotype that is associated with an even greater risk of lifelong cardiovascular and metabolic complications than late-onset diabetes. While this point is not resolved in the present work, their data support previous observations that young people with type 2 diabetes generally have a loss of beta cell insulin secretion, as do older type 2 diabetic patients, at the time of clinical presentation. Moreover, those who develop type 2 diabetes before the age of 40 years are more obese at an earlier age than their older counterparts, have a more deleterious cardiovascular risk profile, and have worse initial and ongoing glycaemic control, despite comparable clinical care [3]. Collectively, these findings support the concept that early-onset type 2 diabetes reflects

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an extreme phenotype. Follow-up studies in early-onset type 2 diabetic patients will reveal whether this population will suffer a disproportionate cardiovascular and metabolic risk burden in the future.

Burns et al. [2] went on to investigate whether modest exercise could improve metabolic defects in early-onset type 2 diabetes, testing the hypothesis that a 12 week exercise training programme would improve insulin sensitivity in both cohorts. Exercise is a leading strategy in the battle against the bulge, and enhances peripheral insulin sensitivity [4]. Acute exercise can increase glucose uptake and metabolism in skeletal muscle via an insulin-independent mechanism that bypasses insulin signalling defects associated with type 2 diabetes [5]. Furthermore, the expression of metabolic and mitochondrial genes involved in glucose and lipid metabolism is increased in a coordinated manner in response to habitual exercise training, which has a positive effect upon whole-body glucose homeostasis [6]. Burns et al. [2] found that non-diabetic obese individuals responded to the exercise training programme with a 20% increase in their maximum volume of oxygen utilisation ($\dot{V}O_{2\max}$). Waist circumference was decreased in the early-onset type 2 diabetic patients after exercise training, but all other clinical parameters were unchanged. Furthermore, there was a greater increase in glucose disposal and a greater reduction in fasting NEFA in the non-diabetic obese group than in the diabetic group, despite identical compliance with the training programme. These findings contrast with previous reports in older individuals with type 2 diabetes or obesity, in whom exercise triggers some level of metabolic improvement in both groups [7]. Burns et al. [2] thus provide new evidence that early-onset obese type 2 diabetic patients are less responsive to the clinical benefits of exercise training.

The idea that there are exercise *responders* and *non-responders* is not new, but is perhaps underappreciated. In a microarray analysis of skeletal muscle biopsies from the HERITAGE Family Study, Teran-Garcia et al. [8] demonstrated differences in gene expression profiles between groups that do or do not exhibit improvements in insulin sensitivity following a 20 week training protocol, and propose that a differentially expressed set of genes may predict the exercise training response. In a study similar to that of Burns et al. [2], involving an older population [9], exercise intervention reversed clinical and metabolic consequences of type 2 diabetes. Fritz et al. [9] compared the expression of genes involved in glucose and lipid metabolism in the skeletal muscle of middle-aged type 2 diabetic patients after a 4 month walking programme. They found that exercise responders, who demonstrated a marked improvement in clinical variables, glucose metabolism, triacylglycerol and $\dot{V}O_{2\max}$, differed from the non-responders in the expression of metabolic genes. These findings suggest that intrinsic genetic differences may dictate the exercise

response and metabolic adaptation in skeletal muscle. Moreover, compelling work from Kelley et al. [10] suggests that exercise in obese/diabetic individuals boosts mitochondrial function [6]. Such findings spark further questions regarding the identification of biomarkers that may be useful in defining responder and non-responder phenotypes.

Burns et al. [2] are among the first to address the effectiveness of an intervention study in early-onset type 2 diabetic subjects aged between 15 and 30 years. The lack of clear benefits from exercise raises additional questions as to the mechanism underlying the failure to respond, and further emphasises the multifaceted phenotype of obesity coupled to type 2 diabetes. Although the authors did not perform a gene expression analysis, the possibility that differential transcript profiles play a role in segregating responders from non-responders is attractive and requires attention. Alternatively, these findings may imply that early-onset obesity and/or type 2 diabetes could shift the metabolic equilibrium beyond the reach of an activity-based improvement (Fig. 1), and cast doubt on the assumption that an exercise training protocol can improve insulin sensitivity in every metabolic situation. It is also possible that the conversion of an early-onset exercise non-responder to a responder may simply involve a more aggressive exercise intervention protocol.

Regardless, this study should not distract attention from the countless individual examples showing that exercise restores dysfunctional metabolic responses and that the benefit of exercise training in the management and

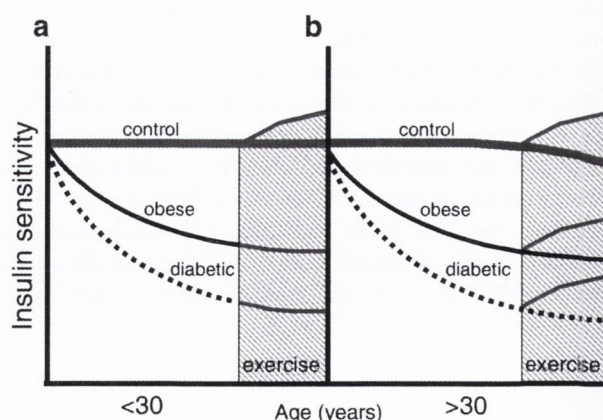


Fig. 1 Age-graded response to aerobic exercise in obese and type 2 diabetic patients. Independent of the age of onset, obesity and diabetes result in a progressive decrease in insulin sensitivity over time (black lines). Exercise intervention (red lines) improves insulin sensitivity and promotes weight loss in states of later-onset (>30 years) obesity and type 2 diabetes (b). This response mimics the observed effects in the non-obese, non-diabetic population (control). Early-onset obesity and type 2 diabetes (a) may render a state of 'exercise unresponsive' upon initiation of a physical training programme

prevention of type 2 diabetes is supported by a substantial body of evidence [4]. Further intervention studies will be required to determine which variables, such as duration, time, distance, aerobic nature and involvement of resistance training, elicit the most useful metabolic responses, and the influence of genetic factors upon the heterogeneity of exercise responsiveness.

In summary, Burns et al. [2] have shown that there are still unresolved issues regarding an appropriate exercise prescription to combat insulin resistance in obesity and type 2 diabetes. Although teasing out the ideal exercise protocol may not be a rapid process, physical activity remains the better option for treatment of metabolic disorders in most cases. A recent review points out that ‘although the magnitude of the effect of exercise on body fatness might be modest in most individuals, exercise can nevertheless be an important public health measure to reduce the prevalence of overweight [individuals]’ [11]. Moreover, the concept of NEAT (non-exercise activity thermogenesis), as introduced by Levine et al. [12], appears to be an important component in weight management as the ‘principal mediator of resistance to fat gain with overfeeding’. In short, daily activity does not have to be performed entirely in the form of a rigid exercise regimen; leisure-time physical activity such as gardening or household chores can also play a useful role in warding off obesity. On present evidence, a two-pronged attack seems justified. First, through meticulous design of future intervention studies in the young obese population, we must identify the best strategy to counteract the potential long-term health burden of type 2 diabetes. Second, emphasis should be placed on a more forceful and profound educational push for lifestyle changes and early intervention. In the words of the Chinese proverb, ‘The journey of a thousand miles begins with a single step.’

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