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TRINITY COLLEGE DUBLIN

# Clinical, metabolic and biochemical responses to a diet and aerobic exercise intervention in early-onset type 2 diabetes mellitus.

By

Declan O'Hanlon

B.Sc. (Physiotherapy), Post Graduate Diploma (Exercise Physiology)

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

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Department of Clinical Medicine

Trinity College

Dublin 2

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# Summary


## Methods

This study was a longitudinal, randomised, controlled trial. Two primary groups of subjects were recruited for comparison: young obese patients (less than 30 years of age) with early-onset type 2 diabetes (YT2), and a control group of body mass index (BMI) matched older patients (>50 years) with later-onset type 2 diabetes (OT2). The study protocol featured a 6 month crossover lifestyle intervention, including a separate 3 month dietary intervention and a 3 month exercise intervention, assigned in random order. The effect of the entire 6 month intervention, the crossover effect, and the effect of the individual 3 month components were all examined. The energy expenditure per week during the exercise intervention was matched with the weekly dietary energy deficit (-2500 kcal/week). Two days of testing were performed on each occasion, with a record of baseline physical activity levels [via the International Physical Activity Questionnaire (IPAQ)] and a maximum oxygen consumption capacity ($VO_2$max) fitness test performed on one day, and fasting measurements taken on the other, including: anthropometric measurements (BMI, waist to hip ratio, fat mass, body fat percentage and fat free mass), fasting blood samples [for measurement of glucose, glycated haemoglobin ($HbA_{1c}$), lipids, adipokines and metabolomics], a two hour oral glucose tolerance test (OGTT), and a biopsy of the vastus lateralis muscle (for measurement of intrinsic mitochondrial function using high resolution respirometry). During the exercise intervention, the participants trained 4 times per week at an intensity corresponding with 70% of their $VO_2$max. The majority of the exercise was performed on a stationary bicycle ergometer, and all training sessions were supervised. The exercise protocol incorporated training progression, with supplementary $VO_2$max tests performed at week 4 and week 8, which were used to formally adjust the training intensity as was appropriate. During the dietary intervention, subjects were provided with three-day food diaries, and were met every two weeks to be weighed, at which time they received nutritional advice regarding the implementation of a reduced calorie, low fat diet.

Chapter 4 Results: Experiment 1: Early-Onset T2DM - Physical Characteristics and Anthropometric Adaptation to Lifestyle Intervention.

Both groups were inactive at baseline and had a similar calorie intake and a similarly high percentage of dietary fat intake. There were no differences in weight, BMI, fat mass or waist circumference between groups. Contrary to previously reported results, the YT2 group responded to lifestyle intervention, with a reduction in waist circumference after the 6 month intervention, and a reduction in waist circumference and fat mass after the exercise intervention. In both groups, when the lifestyle intervention components were examined separately, the exercise intervention was associated with a trend towards greater health benefits.

Chapter 5 Results: Experiment 2: Whole Body and Cellular Aerobic Capacity

There were no significant differences in whole body maximum aerobic capacity ($VO_2max$) or cellular oxidative capacity (intrinsic mitochondrial function) between groups at baseline. However, as an age related reduction in these parameters is expected, this may represent a relative deficit in the YT2 group. The YT2 subjects responded to both the exercise intervention and the 6 month intervention with a significant increase in $VO_2max$ and intrinsic mitochondrial function. There was a trend towards a slower rate of increase in $VO_2max$ in the YT2 group. Participation in the dietary intervention was associated with a lesser mitochondrial adaptation during subsequent exercise.

Chapter 6 Results: Experiment 3: Insulin Resistance and Contributing Factors

The YT2 group had a distinctive lipid profile, with a higher circulating concentration of total fatty acids and several individual fatty acid species (palmitic and oleic acid). The YT2 group also had a lower adiponectin concentration. There was no difference in glycaemic control, insulin sensitivity, or β-cell function between groups at baseline. Both groups obtained a reduction in $HbA_{1c}$ after the 6 month intervention, but there was a trend towards increased adaptation after lifestyle intervention in the older group, with additional improvements in triglyceride profile obtained by the OT2 subjects.

# Acknowledgements

I would like to particularly thank my supervisor Professor John Nolan for giving me the opportunity to take part in this process and for all his guidance, support and assistance throughout.

I would like to thank the study collaborators [Krzysztof Wanic, Agnieszka Pazderska and Syed Ali Shah (the study physicians from the Metabolic Research Unit in St. James's Hospital), Donal O'Gorman and Diane Cooper (from Dublin City University), Noelle Collura, and Fiona Lithander], the other laboratory collaborators (in Trinity College, St. James's Hospital, and Duke University), the staff at the Diabetic Day Centre and the Metabolic Research Unit, and the patients who participated in the study.

Finally, I wish to thank Joy, my parents (Joe and Patricia), my sister (Maria), my extended family, friends and colleagues, for their continued support, without which this would not have been possible.

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## List of Abbreviations

| | |
|---|---|
| α-cells | alpha cells |
| AKT | activating protein kinase B |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| AUC | area under the curve |
| β-cells | beta cells |
| BCAA | branched-chain amino acid |
| BMR | basal metabolic rate |
| BMI | body mass index |
| BSA | bovine serum albumin |
| cm | centimetre |
| CO2 | carbon dioxide |
| CT | computed tomography |
| DE | diet then exercise |
| DEXA | dual-energy X-ray absorptiometry |
| DPP-4 | dipeptidyl peptidase-4 |
| ED | exercise then diet |
| EDTA | ethylenediaminetetraacetic acid |
| ELISA | enzyme-linked immunosorbent assay |
| FCCP | carbonyl cyanide-p-trifluoromethoxyphenylhydrazone |
| FFA | free fatty acid |
| FFM | fat free mass |

| | |
|---|---|
| g | gram |
| GAD | glutamic acid decarboxylase |
| GC/MS | gas chromatography/mass spectrometry |
| GIP | gastric inhibitory polypeptide |
| GLP-1 | glucagon-like peptide-1 |
| GLUT | glucose transporter protein |
| HbA1c | glycated haemoglobin |
| HDL | high-density lipoprotein |
| $^{1}$H-MRS | proton magnetic resonance spectroscopy |
| HOMA-IR | homeostatic model assessment |
| HRmax | maximum heart rate |
| IGI | insulinogenic index |
| IRS-1 | insulin receptor substrate protein |
| IL | interleukin |
| IPAQ | international physical activity questionnaire |
| kcal | kilocalorie |
| kg | kilogram |
| L | litre |
| LDL | low density lipoprotein |
| MRI | magnetic resonance imaging |
| mg | milligram |
| min | minute |
| µg | microgram |

| | |
|---|---|
| ml | millilitre |
| mm | milimetre |
| mmol | millimole |
| MS/MS | targeted tandem mass spectrometry |
| nM | nanomole |
| NS | not significant |
| OGIS | oral glucose insulin sensitivity test |
| OGTT | oral glucose tolerance test |
| OLIGO | oligomycin |
| OT2 | patient with later-onset type 2 diabetes |
| $O_2$ | oxygen |
| PCA | principal components analysis |
| PGC-1α | peroxisome proliferator-activated receptor γ coactivator-1 α |
| PI 3-kinase | phosphatidylinositol 3-kinase |
| $^{31}$P-MRS | phosphorous magnetic resonance spectroscopy |
| P+M | pyruvate and malate |
| P+M+S | pyruvate, malate and succinate |
| QUICKI | quantitative insulin sensitivity check index |
| ROT | rotenone |
| SEM | standard error of the mean |
| TNFα | tumor necrosis factor-alpha |
| T1DM | type 1 diabetes mellitus |
| T2DM | type 2 diabetes mellitus |

| | |
|---|---|
| $VCO_2$ | carbon dioxide production |
| $VO_2$ | oxygen consumption |
| $VO_2max$ | maximum oxygen consumption capacity |
| WHR | waist to hip ratio |
| w/v | weight per volume |
| YT2 | patient with early-onset type 2 diabetes |

# Chapter One

## Background

## 1.1 **Background**

Diabetes is a serious public health issue which is associated with increased morbidity and mortality (predominantly due to cardiovascular disease) (1). Diabetes mellitus refers to a number of metabolic disorders characterised by a chronic elevation in blood glucose concentration (hyperglycaemia) as a result of abnormal nutrient metabolism, because of deficiencies in insulin secretion or action, or both (2). The two main subcategories of diabetes mellitus are, type 1 diabetes mellitus (T1DM: "insulin dependent diabetes mellitus") and type 2 diabetes mellitus (T2DM: "non-insulin dependent diabetes mellitus") (3). T2DM accounts for 90 – 95% of the cases of diabetes (2), and is typically characterised by insulin resistance in the peripheral tissues and a progressive reduction in insulin secretion from the pancreas. T2DM is a progressive heterogeneous condition with a multi-factorial aetiology (2), and several complex metabolic factors also contribute to the characteristic hyperglycaemia, including chronically elevated hepatic glucose output, and other hormonal irregularities such as, elevated plasma glucagon and cortisol concentrations (4).

Typical symptoms of frank and uncontrolled diabetes include polydipsia, polyuria, weight loss, blurred vision and in severe cases, ketoacidosis (2). Diabetes is diagnosed from a blood test, as a fasting (8 hours) plasma glucose concentration $\geq$ 7.0mmol/l, a 120 minute oral glucose tolerance test (OGTT) value $\geq$ 11.1mmol/l after a 75g glucose load, or a random plasma glucose $\geq$ 11.1mmol/l in the presence of classic symptoms of hyperglycaemia. A $HbA_{1c}$ (glycated haemoglobin) value $\geq$ 6.5% is now also considered diagnostic of diabetes (Table 1.1).

Table 1.1. Diagnosis of diabetes. Any one of the following criteria are diagnostic (3).

| |
|---|
| • Fasting plasma glucose concentration ≥ 7.0mmol/l |
| • 120 minute oral glucose tolerance test value ≥ 11.1mmol/l |
| • $HbA_{1c} \geq 6.5\%$ |
| • Random plasma glucose ≥ 11.1mmol/l in the presence of classic symptoms of hyperglycaemia. |

Diabetes has reached epidemic proportions and the predictions are that the incidence of diabetes will continue to increase (5). The current data on worldwide diabetes prevalence have exceeded even the high end of previously predicted numbers, with the number of people worldwide with diabetes having increased from 153 million in 1980 to 347 million in 2008 (6). Predictions of the future increased incidence of diabetes are primarily related to predicted increases in the lifespan of the population and general population growth (7), but the current increased prevalence of diabetes is occurring not just due to changing population demographics (8), but because of the increasing accumulation of lifestyle related risk factors, especially obesity (7, 9).

Insulin resistance is correlated with obesity (10), and approximately 90% of patients with T2DM are obese (11). Although T2DM is known to have a genetic contribution (12, 13), lifestyle-related factors such as inactivity, poor diet and excess fat mass accumulation, are key to the pathogenesis and progression of the disease. T2DM has typically presented in older adulthood, but the greatest rate of the current growth in prevalence is in younger populations (8), and T2DM is now presenting in adolescents and children (14). These trends are of great concern as earlier onset increases patient exposure to the disease, increasing the risk of diabetes related micro- and macrovascular complications (15). However, early-onset

T2DM may also be associated with a disproportionately increased risk as a result of a more accelerated and aggressive pathogenesis (16), as these patients have a strong family history of diabetes (17), and are severely insulin resistant (18).

An increase of physical activity by 150 minutes per week has been shown to reduce the risk of T2DM by 58% (19, 20). Diet and exercise interventions that reduce body fat percentage can increase insulin sensitivity (21), while exercise training increases insulin sensitivity irrespective of weight loss (22). Exercise intervention can increase maximum oxygen consumption capacity ($VO_2max$) and mitochondrial density, the latter correlating with improved peripheral insulin sensitivity (23). Although lifestyle intervention can be used effectively in the prevention (20), and treatment of later-onset T2DM (24), it may be less effective in the management of severely insulin resistant subjects with early-onset T2DM. It has been recently shown that patients with early-onset T2DM did not respond metabolically following a 12 week exercise intervention, demonstrating no weight loss, and no improvement in $VO_2max$, insulin sensitivity or glycaemic control (25, 26).

## 1.2 **Aim**

The overall aim of these studies was to compare baseline pathophysiology between young and older T2DM patients to determine whether the younger group had any distinctive characteristics, and to compare the responses of each group to diet and exercise interventions.

## 1.3 **Objectives**

To achieve this aim, the objectives of the study were to compare between patients with early-onset and later-onset T2DM:

- anthropometrics
- whole body aerobic capacity
- intrinsic mitochondrial function
- insulin sensitivity (estimated from OGTT and HOMA-IR)
- lipid and adipokine profile
- responsiveness to lifestyle intervention (diet and exercise)

as well as:

- to compare dietary and exercise interventions to determine if there was a priming effect related to the sequencing with diet/exercise or with exercise/diet.

To achieve these objectives, two groups of subjects were recruited for the study: a group of younger patients with early-onset T2DM (YT2) and a group of older patients with later-onset T2DM (OT2). Measurements of clinical, biological and metabolic profile were taken at baseline (involving the use of bioelectrical impedance, $VO_2$max, blood sampling, OGTT and a muscle biopsy), and again during and after a 6 month lifestyle intervention, including a crossover 3 month dietary intervention and a 3 month exercise intervention, assigned in a random order.

## 1.4 **Hypothesis**

Based on what is known from the current literature, it was hypothesised that patients with early-onset T2DM would be more centrally obese with a greater fat mass, more insulin resistant, have a worse lipid and adipokine profile, have a low aerobic capacity and reduced intrinsic mitochondrial function. It was also hypothesised that the younger group would not be as responsive to lifestyle intervention, and that they would respond better to diet than to exercise.

## 1.5 **Limitations**

- The protocol used in the current study employed a novel crossover lifestyle intervention design, and while this allowed the priming influence of one intervention on another to be examined, it made the overall interpretation of the results more challenging as it introduced multiple contributing factors and subgroups.
- High resolution respirometry was used to assess intrinsic mitochondrial function as it measures oxidative capacity directly. The inclusion of further tests of mitochondrial function, density and biogenesis will add further value to the current study. These laboratory investigations are currently being conducted in the laboratory of Professor Antonio Zorzano in the Institut De Recerca Biomedica Barcelona as part of a follow up study: the DEXLIFE project (www.DEXLIFE.eu).
- Due to limited serum and plasma sample stocks at the time of metabolomic and adipokine testing apparatus availability, testing was only possible at baseline. It

would have been interesting to examine the effect of lifestyle intervention on these parameters, and this topic will be addressed during the ongoing DEXLIFE study.

- Bioelectrical impedance was used to assess body composition, and while other more accurate tests are available [e.g. dual-energy X-ray absorptiometry (DEXA) or magnetic resonance imaging (MRI)], all tests of body composition were performed under strict fasting conditions to keep hydration status constant so as to optimize the accuracy of the test-retest results.

# Chapter Two:

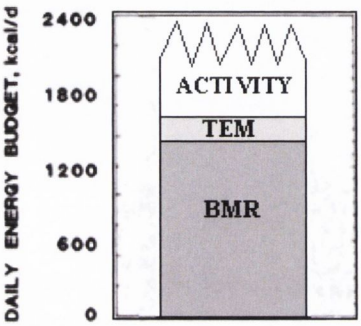
## Literature Review

## 2.1 **Regulation of substrate metabolism**

### 2.1.1 Normal substrate metabolism

The components that contribute to total daily energy expenditure include: basal metabolic rate (BMR), accounting for between 60 – 75% of daily energy expenditure, the thermic effect of feeding (approximately 10% daily energy expenditure), and the thermic effect of activity (approximately 15 - 30% daily energy expenditure), which varies according to physical activity levels (Figure 2.1) (27). The most metabolically active tissues with the greatest rate of energy expenditure which contribute to BMR include skeletal muscle, liver, brain and heart (28). To facilitate biological processes and energy expenditure, micronutrients (e.g. vitamins and minerals) and macronutrients (proteins, fats and carbohydrates) are ingested, and metabolised to their constituent components (amino acids, fatty acids and glucose). Postprandially, glucose is the primary nutrient oxidised, while lipids are predominantly metabolised under basal conditions in the postabsorptive state (29). Glucose intake beyond acute physiological requirements leads to storage as glycogen in the muscle and liver. Further intake results in the conversion of glucose to triglyceride which is stored in adipose tissue and elsewhere. Similarly, lipid and protein intake beyond physiological requirements results in the storage of the excess as fat (28).

Figure 2.1. Total daily energy expenditure: Basal metabolic rate (BMR), Thermic effect of meals (TEM), and activity (modified). *Casper RC, Schoeller DA, Kushner R, Hnilicka J, Gold ST. Am J Clin Nutr. 1991; 53: 1143-50.* (30).

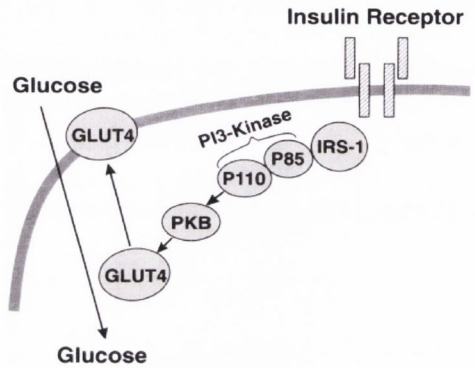



2.1.2 Blood glucose homeostasis

The majority of tissues can metabolise lipids, but the brain and central nervous system, and red blood cells require glucose as an energy source. While the brain can utilise lactate and ketones (a fat derivative produced by the liver), blood glucose concentrations need to be maintained within a narrow range to support essential brain function. Prolonged exposure to hyperglycaemia causes the glycation of tissue proteins as well as a range of other adverse effects, ultimately leading to micro and macrovascular damage. Blood glucose homeostasis is maintained chiefly through the regulation of insulin secretion from within the Islets of Langerhans in the pancreas. The islets make up approximately 1 to 2% of the pancreatic mass (31). The most abundant pancreatic endocrine cells are the β-cells, which account for approximately 80% of islet volume (32), and produce the peptide hormone insulin, while the α-cells produce glucagon.

The pancreatic release of insulin is closely coupled to changes in blood glucose concentrations (33), with secreted insulin increasing glucose uptake by the cells in target tissues, via glucose transporter molecules (31). The main glucose transporter is GLUT4, found in muscle and adipose tissue. Other glucose transporters include GLUT-1 (transporting glucose across the blood-brain barrier), GLUT-2 (transporting glucose into the pancreatic β-cells, and from kidney and intestinal cells into the blood stream), and GLUT-3 (transporting glucose into neurons) (28). Under basal conditions, GLUT4 is contained in cytoplasmic vesicles and is unavailable to extracellular glucose, but when insulin binds to its receptor on the cell membrane, a cascade of biochemical reactions activate the translocation of GLUT4 to the cell surface (34). The binding of insulin to its receptor activates tyrosine kinase, which phosphorylates the insulin receptor substrate proteins (IRS-1). IRS-1 binds to the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase) activating protein kinase B (AKT), which ultimately leads to GLUT4 translocation (35) (Figure 2.2). This results in the uptake of glucose and reduces the original stimulus for insulin secretion, subsequently reducing further insulin release in a negative feedback manner. In the absence of insulin, glucose remains in the bloodstream unable to cross the cell membrane to be metabolised, and when the plasma concentration is above the renal glucose threshold, glucose is eliminated from the body in the urine.

Figure 2.2. Diagram of cell membrane including insulin receptor and GLUT4 translocation pathway (modified). *Kelley DE, Mandarino LJ. Diabetes. 2000; 49: 677-83.* (29).




Insulin also suppresses hepatic glucose output (25), by reducing glycogenolysis (the breakdown of glycogen to form glucose), and gluconeogenesis (the production of glucose from non-carbohydrate fuel e.g. amino acids). In contrast to insulin, glucagon is secreted in response to low blood glucose concentrations (36), raising plasma glucose concentrations by increasing glycogenolysis and gluconeogenesis (37), and conversely, elevated plasma glucose concentrations reduce glucagon release (38). Insulin and glucagon therefore have opposite triggers for secretion and opposite effects on blood glucose concentrations. Other hormones that increase blood glucose concentration include: cortisol (a glucocorticoid produced by the adrenal cortex) (39), growth hormone (40) (secreted from the anterior pituitary), and the catecholamines (41) (adrenaline and noradrenaline, secreted from the adrenal medulla in response to stimulation by the sympathetic nervous system). The catecholamines increase glycogenolysis (41), inhibit insulin secretion (42), and enhance glucagon release (37), stimulating further liver glycogenolysis and gluconeogenesis.

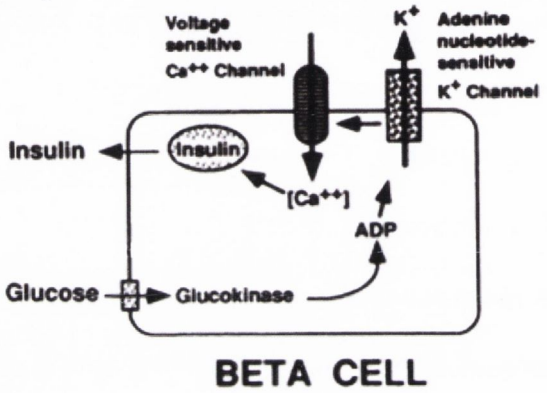
2.1.3 Insulin secretion

Glucose passes through the cell membrane of the pancreatic β-cells via GLUT-2 glucose transporter molecules, and is metabolized by the mitochondria, generating adenosine triphosphate (ATP) molecules. The initial reaction with glucokinase to phosphorylate glucose to glucose-6-phosphate acts as a rate limiting step, with glucokinase serving as a glucose sensor (43). Pyruvate is formed, which enters the mitochondria and generates the ATP that activates the closure of ATP-sensitive potassium channels, depolarising the cell membrane and opening the voltage-dependent calcium channels. The influx of calcium into the β-cells stimulates the release and exocytosis of insulin secretory vesicles from within the cell (44) (Figure 2.3). There is a large rapid first phase insulin response to glucose (33), which is particularly important in the maintenance of blood glucose homeostasis (45), followed by a smaller but more sustained second phase response (33). During the endogenous synthesis of insulin, the larger precursor hormone, proinsulin, is enzymatically hydrolysed to form C-peptide and insulin, and C-peptide concentrations can therefore be used as a surrogate marker of endogenous insulin secretion. The circulating insulin molecules are eventually degraded by the liver.

A larger plasma insulin release occurs in response to the oral administration of glucose than to intravenous glucose (46). This occurs because of signalling from the gastrointestinal tract and the secretion of incretin hormones. Both glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide or GIP) (47), and glucagon-like peptide-1 (GLP-1) increase β-cell insulin secretion in the presence of glucose (48). Insulin secretion is also increased by

other stimuli such as parasympathetic stimulation (42), while hormones such as somatostatin (41), and the catecholamines (42), reduce insulin secretion.

Figure 2.3. Glucose stimulated insulin secretion pathway. *Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G, et al. J Clin Invest. 1993; 92: 2092-8.* (49).




## 2.2 **Pathophysiology of T2DM**

### 2.2.1 Features of T2DM

Insulin resistance is a key metabolic characteristic of T2DM (25), and reduced insulin sensitivity is a predictor for progression to diabetes (50). In an insulin resistant state, the patient is unable to efficiently utilise endogenous insulin, which reduces peripheral glucose uptake and disposal (51), in target tissues, including muscle, adipose tissue and the liver (52). A number of techniques can be used to determine insulin resistance including the euglycaemic–hyperinsulinaemic clamp technique, a number of oral glucose tolerance test models which provide results comparable to those of the clamp technique (53, 54), or tests such as the homeostatic model assessment (HOMA-IR) and the quantitative insulin

sensitivity check index (QUICKI) (55), which only require fasting glucose and insulin samples and are based on mathematical models that utilise estimation algorithms.

Skeletal muscle is an important site of glucose uptake and it is also the primary site of peripheral insulin resistance in T2DM (52). Insulin resistance in the liver reduces suppression of hepatic glucose production, increasing basal hepatic glucose output (56). This arises due to the development of deficiencies within the insulin activated GLUT4 translocation pathways. There is also a reduction in both the number and capacity of GLUT4 molecules in the tissues of patients with T2DM (57). In the prediabetic state insulin resistance is offset by a compensatory increase in β-cell insulin secretion (hyperinsulinaemia) to maintain glycaemic control (58).

Normoglycaemia can be maintained in the pre-diabetic state for as long as increased β-cell output can be sustained. However, β-cell dysfunction is also a feature of T2DM, and as this develops, and the condition deteriorates, deficiencies in insulin secretion become apparent (52). When insulin secretion becomes insufficient, normoglycaemia can no longer be maintained and a state of impaired glucose tolerance and hyperglycaemia develops (51). After progression through pre-diabetes and further deterioration beyond the arbitrarily agreed diagnostic threshold (fasting blood glucose $\geq$ 7.0mmol/l), the patient is considered to have diabetes, and symptoms of polydipsia, polyuria, and weight loss can present (2). Qualitative β-cell dysfunction also develops, whereby a reduction in first phase insulin secretion is seen as another specific early feature of T2DM (59). The β-cells become insensitive to fluctuations in circulating glucose concentrations, reducing glucose-stimulated insulin

secretion (52). Significant pancreatic dysfunction is already present before patients with T2DM ever present for treatment (60), which occurs in part due to a reduction in the volume of GLUT-2 in the β-cells (61), and also because of β-cell destruction (62). Islet apoptosis occurs (62), in addition to the distortion of the islet architecture, with the gradual replacement of normal tissue with fibrous tissue (61). Computed tomography images show that the pancreas of patients with T2DM is smaller than that of healthy individuals (63). Obese individuals have an increased β-cell volume, facilitating compensatory hyperinsulinaemia in response to insulin resistance, but there is a reduction in the β-cell volume of individuals with impaired glucose tolerance and T2DM (64).

### 2.2.2 Obesity

Although genetic contribution is recognised in the aetiology of T2DM (12), and a heritable element has been demonstrated (13), with the offspring of patients with T2DM having insulin resistance and a predisposition towards elevated fasting plasma glucose concentrations (65), lifestyle and environmental factors, particularly obesity, are key to the pathogenesis and progression of the disease (11). Obesity, which is characterized by excess adipose tissue, is correlated with insulin resistance (2, 58), and approximately 90% of patients with T2DM are obese (11). Obesity is also associated with cardiovascular disease (66), and other conditions such as hepatic steatosis (with the prevalence as high as 75%) (67), and increased risk for cancer (especially pancreatic, esophageal and gastrointestinal) (68).

Body mass index (BMI) is measured as weight (in kilograms) divided by the square of height (in meters), and is used as an index of obesity (Table 1). However, central obesity is more

closely correlated with metabolic dysfunction than whole body obesity (69). The fat located in the abdominal region consists of both subcutaneous and intra-abdominal or visceral fat stores (70). Visceral fat contains a high volume of inflammatory macrophages (71), and insulin resistance is associated with visceral fat deposition. Obese T2DM subjects have a greater degree of central obesity than BMI matched control subjects (25). While BMI is correlated with central obesity (72), and visceral fat (73), BMI is a better indicator of whole body adiposity (73). It is possible to have a normal BMI and to be centrally obese (72), and insulin resistant with an abnormally high body fat percentage (74). Waist circumference or waist to hip ratio (WHR) measurements are used as an index of body weight distribution, with waist circumference more strongly correlated with visceral fat than BMI (73). In Caucasian subjects, waist circumference measurements ≥94cm for men and ≥80cm for women, and a WHR >0.9 for men and >0.85 for women are considered to represent central obesity (75). Ethnic differences exist for body fat distribution however, with lower BMI and waist circumference thresholds for Asian populations than for Europeans (Table 1) (75-77).

Table 2.1. Body mass index. Values and classification (77).

| **Classification** | **BMI Europe** | **BMI Asia** |
|---|---|---|
| Underweight | < 18.5 | < 18.5 |
| Healthy weight | 18.5 - 25 | 18.5 - 23 |
| Overweight | 25 - 29.9 | 23 - 27.5 |
| Obese | ≥ 30 | ≥ 27.5 |

Adipose tissue is not just an energy store, but can also be viewed as an endocrine organ, as it secretes several hormones such as resistin, leptin, and adiponectin (78). Resistin is associated with increased insulin resistance (79), while adiponectin has the opposite effect (80). Adiponectin concentrations are inversely associated with adipose tissue volume (80), with concentrations lower in obese subjects than in non-obese subjects (81), and lower in patients

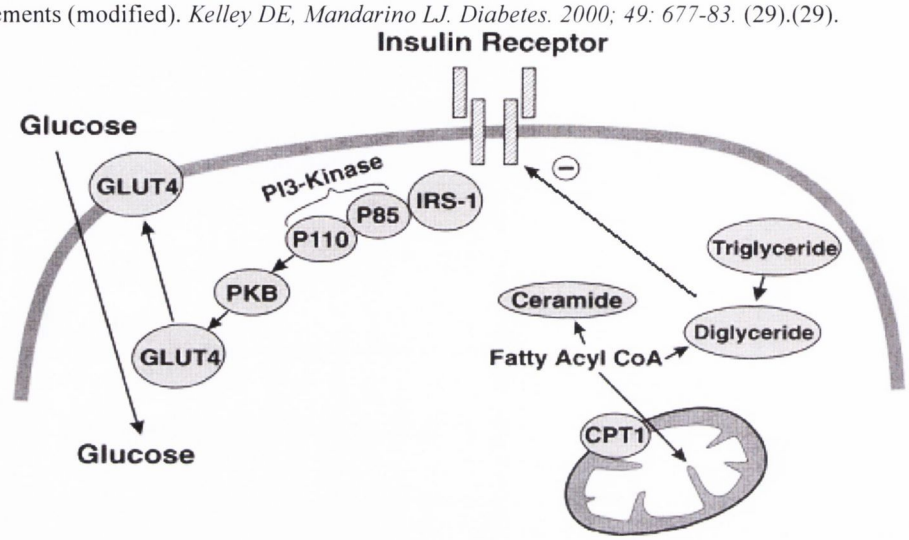
with T2DM than in BMI-matched non-diabetic subjects (82). Leptin increases satiety, with circulating concentrations correlated with adipose tissue volume (83). Leptin concentrations are higher in obese than in non-obese subjects (81), but with no difference between patients with T2DM and BMI-matched non-diabetic controls (82). Cytokines that are involved in inflammatory processes are also secreted by the adipose tissue, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα) (78). Macrophages in visceral adipose tissue may be particularly responsible for the release of these cytokines (78), which contribute to the low grade systemic inflammation associated with atherosclerosis and cardiovascular disease.

### 2.2.3 Lipotoxicity

While adipose tissue is the primary site of lipid storage, fat can also be deposited ectopically, which results in cellular and tissue dysfunction. Dyslipidaemia, including elevated fasting plasma free fatty acid (FFA) and triglyceride concentrations, is a feature of obesity (80). FFA's can accumulate as triglyceride in the cytosol of various non-adipose tissues such as the liver, the pancreas (63), and skeletal muscle (84), and are detectable using proton magnetic resonance spectroscopy ($^{1}$H-MRS) imaging (85), computed tomography (63) or directly by histological examination (86). Insulin resistant subjects demonstrate metabolic inflexibility (29), with reduced switch to lipid oxidation under fasting basal conditions, which maintain elevated plasma FFA concentrations. This cycle is compounded by the fact that the anti-lipolytic properties of insulin are less effective due to insulin resistance, resulting in increased release of lipid from the adipose tissue, further increasing plasma FFA concentrations and intramyocellular lipid accumulation.

The concept of lipotoxicity suggests that obesity and the resultant accumulation of ectopic lipid contributes to the development of insulin resistance (87). Patients with T2DM have increased intramyocellular lipid accumulation, and the degree of lipid accumulation correlates with the degree of insulin resistance (86). However, certain studies have shown no difference in intramyocellular lipid content when subjects with T2DM are compared with BMI matched non-diabetic control subjects (85), indicating that other factors must also contribute to muscle insulin resistance. The infusion of a lipid emulsion as part of a clamp study promptly increases plasma FFA concentrations, but the development of insulin resistance occurs in a delayed manner (88). It is the subsequent accumulation of lipid intermediates during the infusion, such as, ceramide, diacylglycerol and long chain-fatty acyl-coenzyme A, that coincide with the reduction in glucose disposal (88). Lipid intermediates are thought to inhibit insulin signalling (35), and are found in elevated concentrations in the muscle of insulin resistant patients (89) measured from biopsy samples by means of liquid chromatography and mass spectrometry (90). The chronic accumulation of lipid intermediates to harmful levels may lead to insulin resistance by inhibiting the insulin signalling cascade and the translocation of GLUT4, altering IRS-1 phosphorylation and preventing PI 3-kinase-AKT activation (35) (Figure 2.4).

Figure 2.4. Diagram of cell membrane including insulin receptor, GLUT4 translocation pathway, and lipotoxic elements (modified). *Kelley DE, Mandarino LJ. Diabetes. 2000; 49: 677-83.* (29).(29).




2.2.4 Mitochondrial contribution to the pathogenesis of insulin resistance

The mitochondria are the cell organelles (Figure 2.5), that generate most of the energy obtained from ingested nutrients (91), and are found in abundance in metabolically active cells, such as muscle fibres (24). The energy contained within the carbon-hydrogen bonds of food cannot be utilised directly, and must instead be used to form high-energy bonds within molecules of ATP (92), which can then be subsequently released in a controlled manner by hydrolysis, and used to meet physiological needs. Numerous chemical reactions are involved in ATP production, including those of glycolysis (which occurs within the cytosol of the cell), and those of oxidative phosphorylation [including activity within the tricarboxylic acid cycle (Figure 2.6) and the electron transport chain (Figure 2.7) in the mitochondria] (28).

Figure 2.5. Diagram of a mitochondrion displaying the double membrane, the matrix and the cristae (modified). *Rabol R, Boushel R, Dela F. Appl Physiol Nutr Metab. 2006; 31: 675-83.* (93).

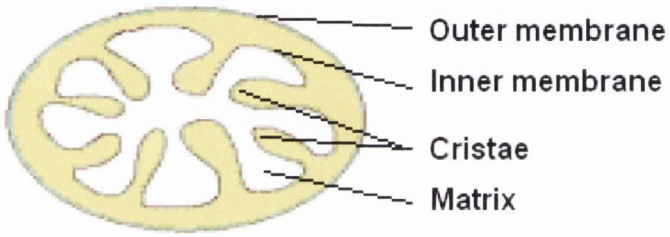



Figure 2.6. Sequence of the Krebs cycle; pyruvate (Pyr), acetyl coenzyme A, oxaloacetate (OAA), citric acid, isocitric acid, α-ketoglutaric acid (α –KG), succinyl coenzyme A (Suc-CoA), succinic acid, fumaric acid, maic acid, oxaloacetic acid (modified). *Ballard JW, Whitlock MC. Mol Ecol. 2004; 13: 729-44.* (91).

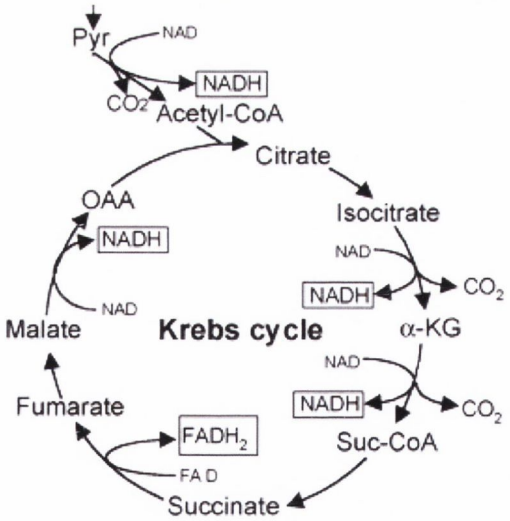
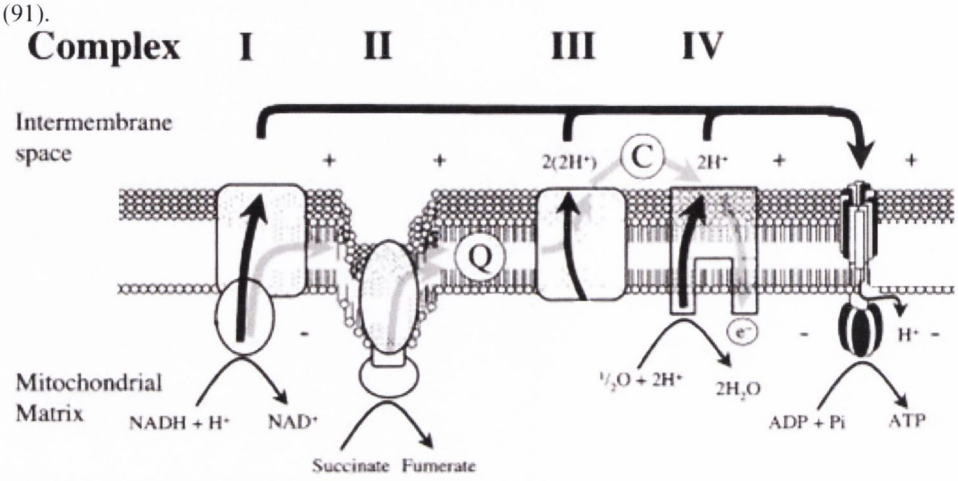


Figure 2.7. The electron transport chain (modified). *Ballard JW, Whitlock MC. Mol Ecol. 2004; 13: 729-44.* (91).




As T2DM is a metabolic disorder relating to the impaired processing of nutrients, and the mitochondria are the sites of substrate oxidation and the endpoints of nutrient metabolism (94), and as the mitochondria dense muscle is the primary site of peripheral insulin resistance (52), the mitochondria have become the subject of increased interest (94). T2DM typically develops in older individuals (8), and it has been shown that mitochondrial oxidative function is reduced with age (51). Therefore as mitochondrial dysfunction correlates with insulin resistance (95), impaired mitochondrial function is thought to be an important contributor to the development of both insulin resistance and T2DM (85).

The accumulation of intramyocellular lipid and lipid intermediates as part of T2DM is consistent with excessive lipid supply or inadequate lipid disposal through mitochondrial oxidation, or both (94). Patients with T2DM also have higher circulating concentrations of acylcarnitines than non-diabetic control subjects (96), and as acylcarnitines are intermediates of lipid oxidation, their accumulation is consistent with incomplete or ineffective lipid

metabolism. Endurance athletes paradoxically have an elevated intramyocellular lipid content, but they have a high oxidative capacity (97), and are very insulin sensitive (86). It therefore appears that a low mitochondrial oxidative capacity in combination with raised FFA concentrations and intramyocellular triglyceride accumulation, leads to the partial processing of triglyceride, resulting in the accumulation of lipid intermediates, which impair insulin action (35). The accumulation of intramyocellular lipid also increases the lipid load on the mitochondria, forcing neutral fatty acids directly into the matrix, instead of entry via the carnitine shuttle, causing them to become deprotonated, which results in the formation of lipid peroxides that damage the mitochondria in a manner of positive feedback (98).

The predominant factor influencing mitochondrial content is physical activity (99), and while some authors have shown reduced mitochondrial density in insulin resistant subjects compared to BMI-matched control subjects, the groups in this study may not have been sufficiently matched, as questionnaires were used to determine activity levels without measuring aerobic fitness (100). The majority of studies show no significant differences in mitochondrial content (85, 95, 99, 101-103), or the volume of mitochondrial dense Type I muscle fibres between patients with T2DM and matched obese control subjects (99, 101). However, insulin resistant subjects have a reduced mitochondrial density when compared to lean subjects (95, 103). Similarly, mitochondrial size (cross-sectional area), examined in muscle biopsies using transmission electron microscopy, is smaller in the skeletal muscle of patients with T2DM compared with lean control subjects, but not compared to BMI-matched controls (95). However, the expression of peroxisome proliferator-activated receptor $\gamma$ coactivator-1 $\alpha$ (PGC-1$\alpha$), a transcription factor coactivator which activates a number of

genes relating to oxidative phosphorylation and mitochondrial biogenesis (104), is reduced in T2DM (105), and in non-diabetic subjects with a family history of T2DM (106).

Studies using magnetic resonance spectroscopy (MRS) show patients with T2DM to have reduced mitochondrial function compared with matched control subjects, measured as longer phosphocreatine recovery half-time (85). This is also shown to be a heritable component of T2DM as it is also observed in the lean non-diabetic offspring of T2DM patients (51). This *in vivo* technique measures collective mitochondrial activity but cannot distinguish between results that occur because of reduced mitochondrial density, or because of lower oxidative capacity per unit volume of mitochondria. As studies have shown normal mitochondrial content in patients with T2DM compared to BMI and $VO_2$max matched controls, the deficiency must relate to intrinsic mitochondrial function, and this has been demonstrated as a lower oxygen flux using high resolution respirometry based techniques in fresh muscle biopsy samples (85, 101, 107). The earliest of these studies (101), recruited two groups of participants: 10 subjects with T2DM and 8 obese BMI matched non-diabetic controls from which muscle biopsies of the vastus lateralis were obtained. The mitochondria were isolated from the biopsy samples and oxygen flux was determined polarographically using an oxygraph respirometer. A true measure of intrinsic mitochondrial function was obtained by normalizing results to citrate synthase activity as an index of mitochondrial density, while maximal physiological oxygen consumption capacity was examined under the influence of pyruvate, malate and ADP, to bring about state 3 respiration, and alamethicin was used to bring about maximal (unphysiological) respiration. The subjects with T2DM obtained significantly lower results, but testing was conducted at 25°C, instead of at body temperature

(37°C), as is usually done. Another study which recruited 10 T2DM patients, 12 obese non-T2DM first-degree relatives of patients with T2DM, and 16 BMI matched non-T2DM control subjects, demonstrated similar results using a different protocol (85). In this case, skinned whole muscle fibres were used, permeabilized with saponin. Oxygen flux was measured in duplicate using a two-chamber Oxygraph (OROBOROS Instruments), and results were normalized to mtDNA copy numbers. Malate and glutamate were added as substrates to examine complex I, and succinate was used to examine both complex I and II, while ADP was added to bring about state 3 respiration, and FCCP was used to determine maximal respiratory capacity. The results showed that basal respiration was 35% lower among T2DM subjects, and that maximal uncoupled respiration was 31% lower, compared with control subjects, while first-degree relatives had intermediate values. As in the case of the previous study, the number of subjects recruited in each group was small, but protocols such as these involving invasive procedures including muscle biopsies tend to attract fewer participants, and the numbers in question were large enough to clearly demonstrate significantly different results between groups.

The exact mechanisms responsible for reductions in intrinsic mitochondrial function are not fully understood, but are thought to relate to an abnormal mitochondrial ultrastructure (85). This might be partly explained by the lower concentration of Mitofusin-2 detected in patients with T2DM, a mitochondrial membrane protein that facilitates mitochondrial fusion, allowing networks of mitochondria to be formed (102). These lower levels of cellular oxidative capacity are mirrored by a lower whole body oxidative capacity ($VO_2max$) in patients with T2DM compared to matched non-diabetic control subjects (108, 109). Other

factors contributing to this lower $VO_2$max include an underlying lower maximal cardiac output (108, 110), impaired peripheral vasodilation (111), and reduced skeletal muscle oxygen extraction (108).

### 2.2.5 Metabolomics and branched-chain amino acids

"Metabolomics" refers to the study and quantification of combinations of metabolites to classify chemical phenotypes, and to explain physiological mechanisms that predict disease. The metabolic environment, composed of all the constituent metabolites of the blood and tissues, is reflective of health status, and different disorders have a distinctive "metabolic signature", or "chemical fingerprint". An extensive panel of metabolites can be measured using mass spectrometry (112). Serum or plasma are tested using gas chromatography and targeted tandem mass spectrometry (113), with gas chromatography used to separate mixed compounds during vaporisation at high temperature (114), and mass spectrometry used to detect and quantify the separated molecules within the sample. The metabolites that are conventionally examined include: total free fatty acids, individual free fatty acid species, lipid derived metabolites (e.g. ceremide, diglyceride), acylcarnitines, amino acids, organic acids (e.g. pyruvate, lactate, citrate), hormones, cytokines, and other conventional metabolites (e.g. ketones) (112). By testing for a broad array of analytes with a diverse array of properties, the diagnostic and prognostic power of the technique is increased, however, the large number of metabolites examined adds to the complexity of the subsequent analysis. The principal components analysis (PCA) statistical technique can be used to reduce the multidimensional nature of the data, consolidating it into clusters or "factors" that are correlated with one another.

Recent studies have shown that, in addition to the accumulation of lipids, the abnormal metabolism and accumulation of other substrates, such as amino acids, are also associated with insulin resistance. To date, PCA analysis suggests the factor with the greatest correlation with insulin resistance is the metabolite cluster containing branched-chain amino acids (BCAA's) (the essential amino acids: leucine, isoleucine and valine) (115). Patients with T2DM have higher concentrations of circulating BCAA's than BMI matched control subjects, with BCAA concentrations correlating with $HbA_{1c}$ (116). Obese non-diabetic subjects have higher circulating concentrations of BCAA's than lean subjects (112), and those with the greatest degree of insulin resistance have the highest BCAA concentrations (112, 117).

BCAA's are not merely a marker or consequence of the disease, but may contribute to the pathogenesis of T2DM, as the intravenous infusion of BCAA's in healthy subjects reduces glucose disposal and glycogen synthesis (118). Impaired glucose disposal is thought to occur because of reduced insulin action and not because of nutrient competition during metabolism (118). Insulin sensitivity appears to be reduced because of impaired insulin receptor action (119) [by activation of the mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinase beta-1 (S6K1) (112), and the serine phosphorylation of IRS-1 (119)], resulting in impaired function within the insulin signalling cascade (119). An amino acid infusion also inhibits the suppression of endogenous glucose production by the liver (119), increasing gluconeogenesis and hyperglycaemia (120). Animal studies confirm the contribution of BCAA's, with high fat diets supplemented with BCAA's in rats inducing insulin resistance (112), while the removal of leucine from the diet increases insulin sensitivity (121).

However, human studies show that elevated concentrations of BCAA's among insulin resistant individuals do not occur because of differences in dietary protein consumption (112, 115). This phenomenon must therefore be the result of either reduced anabolic use for protein synthesis or reduced amino acid metabolism and oxidation (115). Not only are BCAA concentrations correlated with insulin resistance, but elevated BCAA concentrations are also present prior to the development of insulin resistance, making it a potentially important prognostic biomarker (122). This was demonstrated in a cohort from the Framingham Heart Study where normoglycaemic individuals were followed up after 12 years, with those who had elevated BCAA concentrations at baseline having developed T2DM.

## 2.3 **Early-onset T2DM**

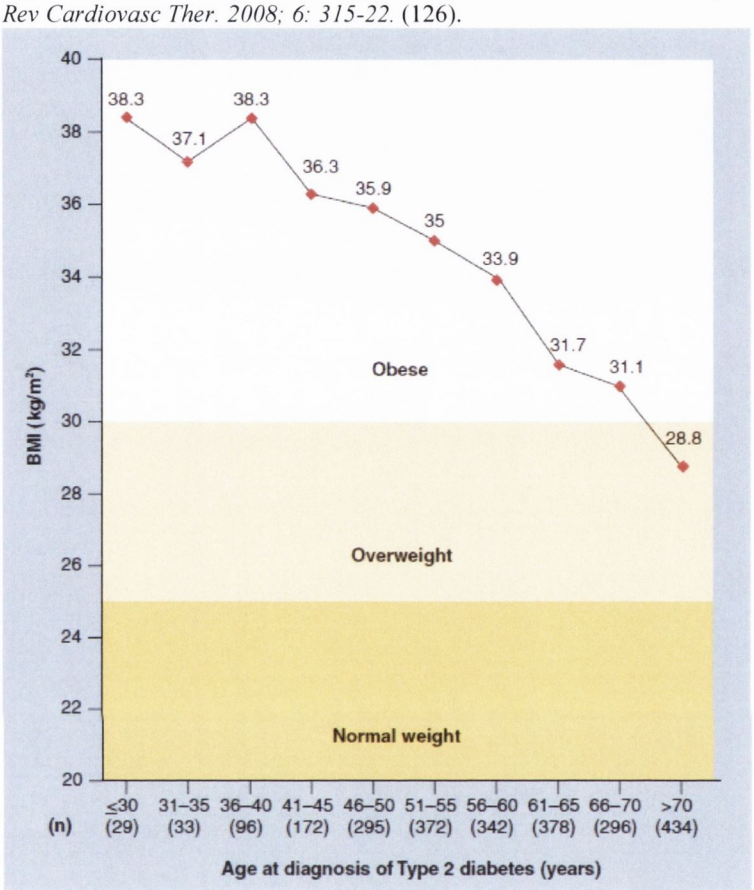
### 2.3.1 The aetiology of early-onset T2DM

T2DM has traditionally been seen as a disease of middle aged and older adults, but the age of onset continues to fall (123). The greatest prevalence of diabetes still exists within older subjects, but the greatest rate of growth in prevalence is now in younger populations (8). Global population-based records are sparse (123), but in countries with a high prevalence of T2DM, 20% of the cohort with diabetes is now comprised of young people with early-onset T2DM (124). T2DM is presenting at an increasingly younger age, and has even been described in adolescents and children (14). This phenomenon was initially identified in ethnic minorities (14), and minority groups still have the highest incidence of T2DM among adolescents (125), but early-onset T2DM has now become widespread (14).

There is very little published data on the pathophysiology of early-onset T2DM. Patients with early-onset T2DM have been shown to have a greater WHR than BMI matched obese control subjects without diabetes (25), and are more obese at the time of diagnosis than subjects with later-onset T2DM (18). Among patients with T2DM, those with the youngest age of onset are the most obese (126) (Figure 2.8). Socioeconomic factors are correlated with the high incidence of T2DM among adolescents (125), and similarly an increase in childhood obesity is occurring particularly in children from low income families (127). Commonly used indices of socioeconomic status include education, income and occupation (128), and there is an inverse relationship between these variables and BMI, with the relationship particularly strong among Caucasian populations (129). Differences in lifestyle partly account for the differences seen between social classes. Those categorised as being within lower social classes report lower levels of physical activity (130, 131), and a more unhealthy diet with the consumption of large quantities of breads, oils, fats and sugars (131). It is suggested that healthier diets are consumed by individuals who are more highly educated and more affluent (132). This may relate to the ability to make healthier food choices and a greater knowledge of health related issues, but the issue of economic assess to healthier foods may also be one of the contributing factors, as certain nutrient dense foods are often more expensive (132). It is suggested that access to specific food sources also influences the type of food consumed (133), and there is a higher prevalence of obesity and chronic disease in deprived neighbourhoods (134), where the residents have less access to supermarkets, and greater access to fast-food restaurants and convenience stores that sell cheap energy-dense food, particularly in urban areas (133). In certain communities, it is difficult to distinguish between racial/ethnic factors associated with minority groups, and specific socioeconomic variables,

and there appears to be an overlap between the two (128). It is a complex relationship, with possible contribution from ethnically distinctive lifestyle and dietary habits, social and physical environments, cultural norms, and race specific attitudes towards body image (128). Employment status is used as a determinant of socioeconomic status, and there is a high prevalence of metabolic risk among non-skilled workers and those working in manual labour jobs, such as construction work (135). Despite the inherent degree of physical activity associated with the work, there is a mismatch between daily physical activity levels and total energy intake. It is speculated that this is compounded by a lack of food choices in the workplace, and that workers from similar industries may also share similar lifestyles and values which reinforce each others actions (135). It is also speculated that once established, there could also be further interaction between obesity and certain socioeconomic variables, and that obesity could limit certain occupational and social opportunities (128). Another issue of note are the health inequalities that exist between different socioeconomic groups. Despite the use of schemes such as medical cards, inequalities in access to health services are noted, and access to appropriate medical care is predominantly reported among individuals of higher social classes with private health insurance (136). The root causes of obesity among lower social classes are multifactorial and complex, and interestingly, while lower socioeconomic status is associated with obesity and T2DM in developed countries, higher socioeconomic status is a risk factor in developing countries (123).

Figure 2.8. Relationship between age of diagnosis with T2DM and subject BMI. *Song SH, Hardisty CA. Expert Rev Cardiovasc Ther. 2008; 6: 315-22.* (126).




It has been hypothesised that more extreme underlying oxidative and metabolic deficiencies might be responsible for early-onset T2DM. The content of the mitochondrial fusion protein, Mitofusin-2, is low in patients with early-onset T2DM, and these patients do not show the adaptive increase in Mitofusin-2 seen in control subjects after 12 weeks of aerobic exercise training, furthermore, these subjects do not demonstrate the increase in expression of PGC-1α in response to acute exercise that matched control subjects produce (102). Although this study included both an acute and chronic exercise protocol, PGC-1α related data was

unfortunately available only after the acute exercise protocol, which does not give a complete picture of adaptation, or the lack thereof, among the early-onset T2DM subjects. Similarly, there was no increase in Mitofusin-2 in the early-onset T2DM group, but as exercise is the main stimulus for mitochondrial biogenesis, and it was demonstrated in the study that the exercise intervention was ineffective in a number of other respects, no increase in the concentration of the fusion protein would have been expected, and the real extent of any inherent deficiency is unclear.

Patients with early-onset T2DM typically have a strong family history of diabetes (17), and a stronger family history than patients with later-onset T2DM (137). While familial lifestyle could explain some of the prevalence, genetic predisposition to T2DM is evident as the lean non-diabetic offspring of patients with T2DM have the risk factors of increased intramyocellular lipid content and lower mitochondrial function (51). The recent widespread increase in the prevalence of diabetes has increased faster than can be explained by genetics alone, however, epigenetic factors could also explain some of the increased disease prevalence. Gestational diabetes is a variant of T2DM that first presents in women during pregnancy (especially when overweight) (3), and increases the mother's risk of developing T2DM later in life (138), however, foetal intrauterine exposure to hyperglycaemia during pregnancy (139) also increases the risk of the offspring developing T2DM in later years (140), possibly through DNA methylation (141). In addition, infants of either abnormally high or low birth weight also have a higher risk of developing T2DM, with the data forming a "U-shaped" curve (142), and with infants of low birth weight who subsequently rapidly

gain weight at greatest risk (143). Early infant nutrition is also of importance as breast-feeding appears to provide a degree of protection against T2DM (144).

### 2.3.2 Early-onset T2DM as a high risk phenotype

The prevalence of diabetes complications is related to both glycaemic control and the duration of diabetes (145). The increased prevalence of early-onset T2DM is of great concern because the earlier onset increases patient exposure to the disease, increasing the associated risk (15). Hyperglycaemia, in combination with other cardiovascular risk factors (e.g. hypertension and dyslipidaemia), causes progressive mircovascular and macrovascular damage, which can progress to the development of diabetes complications (146). Macrovascular complications include coronary artery disease, cerebrovascular disease and peripheral vascular disease (146). Microvascular complications can include retinopathy (147) [which can cause blindness (146)], as well as nephropathy [detected as proteinurea (148), which can eventually result in renal failure (146)]. The third major microvascular complication is neuropathy, which in conjunction with peripheral vascular disease impairs wound healing, and can ultimately cause foot ulceration, neuropathic osteoarthropathy and amputation (146). Neuropathy can also affect the autonomic nervous system, causing a range of further clinical syndromes (149). Patients with early-onset T2DM develop similar vascular complications to older patients with T2DM but do so 20 years earlier (145). For example, patients with early-onset T2DM have been reported to develop diabetes complications by their mid thirties (15). The societal implications of this are serious, as the associated cost increases the burden on health care systems, as well as removing young productive individuals from the workforce (123).

Early-onset T2DM may also be associated with a disproportionately increased risk as a result of a more accelerated and aggressive pathogenesis, and may not simply represent an otherwise equivalent disease to later-onset T2DM (16). It is reported that patients with early-onset T2DM are severely insulin resistant, with a substantial reduction in β-cell function (25), however, these comparisons were made with age and BMI-matched obese non-T2DM control subjects and not with subjects with later-onset T2DM, and by virtue of the fact that the controls do not have a diagnosis of T2DM, a substantial metabolic difference would be expected, making it difficult to confirm if early-onset T2DM is a more aggressive form of T2DM. Patients with early-onset T2DM do however have the greatest insulin or oral anti-diabetic agent requirements (124), and worse glycaemic control upon presentation and in response to treatment than subjects with later-onset T2DM (18). These subjects typically have a higher triglyceride (25), and lower high-density lipoprotein (HDL) cholesterol concentration than obese control subjects without diabetes matched for BMI (17), and raised markers of endothelial dysfunction (18). Although lifestyle intervention can be used effectively in the prevention (20), and treatment of later-onset T2DM (24), it may be less effective in the management of early-onset T2DM. It has been shown that patients with early-onset T2DM did not respond metabolically after a 12 week exercise intervention, demonstrating no weight loss, and no improvement in $VO_2$max, insulin sensitivity or glycaemic control (25). In that study however, it could be argued that this may not actually constitute a series of deficiencies, as the lack of increase in $VO_2$max alone may explain the rest of the results. The lack of increase in $VO_2$max may suggest that the training protocol should have been more robust, because although the exercise was supervised, and the

attendance rate was 95%, the intervention could have benefited from the incorporation of progression and a greater target exercise volume. Furthermore, the T2DM cohort were compared to a group of obese non-T2DM control subjects, and this group did not obtain any improvements in insulin sensitivity calculated from OGIS, or glucose disposal determined from the use of the gold standard hyperinsulinaemic–euglycaemic clamp technique. It is therefore difficult to establish the extent to which the observed lack of adaptation was due to underlying metabolic deficiencies, or to an intervention that could have included more intensive training.

Dietary compliance has also been reported to be poor among these young patients (150). A study examining children and adolescents (10 to 17 years of age) with T2DM as part of the TODAY Study, showed at follow up (2 years after initial contact), that there was poor compliance with treatment (lifestyle intervention and the use of medication), and an increase in cardiovascular risk factors (26). These young patients have different needs to older patients, and are subjected to different environmental, economic and social pressures, and it has been reported that many of them ignore their condition completely (124).

## 2.4 **Management of T2DM**

### 2.4.1 Medical management of T2DM

Medication is required in the management of T2DM depending on the severity of hyperglycaemia, or subsequently as the disease develops further, with the oral glucose lowering medication: metformin (a biguanide), used as the first line drug treatment to reduce hepatic glucose output (151). As T2DM is a progressive disease, the majority of patients

require further combination therapy over time, with the addition of sulfonylureas or insulin (151). Long-acting exogenous insulin is used to raise basal insulin concentrations, with prandial short-acting insulin supplementation added as is required, while sulfonylureas enhance endogenous insulin release by targeting the ATP-controlled potassium channels of the β-cells. Additional treatment options include the use of incretin based therapies [e.g. GLP-1 analogues and dipeptidyl peptidase (DPP)-4 inhibitors, which facilitate increased insulin secretion in the presence of glucose], thiazolidinediones (which enhance insulin sensitivity and lipid redistribution) (151), and bariatric surgical procedures (e.g. Roux-en-Y gastric bypass surgery) in the case of extremely obese patients, which can dramatically improve diabetes status, completely resolving symptoms in majority proportion of cases (152), even before weight loss occurs (153). While other medications are often required to manage the concomitant features of T2DM (e.g. therapies to treat hypertension and dyslipidaemia) (154), non-medical based lifestyle intervention including diet and exercise, remains the first line intervention and can be used effectively in the prevention (20), and treatment of T2DM (24).

### 2.4.2 Weight loss during dietary intervention

Nutrient intake type and volume can be manipulated to manage obesity, the main risk factor for T2DM. A number of dietary strategies can be used, which include the following:

- Low-fat (<30% of total calorie intake) reduced calorie diets high in fibre [which add dietary bulk, slowing gastric emptying, and increasing satiety (155)], are most commonly prescribed (156-158).

- "Mediterranean diets": are moderate-fat diets (35% of total calorie intake from fat, including a high monounsaturated to saturated fat ratio), high in vegetable (159), and fish content (158), and low in red meat (159).
- Low-carbohydrate diets: such as the Atkins diet, contain a higher proportion of protein, and a lower proportion of carbohydrate than standard diets (<20g per day) (159), which some authors suggest increases satiety as a result of a ketogenic state (160), and has an increased thermogenic effect, facilitating increased weight loss (161).
- Very-low-calorie diets: restrict daily energy intake to less than 800kcal (156), are composed primarily of a liquid formula, and are performed for a maximum of 3 - 4 months (157), under medical supervision (162).
- Vegetarian diets: have the worst levels of compliance (163).

A hypocaloric diet produces weight loss (159, 164), and reduces BMI (165), but an initial period of rapid weight loss during the first two weeks can be accounted for by a reduction in total body water as the depletion of glycogen stores is accompanied by an associated reduction in fluid by diuresis (166). However, sustained dietary intervention also reduces fat mass (167), waist circumference (159), abdominal subcutaneous and visceral fat mass (167), and pancreatic and hepatic triglyceride content (168).

The initial weight management goal is to stop the progression of weight gain, before proceeding to weight loss, followed by the prevention of weight regain [weight maintenance within 5lb (2.3 kg) of current weight] (156). Clinically significant weight loss is considered

to be a reduction of 5 - 10% body weight (157), but greater volumes of weight loss are required to maximise sustained health benefits (157). A reduction in calorie intake by 500 - 1,000 kcal/day below energy requirements, usually reducing energy intake to between 1300 kcal/day (169) and 1800 kcal/day (159), to produce a 1 to 2lb (0.45 – 0.91 kg) weekly weight loss (156), is considered safe for individuals who are not being medically supervised (157). Greater calorie restrictions create a greater negative energy balance and produce increased weight reduction (170). Weight loss varies depending on the calorie restriction imposed, the intervention duration, and participant compliance, with weight loss of 11kg (171) to 15kg possible within 4 months (172), and up to 20.5kg in 6months (170). The weight loss from dietary intervention is reduced over time however (164), as motivation and compliance reduce, and with the worst compliance associated with the most restrictive diets (163). Some studies show that between 5kg (164) – 6.7kg weight loss is maintained after 1 year (171, 173), but that dietary adherence is reduced over time, with compliance as low as 50% in some cases (163), and nearly half of the weight lost regained after 12 months (171, 173). A meta-analysis using a five year follow-up after dietary intervention, demonstrated that a 3kg weight loss was maintained, representing approximately 3% of original body weight, and 20% of the original weight loss (174). The degree of attendance at support sessions is strongly associated with successful long term weight loss (175), and when patients are asked to fill out food diaries, weight loss is greatest among those with the greatest frequency of self monitoring (176), and with the most complete dietary records (176). However, obese subjects overestimate their energy expenditure, and underestimate their calorie intake (177). Obese subjects underreport their calorie intake to a greater extent than lean control subjects

(178), and patients with T2DM underreport to a greater extent than BMI-matched obese subjects (179).

### 2.4.3 Weight loss during exercise

Low levels of self reported leisure time physical activity are associated with an elevated BMI (180), and there is an inverse correlation between pedometer measured steps taken per day and obesity levels (181). Aerobic exercise provides a means by which total daily energy expenditure can be increased (182), facilitating weight loss (183), and a reduction in fat mass in patients with T2DM (24). Exercise levels of 150 minutes per week are associated with general health benefits, but for weight loss among obese patients and weight loss maintenance, much larger volumes of exercise (up to 7 hours per week) are required (184). Regular physical activity is also considered a predictor of sustained long term weight loss maintenance, with those performing the greatest volumes of exercise experiencing least weight regain (174).

Some studies have shown dietary intervention to be superior to exercise for weight reduction (185), perhaps because the gains obtained from exercise would be quickly eroded if dietary intake was neglected. However, during dietary intervention, there is a reduction in fat free mass (FFM) in addition to a reduction in fat mass, with approximately 15% of the weight loss in the form of FFM (185), and as FFM contributes to BMR, and BMR is the greatest contributor to daily energy expenditure, continued weight loss would be hindered. However, when dietary intervention is combined with regular exercise, FFM can be preserved (185).

Furthermore, high intensity aerobic exercise increases FFM (186), with even greater muscle hypertrophy associated with resistance training (187).

Exercise training intensity is not directly related to fat loss (188). Low intensity exercise predominantly utilises fat metabolism (189), but during high intensity exercise, the demand for ATP is higher, the rate of energy production from oxidative phosphorylation becomes insufficient, and there is increased reliance on anaerobic glucose metabolism (glycolysis) (28). However, despite increased reliance on fat oxidation during low intensity exercise, 24 hour fat oxidation is similar when exercise interventions are matched for energy expenditure (182). A period of increased fat oxidation above resting levels persists after the completion of exercise (190), and the greater the exercise intensity, the greater the residual oxygen consumption, consistent with a more prolonged recovery period (191). However, the direct energy cost of an acute bout of exercise remains the major contributor to the exercise associated energy expenditure (191, 192). A greater degree of energy expenditure per unit time occurs during high intensity exercise, but there is no difference in fat loss when the duration of low intensity training is extended to match the energy expenditure of higher intensity training (193). High intensity exercise is therefore a more time efficient way of training, but is associated with less compliance irrespective of fitness status or BMI, and a greater rate of injuries compared with lower intensity exercise (194).

### 2.4.4 Insulin sensitivity response to exercise

Lifestyle modification incorporating an increase of physical activity by 150 minutes per week has been shown to reduce the risk of T2DM by 58% (19). The study demonstrating this

(The Diabetes Prevention Program) is one of the large, randomized, controlled, landmark diabetes prevention studies. In this case, 3234 non-diabetic subjects at high risk of T2DM with elevated fasting glucose concentrations were randomly assigned to one of three groups: placebo, drug therapy (metformin twice per day) or lifestyle intervention. All of the participants were obese (mean BMI: 34.0), the mean age was 51 years, and the cohort comprised both males and females from various representative groups, including ethnic minorities, recruited from 27 centres throughout the United States. The target for the participants in the lifestyle intervention group was to meet a physical activity level of at least 150 minutes per week, and a 7% reduction in baseline body weight by 24 weeks, which was achieved by three quarters of the participants. The mean follow-up time was 2.8 years, at which time the incidence of diabetes was 11.0 cases per 100 person-years in the placebo group, 7.8 in the drug therapy group, and 4.8 in the lifestyle group.

Physiological substrate selection during acute exercise is determined by the rate of energy expenditure, with glucose predominantly metabolised during high intensity exercise. This does not reduce blood glucose levels however, as high intensity exercise is associated with catecholamine release and results in increased glycogenolysis (28). Prolonged periods of moderate intensity exercise reduce acute blood glucose concentrations, increasing the risk of hypoglycaemia among patients taking insulin or insulin secretagogue medication (187).

A short term immediate increase in insulin sensitivity occurs after a single bout of exercise and lasts from between 24 - 72 hours depending on the intensity and duration of the session (187), and acute improvements in insulin sensitivity are possible after as little as 20 minutes

of exercise at an intensity corresponding with 70% of maximum capacity (195). Plasma insulin concentrations are reduced at the onset of an acute bout of exercise, and remain depressed throughout (196), however, glucose uptake is possible during exercise as GLUT4 is mobilised to the muscle cell membrane via insulin independent pathways (28). While an acute bout of exercise transiently increases insulin sensitivity and glucose uptake, regular exercise training leads to a sustained increase in insulin sensitivity (22), reducing $HbA_{1c}$ (24, 197). The intensity of regular aerobic exercise training is related to the degree of improvement in insulin sensitivity (188). Resistance training also increases insulin sensitivity (198), and the muscle hypertrophy it induces also provides a greater surface area for glucose disposal. Weight loss associated with lifestyle intervention increases insulin sensitivity (21), and reduces lipotoxic conditions [free fatty acids and their metabolites (199), as well as acylcarnitines (200)], but exercise increases insulin sensitivity irrespective of weight loss (22). There is an increase in PGC-1α expression after an acute bout of exercise (25), which facilitates mitochondrial biogenesis and an increase in mitochondrial volume (99), and patients with T2DM can increase their mitochondrial density using a variety of protocols [e.g. 4 (24), or 10 (99) months of walking, or 12 weeks of stationary cycling (107)]. In addition to an increase in mitochondrial volume (99), which is correlated with an increase in insulin sensitivity (23), exercise also increases mitochondrial function (21), increasing the activity of aerobic enzymes, including succinate dehydrogenase (201), and cytochrome oxidase (202).

2.4.5 Impact of dietary intervention on diabetes status

Diet induced weight loss increases insulin sensitivity, with as little as a 10% reduction in weight leading to improvements (21). The maintenance of weight loss reduces the risk of developing T2DM (203), with a 4.5kg diet induced reduction in weight (or a 5% reduction of baseline body weight) having been shown to reduce the risk of developing T2DM over 2 years by 30% in obese patients with a family history of T2DM (204). Patients with T2DM who adhere to dietary restrictions and reduce their BMI can effectively reduce daily blood glucose values, $HbA_{1c}$, and reduce their reliance on medication (165). A reduction in carbohydrate ingestion reduces excursions in blood glucose concentration and reduces exogenous insulin requirements in patients with established T2DM. A Mediterranean diet offers an effective alternative to a low-fat reduced calorie diet, demonstrating improvements in plasma glucose concentrations in patients with T2DM (159), while it has been demonstrated that a very-low-calorie diet can normalise fasting blood glucose concentration and insulin sensitivity in patients in as little as 7 days (168).

Improvements in insulin sensitivity after dietary intervention occur in a different manner from those associated with exercise, as there is no increase in mitochondrial content, and in fact certain authors report that mitochondrial size is reduced, perhaps as a direct response to restricted energy metabolism (21). Similarly, there is no improvement in mitochondrial aerobic enzyme activity, with no change in the activity of NADH-oxidase (21), the electron transport chain enzymes: succinate dehydrogenase (205), and cytochrome c oxidase, or the β-oxidative enzyme: beta-hydroxyacyl CoA dehydrogenase (172). However, in response to dietary weight loss, there is an increase in adiponectin concentration (82), and a reduction in

intramyocellular lipid content, and the concentration of lipid intermediates (e.g. diglyceride) (205), and BCAA's (113).

### 2.4.6 Cardiovascular adaptations to exercise

Patients with T2DM are at increased risk of developing cardiovascular disease (1, 15), and while strict glycaemic control delays the development of microvascular complications, a "U-shaped" curve exists, with excessively tight control using medication increasing the risk of hypoglycaemia (206), and increasing the prevalence of cardiovascular events (207). Lifestyle intervention incorporating regular exercise training improves cardiovascular risk factor status, including a reduction in LDL-cholesterol (197), plasma triglycerides (202), vascular inflammatory markers (C-reactive protein), proinflammatory cyctokines (IL-6 and IL-18) (169), and blood pressure, and an increase in peripheral circulation (208). Those with a higher $VO_2$max are at lower risk for the development of cardiovascular events (209), and endurance training can be used effectively to increase aerobic capacity in patients with T2DM (24). The greatest contributing factor to an increase in $VO_2$max, is an increase in cardiac output (210), which occurs as a result of an increase in stroke volume (211), irrespective of weight loss (212). Resistance training is not associated with improvements in $VO_2$max (192). Some authors state that it is possible to increase $VO_2$max using extended periods of low intensity aerobic exercise training (197), but it is generally agreed that the intensity of training is directly proportional to improvements in $VO_2$max, with higher intensity training producing greater increases in aerobic fitness (188, 210). Moderate intensity activity (50 – 70% maximum heart rate, or 40 – 60% $VO_2$max) is effective, but high

intensity interval training at intensities approaching maximum capacity increases $VO_2$max to the greatest extent (213), with results detectible in as little as 2 weeks in some cases (214).

### 2.4.7 Impact of dietary intervention on cardiovascular risk factors

Unlike with exercise training, there is no improvement in $VO_2$max after dietary intervention (21). However, diet induced weight loss improves cardiovascular risk by improving lipid profile, including a reduction in serum triglyceride, free fatty acids (169), total cholesterol (215), LDL-cholesterol and very-low-density-lipoprotein cholesterol (216), and an increase in HDL-cholesterol concentration (169). Low-fat diets reduce LDL-cholesterol (175), and diets with the lowest concentrations of saturated fat, and trans fatty acids, reduce total and LDL-cholesterol to the greatest extent (217). Dietary soluble fibre also makes a small but significant contribution to a reduction in LDL-cholesterol (218). HDL-cholesterol is increased and triglyceride is reduced to the greatest extent with low-carbohydrate diets (159, 175). Diet induced weight loss reduces the circulating concentration of C-reactive protein, IL-6 and IL-18 (169), in addition to reducing blood pressure (219). A 5kg reduction in body weight is associated with a 4.4mmHg systolic and a 3.6mmHg diastolic reduction in blood pressure (probably as a result of a reduction in sympathetic nervous system activity) (219). A reduction in salt intake, by reducing the direct addition of salt to food and to the cooking process, and by reducing the consumption of processed foods with a high salt content (158), can reduce blood pressure within 5 weeks, reducing the risk of heart disease and stroke (220).

## 2.5 **Summary**

The increasing prevalence of early-onset T2DM is of great concern, as earlier onset of the disease increases patient exposure to hyperglycaemia, increasing the risk of diabetes complications. There is little data available on the pathophysiology of early-onset T2DM, but it has been hypothesised that it may be a more extreme phenotype with a more aggressive pathogenesis and a disproportionately increased risk of complications. It has been shown that patients with early-onset T2DM have poor glycaemic control and are severely insulin resistant compared to obese non-T2DM control subjects. The experiments in the following chapters directly compare subjects with early (YT2) and later-onset T2DM (OT2) to determine if they are equivalent conditions, using measurements of fasting glucose concentration and indices of insulin resistance and β-cell function derived from oral glucose tolerance test data.

As obesity (particularly central obesity) is correlated with insulin resistance and the development of T2DM, and as patients with early-onset T2DM have been shown to have a particularly elevated BMI and waist circumference, the question remains as to whether early-onset T2DM can be accounted for purely on the basis of obesity status. The first comparison performed in the current study therefore examines anthropometric measurements from YT2 and OT2 subjects, including measurements of weight, BMI, body composition and fat distribution. Obesity is associated not only with an excess of fat deposition, but also with dyslipidaemia and elevated concentrations of FFA's and triglyceride. The lipotoxicity hypothesis suggests that an excess of circulating lipid can result in intramyocellular lipid

accumulation, interfering with insulin signalling. The lipid profile of subjects in the current study was therefore of interest, to determine if there were differences between groups that could explain the earlier onset of T2DM among the younger subjects. Standard lipid profiling was performed for FFA's, triglyceride and HDL cholesterol, in addition to the use of metabolomic testing using mass spectrometry to measure an extensive panel of other lipid species. As the concentration of BCAA's (leucine, isoleucine and valine) has also previously been shown to correlate with insulin resistance, and to cause insulin resistance in a similar manner to an excess of lipid, BCAA's were also measured as part of the current study. Furthermore, the adipose tissue derived adipokines: leptin and adiponectin, were measured as leptin relates to satiety, and adiponectin concentration is correlated with insulin sensitivity.

The lipotoxicity hypothesis suggests that it is specifically the production of the lipid intermediates that results in the impaired translocation of GLUT4, and that their production is likely to be as a result of impaired mitochondrial function. The majority of studies show no significant differences in mitochondrial content or size between patients with T2DM and BMI-matched controls, but a comparison of intrinsic mitochondrial function between both younger and older subjects with T2DM was of interest in the current study, using fresh muscle biopsy samples and high resolution respirometry based techniques to examine oxygen flux at different points in the electron transport chain. As it has previously been shown that YT2 subjects have a reduced PGC-1α content, it was hypothesised that intrinsic mitochondrial function would be lower in this cohort. Of relevance also, are acylcarnitines concentrations, as they are intermediates of lipid metabolism with their abundance reflecting mitochondrial oxidative status. The predominant factor influencing mitochondrial capacity is

physical activity, so it was of further interest to compare maximal oxidative capacity ($VO_2max$) between subjects. $VO_2max$ is naturally higher in younger subjects than in older subjects, but as patients with early-onset T2DM are considered to be particularly metabolically compromised, it was of interest to compare the capacity of both older and younger subjects, and to see if whole body oxidative capacity was correlated with cellular mitochondrial capacity.

Exercise training can be used to increase $VO_2max$, and lifestyle intervention remains the first line intervention used in an attempt to prevent and treat T2DM. Aerobic exercise facilitates increased energy expenditure and therefore weight loss, with high intensity exercise providing the greatest rate of energy expenditure. Exercise training has been shown to increase insulin sensitivity, $VO_2max$ and mitochondrial biogenesis irrespective of weight loss however, with higher intensity training increasing insulin sensitivity and $VO_2max$ to the greatest extent. Interestingly, it was recently shown that patients with early-onset T2DM were less responsive to exercise training than young obese non-T2DM subjects, so one of the areas of focus in the experiments to follow, is the comparison of adaptation to a high intensity exercise intervention between YT2 and OT2 subjects.

The other component of lifestyle intervention is dietary intervention, which can also be used to create a negative energy balance to facilitate weight loss. The most commonly used dietary intervention is a low-fat (<30% of total calorie intake) reduced calorie diet that is high in fibre. A 10% reduction in body weight achieved in this manner can increase insulin sensitivity, and the maintenance of weight loss reduces the risk of developing T2DM among

high risk subjects. A reduction in carbohydrate ingestion reduces excursions in blood glucose concentration and patients who adhere to dietary restrictions can effectively reduce their fasting blood glucose concentration and $HbA_{1c}$. The current study therefore also examines the response of both YT2 and OT2 subjects to a separate calorie restricted dietary intervention. The overall objective was to determine if there were differences between groups at baseline and any differences in their ability to adapt to lifestyle intervention.

# Chapter Three:

## Methods

3.1 **Participants**

Two groups of subjects were recruited and examined as part of the primary study analysis: young patients (less than 30 years of age) with early-onset type 2 diabetes (YT2), and a control group of BMI-matched older (>50 years) patients with later-onset type 2 diabetes (OT2). Two additional reference groups were recruited for baseline examination: an age and BMI matched young obese non-diabetic control group (YOb), and a matched older obese non-diabetic control group (OOb). The participants with T2DM were recruited from outpatient clinics at St. James's Hospital Dublin, a large general hospital based in the city centre, serving the needs of patients from a predominantly lower socioeconomic background. All T2DM participants lived locally within the catchment area of the hospital, and were recruited by the study research physicians and the study exercise physiologist (the author). In addition, the nurses in the Diabetes Day Centre adjacent to the Metabolic Research Unit in St. James's Hospital were informed of the study inclusion and exclusion criteria for subjects, and they helped to identify patients with T2DM that matched the age and BMI requirements. Obese non-diabetic subjects were recruited by the staff of The Department of Health and Human Performance in Dublin City University by local advertisement. Sampling was performed by a process of rolling recruitment. Given the limited capacity of the exercise laboratory where exercise training took place during the intervention, only 3 subjects could participate in each arm of the intervention at any given time. When a subject completed the intervention, the next available and suitable candidate that could be identified was approached. Although the prevalence of early-onset T2DM is increasing, there are still relatively few patients with the condition, and effectively every YT2 patient that came to the hospital for review during the duration of the study was invited to participate. The patients

had stable blood glucose concentrations controlled either with diet alone or with diet and Metformin. Medication doses were not changed throughout the course of the study. Potential subjects were told about the nature and purpose of the study, including the benefits, risks and possible discomforts associated with the study procedures, and were provided with written information about the project. If the subject agreed to participate, written informed consent was obtained and candidates were medically screened by a doctor. This included a medical history, a routine physical examination, measurement of blood pressure, heart rate, and a resting 12-lead electrocardiogram. Ethical approval was obtained for all procedures from the local ethics committee (the Research Ethics Committee of St. James's Hospital Dublin, and The Adelaide and Meath Hospital, Dublin, Incorporating The National Children's Hospital).

### 3.1.1 Inclusion Criteria

Participants were included if they had type 2 diabetes, with oral glucose tolerance tests used to confirm the diagnosis. Further inclusion criteria required patients to be obese (BMI > 30), weight stable and sedentary for at least one month prior to the study.

### 3.1.2 Exclusion Criteria

Patients with pre-diabetes or type 1 diabetes mellitus were excluded. Patients who were glutamic acid decarboxylase (GAD) antibody-positive and who had a fasting C-peptide less than 2.5ng/ml were excluded. Participants were also excluded if they were aged between 30 and 50 years or if they took part in any regular, formal exercise or were classified as having high daily physical activity levels by the International Physical Activity Questionnaire (IPAQ), or had a maximum aerobic capacity greater than 50ml/kg/min. Further exclusion

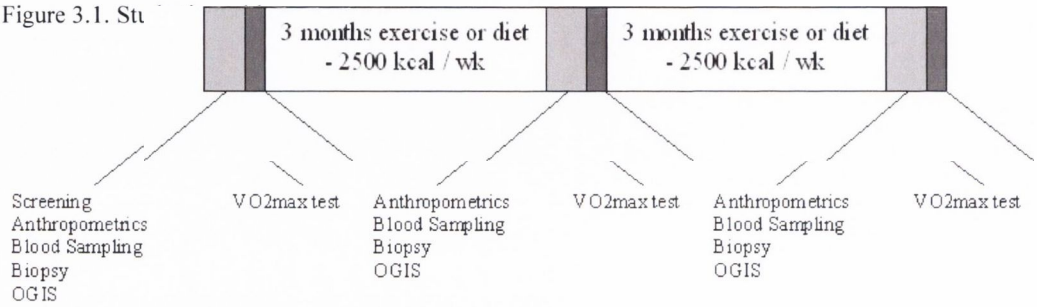
criteria included pregnant women or those women planning to become pregnant, those with other underlying metabolic disorders such as polycystic ovary syndrome, any health condition or musculoskeletal disorder which would prevent full participation in the intervention, or individuals who were unable to stay in the same geographic location for the duration of the study.

### 3.2 **Experimental protocol**

The study was a longitudinal, randomised, controlled, crossover trial, with testing carried out over a 3 year period. The study protocol featured a 6 month lifestyle intervention, including a separate 3 month dietary intervention and a 3 month exercise intervention, assigned in random order (Figure 3.1). Subjects were randomised to diet or exercise in an alternate manner based on the consecutive order of their appearance. The energy expenditure per week during the exercise intervention was matched with the weekly dietary energy deficit: -2500 kcal per week. Subjects were instructed to adhere strictly to the protocol, and not to change their background physical activity levels during the interventions or dietary habits outside of those prescribed, a point which was strongly reinforced throughout the course of the study. After initial screening, baseline testing was performed. Two days of testing were performed on each occasion, with a maximum oxygen consumption capacity ($VO_2max$) fitness test performed on one day, and fasting measurements on the other, including: anthropometric measurements, fasting blood samples (for glucose, insulin, $HbA_{1c}$, lipid and adipokine measurement), a two hour oral glucose insulin sensitivity test (OGIS) and a biopsy of the vastus lateralis muscle (for measurement of intrinsic mitochondrial function). Subjects fasted for 10 hours overnight and all procedures were conducted at the test centre from 8am.

Testing was repeated after 3 months at the crossover between interventions, and after a subsequent 3 months following the second intervention at the end of the study.

Figure 3.1. Stu




## 3.3 **Anthropometric measurements**

Anthropometric measurements, including weight were recorded at baseline to allow for comparison between groups, as obesity is correlated with the prevalence of T2DM (11). Measurements were also made after each intervention, to allow the effect of each to be examined. These measurements were taken on each occasion by the study exercise physiologist. A measurement of subject height was also performed so that BMI could be calculated (subject's weight in kilograms divided by the square of their height in meters), which provides more information than weight alone, and is directly related with adiposity (73), and obesity (72). The reliability of weight and height measurements is considered to be excellent (r=0.99) (221), but several following steps were also taken to ensure the accuracy of testing: anthropometric measurements were taken under fasting conditions, with height measured in the standing position in bare feet using a stadiometer (Seca 220, Height Measure, Hamburg, Germany), to the nearest 0.1cm, and with the mean of two measurements used. Fasting weight was measured to the nearest 0.1kg using a calibrated

clinical scales, with measurements made in light clothing and bare feet, and the same outfit worn for subsequent measurements.

Weight and BMI are correlated with obesity, but cannot differentiate between fat mass and FFM, so a bioelectrical impedance device was also used (Tanita Body Composition Analyser, Model TBF-300, Tanita Corp, Tokyo, Japan) (Figure 3.2). In addition to measurements at baseline, the use of bioelectrical impedance was also important as adaptation in response to lifestyle intervention can include a reduction in fat mass as well as changes in FFM (which can increase in response to exercise and reduce after diet). The correlation between measurements obtained using bioelectrical impedance and hydrostatically determined fat mass readings ranges from 0.71 to 0.76. Bioelectrical impedance is considered to be highly reliable (r=0.957 – 0.987) (222), and accurate in the detection of changes that occur in response to lifestyle intervention (222). The other benefit of bioelectrical impedance measurements is that its use has application in clinical settings as it is inexpensive, and as little training is required. The main factor to consider when using bioelectrical impedance is subject hydration status, as dehydration can lead to an overestimation of fat mass. Taking this into account, all measurements were made under fasting conditions to standardise the procedure. This may affect the validity of the absolute values, but allows for an accurate relative comparison of subjects, and for a reliable pre-post intervention comparison.

Figure 3.2. Tanita bioelectrical impedance scale.

In addition to measurements of body composition, measurements of fat distribution were also of interest, as central obesity is more closely correlated with metabolic dysfunction than whole body adiposity (69), and waist circumference is a measurement that is strongly correlated with visceral fat content (73). The interclass correlation for the reproducibility of waist circumference measurements can be as high as 0.99 (223), with reliability at its highest when staff have been trained (221). Therefore, all measurements were taken by an experienced therapist (the study exercise physiologist), and taken by the same person on each occasion (pre and post intervention). To further ensure the reliability of the measurements, waist and hip circumference were measured to the nearest 0.1cm with an inelastic plastic fibre 150cm anthropometric measuring tape, from which waist to hip ratio (WHR) was calculated (waist circumference measurement in centimetres divided by hip circumference measurement in centimetres), with the mean of two measurements used on each occasion. Waist measurements were taken around the abdomen at the narrowest point between the iliac crests and the lowest rib margin, just above the umbilicus. The subjects were asked to exhale gently and to relax their abdomen. The measuring tape was held taut around the

measurement site without squeezing in against the skin, ensuring that the tape was maintained in a horizontal position. Hip circumference was measured around the femoral greater trochanters and around the gluteal mass.

### 3.4 **IPAQ**

The International Physical Activity Questionnaire - Long Format (Appendix I) was administered via interview. The questions were asked according to the order and wording of the "Self-Administered Format". The subject was asked to recall physical activity that they had participated in over the previous 7 days, including: job, household, recreation, and transport related activity. The questions examined activity frequency, duration and intensity. The IPAQ was used to ensure that the subjects met the inclusion criteria of being sedentary (having previously been asked if they considered themselves to be sedentary, and if they participated in any regular, formal exercise), and was therefore only used at baseline and not used as an outcome measure. For this reason, accelerometry was not used as the only information that was required, was that which confirmed that the subjects were below the activity threshold that classified them as sedentary. If on the other hand, the protocol had included a random subject sample from the general population for comparison, it would have been necessary to establish exactly how active the participants were. Had the study included a home based exercise intervention, it would have been useful to include an objective measure of physical activity to determine compliance with the protocol (224), but in the current study, all of the exercise was supervised. The long form IPAQ questionnaire is considered to have acceptable validity (225), and although there is only modest correlation with objective measurements of physical activity ($r=0.33$), it is comparable to other self

reported methods, and has a high degree of test-retest reliability (r=0.8) (226, 227). The use of accelerometry could have added to the subject burden (228), when the protocol was already lengthy and included many invasive procedures. Patients with T2DM are traditionally a less compliant cohort of subjects and to obtain usable data from accelerometers would have relied on the subjects to remember to wear them each day, to put them back on if taken off at night or while washing, to reposition them correctly, and to return them in a timely manner at the end of the testing period so that they could be given to the next participant.

### 3.5 **Maximum oxygen consumption capacity testing**

A maximum oxygen consumption capacity ($VO_2$max) test was used to assess aerobic capacity and fitness. $VO_2$max testing is the gold standard measurement of aerobic capacity, from which all other fitness tests are extrapolated, with a coefficient of variation of 3.4% for $VO_2$max values (229). T2DM is a metabolic disorder characterised by abnormal nutrient metabolism, and $VO_2$max was therefore of interest as it is a measure of maximal metabolic capacity. It was also of interest at baseline as an index of physical capacity and activity status, as inactivity is correlated with metabolic and cardiovascular risk (209). One of the components that contributes to $VO_2$max is peripheral oxygen extraction, and it was of particular interest to compare $VO_2$max between groups as mitochondrial function contributes to both oxygen uptake and insulin sensitivity, with aerobic exercise acting as the primary stimulatory factor for mitochondrial biogenesis. The collection of this data would allow for further examination of correlations between $VO_2$max, insulin sensitivity and mitochondrial function. Despite the fact that it was expected that the $VO_2$max of the older group would

automatically be lower because of assumed age related differences in cardiac function, it was important to examine all factors that could contributed to the severe insulin resistance previously reported among young patients with T2DM (25). Similarly, $VO_2$max was of interest as an outcome measure to determine the effectiveness of the exercise intervention [as $VO_2$max is expected to increase in response to regular aerobic exercise (24)], in the context of which, other elements of physiological adaptation (or the lack thereof) could be interpreted.

Treadmill based $VO_2$max protocols often attach patients to ceiling-mounted harnesses to ensuring subject safety (230), but this is a difficult apparatus to install, and the ceilings of many building cannot support them. A bicycle ergometer was therefore used in the current study, which was also beneficial as the training was intended to be bicycle based to target the quadriceps muscle group, from which the muscle biopsy for mitochondrial isolation was to be obtained. The maximal progressive incremental test to exhaustion was performed in the exercise laboratory in St. James's Hospital by the study exercise physiologist, using a computer controlled electromagnetically braked medical assessment bicycle ergometer (Ergoselect 100, Ergoline, Germany). Subjects were instructed to be appropriately rested (not to have engaged in any vigorous physical activity the day before), and adequately fed and hydrated prior to testing. Before testing, subjects were familiarised with the test procedure and the bicycle ergometer. Subjects were instructed to maintain a cycling cadence of 70 to 80 revolutions per minute, so that consistent steady state heart rate and $VO_2$ data could be collected. The power output of the subject was kept constant throughout each stage of the test

by the ergometer apparatus which had the capacity to adjust the resistance applied to the flywheel to compensate for any fluctuation in cadence.

The same $VO_2$max test protocol was used for each subject during testing at baseline. The workload at level 1 was set at 50 Watts and was then increased by 25 Watts every 3 minutes until volitional exhaustion. If subjects performed well during the test and were able to continue cycling for an extended period, or if subjects appeared to progress well during the exercise training intervention, subsequent tests were individually redesigned to reduce the test time by increasing the initial load, and the resistance added at each subsequent level. The goal was for the test to last for 8 to 17 minutes (231), to avoid an excessive increase in body temperature and any resultant cardiovascular drift. To further assist with this, the windows in the exercise laboratory were kept open and the subjects were cooled with an electric fan throughout.

Blood pressure and heart rate were measured at rest (after resting for five minutes, seated in an upright position on the ergometer), and throughout the exercise test, at the end of each 3 minute level. Blood pressure was measured for safety reasons, and measurements were taken manually from the left arm with a sphygmomanometer and stethoscope. Testing was terminated if the subject's systolic blood pressure rose above 250mmHg, if their diastolic blood pressure rose above 120mmHg, or if they reported light headedness, chest pain or nausea.

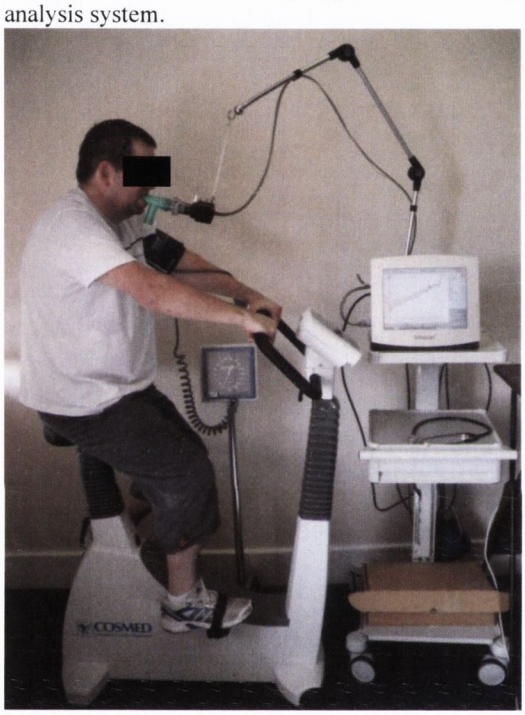
Heart rate was measured using a heart rate monitor chest strap sensor and watch receiver (Cardiosport Go Heart Rate Monitor, Healthcare Technology Ltd., UK). Ultrasound gel (Bluescan Ultrasound Transmission Gel, LiNA Medical, Denmark) was applied to the strap sensors to improve contact with the skin and to facilitate heart rate detection, with the strap worn immediately below the pectoral region. A heart rate figure was recorded during the last 15 seconds of each exercise stage, and the highest recorded figure towards the end of the test was considered the maximum heart rate (HRmax).

The subjects were encouraged verbally throughout the test, particularly if there was any reduction in cycling cadence. Cycling continued until volitional exhaustion e.g. until the subject complained of excessive shortness of breath or leg muscle fatigue. The test was considered maximal and the data usable if two of the following criteria were satisfied: a HRmax equal to or greater than 95% of predicted HRmax (220 - age), a respiratory exchange ratio (the ratio of carbon dioxide exhaled to oxygen consumed) greater than 1.1 (indicative of anaerobic metabolism and fatigue), or a levelling off in oxygen consumption in spite of further increases in power output. If the test did not meet these criteria, or if the subject stopped for any reason other than exhaustion, the test was repeated several days later.

Open-circuit indirect calorimetry was employed throughout the test and a system of respiratory gas analysis was used to measure ventilation (L/min), oxygen consumption ($VO_2$: L/min and ml/kg/min), and carbon dioxide production ($VCO_2$: L/min). An Innocor metabolic analysis system (INN00500, Innovision, Denmark) (Figure 3.3) was utilised, which allowed automated on-line breath-by-breath measurements to be made. While cycling, the subject

breathed in and out through a silicone rubber mouthpiece (Hans Rudolf, Inc. USA), which was attached to the apparatus, they wore a nose clip (Hans Rudolf, Inc. USA), and were instructed to maintain a good seal around the mouth piece with their lips throughout.

Figure 3.3. $VO_2$max testing apparatus, featuring an Ergoselect bicycle ergometer and Innocor metabolic analysis system.




Measurements of ventilation were made at the level of the mouthpiece using a pressure differential flowmeter (pneumotachometer) to measure respiratory flow and expired gas volumes. The pneumotachometer consisted of a cylinder through which the subject breathed, within which a nylon mesh formed a screen which provided a small, fixed, known degree of airflow resistance. The pressure on each side of the screen was measured and the reduction in pressure caused by the screen was used to calculate airflow. Simultaneous measurements of expired oxygen ($O_2$) and carbon dioxide ($CO_2$) concentrations were measured within the

main body of the Innocor machine by a laser diode absorption spectroscopy gas analyser. The gas sensor and flowmeter were calibrated prior to each test, and ambient room conditions (temperature, humidity and atmospheric pressure) were also recorded and taken into account (Appendix II). From the measurements of ventilation and expired gas concentrations, oxygen consumption and maximum oxygen consumption were determined, where oxygen consumption was taken as the difference between ambient $O_2$ and expired $O_2$ concentrations. Fifteen second $VO_2$ figure averages were calculated and the mean of the four highest consecutive values was taken as $VO_2max$.

### 3.6 **Blood Sampling**

Blood sampling was performed under fasting conditions, to standardize the procedure so that the underlying metabolic state could be examined without contamination from any recently ingested food. An intravenous cannula was inserted into the forearm of the subjects by the study physician, and blood samples were drawn by the study exercise physiologist. The samples taken after the exercise intervention were taken 48 hours after the last exercise session to limit the effects of acute exercise (22). Blood samples were taken for measurement of glucose, insulin, $HbA_{1c}$, C-peptide, GAD antibodies, HDL-cholesterol, serum triglyceride, nonesterified free fatty acids, leptin, adiponectin, and metabolomic examination of acylcarnitines, amino acids, and individual free fatty acids. All blood samples were sent straight to the local hospital laboratory for testing, with the exception of blood samples that were retained for examination of leptin, adiponectin, and metabolomics. These samples that were retained were centrifuged at 3000 rpm for 15 minutes at 4°C (Centrifuge 5702 R, Eppendorf, Hamburg, Germany). The supernatant was removed and placed in labelled

aliquot containers, which were stored in a freezer (Platinum 500 Freezer, AS Biomedical Division, Angelantoni Industrie, Italy) at -80°C.

### 3.7 **Oral glucose tolerance test**

After the initial blood sampling, subjects began a 2 hour oral glucose tolerance test (OGTT) by consuming a 200ml drink containing 87ml of water and 113ml of Polycal high energy carbohydrate liquid drink supplement (Nutricia Zoetermeer, The Netherlands), to provide a 75g glucose load. Subsequent blood samples were taken 30, 60, 90 and 120 minutes after ingestion of the drink. Insulin sensitivity was measured using oral glucose insulin sensitivity (OGIS) testing, from the OGTT data (53). This test was used as insulin resistance is one of the main features of T2DM and the precursor step to the establishment of disease, and a comparison of the severity of disease between groups was one of the main study objectives. The OGIS test is correlated with the gold standard euglycaemic–hyperinsulinaemic clamp technique ($r=0.77$, $p<0.0001$), and the coefficient of variation is 6.4% for clamp studies and 7.1% for OGIS (53). The OGIS technique was chosen as it does not require the infusing and continued titration of insulin, or the associated close medical supervision of clamp studies. Glucose data from minutes 0, 90 and 120, and insulin data from minutes 0 and 90, were imported into spreadsheets available on the World Wide Web at http://webmet.pd.cnr.it/ogis/. The spread sheets incorporated the following formula:

$$Cl_{OGTT} = p_4 \frac{\frac{p_1 D_0 - V[G(120) - G(90)]/60}{G(120)} + \frac{p_3}{G(0)}}{I(90) - I(0) + p_2}$$

- **$Cl_{OGTT}$**: glucose clearance from OGTT.
- **G(0), G (90), G(120)**: glucose concentration (mg/ml) at time 0min, 90min. and 120min respectively.
- **I(0), I(90)**: insulin concentration (μ/ml) at time 0min and 120min.
- **V**: glucose distribution volume, assumed: 10 $l/m^2$.
- **$D_0$**: oral glucose dose ($g/m^2$).
- **P values**: parameters. For SI units. $p_1$=2.89, $p_2$=1618, $p_3$=779, $p_4$=2642.

To facilitate further examination of diabetes status, other calculations performed to examine insulin resistance included the homeostatic model assessment (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI), which is the log of HOMA-IR (232). HOMA is correlated with the clamp technique ($r= -0.75$, $p<0.0001$), as is QUICKI (53). The formulae used are as follows:

$$\text{HOMA} = \frac{\text{Fasting Glucose (mmol/L) x Fasting Insulin (μU/mL)}}{22.5}$$

$$\text{QUICKI} = \frac{1}{\text{Log (Fasting Insulin, pmol/L)} + \text{Log (Fasting Glucose, mmol/L)}}$$

Fasting β-cell function was assessed as the ratio of glucose to insulin [insulin (μU/ml) / glucose (mmol/l)]. Insulin secretion was examined as insulin concentration area under the curve (AUC) during the OGTT, and dynamic β-cell function was calculated using the insulinogenic index (IGI), as AUC for insulin divided by AUC for glucose. To take insulin resistance into account during the measurement, the disposition index was also used (OGIS x IGI).

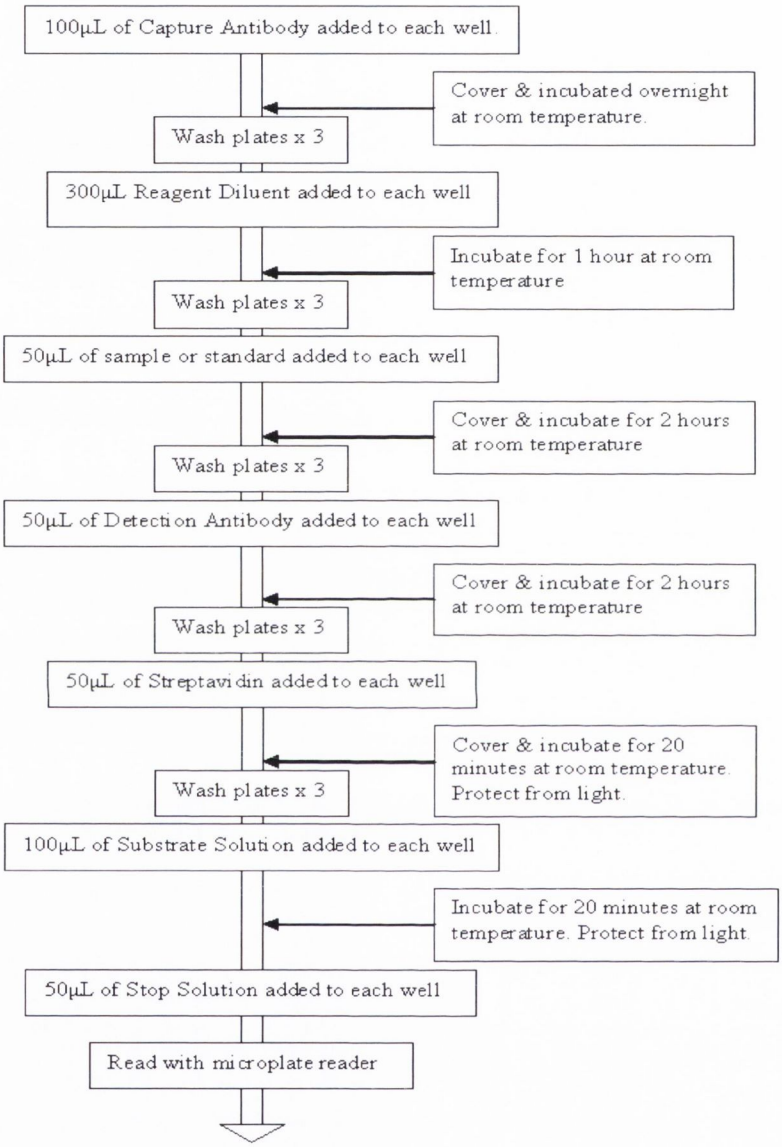
### 3.8 **Glucose, insulin and lipid testing**

Glucose, insulin, $HbA_{1c}$, C-peptide, GAD antibodies, HDL-cholesterol, and serum triglyceride were measured by the staff working in the St. James's Hospital biochemistry laboratory, using hospital grade equipment and diagnostic testing protocols. Glucose measurement is one of the most relevant measurements of the study as T2DM is characterized by hyperglycaemia, and one of aims of the study was to compare the severity of diabetes between group, using glucose to examine current disease status, and $HbA_{1c}$ to examine glycaemic control over the previous 4 to 12 weeks. Lipid measurements (standard and metabolomic) were of interest to determine their contribution to lipotoxic conditions. HDL-cholesterol, serum triglyceride and nonesterified free fatty acids were some of the main lipids of interest, as it had previously been shown there were no differences in LDL and total cholesterol between YT2 and OT2 subjects (18). A glucose oxidase technique was used to measure plasma glucose concentration (bio Merieux kit; Hitachi Modular), while commercially available flouroimmunoassays were used to measure serum insulin and C-peptide (Auto-Delfia, Wallac-Oy, Finland). A $HbA_{1c}$ analyser (Hi-Auto A1c HA 8140; Menarini, Florence Italy) was used to quantify $HbA_{1c}$ percentage, and a direct radioligand assay was used to test for GAD antibodies. Enzymatic methods were used to test for plasma triacylglycerols (Human liquicolor kits; Hitachi Modular; Roche Diagnostics, Basel Switzerland) (25), and HDL-cholesterol (Randox direct kits; Hitachi Modular). A spectrophotometric assay was used to test for serum free fatty acids (Randox Laboratories, Antrim, UK) (25).

## 3.9 **Adipokine ELISA procedures**

The enzyme-linked immunosorbent assay (ELISA) procedure (Figure 3.4) was used to test serum samples for adipokines, and was performed by the study physician and exercise physiologist. As obesity and lipotoxicity are considered to be key features of T2DM, adipose tissue derived cytokines were identified as candidate biomarkers of interest to provide another avenue of investigation in an attempt to identify possible contributory causes for the earlier onset of disease in the YT2 group. Leptin and adiponectin were selected as their function is thought to relate to satiety and insulin sensitivity respectively. Commercially available assay kits were used for the detection of adiponectin (Human Adiponectin, DuoSet ELISA Development System, Catalogue No. DY1065, R&D SYSTEMS, Minneapolis), and leptin (Human Leptin, DuoSet ELISA Development System, Catalogue No. DY398, R&D SYSTEMS, Minneapolis). The validity and reliability of ELISA results are dependent upon adherence to the correct experimental technique, and so the manufacturers kit instructions were strictly adhered to. Pipettes were calibrated in advance of use, and pipette technique was practised extensively in advance of testing. The Capture Antibodies for each of the proteins for detection were reconstituted and diluted in phosphate buffered saline to their respective working concentrations, and added to coat each well of an ELISA microplate (96 Well ELISA Microplates with F-Bottom/ST, Crystal-Clear, PS, MICROLON 600 High Binding, Catalogue no. 655061G, Greiner Bio-One, Germany). The plates were covered with adhesive plastic and incubated at room temperature overnight. Between each step, the plates were decanted and washed 3 times with wash buffer [a mild detergent composed of 250μL Tween 0.05%, 50ml PBS(x10), 450ml deionised water per wash bottle] using a laboratory squirt bottle. All residual fluid was removed from the wells by blotting with paper towels.

Figure 3.4. Adiponectin assay procedure.




A non-reacting protein (Reagent Diluent: 1% bovine serum albumin, in PBS) was added to block any uncoated plastic that would act as a potential nonspecific binding site within the well. The plates were incubated for 1 hour at room temperature, before the addition of either serum samples or Standard in triplicate. The Standard was diluted through a process of serial

dilution to form a standard curve. The plates were covered and incubated for 2 hours at room temperature, before the addition of an enzyme-linked Detection Antibody. This was followed by the addition of enzyme labelled streptavidin and Substrate Solution, which activated the bound detection antibody, producing a colour change in proportion with the concentration of the bound antigen. The colour change development was halted by the addition of a Stop Reagent (sulphuric acid; $H_2SO_4$ 7.7%), before the optical density of the colour was determined using a microplate reader (VersaMax, Molecular Devices LLC, USA). The standard curve was used to calculate the protein concentration in each sample.

### 3.10 **Metabolomics**

Metabolomic examination of blood plasma was performed in the laboratory of one of the study collaborators: Professor Chris Newgard at Duke University Medical Centre (the Department of Pharmacology and Cancer Biology, Duke University, Durham, North Carolina, USA). The use of metabolomic studies provided the opportunity to look for additional biomarkers contributing to lipotoxic conditions, beyond the standard lipids tested for as part of routine hospital based screening. Metabolomic examination was performed using targeted tandem mass spectrometry (MS/MS) (Quattro Micro instrument) and gas chromatography/mass spectrometry (GC/MS) (Trace DSQ instrument, Thermo Electron Corporation, Texas), to examine a series of fatty acids, acylcarnitines, and amino acids, which had been identified as candidate biomarkers correlating with insulin resistance (112). This testing procedure is considered reliable with coefficients of variation in replicate assays of major analytes of less than 15% (114). Amino acids and acylcarnitines were removed by precipitation with methanol. For acylcarnitines the supernatants were dried and esterified

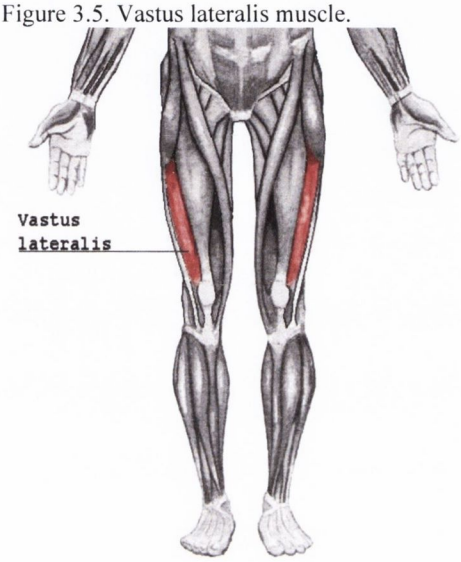
with hot acidic methanol, while for amino acids the supernatants were dried and esterified with hot n-butanol. Nonesterified free fatty acids were measured by methylating plasma samples with iodomethane and purifying using solid-phase extraction. Analysis was performed by capillary GC/MS. For the measurement of total fatty acid species, fatty residues from plasma samples were transesterified in a solution of 4% w/v acetyl chloride in methanol, before analysing using GC/MS. Stable-isotope-dilution was used in each case, whereby stable-isotope internal standards were added to facilitate quantification, by pairing unknown metabolites with their labelled match (113).

### 3.11 **Muscle biopsy**

Muscle biopsies were taken from the distal aspect of the vastus lateralis muscle (Figure 3.5) (by the study physician, assisted by the exercise physiologist), so that intrinsic mitochondrial oxidative capacity could be examined, as impaired mitochondrial function is though to contribute to the lipotoxic conditions that lead to insulin resistance. A baseline comparison between groups, and the response of each group to lifestyle intervention was therefore of interest. The vastus lateralis muscle was chosen as the site for the biopsy as it is a large assessable muscle that can be easily exposed to exercise, with no major arteries or nerves running through it. Measurement of mitochondrial enzyme activity is often used to estimate mitochondrial capacity, but this is a surrogte appoximation only, while MRS can be used to reliably measure phosphocreatine recovery half-time as an index of mitochondrial capacity (85), but this technique cannot distinguish between results that are influenced by mitochondrial density, or oxidative capacity per unit volume of mitochondria. The use of respirometry was therefore chosen as it is the gold standard technique and directly measures

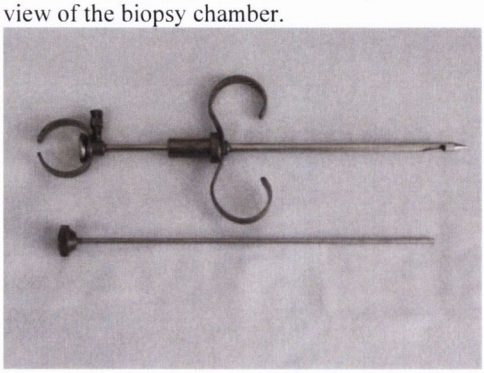
oxidative capacity in isolated mitochondria. Furthermore high resolution respirometry was used to ensure that the reliability and validity of the results (233).

Figure 3.5. Vastus lateralis muscle.

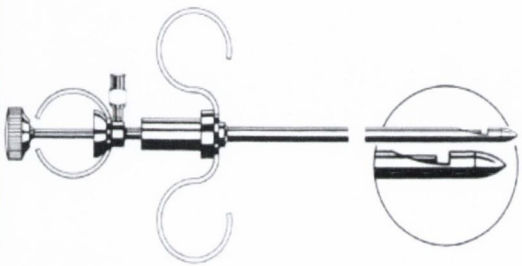



Subjects fasted overnight prior to the procedure. Subjects wore shorts to provide access to the leg, the target incision site was identified, and the area was shaved and cleaned with iodine. Sterile equipment was used and an aseptic technique was adhered to throughout. The biopsy was taken under local anaesthetic using 5 - 7ml of 1% w/v Lidocaine (Lidocaine Hydrochloride, Braun Medical Ltd., Ireland). When the site was sufficiently anaesthetised, an incision was made with a size 10 disposable scalpel (Swann-Morton Ltd., Sheffield, England) approximately 0.5cm in length. A 5mm x 120mm percutaneous biopsy needle (Popper and Sons, Inc., USA) (Figure 3.6), was inserted through the guide incision and into the muscle.

Figure 3.6a and 3.6b:
3.6a. Muscle biopsy needle, featuring inner cutting cylinder. 3.6b. Muscle biopsy needle, featuring a close-up view of the biopsy chamber.



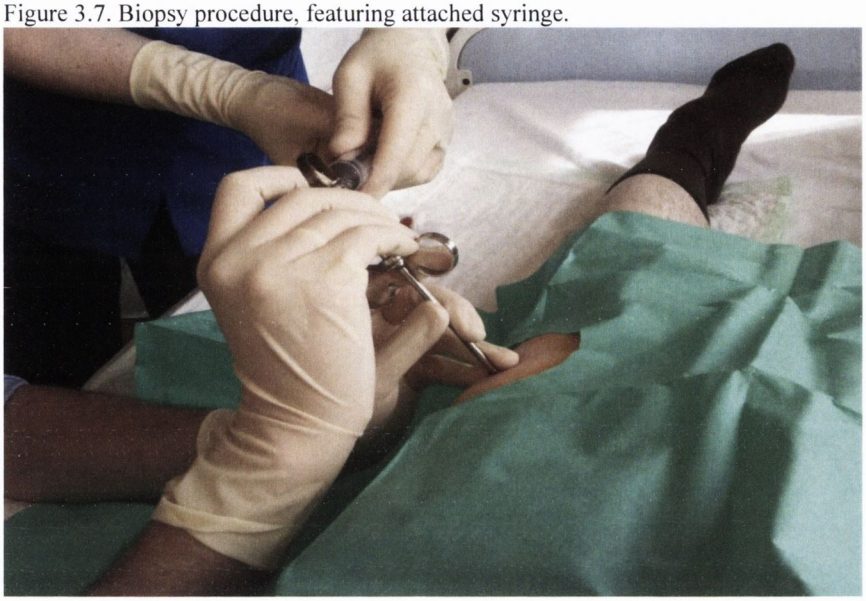
a.



b.

The inner cutting cylinder of the needle was drawn up several centimetres to open the biopsy chamber window. The surrounding muscle was compressed manually against the needle, and a 20ml syringe (BD Plastipak, Ireland) was attached to the suction port of the needle and used to generate a vacuum (Figure 3.7), to allow a larger muscle sample to be drawn into the chamber (213). While the suction was being applied, the inner cylinder of the needle was pushed down sharply to close the window and to cut the sample. The needle was rotated several degrees and the procedure was repeated to allow cutting from a different angle. Approximately 100mg of muscle tissue was obtained on each occasion.

Figure 3.7. Biopsy procedure, featuring attached syringe.



Afterwards, the surrounding skin was cleaned with sterile water, and the incision site was sealed with two Steri-Strips (6mm x 38mm Steri-Strips, 3M Health Care, USA). This was covered with sterile gauze, wrapped in elastic crepe bandage (7.5cm x 4.5m bandage, Novalast, Midland Bandages Ltd, Ireland), and secured with a dressing clip, to reduce swelling. The biopsy needles were autoclaved after use. Biopsies taken at subsequent visits were taken as close to the original site as possible. The biopsy taken after the exercise intervention was taken 48 hours after the last exercise session so that the acute effects of exercise would not be confused with the chronic effects of the intervention.

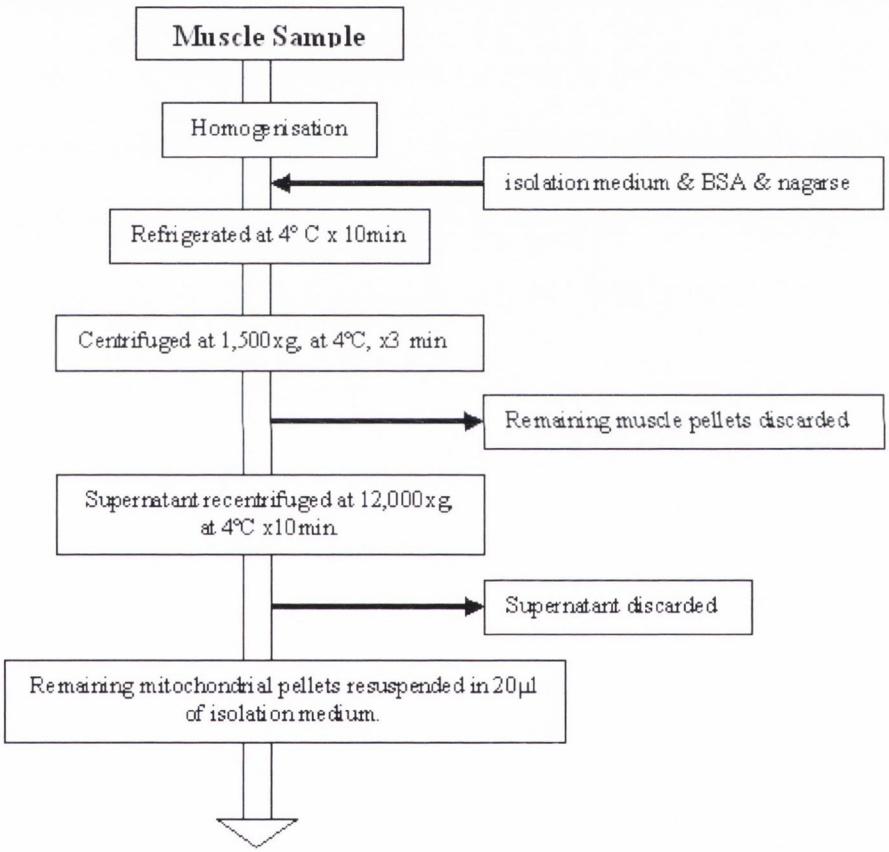
## 3.12 **Preparation of mitochondria from muscle biopsy samples**

Mitochondrial testing was conducted using fresh muscle in a biochemistry laboratory offsite, and was performed by the study physician and exercise physiologist. The muscle was placed

in an eppendorf tube containing a chilled buffering isolation medium [100mM sucrose, 9mM ethylenediaminetetraacetic acid (EDTA), 100mM Trizma, 46mM KCl], packed in ice as it was transported, and tested within 2 hours of acquisition, to ensure that any sample degradation was kept to a minimum before processing. To examine intrinsic mitochondrial oxidative capacity, the mitochondria were isolated from the muscle sample (Figure 3.8). The sample was placed on a glass tile that had been chilled with ice and that contained several drops of chilled isolation medium solution, containing 0.5% bovine serum albumin (BSA) and 0.02% nagarse (bacterial proteinase, Type XXIV). The sample was finely chopped with a scalpel blade and divided into two eppendorf tubes, each containing 1ml of the above isolation medium solution, and placed in a fridge on a roller at 4°C for ten minutes. The samples were then spun in a centrifuge at 1,500 x g, at 4°C, for three minutes, after which any BSA or fat that had accumulated at the top of the eppendorf tubes were removed with a spatula. The supernatant was then removed with a pipette, placed in two new eppendorf tubes and stored on ice. The process was repeated by adding 1ml of isolation medium solution to each of the remaining muscle pellets. The pellets were disrupted using a vortex shaker and were refrigerated on a roller and centrifuged as before. The supernatant was again removed and two additional eppendorf tubes were filled. The remaining muscle pellets were discarded. The four eppendorf tubes were centrifuged at 12,000 x g, at 4°C, for ten minutes. The supernatant was discarded and the remaining mitochondrial pellets were retained. The pellets were resuspended in 20 µls of isolation medium (without the addition of BSA or nagarse) and combined together. This solution was divided into two eppendorf tubes, each containing 1 ml of isolation medium that did not contain proteinase, and the solution was "washed" by re-centrifuging at 12,000 x g, at 4°C, for ten minutes, to remove any remaining

nagarse, to maintain the integrity of the inner mitochondria membrane. The supernatant was discarded, and the mitochondrial pellets were resuspended in 20 µls of isolation medium. The mitochondrial protein concentration of the sample was measured by comparison with the results of a standard curve generated from known concentrations of BSA.

Figure 3.8. Mitochondrial isolation

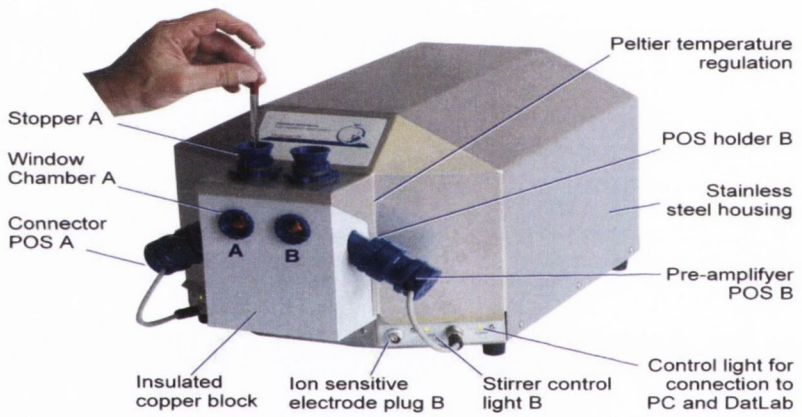



## 3.13 **Respirometry**

High resolution respirometry was used to examine intrinsic mitochondrial function, using an Oxygraph-2k respirometer, (OROBOROS INSTRUMENTS, Austria) (85) (Figure 3.9). Prior to testing, both chambers of the respirometer were washed with 70% ethanol in triplicate, and

then washed with deionised water in triplicate to increase the validity of the results by ensuring that there was no residual material in the analyser from previous tests, and to provide a standardized sterile environment for testing. After the deionised water was removed from the chambers, 2mls of Oxygraph buffer (KHE assay medium: 120mM KCl, 5mM Hepes, 1mM EGTA, 2mM Pi, 2mM $Mg^{2+}$, 0.1% BSA) was added to each, and the system was calibrated using 100% oxygen.

Figure 3.9. Oxygraph-2k respirometer.




The mitochondria were added (17µl of mitochondrial supernatant) to both chambers (to facilitate testing in duplicate and to increase the reliability of the results) and allowed to stabilise for ten minutes, for basal rates of oxygen consumption to be recorded. This was followed by the addition of a series of different substrates and inhibitors, to examine various enzymatic pathways and different aspects of oxidative phosphorylation (Figure 3.10). Twenty microlitres of pyruvate and malate (10mM) were added and allowed to react for 15 minutes. Pyruvate acted as the first substrate, and malate (an intermediate of the tricarboxylic acid cycle) was added as it becomes diminished by the process (99). Complex II (succinate

dehydrogenase) was inhibited by this preparation, allowing oxygen flux through Complex I (NADH dehydrogenase) to be examined (State 2 respiration). 20 µls of Succinate (10mM) was added and allowed to react for 15 minutes. Succinate, another intermediate of the tricarboxylic acid cycle, was added to activate Complex II, allowing both Complex I and II to be examined (85). 2 µls of Rotenone (1µM) was added and the solution was allowed to react for a further 15 minutes. Rotenone inhibited Complex I so that activity through Complex II could be examined independently (234). 2 µls of adenosine diphosphate (ADP) (10µM) was added, until the rate of oxygen consumption returned to the level it had been at prior to the addition of Rotenone. A further 2 µls of ADP (100µM) was then added and allowed to react for 10 minutes. This caused a sharp increase in oxygen flux, producing maximal physiological respiration (State 3) (85, 99) (Figure 3.11). 1 µl of oligomycin (1µg/ml) was added and allowed to process for a further 10 minutes. Oligomycin inhibits ATP synthase, reducing oxygen consumption, and demonstrating the degree to which ATP production was coupled with oxygen consumption (85). Finally 2 µls of Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (100nM) was added and recordings were made until no further increase in oxygen consumption occurred. FCCP was added as an uncoupling agent (85), uncoupling respiration from the limitations imposed by the process of phosphorylation, and bringing about maximal electron transport chain activity and a large unphysiological increase in oxygen flux. Some study protocols induce State 3 respiration immediately, before the addition of other substrates, as State 3 and maximally uncoupled respiration are the conditions of most interest (99), but the stimulation/inhibition protocol used in the current study allowed a more sensitive examination of the individual enzymes of the electron transport chain to take place, before maximal stimulation was brought about

(85). Oxygen consumption was calculated relative to the protein concentration of the sample (normalised per milligram of mitochondrial protein). During all procedures, testing was conducted at 37° to replicate *in vivo* conditions as closely as possible, and the substrates were used in saturating concentrations with oxygen levels kept elevated to ensure that these factors did not impose any limitations upon respiration.

Figure 3.10. Respirometry protocol.

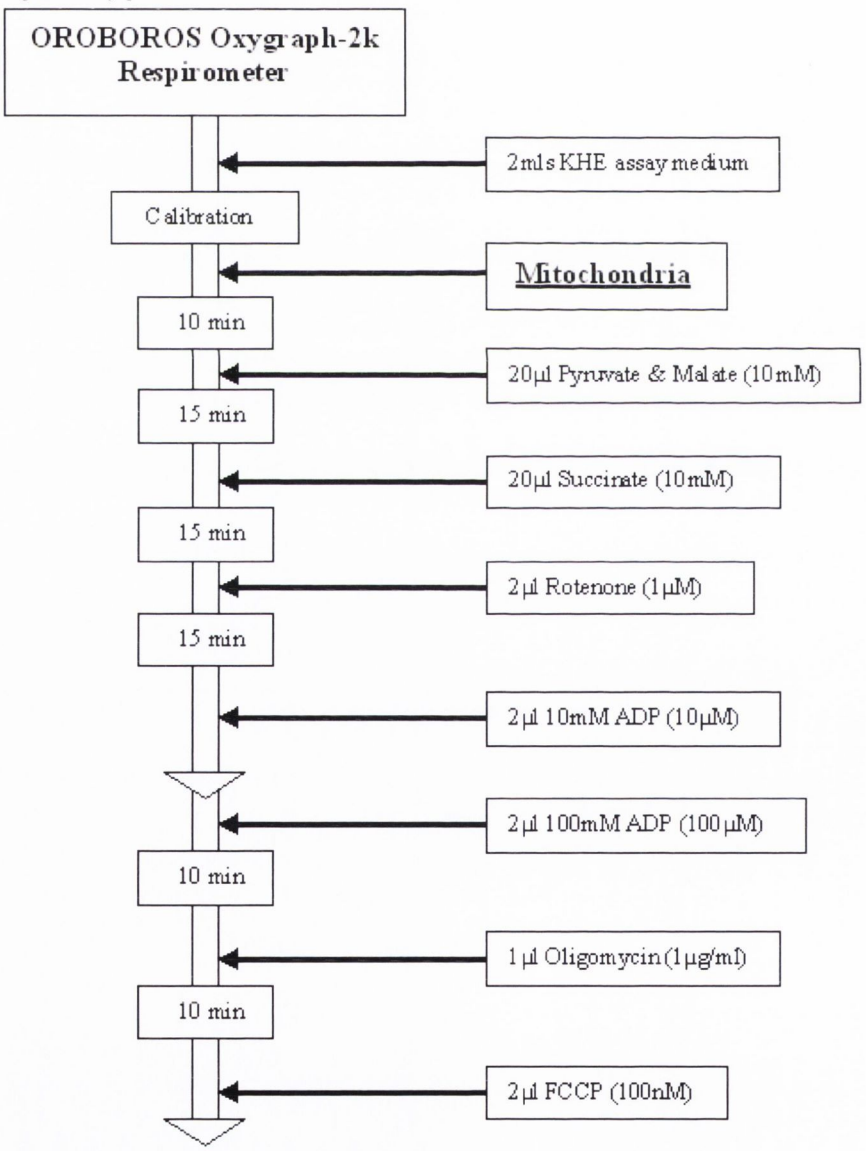
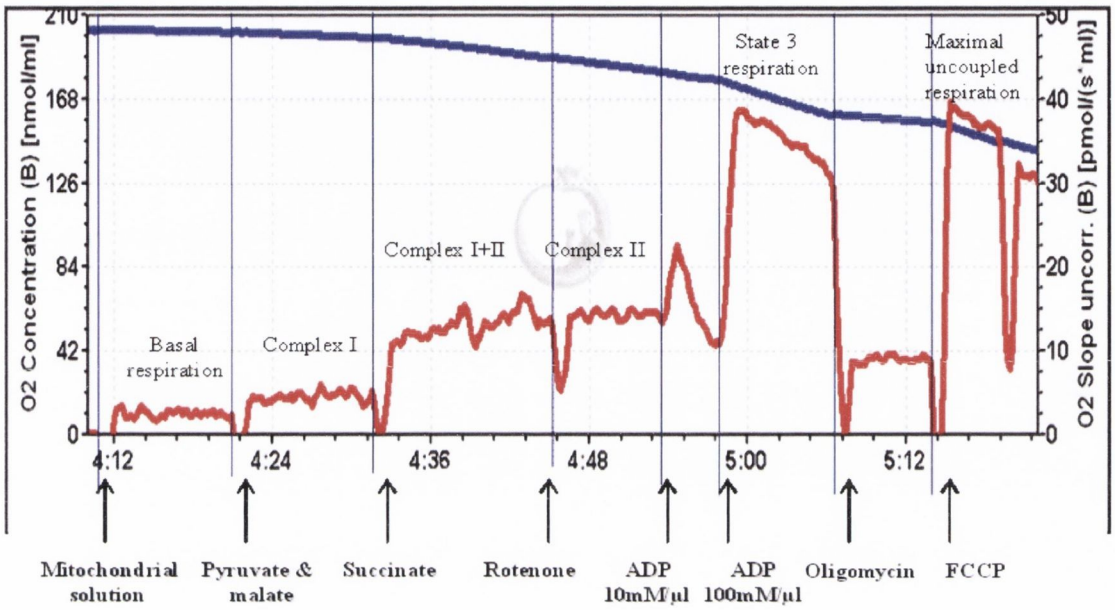


Figure 3.11. Oxygraph trace.




## 3.14 **Exercise intervention**

During the exercise intervention, subjects trained 4 times per week for 3 months at an intensity corresponding with 70% of their $VO_2$Max, expending 625 kcal per session (-2500 kcal per week). All training sessions were supervised by the study exercise physiologist, and performed in an exercise laboratory in St. James's Hospital. A minimum of two thirds of the training time per session was spent exercising on a stationary bicycle ergometer (Stratus System 3300 CE, Stair Master Sports / Medical Products, Inc. Washington, USA), and approximately one third of the time was spent exercising on a treadmill (Trotter, 645 CR, Massachusetts, USA). The emphasis of the training was placed on cycling in an attempt to specifically target the muscle group involved in the biopsy (the quadriceps muscles), and as the $VO_2$max test was bicycle based. The treadmill was used to

break up the bicycle ergometer training while continuing to provide an exercise stimulus of the same intensity. The target cycling workload and training heart rate were obtained by extrapolation from graphed $VO_2$max test data. To calculate the target treadmill speed in kilometres per hour, the American College of Sports Medicine's training formula was used (235):

$$\textbf{[Speed][0.1] + [Speed][Percentage Gradient][1.8] = [VO}_2\textbf{ (ml.kg}^{-1}\textbf{.min}^{-1}\textbf{) – 3.5][0.0597]}$$

The training time per session varied between subjects and was dependent on their absolute rate of energy expenditure. Energy expenditure per minute was calculated for exercise at 70% of each individual's $VO_2$max, and was used to determine the time required to expend 625kcal. Those with a higher $VO_2$max exercised at a higher absolute power output while exercising at 70% $VO_2$max, and expended the 625kcal per session more quickly. The training time also incorporated and accounted for the energy expenditure associated with a 5 minute warm up and a 5 minute cool down at approximately 50% $VO_2$max. Metabolic equations that estimate oxygen consumption during exercise can overestimate energy expenditure (236), so it is more beneficial to use direct measures of oxygen consumption when they are available, to reduce the degree of estimation and extrapolation. The formula used to calculate energy expenditure was a derivative of the Weir equation (237) and incorporated the individualized oxygen consumption and carbon dioxide production values corresponding with exercise at 70% maximum capacity, obtained during $VO_2$max testing:

$$\textbf{kcal = 3.941 x O}_2\textbf{ (litres) + 1.106 x CO}_2\textbf{ (litres)}$$

On the first day of training the validity of the calculated target work load and the associated rate of energy expenditure were confirmed by performing a measurement of steady-state oxygen consumption and carbon dioxide production using the Innocor metabolic analysis system as the subjects cycled while wearing a heart rate monitor. After the subjects had been exercising for 15minutes, a 10 minute gas sample was recorded and the corresponding heart rate noted. The exercise load was then manipulated slightly if necessary to ensure that the rate of oxygen consumption was at 70% $VO_2$max, and that the planned training time would yield the correct volume of energy expenditure (625kcal). Heart rate data was monitored during subsequent training sessions.

In addition to the $VO_2$max tests performed at baseline, between interventions and at the end of the study, two supplementary $VO_2$max tests were also performed during the exercise intervention: one at week 4, and another at week 8. The data these tests provided was used to formally adjust the exercise workload if any training related improvements in fitness occurred (238), to ensure that the training stimulus remained optimal, and was maintained at 70% of a potentially ever increasing $VO_2$max. If there was an increase in $VO_2$max and the training workload was increased, and the trained time was recalculated and reduced. There are a number of factors that influence energy expenditure rate, including gender, age and weight, but for the most part, the majority of these differences can be accounted for by difference in FFM (239). High intensity exercise, such as that employed in the current study is often associated with an increase in FFM (186), and a reduction in fat mass, which would affect energy expenditure rate, but by reassessing $VO_2$max and normalizing to body weight, and by taking direct measurements of steady-state oxygen consumption during exercise,

these variables were taken into account. Prior to the exercise intervention, the subjects were instructed to maintain an unchanged dietary intake throughout, and they were provided with food diaries to fill out every two weeks during the intervention. Subjects who were assigned to the exercise arm of the study first, were also instructed to return to their pre-study sedentary level of activity upon completion of the intervention and commencement of the dietary intervention.

### 3.15 **Dietary intervention**

During the 3 month dietary intervention, subjects were met every two weeks by the research dietician on the study, they were weighed and received nutritional advice to assist them to reduce their caloric intake by 357 kcal per day (-2500 kcal per week), to match the energy expenditure of the exercise intervention. In order to create an energy deficit by applying a calorie restriction, daily calorie requirements were first established, using the Schofield equation (240) to estimate basal metabolic rate (Table 3.1). Total energy requirements were calculated by multiplying estimated BMR by a coefficient to take into account activity status (Table 3.2). Advice was sought from a research dietician, and a reduced calorie, low fat, high fibre diet was promoted among subjects, in which specific nutrient intake, food portion size and cooking methods were addressed. Meal plans were discussed with participants, based on individual food preferences. The subjects were provided with three-day food diaries (MRC Human Nutrition Research food diary, UK), which were to be completed once every two weeks, and to include details of nutrient intake on two midweek days and one weekend day. The returned diaries were processed using dietary analysis software (WISP, version 3, Tinuviel software, United Kingdom) (241), and the subjects were instructed based on the

data generated. The subjects who were assigned to the dietary arm of the study first were instructed not to change their physical activity habits during the intervention, and to increase their nutritional intake back to the pre-study level at the time of transition to the exercise intervention.

Table 3.1. BMR (kcal) equations. *Schofield WN. Hum Nutr Clin Nutr. 1985; 39; Suppl 1: 5–41* (240).

| **Age** | **Male** | **Female** |
|---|---|---|
| **18 - 29 years** | 15.1 x Weight (kg) + 692 | 14.8 x Weight (kg) + 487 |
| **30 - 59 years** | 11.5 x Weight (kg) + 873 | 8.3 x Weight (kg) + 846 |

Table 3.2. Activity coefficients. *Schofield WN. Hum Nutr Clin Nutr. 1985; 39; Suppl 1: 5–41* (240).

| **Activity Status** | **Male** | **Female** |
|---|---|---|
| **Sedentary** | 1.3 | 1.25 |
| **Lightly active** | 1.6 | 1.5 |
| **Moderately active** | 1.7 | 1.6 |
| **Very active** | 2.1 | 1.9 |

### 3.16 **Statistical analysis**

Statistical analysis was performed by the author, using SPSS statistical software (version 16). The data are expressed as Mean ± the Standard Error of the Mean (SEM). A Shapiro-Wilk test was used to test data set distribution, with values greater than 0.05 confirming the null hypothesis that the data came from a normally distributed population. Differences between groups were compared using unpaired *t*-tests for normally distributed data and Mann–Whitney U tests for nonparametric data. To examine the effect of the intervention, and the interaction of age with intervention, a two-way repeated measures analysis of variance (ANOVA) was used, with time used as the within-subject factor (including 2 levels: pre and post intervention data), and age (or group) used as the between-subject factor. To examine the effect of the intervention in each group independently, paired *t*-tests were used for normally distributed data and Wilcoxon signed rank tests for nonparametric data.

Correlations were performed using Pearson and Spearman tests for normally distributed and nonparametric data respectively. Statistical significance was set at $p<0.05$.

# Chapter Four:

## Experiment 1: Early-Onset T2DM: Physical Characteristics and Anthropometric Adaptation to Lifestyle Intervention.

## 4.1 **Introduction**

The prevalence of early-onset T2DM is increasing as is the prevalence of obesity. While it has been suggested that early-onset T2DM may be a unique phenomenon and different from the more usual later presentation of T2DM, some obvious anthropometric differences existed between groups in previous studies which could partly explain these differences and the earlier onset of disease in the younger group, e.g. differences in waist circumference. Similarly, it has been reported that patients with early-onset T2DM are not as responsive to lifestyle intervention and do not demonstrate the weight loss that would be expected. It is not clear whether this lack of adaptation represents metabolic deficiency, suboptimal lifestyle intervention protocols, a lack of adherence, or a combination of these factors.

## 4.2 **Aims**

The aim of this experiment was to compare anthropometric measurements between patients with early-onset T2DM (YT2) and later-onset T2DM (OT2), and to examine the effect of a 6 month crossover lifestyle intervention, including a separate 3 month dietary intervention and a 3 month supervised, progressive exercise intervention. Additional objectives were to examine the effect of the intervention sequence, and to determine which individual intervention was most beneficial.

## 4.3 **Results**

### 4.3.1 Baseline

A total of seventy-three subjects were screened from diabetes clinics in St. James's Hospital for inclusion in the study, of which ten subjects were excluded: 3 were found to have T1DM, 1 had polycystic ovary syndrome, and 6 did not meet the age requirement. The remaining sixty-three subjects with T2DM were recruited for baseline examination: 23 YT2 and 40 OT2 subjects (Table 4.1). In the YT2 group, 26% of the subjects were female, while in the OT2 group 24% of the subjects were female. There were no significant differences in body weight, BMI, body fat percentage, fat mass or FFM between groups at baseline (Table 4.1). Although there was no significant difference in waist or hip circumference between groups, the YT2 group had a slightly lower WHR than the OT2 group (0.98±0.01 vs 1.02±0.01, $p$=0.002). An additional 30 obese control subjects were recruited for baseline examination: 14 YOb and 16 OOb subjects. There was no significant difference in age between the YT2 and the YOb group (27.9±0.9 vs 24.7±1.5 years, $p$=ns), or between the OT2 and the OOb group (57.2±1.2 vs 54.5±1.5 years, $p$=ns). There was no significant difference between the BMI of the YOb (36.7±1.5) or the OOb group (33.4±0.9), when compared with the T2DM groups. Similarly, the T2DM subjects did not have a greater body fat percentage than the non-T2DM subjects.

Table 4.1. Baseline anthropometric data. BMI: body mass index, FFM: fat free mass, WHR: waist to hip ratio, NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 | OT2 | YT2 v OT2 |
|---|---|---|---|
| **N** | **23** | **40** | |
| **M : F** | **17 : 6** | **31 : 9** | |
| **Age** | **27.9** (0.9) | **57.2** (1.2) | **0.01** |
| **Height (cm)** | **174.9** (2.4) | **168.2** (2.1) | **0.04** |
| **Weight (kg)** | **110.2** (5.0) | **102.4** (2.4) | NS |
| **BMI (kg/m2)** | **36.3** (1.9) | **36.9** (1.5) | NS |
| **Body fat %** | **36.1** (2.1) | **37.9** (1.1) | NS |
| **Fat Mass (kg)** | **41.1** (4.0) | **38.7** (1.6) | NS |
| **FFM (kg)** | **68.7** (2.5) | **63.1** (1.7) | NS |
| **Waist (cm)** | **113.7** (3.4) | **114.8** (1.6) | NS |
| **Hip (cm)** | **114.9** (3.0) | **112.8** (1.5) | NS |
| **WHR (cm)** | **0.98** (0.01) | **1.02** (0.01) | **0.002** |

There was no difference in daily energy intake between the T2DM groups at baseline (Table 4.2). The nutrient proportion of total energy intake for the YT2 group was 45.4±1.6% carbohydrate, 38.7±1.6% fat, and 15.9±0.8% protein, and for the OT2 group was 41.9±1.4% carbohydrate, 40.4±1.2% fat and 17.7±0.5% protein, with no difference between groups. In both cases this is a lower carbohydrate, and a higher fat intake than the recommended proportions (55% carbohydrate, 30% fat and 15% protein). While this is likely to merely reflect an unhealthy diet, there is the possibility that these patients were trying to adhere to a previously prescribed diabetes diet, reducing their carbohydrate intake in an attempt to control their blood glucose concentration. There were also no differences in saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, sugar, starch, or fibre intake between groups. There was no significant difference between the calorie intake or proportion of nutrient intake between the T2DM subjects and the non-T2DM subjects.

Table 4.2. Baseline nutrient intake. Total energy intake, Carbohydrate, Fat and Protein intake. NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 (n = 23) | OT2 (n = 40) | YT2 v OT2 |
|---|---|---|---|
| **Calories (kcal/day)** | **2256.7** (221.3) | **2371.8** (167.2) | NS |
| **Carbs (g/day)** | **256.3** (24.4) | **238.3** (16.1) | NS |
| **Fat (g/day)** | **101.0** (13.4) | **101.6** (6.3) | NS |
| **Protein (g/day)** | **86.6** (6.9) | **99.9** (5.6) | NS |

4.3.2 Six month effect of combined interventions

Of the 63 T2DM subjects recruited for the study, 21 either agreed only to participate in baseline examination or dropped out after baseline testing (5 YT2 and 16 OT2), 5 dropped out during the first intervention (4 YT2 and 1 OT2), 8 dropped out upon completion of the first intervention (4 YT2 and 4 OT2), and 4 dropped out during the second intervention (4 OT2). There were no anthropometric differences between those who dropped out and those who completed the intervention. The remaining 25 subjects completed both the exercise and dietary intervention over a 6 month period: 10 YT2 and 15 OT2 subjects. One of the objectives of the lifestyle intervention was to examine the priming effect of intervention sequence, to determine if exercise followed by diet (ED) or diet followed by exercise (DE) was more effective. This complex design created multiple subgroups reducing the power of the resultant ANOVA analysis, which did not show any significant differences between groups. To explore the results further, all individual pre-post intervention subgroup results were analysed using paired *t*-tests, or the nonparametric equivalent where appropriate (Table 4.3). Four YT2 and 8 OT2 subjects completed the 6 month intervention, participating in the exercise intervention prior to the dietary intervention, while another 6 YT2 and 7 OT2 subjects completed the dietary intervention prior to the exercise intervention.

Table 4.3. Anthropometric data pre and post 6 month intervention, incorporating intervention sequence. ED: Exercise intervention performed before dietary intervention. DE: Dietary intervention performed before exercise intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$*.

| | YT2 | | | | OT2 | | | |
|---|---|---|---|---|---|---|---|---|
| | ED | | DE | | ED | | DE | |
| N | 4 | | 6 | | 8 | | 7 | |
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Weight (kg) | 100.7 (7.6) | 96.8 (6.4) | 131.8 (12.3) | 126.3* (12.3) | 97.1 (4.7) | 93.6* (4.9) | 102.5 (6.7) | 96.8* (7.8) |
| BMI (kg/m2) | 30.7 (1.8) | 29.6 (1.7) | 42.5 (4.9) | 40.7* (4.9) | 31.8 (1.7) | 30.6* (1.5) | 36.7 (1.9) | 34.6* (2.3) |
| Body Fat % | 29.3 (2.6) | 27.3 (2.5) | 41.9 (3.8) | 40.4 (4.2) | 35.1 (3.3) | 33.7 (3.3) | 43.3 (2.2) | 41.5 (2.8) |
| Fat Mass (kg) | 30.0 (4.3) | 26.6 (3.6) | 58.5 (10.9) | 54.6 (10.6) | 34.6 (4.1) | 32.1* (4.1) | 44.7 (4.8) | 41.0 (5.6) |
| FFM (kg) | 70.7 (3.8) | 70.1 (4.2) | 75.8 (3.9) | 74.5 (4.3) | 62.4 (3.1) | 61.5 (3.1) | 57.8 (3.1) | 55.9 (3.5) |
| Waist (cm) | 108.3 (5.2) | 100.3 (4.7) | 119.4 (7.3) | 115.0* (7.0) | 108.8 (3.4) | 105.4* (3.4) | 117.0 (3.3) | 109.3* (5.3) |
| Hip (cm) | 112.0 (3.2) | 107.4 (2.2) | 120.3 (6.4) | 118.1 (7.3) | 110.1 (3.2) | 107.6* (3.0) | 115.7 (4.9) | 111.6* (4.6) |
| WHR (cm) | 0.97 (0.03) | 0.93 (0.03) | 0.99 (0.02) | 0.97 (0.02) | 0.99 (0.02) | 0.98 (0.02) | 1.02 (0.03) | 0.98 (0.03) |

The OT2 group obtained health benefits from the 6 month intervention irrespective of the sequencing of the diet and exercise interventions, with a reduction in weight, BMI and waist circumference in each case (Table 4.3). There was a similar reduction in mean fat mass in each OT2 group, but this only reached statistical significance when the exercise intervention was performed first (-2.5±0.8kg, $p$=0.01). There were no significant changes in the YT2 group when the exercise intervention was performed prior to the dietary intervention, but when the dietary intervention was performed first, there was a significant reduction in weight

(-5.5±1.6kg, $p$=0.02), BMI (-1.77±0.5kg.m$^{-2}$, $p$=0.02), and waist circumference (-4.4±1.1cm, $p$=0.008) (Table 4.3). However, it is acknowledged that the small number of YT2 subjects that completed the exercise intervention prior to the diet reduces the power of this sub-analysis.

Because of the small number of subjects in each subgroup, the data was also examined in a number of different ways to facilitate greater understanding of the effect of the intervention, including an examination of the combined 6 month intervention data without regard to the sequence of the sub-interventions, to increase the number of subjects per group (Table 4.4). When examined in this manner, there was no difference in weight, BMI, fat mass, waist circumference, or WHR between groups at baseline. After the intervention, there was a significant reduction in fat mass in the OT2 group only (-3.1±0.9kg, $p$=0.006). There was a similar reduction in mean fat mass in the YT2 group but this did not reach statistical significance. However, the YT2 group did respond to the intervention with a significant reduction in weight (-4.8±1.4kg, $p$=0.007), BMI (-1.6±0.4kg.m$^{-2}$, $p$=0.007), and waist circumference (-6.0±2.0cm, $p$=0.02).

Table 4.4. Anthropometric data pre and post 6 month intervention, irrespective of sequence. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 | | OT2 | |
|---|---|---|---|---|
| **N** | **10** | | **15** | |
| **M : F** | **9 : 1** | | **10 : 5** | |
| **Age** | **28.6** (1.2) | | **54.8** (1.2)† | |
| **Height (cm)** | **178.6** (2.6) | | **171.2** (2.6) | |
| | **Pre** | **Post** | **Pre** | **Post** |
| **Weight (kg)** | **119.3** (9.2) | **114.5** (8.9)* | **99.6** (3.9) | **95.1** (4.3)* |
| **BMI (kg/m2)** | **37.8** (3.5) | **36.2** (3.4)* | **34.1** (1.4) | **32.5** (1.4)* |
| **Body Fat %** | **36.3** (2.6) | **34.6** (4.8) | **38.9** (2.3) | **37.4** (2.4)* |
| **Fat Mass (kg)** | **45.8** (7.8) | **42.3** (7.6) | **39.3** (3.3) | **36.2** (3.5)* |
| **FFM (kg)** | **73.5** (2.7) | **72.6** (3.0) | **60.3** (2.2)† | **58.9** (2.4)* |
| **Waist (cm)** | **114.4** (4.8) | **108.4** (4.9)* | **112.6** (2.5) | **107.2** (3.0)* |
| **Hip (cm)** | **116.6** (3.9) | **113.3** (4.4) | **112.7** (2.9) | **109.5** (2.6)* |
| **WHR (cm)** | **0.98** (0.02) | **0.96** (0.02)* | **1.00** (0.02) | **0.98** (0.02) |

### 4.3.3 Exercise intervention

Another of the study objectives was to examine the exercise and dietary interventions separately to determine the independent benefits of each. The exercise intervention was therefore examined in isolation to establish its individual contribution. In addition to the subjects described above who completed the 6 month intervention, one additional OT2 subject completed the exercise intervention without completing the diet. Therefore 26 subjects completed the exercise intervention: 10 YT2 and 16 OT2 subjects. The participating groups had a similar weight, BMI, body fat percentage, fat mass, waist circumference, and WHR at baseline. There was no change in self-reported dietary habits in either group throughout the exercise intervention. The attendance rate at supervised exercise training sessions for those who completed the intervention was 87.9±3.7% for the YT2 group, and

95.8±1.7% for the OT2 group, with no significant difference between groups. Mean initial training time per session was 67±2.4 minutes.

After the exercise intervention, there was a reduction in mean body weight and BMI in each T2DM group, but this did not reach statistical significance in the YT2 group (Table 4.5). However, the YT2 subjects obtained a significant reduction in body fat percentage (-2.0±0.7%, $p$=0.02), fat mass (-2.6±0.9kg, $p$=0.02), waist circumference (-4.2±1.5cm, $p$=0.02) and WHR (from 0.99±0.02 to 0.96±0.02, $p$=0.02). The cohort examined included both those who had completed the exercise intervention as their first or second intervention, but there was no additional priming effect from preceding the exercise intervention with diet.

Table 4.5. Anthropometric data pre and post exercise intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 16) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Weight (kg)** | **116.8** (8.7) | **115.2** (8.8) | **99.0** (4.0) | **97.7** (4.2)* |
| **BMI (kg/m2)** | **37.0** (3.4) | **36.5** (3.4) | **33.9** (1.4) | **33.4** (1.4)* |
| **Body Fat %** | **35.4** (2.9) | **33.4** (3.2)* | **38.1** (2.2) | **36.5** (2.3)* |
| **Fat Mass (kg)** | **43.4** (6.6) | **40.8** (6.9)* | **38.3** (3.2) | **36.1** (3.2)* |
| **FFM (kg)** | **73.4** (2.8) | **74.4** (2.6) | **60.7** (2.5) † | **61.6** (2.8) |
| **Waist (cm)** | **113.6** (5.0) | **109.4** (4.6)* | **111.3** (3.0) | **109.3** (2.9)* |
| **Hip (cm)** | **114.8** (3.9) | **114.3** (4.2) | **111.8** (2.7) | **111.3** (2.5) |
| **WHR (cm)** | **0.99** (0.02) | **0.96** (0.02)* | **1.0** (0.02) | **0.98** (0.02) |

4.3.4 Dietary intervention

In addition to the 25 subjects who completed the entire 6 month intervention, an additional 4 YT2 and 7 OT2 subjects completed the dietary intervention without completing the exercise intervention, bringing the total number of subjects who completed the dietary intervention to 36: 14 YT2 and 22 OT2 subjects. The participating groups had a similar body weight, BMI, body fat percentage, fat mass, waist circumference, WHR and nutrient intake at baseline.

During the dietary intervention, there was a significant reduction in self-reported daily energy intake (YT2: -812.0±258.3kcal, $p$=0.01, and OT2: -742.5±167.6kcal, $p$=0.001), and a reduction in carbohydrate (YT2: -102.1±26.4g, $p$=0.03, and OT2: -56.1±15.2g, $p$=0.001), fat (YT2: -39.4±17.2g, $p$=0.03, and OT2: -38.2±7.2g, $p$=0.001) and protein (YT2: -17.9±7.6g, $p$=0.04, and OT2: -20.4±5.5g, $p$=0.001) intake in both groups, with no significant differences between groups (Table 4.6).

Table 4.6. Nutrient intake pre and post dietary intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | **YT2 (n = 14)** | | **OT2 (n = 22)** | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Calories (kcal/day)** | **2340.7** (267.2) | **1528.7** (173.8)* | **2408.6** (181.4) | **1666.1** (65.4)* |
| **Carbs (g/day)** | **277.9** (30.5) | **175.8** (20.7) * | **240.4** (17.6) | **184.4** (8.1)* |
| **Fat (g/day)** | **103.5** (16.7) | **64.1** (8.1)* | **102.4** (6.8) | **64.2** (3.1)* |
| **Protein (g/day)** | **85.3** (6.9) | **67.4** (7.8)* | **101.4** (6.0) | **81.0** (2.6)* |

After the intervention, a similar significant reduction in body weight was observed in the YT2 (-2.5±1.0kg, $p$=0.03) and the OT2 (-2.9±0.5kg, $p$=0.001) groups, but with no significant fat loss in either group (Table 4.7). There was a significant reduction in waist circumference

in the OT2 group (-3.3±1.0cm, $p$=0.001), but the reduction in the YT2 group did not reach statistical significance. The reduction in waist circumference in the OT2 group coincided with a reduction in FFM (-1.6±0.5kg, $p$=0.006). There was no priming benefit associated with preceding the dietary intervention with exercise. Instead, in both groups there was a reduction in FFM (YT2: -2.8±0.7kg, $p$=0.03, OT2: -2.4±0.8kg, $p$=0.02) after the dietary intervention, when it was performed as the second intervention after exercise.

Table 4.7. Anthropometric data pre and post dietary intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 14) | | OT2 (n = 22) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Weight (kg)** | **113.0** (7.0) | **110.6** (6.7)* | **101.2** (3.2) | **98.3** (3.2)* |
| **BMI (kg/m2)** | **35.8** (2.7) | **35.0** (2.6)* | **34.8** (1.0) | **33.8** (1.0)* |
| **Body Fat %** | **32.6** (3.2) | **32.9** (3.1) | **38.8** (1.8) | **38.5** (1.7) |
| **Fat Mass (kg)** | **40.2** (7.1) | **39.4** (6.6) | **39.7** (2.5) | **38.4** (2.6) |
| **FFM (kg)** | **75.3** (2.5) | **73.3** (2.7) | **61.5** (1.9)† | **59.9** (1.8)* |
| **Waist (cm)** | **109.8** (4.4) | **107.7** (4.7) | **113.4** (2.1) | **110.1** (2.4)* |
| **Hip (cm)** | **113.8** (3.4) | **111.7** (3.3) | **113.0** (2.1) | **111.3** (2.1)* |
| **WHR (cm)** | **0.96** (0.01) | **0.96** (0.02) | **1.01** (0.02) | **0.99** (0.01) |

## 4.5 **Discussion**

The main finding of this experiment was the observation that the YT2 group responded to lifestyle intervention. The YT2 group had a significant reduction in waist circumference after the 6 month intervention, and after the exercise intervention they had a similar degree of adaptation to the OT2 group, with a significant reduction in fat mass and waist circumference, in contrast to previous findings in a similar patient group studied at our centre (25). In that study it was found that the subjects with early-onset T2DM failed to show any weight loss or improvements in insulin sensitivity after 12 weeks of supervised exercise at

70% $VO_2$max. There are important differences between the intervention protocol in that study and in the current study in relation to training volume. In the other study, the volume of exercise may have been insufficient to bring about detectable physiological adaptation, given the low absolute training intensity because of the low aerobic capacity of the subjects. Each subject exercised for 1 hour at 70% $VO_2$max, but those with the lowest $VO_2$max would therefore have performed a smaller absolute volume of exercise. In the current study, the volume of exercise per training session was controlled and kept constant (625kcal per session), with the training time adjusted on an individual basis, based on $VO_2$max.

Although the YT2 group responded to lifestyle intervention, there are trends in the data to suggest that the OT2 group may be better responders to lifestyle intervention, as they were the only group to obtain a significant reduction in fat mass after the 6 month intervention, and a reduction in waist circumference after the dietary intervention. The mean changes in fat mass and waist circumference after the 6 month intervention and the dietary intervention respectively, were similar in both groups, but did not reach statistical significance in the YT2 group. These trends are in line with previously reported observations, but it is acknowledged that a larger sample size may have negated this trend.

Because of the dropout rate, and the resultant number of subjects in each group that were created by forming further subgroups, it was difficult to ascertain the true priming effect of the intervention sequence. There was a tend towards increased 6 month benefit among the YT2 subjects who performed the dietary intervention prior to the exercise intervention, but the analysis is limited by the sample size in the group who performed the exercise

intervention first. In addition to examining the 6 month effect of intervention sequence, the 3 month effect of a single intervention was also studied, both when it was performed as the first intervention and also when it was performed as the second intervention. During the 3 month exercise intervention, there was no additional priming benefit associated with preceding the intervention with diet. It could have been hypothesized that the reduction in adiposity associated with weight loss during the dietary intervention, could have carried over during the exercise intervention, facilitating increased oxidative capacity and weight loss. However, there was no statistically significant fat loss during the dietary intervention which could have countered this. It could also have been hypothesized that having taken part in the dietary intervention for 3 months, that subjects might have not completely returned to their pre-intervention calorie intake during the exercise intervention, leading to additional weight loss. However, food diaries were carefully maintained throughout and there was no evidence to suggest a difference between baseline calorie intake, and calorie intake during the exercise intervention. Similarly, during the 3 month dietary intervention, there was no priming benefit associated with preceding the intervention with exercise. Instead of a reduction in fat mass, there was a reduction in FFM in both groups after the dietary intervention, when it was performed as the second intervention. The reduction in FFM was not merely a reduction back towards baseline after an increase during the exercise intervention, as there was no significant change as a result of exercise training. A diet induced reduction in FFM has negative consequences as it reduces basal metabolic rate (243), reducing total daily energy expenditure, which would hinder further weight loss. It has previously been demonstrated that as much as 15% of diet induced weight loss can be composed of FFM (185). The lack of reduction in fat mass during the dietary intervention may represent a form of inappropriate

substrate selection for oxidation, or "metabolic inflexibility", which has been shown to be a feature of insulin resistance (29).

Fat loss after the exercise intervention was less than anticipated, suggesting that the participants did not remain calorie neutral throughout as they reported, or that they reduced the rest of their total daily physical activity. It has been noted that during exercise interventions, that general physical activity levels outside of the programme can reduce initially because of fatigue, but that it can increase above starting levels as fitness is increased (244). It was not suspected that there was any additional increase in total daily physical activity levels in the current study however, as weight loss was well below what had been predicted. During the dietary intervention, there was a significant reduction in self-reported daily calorie intake in both the YT2 and the OT2 groups, but in both cases the weight lost was less than the predicted 3.9kg, based on the associated 12 week target energy deficit of 2500kcal/week. Better compliance with the diet would have been expected as the calorie restriction in question was not severe. Given the reported reduction in daily calorie intake during the dietary intervention, the associated weight loss should have been 8.8kg in the YT2 group and 8.1kg in the OT2 group after 12 weeks, highlighting the underestimation of calorie intake by subjects, and the overestimation of the calorie deficit they generate. It has been similarly observed previously that obese patients, and particularly patients with T2DM, under-report their daily calorie intake (178, 179). Given the lack of fat loss as a result of the dietary intervention, the exercise intervention appears to have been associated with greater health benefit.

Weight is the main variable used in clinical settings to monitor changes in patient anthropometric profile. These study results highlight the importance of using additional measures, as there was a reduction in body weight and BMI in both groups after the dietary intervention, but this was not accompanied by a significant reduction in fat mass. Similarly, there was no significant reduction in weight or BMI in the YT2 group after the exercise intervention, but there was a reduction in fat mass and waist circumference. Furthermore, after the 6 month intervention, there was a reduction in waist circumference in the YT2 group, and despite no significant reduction in fat mass, the results suggest a healthier redistribution of adipose tissue which would otherwise have been undetected if body weight were to have been the only outcome measure used.

## 4.6 **Conclusion**

It has been suggested by other authors that early-onset T2DM has a more aggressive pathogenesis and unique characteristics different to that of later-onset T2DM (16). The current experiment demonstrated that both groups of subjects were equally obese at baseline, and had a high proportional fat intake, with no noteworthy anthropometric differences between groups. Interestingly, the YT2 group responded to lifestyle intervention, especially exercise intervention, contrary to previous findings.

# Chapter Five:

## Experiment 2: Whole Body and Cellular Aerobic Capacity in Early-Onset T2DM.

## 5.1 **Introduction**

Mitochondrial dysfunction is thought to contribute to the pathogenesis of both insulin resistance and T2DM. The concept of lipotoxicty has been implicated in this process, whereby lipid intermediates accumulate and interfere with the insulin signalling cascade, because of excessive lipid ingestion and / or because of impaired mitochondrial function and inadequate lipid oxidation and disposal. Patients with T2DM have been shown to have low levels of whole body oxidative capacity and aerobic fitness, and it has been demonstrated that patients with early-onset T2DM have a blunted $VO_2$max response to exercise. It has also been previously shown that patients with T2DM have reduced intrinsic mitochondrial oxidative capacity, and that patients with early-onset T2DM have abnormalities in some mitochondrial responses to aerobic exercise when compared to BMI matched obese non-diabetic subjects, but it remains to be seen whether impaired mitochondrial function contributes to differences between early and later-onset T2DM.

## 5.2 **Aims**

The aim of this experiment was to compare whole body and cellular oxidative capacity ($VO_2$max and intrinsic mitochondrial function respectively), between patients with early-onset T2DM and later-onset T2DM. A further objective was to examine adaptations in these parameters in response to lifestyle intervention.

## 5.3 **Results**

### 5.3.1 **Whole Body Oxidative Capacity**

#### 5.3.1.1 Baseline

These results are based on the same cohort described in Chapter 4. At baseline, the OT2 group had a lower maximum heart rate than the YT2 group, but there was no difference in daily physical activity level, $VO_2$max, maximum exercise load, respiratory exchange ratio (RER) at $VO_2$max, or anaerobic threshold between groups (Table 5.1). Among the non-T2DM subjects, $VO_2$max was significantly higher in the YOb group than in the OOb, with a 23.6% difference between groups ($p$=0.02). There were no differences in $VO_2$max, baseline physical activity levels, or anaerobic threshold between the T2DM subjects who completed baseline studies only, those who completed the lifestyle intervention, and those who dropped out. In the entire cohort, daily physical activity level (IPAQ, MET-minutes/week) was negatively correlated with waist circumference ($r$=-0.44, $p$=0.01) and fat mass ($r$=-0.48, $p$=0.002), but there was no correlation with $VO_2$max.

Table 5.1. Baseline $VO_2$max related data. IPAQ = International Physical, RER: respiratory exchange ratio, AT: anaerobic threshold, NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 (n = 23) | OT2 (n = 40) | YT2 v OT2 |
|---|---|---|---|
| **IPAQ (MET–min/wk)** | **1504.1** (569) | **1682.9** (616) | NS |
| **VO2max (ml/kg/min)** | **23.8** (1.3) | **21.9** (1.1) | NS |
| **HRmax (bpm)** | **179.6** (3.1) | **163.7** (3.3) | **0.001** |
| **Load Max (Watts)** | **136.3** (9.1) | **119.3** (7.3) | NS |
| **RER at Max** | **1.14** (0.01) | **1.18** (0.02) | NS |
| **VO2 at AT (ml/kg/min)** | **17.9** (1.1) | **17.6** (1.0) | NS |
| **AT as a % of VO2max ml/kg/min (%)** | **75.8** (2.4) | **80.9** (1.6) | NS |
| **Load at AT** | **105.6** (8.8) | **95.8** (6.2) | NS |

5.3.1.2 Six month effect of combined interventions

Among those who completed the 6 month intervention, there was no difference in $VO_2max$ or anaerobic threshold between the YT2 and OT2 groups at baseline. After the 6 month intervention, the YT2 group responded with a significant increase in $VO_2max$ ($l.min^{-1}$) (+15.2±4.2%, $p$=0.006), as did the OT2 group (+9.8±2.2%, $p$=0.001), with no significant difference between groups (Table 5.2). While there was also an increase in the cycling load at $VO_2max$, and the load at anaerobic threshold in both groups, interestingly there was also a reduction in the percentage of $VO_2max$ at which anaerobic threshold occurred in the YT2 group (-8.9±2.7%, $p$=0.02).

Table 5.2. $VO_2max$ related data pre and post 6 month intervention, irrespective of sequence. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 15) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **VO2max (l/min)** | **2.77** (0.1) | **3.19** (0.1)* | **2.15** (0.1) | **2.36** (0.1)* |
| **HRmax (bpm)** | **182.0** (5.9) | **169.9** (5.6)* | **166.0** (2.8)† | **157.8** (5.3) |
| **Load Max (Watts)** | **145.6** (11.1) | **198.3** (13.5)* | **121.4** (9.8)† | **145.6** (10.2)* |
| **VO2 at AT (L/min)** | **2.26** (0.1) | **2.35** (0.2) | **1.82** (0.2) | **1.97** (0.2) |
| **AT as a % of VO2max L/min (%)** | **83.8** (3.1) | **74.9** (3.5)* | **83.5** (3.2) | **83.8** (1.7) |
| **Load at AT** | **120.7** (10.1) | **158.6** (16.6)* | **105.0** (13.6) | **122.3** (15.1)* |

When the subgroups and intervention sequence of the 6 month intervention were further examined, it was revealed that the OT2 group obtained an increase in $VO_2max$ irrespective of the sequencing of the diet and exercise interventions (Table 5.3). The YT2 group did not

obtain any significant benefit from performing the exercise intervention prior to the dietary intervention, but when the dietary intervention was performed first, a significant increase in $VO_2$max (l/min) (+18.9±3.5%, $p$=0.003), and cycling load at $VO_2$max (+66.7±11.4Watts, $p$=0.01) was observed. However, it is acknowledged as before that the small number of subjects that completed the exercise intervention prior to the diet limits the statistical power of this sub-analysis. In addition, any increase in $VO_2$max or cycling load obtained when the exercise intervention was performed second is likely to be a reflection of the protocol design and not any form of metabolic priming, as a greater degree of exercise stimulus would be provided prior to the last $VO_2$max test. Similarly, any exercise related adaptation resulting from performing exercise during the first 3 months would be expected to diminish naturally when the exercise stimulus is removed during the second 3 months of dietary intervention.

Table 5.3. $VO_2$max related data pre and post 6 month intervention, incorporating intervention sequence. ED: Exercise intervention performed before dietary intervention. DE: Dietary intervention performed before exercise intervention. IPAQ = International Physical, RER: respiratory exchange ratio, AT: anaerobic threshold. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 | | | | OT2 | | | |
|---|---|---|---|---|---|---|---|---|
| | ED (n = 4) | | DE (n = 6) | | ED (n = 8) | | DE (n = 7) | |
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| VO2max (l/min) | 2.60 (0.3) | 2.78 (0.3) | 2.86 (0.1) | 3.40* (0.1) | 2.27 (0.2) | 2.35* (0.2) | 2.01 (0.1) | 2.38* (0.1) |
| HRmax (bpm) | 174.5 (0.5) | 166.5 (6.5) | 185.0 (8.1) | 171.2* (7.8) | 166.7 (4.1) | 156.3* (7.4) | 164.8 (3.9) | 160.5 (7.8) |
| Load Max (Watts) | 151.7 (15.9) | 176.7 (26.8) | 142.5 (15.5) | 209.2* (14.9) | 132.1 (17.9) | 143.4 (18.3) | 110.7 (7.4) | 147.9* (10.5) |
| VO2 at AT (L/min) | 1.89 (0.2) | 1.98 (0.4) | 2.40 (0.1) | 2.50 (0.2) | 1.95 (0.2) | 2.04 (0.3) | 1.61 (0.1) | 1.84 (0.2) |
| AT as a % of VO2max L/min (%) | 81.9 (8.4) | 74.9 (2.5) | 84.6 (3.6) | 74.9 (5.0) | 83.1 (4.8) | 84.1 (2.8) | 84.3 (4.6) | 83.2 (1.0) |
| Load at AT | 112.5 (12.5) | 152.5 (27.5) | 124.0 (13.7) | 161.0 (22.3) | 118.0 (19.7) | 135.7 (21.4) | 83.3 (8.3) | 100.0 (14.4) |

5.3.1.3 Exercise intervention

When the individual interventions were examined, there was no difference in $VO_2max$ ($ml.kg^{-1}.min^{-1}$) or anaerobic threshold between the YT2 and OT2 groups at baseline among those who completed the exercise intervention. After the exercise intervention, there was a significant increase in $VO_2max$ ($l.min^{-1}$) in each group (YT2: +14.8±2.3%, $p$=0.001, and OT2: +15.9±2.6%, $p$=0.001) with no significant difference between groups (Table 5.4). Changes in $VO_2max$ were not correlated with changes in weight or fat mass. There was a similar increase in maximal cycling load (YT2: +33.9±4.8%, $p$=0.001, OT2: +37.6±5.1%, $p$=0.001), and the load at which anaerobic threshold occurred (YT2: +35.2±11.1%, $p$=0.006, OT2: +29.8±6.9%, $p$=0.01) in both groups. After the intervention, there was an increase in the rate of oxygen consumption at which anaerobic threshold occurred in both groups, but this only reached statistical significance in the OT2 group. There was no additional priming benefit associated with performing the dietary intervention prior to the exercise intervention.

Table 5.4. $VO_2max$ related data pre and post exercise intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 16) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **VO2max (l/min)** | **2.9** (0.1) | **3.33** (0.1)* | **2.2** (0.1) | **2.6** (0.1)* |
| **HRmax (bpm)** | **182.1** (5.4) | **175.4** (5.7) | **165.2** (4.5) | **158.9** (4.7)* |
| **Load Max (Watts)** | **155.5** (6.3) | **209.0** (12.3)* | **118.8** (8.7) | **160.6** (9.8)* |
| **VO2 at AT (L/min)** | **2.16** (0.2) | **2.42** (0.1) | **1.74** (0.1) | **2.05** (0.1)* |
| **AT as a % of VO2max L/min (%)** | **75.3** (4.4) | **72.9** (3.2) | **82.7** (2.3) | **83.4** (1.1) |
| **Load at AT** | **120.0** (11.7) | **155.2** (13.7)* | **101.4** (10.1) | **131.0** (12.8)* |

Further examination of temporal changes in $VO_2max$ during the exercise intervention suggests a trend towards a slower rate of progression in the YT2 group over the 12 week

period. In the OT2 group, $VO_2$max ($l.min^{-1}$) increased rapidly over the first 8 weeks before plateauing, with a significant increase between baseline and week 4 (+7.7±1.5%, $p$=0.001), and between week 4 and 8 (+5.9±1.6%, $p$=0.002), but none between week 8 and 12 (+1.6±2.0%, $p$=NS) (Figure 5.1). However, in the YT2 group there was an earlier plateau, with a similar significant increase in $VO_2$max between baseline and week 4 (+8.7±1.9%, $p$=0.001), but a more gradual increase between week 4 and 12, and no significant change between week 4 and 8 (+2.2±2.1%, $p$=NS), or between week 8 and 12 (+3.7±2.8%, $p$=NS) (Figure 5.2). There was no difference in compliance with the exercise intervention between groups, at any of the time points.

Figure 5.1. Temporal changes in $VO_2$max during the exercise intervention in the OT2 group. Data expressed as Mean ± Standard Error of Mean. Significantly different from previous test, $P<0.05$ *

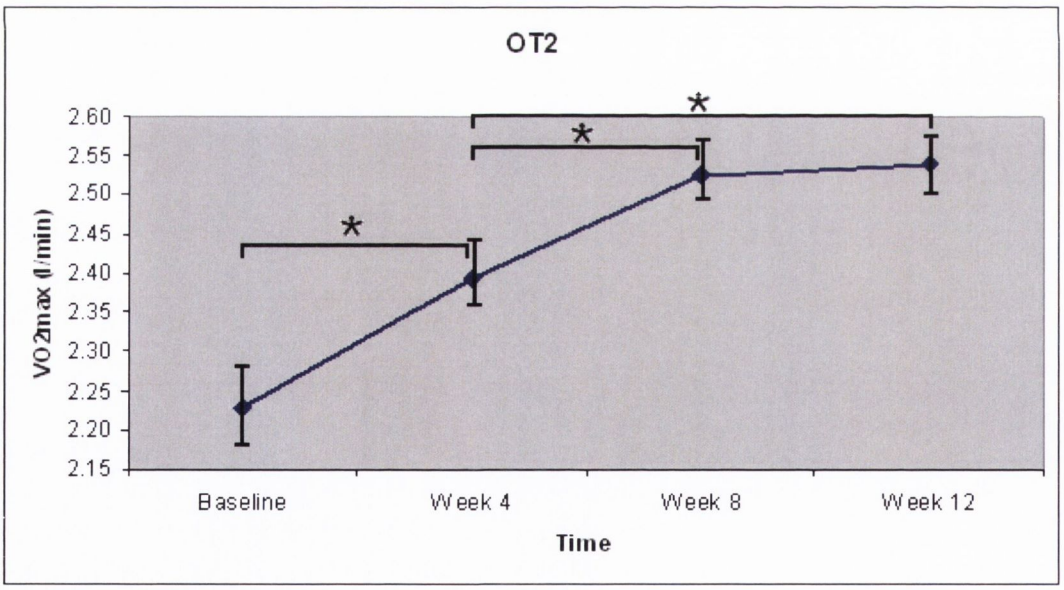
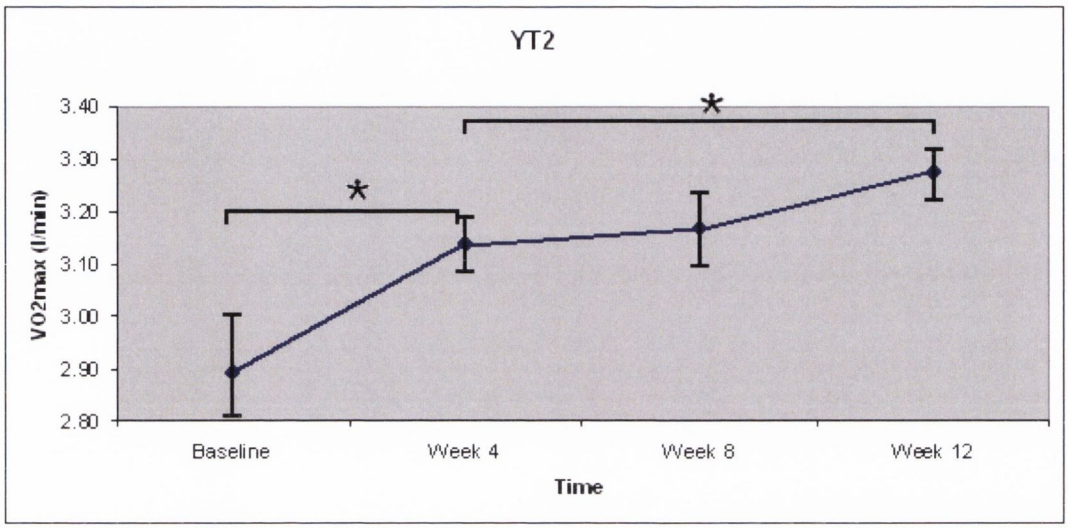


Figure 5.2. Temporal changes in $VO_2$max during the exercise intervention in the YT2 group. Data expressed as Mean ± Standard Error of Mean. Significantly different from previous test, $P<0.05$ *




5.3.1.4 Dietary intervention

When the dietary intervention was examined in isolation, there was no difference in $VO_2$max ($ml.kg^{-1}.min^{-1}$) or anaerobic threshold between the YT2 and OT2 groups at baseline among those who completed the intervention. After the dietary intervention, there was no change in $VO_2$max in the YT2 group, but there was a reduction in the OT2 group (Table 5.5). The reduction in $VO_2$max in the OT2 group was attributable to the subjects who had completed the exercise intervention prior to the diet.

Table 5.5. $VO_2$max related data pre and post dietary intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 14) | | OT2 (n = 22) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **VO2max (l/min)** | **2.92** (0.1) | **2.98** (0.1) | **2.3** (0.1) | **2.15** (0.1)* |
| **HRmax (bpm)** | **179.9** (4.9) | **176.3** (5.0) | **164.9** (3.1)† | **162.1** (3.5) |
| **Load Max (Watts)** | **161.8** (12.5) | **166.8** (8.6) | **134.2** (11.1) | **123.4** (9.0) |
| **VO2 at AT (L/min)** | **2.20** (0.1) | **2.06** (0.1) | **1.93** (0.2) | **1.76** (0.1)* |
| **AT as a % of VO2max L/min (%)** | **75.9** (4.1) | **69.7** (3.9) | **84.1** (2.4) | **82.0** (1.3) |
| **Load at AT** | **127.5** (9.6) | **129.0** (12.0) | **110.0** (11.5) | **106.3** (9.2) |

### 5.3.2 **In vitro oxidative capacity in mitochondria from muscle biopsies**

#### 5.3.2.1 Baseline

Intrinsic mitochondrial oxidative capacity was measured at baseline using high resolution respirometry from mitochondria isolated from muscle biopsy samples, and revealed no differences between the YT2 and the OT2 groups under any of the substrate conditions (Table 5.6). There was no respirometry data available from the non-T2DM subjects. The following correlations were observed in the combined T2DM cohort:

- pyruvate and malate (P+M) based oxidative flux was:
    - positively correlated with $VO_2$max (ml/kg/min) ($r$=0.48, $p$=0.02).
    - positively correlated with daily physical activity levels (MET-min/wk) ($r$=0.43, $p$=0.02).
    - negatively correlated with body fat percentage ($r$=-0.38, $p$=0.045).

- pyruvate, malate and succinate (P+M+S) based oxidative flux was:
    - positively correlated with QUICKI ($r$=0.57, $p$=0.001).
    - negatively correlated with HOMA-IR ($r$=-0.24, $p$=0.004).
    - negatively correlated with fasting blood glucose concentration ($r$=-0.18, $p$=0.04).

Table 5.6. Baseline mitochondrial oxidative flux under the influence of pyruvate and malate (P+M), after the addition of succinate (P+M+S), rotenone (ROT), ADP (adenosine diphosphate), oligomycin (OLIGO), and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP). NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 (n = 10) | OT2 (n = 20) | YT2 v OT2 |
|---|---|---|---|
| **P+M** (pmol $O_2$/ml/mg of protein) | **58.8** (14.2) | **59.0** (9.4) | NS |
| **P+M+S** (pmol $O_2$/ml/mg of protein) | **214.6** (21.1) | **225.4** (16.5) | NS |
| **ROT** (pmol $O_2$/ml/mg of protein) | **239.8** (29.7) | **239.8** (19.6) | NS |
| **ADP** (pmol $O_2$/ml/mg of protein) | **676.3** (101.9) | **559.2** (78.2) | NS |
| **OLIGO** (pmol $O_2$/ml/mg of protein) | **221.5** (29.6) | **233.9** (26.4) | NS |
| **FCCP** (pmol $O_2$/ml/mg of protein) | **801.5** (93.6) | **794.0** (83.0) | NS |

5.3.2.2 Six month effect of combined interventions

There were no differences in intrinsic mitochondrial function between groups at baseline, among those who completed the six month intervention. After the six month intervention, there was a mean increase in all mitochondrial related parameters in both groups, but significant results were only obtained by the YT2 group (ADP: +673.8±222.3 pmol $O_2$/ml/mg of protein, $p$=0.03, and Oligomycin: +238.7±99.8 pmol $O_2$/ml/mg of protein, $p$=0.046) (Table 5.7). When the groups were further divided to examine the effect of sequence, the numbers in each subgroup were too low to show any changes. To increase the power, the data from the YT2 and the OT2 groups were combined and then re-divided into

two groups based on intervention sequence: those who completed the 6 month intervention starting with the exercise intervention, and those who completed the intervention starting with diet. These results revealed an increase in P+M+S (+177.9±78.1 pmol $O_2$/ml/mg of protein, $p$=0.04), rotenone (+183.9±75.8 pmol $O_2$/ml/mg of protein, $p$=0.03), and ADP (+561.1±173.6 pmol $O_2$/ml/mg of protein, $p$=0.007) based oxidative flux, only in the subgroup of T2DM subjects who performed the exercise intervention prior to the dietary intervention.

Table 5.7. Mitochondrial oxidative flux pre and post 6 month intervention under the influence of pyruvate and malate (P+M), after the addition of succinate (P+M+S), rotenone (ROT), ADP (adenosine diphosphate), oligomycin (OLIGO), and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP).
Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 6) | | OT2 (n = 8) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **P+M** (pmol $O_2$/ml/mg of protein) | **68.0** (22.5) | **109.7** (42.6) | **60.9** (17.8) | **112.9** (25.3) |
| **P+M+S** (pmol $O_2$/ml/mg of protein) | **236.6** (21.1) | **436.2** (132.2) | **221.7** (28.2) | **383.3** (72.5) |
| **ROT** (pmol $O_2$/ml/mg of protein) | **277.3** (33.0) | **487.8** (144.0) | **237.2** (38.7) | **401.2** (67.9) |
| **ADP** (pmol $O_2$/ml/mg of protein) | **612.4** (97.1) | **1286.2** (206.3)* | **640.8** (145.8) | **1117.4** (135.5) |
| **OLIGO** (pmol $O_2$/ml/mg of protein) | **232.0** (37.5) | **470.6** (124.7)* | **238.1** (43.3) | **379.1** (109.2) |
| **FCCP** (pmol $O_2$/ml/mg of protein) | **754.2** (64.7) | **1290.5** (236.6) | **787.2** (151.9) | **1165.2** (124.3) |

5.3.2.3 Exercise intervention

When the individual interventions were examined, there were no differences at baseline between the YT2 and the OT2 groups who completed the exercise intervention. After the exercise intervention, there was a similar mean increase in all mitochondrial related parameters in both groups, but with significant results only in the YT2 group (ADP based oxygen flux: +709.8±169.4 pmol $O_2$/ml/mg of protein, $p$=0.009) (Table 5.8). However, there

was a significant increase in oxidative flux among the subgroup of OT2 subjects who performed the exercise intervention as their first intervention (P+M based flux: +50.0±18.0 pmol $O_2$/ml/mg of protein, $p$=0.03, P+M+S: +261.8±68.6 pmol $O_2$/ml/mg of protein, $p$=0.009, ADP: +795.0±203.4 pmol $O_2$/ml/mg of protein, $p$=0.01, and FCCP: +814.1±285.6 pmol $O_2$/ml/mg of protein, $p$=0.04), with no change among those who performed exercise after the dietary intervention. In the entire cohort, an increase in ADP oxygen flux was correlated with a reduction in fasting blood glucose concentration ($r$=-0.55, $p$=0.034).

Table 5.8. Mitochondrial oxidative flux pre and post exercise intervention under the influence of pyruvate and malate (P+M), after the addition of succinate (P+M+S), rotenone (ROT), ADP (adenosine diphosphate), oligomycin (OLIGO), and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP).
Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 6) | | OT2 (n = 12) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **P+M** (pmol $O_2$/ml/mg of protein) | **56.5** (10.3) | **100.4** (35.5) | **74.8** (17.6) | **93.6** (17.1) |
| **P+M+S** (pmol $O_2$/ml/mg of protein) | **235.5** (31.7) | **478.2** (126.3) | **293.8** (60.3) | **408.9** (55.3) |
| **ROT** (pmol $O_2$/ml/mg of protein) | **243.5** (31.5) | **523.7** (138.4) | **302.4** (65.1) | **408.5** (43.2) |
| **ADP** (pmol $O_2$/ml/mg of protein) | **669.8** (164.9) | **1379.6** (199.8)* | **785.2** (182.2) | **1155.8** (99.2) |
| **OLIGO** (pmol $O_2$/ml/mg of protein) | **236.6** (43.2) | **547.3** (125.0)* | **254.2** (44.4) | **371.8** (69.3) |
| **FCCP** (pmol $O_2$/ml/mg of protein) | **932.2** (200.5) | **1368.4** (203.1) | **900.9** (171.6) | **1323.1** (109.5) |

5.3.2.4 Dietary intervention

There were no differences at baseline among those who completed the dietary intervention. After the dietary intervention, there were no significant changes in respirometry based intrinsic mitochondrial function in either group (Table 5.9). There was also no benefit associated with preceding the intervention with exercise. However, changes in oxygen flux under the influence of P+M+S were negatively correlated with changes in body fat

percentage ($r$=-0.59, $p$=0.008), fat mass ($r$=-0.48, $p$=0.04), and fasting glucose concentration ($r$=-0.54, $p$=0.02).

Table 5.9. Mitochondrial oxidative flux pre and post dietary intervention under the influence of pyruvate and malate (P+M), after the addition of succinate (P+M+S), rotenone (ROT), ADP (adenosine diphosphate), oligomycin (OLIGO), and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP).
Data expressed as Mean and (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 7) | | OT2 (n = 12) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **P+M** (pmol $O_2$/ml/mg of protein) | **80.3** (20.1) | **92.1** (24.5) | **77.2** (16.6) | **114.1** (19.6) |
| **P+M+S** (pmol $O_2$/ml/mg of protein) | **289.8** (60.2) | **269.2** (46.7) | **291.8** (50.7) | **362.2** (67.7) |
| **ROT** (pmol $O_2$/ml/mg of protein) | **339.7** (71.4) | **300.7** (58.9) | **311.2** (51.6) | **371.0** (77.2) |
| **ADP** (pmol $O_2$/ml/mg of protein) | **902.3** (214.0) | **967.6** (183.6) | **840.8** (156.2) | **977.9** (173.7) |
| **OLIGO** (pmol $O_2$/ml/mg of protein) | **365.2** (98.8) | **327.8** (68.1) | **291.3** (37.7) | **263.8** (49.3) |
| **FCCP** (pmol $O_2$/ml/mg of protein) | **1048.2** (196.8) | **1194.6** (211.0) | **1148.4** (159.3) | **1022.5** (168.8) |

## 5.4 **Discussion**

As impaired mitochondrial function has previously been shown to be a feature of T2DM and as it is thought to contribute to the development of insulin resistance, it was hypothesised that the YT2 group would have reduced intrinsic mitochondrial capacity compared with the OT2 group, which might contribute in some way to the earlier onset of the disease. However, interestingly there was no detectable difference in intrinsic mitochondrial oxidative function between T2DM groups in the current study at baseline. Similarly there was no difference in maximum whole body oxidative capacity between T2DM groups at baseline. However, baseline $VO_2$max was extremely low in both groups. It has previously been shown that there is an age associated reduction in $VO_2$max with the difference between younger and older groups as high as 25 – 30% (245), as was observed among the control subjects in the current

study (as maximum heart rate and stroke volume are reduced over time), but as $VO_2$max was the same in both T2DM groups, this could reflect a relative deficiency in this younger cohort. Furthermore, due to oxidative stress over time, mitochondrial function is believed to be reduced with age, and as there was no difference between the younger and the older groups in the current study, this could also reflect a relative deficit in the YT2 group. Studies that examine intrinsic mitochondrial function normalize the results to mitochondrial density using an array of different markers which prevents comparison with control subjects from other studies to put these results into context. However, studies that compare both T2DM and non-T2DM subjects, consistently demonstrate reduced intrinsic mitochondrial function among controls (85, 101, 107). Although there was no difference in intrinsic mitochondrial function per unit volume of protein, there could still be a difference in mitochondrial volume or density between groups (measured using mitochondrial DNA copy-number, citrate synthase, or cardiolipin), which would effect overall mitochondrial function. However, it has been recently shown that there was no difference between YT2 subjects and matched young obese non-diabetic subjects, when porin was used as a marker of mitochondrial volume (102).

Another interesting observation was the fact that the YT2 group responded well to the exercise intervention, with a 15% increase in $VO_2$max, and a significant increase in intrinsic mitochondrial function. At baseline, intrinsic mitochondrial function was positively correlated with both $VO_2$max and daily physical activity level (MET-min/wk), and after exercise there was an improvement in whole body oxidative capacity that was mirrored at a cellular level. This is in contrast to previous findings in a similar patient group where no improvements in $VO_2$max were detected after a 12 week exercise intervention (25), but there

were some important differences between the intervention protocol in that study and in the current study in relation to training progression and specificity. In the current study, $VO_2$max was formally reassessed at week 4 and 8, and in the event of an interim increase in $VO_2$max, the training intensity was increased to 70% of the new $VO_2$max to ensure that the subjects were never under-training. In addition, unlike in the previous study, the majority of exercise training in the current study was performed on a bicycle ergometer, as the $VO_2$max tests were performed on a bicycle ergometer, and as training and testing are both mode-specific.

Previous studies have suggested that "exercise resistance" is a feature of early-onset T2DM, and although the YT2 group responded well, there are trends within the data to suggest that the rate of increase in $VO_2$max may be slower in the YT2 group. It was also observed that after the exercise intervention, anaerobic threshold occurred at a significantly greater rate of oxygen consumption in the OT2 group only. A later occurrence of anaerobic threshold is an indicator of aerobic adaptation, as greater work can be performed before the accumulation of lactic acid, which reduces exercise tolerance. Similarly, after the six month intervention, anaerobic threshold occurred at a lower percentage of $VO_2$max in the YT2 group.

Most lifestyle recommendations for health, diabetes management, and weight loss suggest a combination of diet and exercise (157). In the current protocol, the interventions were performed separately in order to make it possible to separately examine the effects of each modality. The exercise protocol was consistent with current exercise recommendations for diabetes: moderate to vigorous exercise (>60% $VO_2$max), at least three times per week (with no more than two consecutive rest days between sessions), for a minimum of 150 minutes

per week, with more required for weight loss (184). Physical activity guidelines report that general daily unstructured physical activity contributes to health status (184), and while baseline daily physical activity levels in the current study correlated negatively with fat mass, there was no correlation with $VO_2$max, demonstrating the need for formal higher intensity exercise training for optimal cardiovascular health and fitness. Improvements in $VO_2$max after the exercise intervention were also not correlated with weight loss, highlighting the fact that optimal anthropometric results are more difficult to obtain with exercise training if dietary intake is not also addressed.

The results show trends towards greater benefits in terms of mitochondrial function and aerobic fitness associated with the exercise intervention than with the dietary intervention. It could have been speculated that there could have been an increase in mitochondrial function as a result of the reduced calorie, low fat diet, which could have reduced "lipotoxic" conditions and the production of reactive oxygen species which hinder mitochondrial function, but interestingly, the dietary intervention blunted any mitochondrial adaptation.

## 5.5 **Conclusion**

It had been hypothesised that the YT2 group would have a lower intrinsic mitochondrial capacity than the OT2 group, which would explain the earlier onset of disease. Interestingly, there was no difference in intrinsic mitochondrial function or $VO_2$max between groups at baseline. However, the YT2 $VO_2$max values were very low, and this may still reflect a relative deficiency in this group, as age associated differences would have been expected. Another important finding was the fact that, contrary to previous observations, the YT2

group responded well to lifestyle intervention. Improvements in $VO_2$max and mitochondrial function were associated with the exercise intervention, but not with the dietary intervention.

# Chapter Six:

## Experiment 3: Insulin Resistance and Contributing Factors in Early-Onset T2DM.

## 6.1 **Introduction**

It has been reported that early-onset T2DM is difficult to manage both medically and with lifestyle intervention, leading to an increased risk of diabetes complications as a result of inadequate glycaemic control. It has also been shown using the euglycaemic–hyperinsulinaemic clamp technique that YT2 patients are severely insulin resistant. Possible explanations include:

- early onset of obesity
- rapid weight gain
- sustained obesity
- poor nutrition
- lack of exercise
- or a combination of these factors

A number of other underlying factors, including lipotoxicity, have also been implicated in the pathogenesis of insulin resistance and T2DM, but it remains unclear what role these play in early-onset T2DM.

## 6.2 **Aims**

The aim of this experiment was to compare insulin resistance and lipid profile in patients with early and later-onset T2DM, using a number of indices of insulin resistance, and using both standard and novel markers of lipid profile. An additional objective was to examine acylcarnitines, BCAA's, and adipokines which have been shown to be correlated with obesity and insulin resistance, and a further objective was to assess the responsiveness of these parameters to lifestyle intervention.

## 6.3 **Results**

### 6.3.1 **Oral Glucose Tolerance Test Data**

#### 6.3.1.1 Baseline

These results are based on the same cohort described in Chapter 4 and 5. At baseline, there were no differences in fasting blood glucose, $HbA_{1c}$, or indices of insulin sensitivity (OGIS-2hr and QUICKI) or insulin resistance (HOMA-IR) between the YT2 and the OT2 groups (Table 6.1). Fasting glucose concentration was significantly higher among the T2DM subjects than among the obese control subjects (YOb: 5.0±0.16, $p$=0.001, OOb 5.0±0.17, $p$=0.001), and the non-T2DM subjects were also less insulin resistant, with higher OGIS-2hr (YOb 395.4±23.7ml/min/$m^2$, $p$=0.001, OOb 361.6±24.27ml/min/$m^2$, $p$=0.02) and QUICKI results (YOb 0.376±0.015, $p$=0.001, OOb 0.408±0.011, $p$=0.02).

There were no differences in β-cell function between the younger and older groups with T2DM (Table 6.1). There was no difference in fasting β-cell function between the T2DM and non-T2DM subjects, but dynamic β-cell function (IGI total AUC) was higher in the YOb group compared to the YT2 group (12.44±1.69 vs 4.75±0.85, $p$=0.001), and higher in the OOb group compared to the OT2 group (13.04±2.54 vs 3.77±0.45, $p$=0.001). Similarly, disposition index results were higher in the YOb group compared to the YT2 group (53.96±7.91 vs 14.38±2.56, $p$=0.001), and higher in the OOb group compared to the OT2 group (51.46±8.68 vs 11.40±1.55, $p$=0.001).

An examination for various potential correlations was performed in the entire cohort, revealing the following:

- OGIS-2hr was positively correlated with $VO_2$max (ml/kg/min) ($r$=0.42, $p$=0.01), and negatively correlated with body fat percentage ($r$=-0.44, $p$=0.003).
- QUICKI was negatively correlated with waist circumference ($r$=-0.34, $p$=0.043).
- Fasting blood glucose concentration was positively correlated with waist circumference ($r$=0.32, $p$=0.03), and BMI ($r$=0.29, $p$=0.049).
- HOMA-IR was positively correlated with waist circumference ($r$=0.45, $p$=0.002), and body fat percentage ($r$=0.32, $p$=0.03), and negatively correlated with $VO_2$max (ml/kg/min) ($r$=-0.4, $p$=0.009).

Table 6.1. Baseline OGTT data. NS = non-significant. Data expressed as Mean and (Standard Error of Mean).

| | YT2 (n = 22) | OT2 (n = 40) | YT2 v OT2 |
|---|---|---|---|
| **Glucose** (mmol/l) | **8.9** (0.7) | **9.6** (0.6) | NS |
| **HBA1c** (%) | **8.1** (0.4) | **7.5** (0.2) | NS |
| **Insulin** (μU/ml) | **31.5** (7.2) | **27.0** (4.5) | NS |
| **OGIS-2hr** (ml/min/m$^2$) | **268.7** (14.2) | **286.2** (15.4) | NS |
| **QUICKI** | **0.30** (0.01) | **0.31** (0.01) | NS |
| **HOMA-IR** | **13.4** (3.4) | **12.6** (2.7) | NS |
| **B-cell fasting** [(μU/ml)/(mmol/l)] | **0.197** (0.04) | **0.152** (0.02) | NS |
| **Insulin Secretion** (AUC I total) | **10785.0** (1744.2) | **9250.4** (1015.8) | NS |
| **B-cell function** (IGI total AUC) | **4.75** (0.85) | **3.77** (0.45) | NS |
| **Disposition Index** (OGIS x IGI total) | **14.38** (2.56) | **11.40** (1.55) | NS |

6.3.1.2 Six month effect of combined interventions

There were no OGTT based differences at baseline, among the YT2 and OT2 subjects who completed the six month intervention. The six month intervention resulted in a significant

reduction in $HbA_{1c}$ in both the YT2 and OT2 groups (YT2: from 8.3±0.6 to 6.6±0.5%, $p$=0.04, and OT2: from 7.7±0.3 to 6.9±0.3%, $p$=0.03), with no significant difference between groups. There was also a significant increase in QUICKI (from 0.33±0.01 to 0.35±0.01, $p$=0.02) in the OT2 group, and while there was a similar mean change in the YT2 group, these results did not reach statistical significance (Table 6.2). The 6 month adaptation in the OT2 group was attributable to the subgroup of patients who performed the diet before exercise. After the combined 6 month intervention, there was no significant change in β-cell function in the YT2 group, but there was a significant increase in dynamic β-cell function (IGI total AUC) in the OT2 group (from 3.1±0.5 to 5.0±1.1, $p$=0.02), and also an increase in disposition index values (from 9.8±1.9 to 15.8±3.5, $p$=0.02).

Table 6.2. OGTT data pre and post 6 month intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 15) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Glucose** (mmol/l) | **8.7** (0.6) | **7.9** (1.0) | **9.7** (0.7) | **7.9** (0.6) |
| **HBA1c** (%) | **8.3** (0.6) | **6.6** (0.5)* | 7.7 (0.3) | **6.9** (0.3)* |
| **Insulin** (μU/ml) | **42.2** (13.9) | **34.2** (13.8) | **26.2** (7.0) | **32.1** (15.1) |
| **OGIS-2hr** (ml/min/m$^2$) | **248.1** (17) | **311.2** (31) | **291.2** (19) | **303.0** (13) |
| **QUICKI** | **0.32** (0.01) | **0.34** (0.01) | **0.33** (0.01) | **0.35** (0.01)* |
| **HOMA-IR** | **16.9** (5.9) | **15.2** (8.2) | **14.1** (4.6) | **11.3** (5.7) |
| **B-cell fasting** [(μU/ml)/(mmol/l)] | **0.27** (0.08) | **0.23** (0.06) | **0.14** (0.03) | **0.24** (0.10) |
| **Insulin Secretion** (AUC I total) | **12989** (3391) | **13989** (3496) | **7966** (1306) | **11094** (2559) |
| **B-cell function** (IGI total AUC) | **5.8** (1.6) | **7.7** (2.1) | **3.1** (0.5) | **5.0** (1.1)* |
| **Disposition Index** (OGIS x IGI total) | **16.9** (4.0) | **23.8** (5.6) | **9.8** (1.9) | **15.8** (3.5)* |

6.3.1.3 Exercise intervention

When the effects of the intervention components were examined in isolation, there were no OGTT based differences between groups at baseline among those who completed the exercise intervention. After the exercise intervention, there was a significant increase in QUICKI (from 0.33±0.01 to 0.36±0.02, $p$=0.047) in the YT2 group, and no significant change in β-cell function in either group (Table 6.3).

Table 6.3. OGTT data pre and post exercise intervention. Data expressed as Mean and (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 15) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Glucose** (mmol/l) | **8.21** (0.9) | **7.34** (0.9) | **8.81** (0.6) | **7.81** (0.6) |
| **HBA1c** (%) | **7.46** (0.6) | **6.56** (0.3) | **7.22** (0.3) | **7.01** (0.3) |
| **Insulin** (μU/ml) | **32.94** (9.9) | **21.01** (5.8)* | **24.24** (6.7) | **31.54** (14.1) |
| **OGIS-2hr** (ml/min/m$^2$) | **282.5** (23) | **300.3** (34) | **300.9** (15.5) | **306.0** (14) |
| **QUICKI** | **0.33** (0.01) | **0.36** (0.02)* | **0.35** (0.01) | **0.35** (0.01) |
| **HOMA-IR** | **12.12** (3.8) | **7.24** (1.8) | **11.63** (3.9) | **11.23** (4.8) |
| **B-cell fasting** [(μU/ml)/(mmol/l)] | **0.24** (0.07) | **0.17** (0.05) | **0.14** (0.03) | **0.23** (0.10) |
| **Insulin Secretion** (AUC I total) | **11946** (2930) | **11759** (3436) | **9337** (1406) | **10472** (2137) |
| **B-cell function** (IGI total AUC) | **6.2** (1.7) | **7.1** (2.1) | **3.9** (0.7) | **4.6** (1.0) |
| **Disposition Index** (OGIS x IGI total) | **18.1** (4.2) | **22.3** (5.7) | **12.2** (2.3) | **15.8** (3.4) |

In the entire cohort, the following correlations were observed:

Changes in weight after the exercise intervention were:

- correlated with changes in HOMA-IR ($r$=0.45, $p$=0.02).

Changes in waist circumference after the exercise intervention were:

- positively correlated with changes in:
    - HOMA-IR ($r$=0.54, $p$=0.006).
    - $HbA_{1c}$ ($r$=0.44, $p$=0.027).
- negatively correlated with changes in:
    - OGIS-2hr ($r$=-0.59, $p$=0.004).
    - QUICKI ($r$=-0.59, $p$=0.004).

6.3.1.4 Dietary intervention

There were no OGTT based differences between groups at baseline among those who completed the dietary intervention. After the dietary intervention, there was a significant reduction in $HbA_{1c}$ (from 7.76±0.3 to 7.21±0.3%, $p$=0.02) in the OT2 group only (Table 6.4). There was a similar level of reduction in mean $HbA_{1c}$ in the YT2 group, but this did not reach statistical significance. The dietary intervention resulted in a significant increase in dynamic β-cell function (IGI total AUC) in the OT2 group only (from 4.0±0.7 to 5.0±0.9, $p$=0.02). There was no priming effect associated with preceding the intervention with exercise.

Table 6.4. OGTT data pre and post dietary intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 15) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **Glucose** (mmol/l) | **8.99** (1.2) | **8.25** (1.0) | **9.91** (0.6) | **8.40** (0.6) |
| **HBA1c** (%) | **7.88** (0.6) | **6.89** (0.4) | **7.76** (0.3) | **7.21** (0.3)* |
| **Insulin** (μU/ml) | **33.78** (12.1) | **37.35** (13.2) | **27.39** (5.2) | **32.20** (10.9) |
| **OGIS-2hr** (ml/min/m$^2$) | **256.7** (22) | **306.2** (21) | **265.3** (14) | **281.1** (12) |
| **QUICKI** | **0.34** (0.02) | **0.33** (0.01) | **0.33** (0.01) | **0.34** (0.01) |
| **HOMA-IR** | **13.42** (5.5) | **16.56** (8.2) | **15.25** (4.0) | **12.68** (4.0) |
| **B-cell fasting** [(μU/ml)/(mmol/l)] | **0.22** (0.07) | **0.25** (0.07) | **0.20** (0.08) | **0.22** (0.08) |
| **Insulin Secretion** (AUC I total) | **11563** (3139) | **12704** (2820) | **10007** (1648) | **11234** (1858) |
| **B-cell function** (IGI total AUC) | **5.7** (1.5) | **6.6** (1.5) | **4.0** (0.7) | **5.0** (0.9)* |
| **Disposition Index** (OGIS x IGI total) | **17.0** (3.9) | **19.7** (4.0) | **12.2** (2.4) | **15.1** (2.7) |

In the entire cohort, a change in weight after the dietary intervention was:

- positively correlated with a change in:
    - $HbA_{1c}$ ($r$=0.53, $p$=0.002).
    - HOMA-IR ($r$=0.47, $p$=0.01).
- negatively correlated with a change in:
    - QUICKI ($r$=-0.59, $p$=0.001).
    - OGIS-2hr ($r$=-0.43, $p$=0.03).

### 6.3.2 **Lipids, metabolomics and other parameters**

#### 6.3.2.1 Baseline

At baseline, the YT2 and the OT2 groups had similar HDL-cholesterol (YT2: 0.96±0.1mmol/l vs OT2: 1.05±0.03mmol/l, $p$=ns), triglyceride (YT2: 2.01±0.2mmol/l vs OT2: 2.11±0.2mmol/l, $p$=ns), and FFA concentrations (YT2: 0.75±0.1mmol/l vs OT2: 0.72±0.1mmol/l, $p$=ns) (Table 6.5). There were no significant differences between the T2DM and the non-T2DM subjects with regard to HDL-cholesterol or FFA concentrations, but triglyceride was lower among the obese control subjects (combined non-T2DM cohort: 1.22±0.16 vs T2DM cohort: 2.08±0.12 mmol/l, $p$=0.001). An examination for various potential correlations was performed in the entire cohort at baseline, revealing the following:

- fasting blood glucose concentration was positively correlated with:
    - FFA concentration ($r$=0.58, $p$=0.02).
    - triglyceride concentration ($r$=0.29, $p$=0.04).
- OGIS-2hr was negatively correlated with
    - FFA concentration ($r$=-0.73, $p$=0.003).
    - triglyceride concentration ($r$=-0.33, $p$=0.03).

Table 6.5. Baseline lipid profile data. NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | **YT2 (n = 22)** | **OT2 (n = 40)** | **YT2 v OT2** |
|---|---|---|---|
| **HDL** (mmol/l) | **0.96** (0.1) | **1.05** (0.03) | NS |
| **Trigs** (mmol/l) | **2.01** (0.2) | **2.11** (0.2) | NS |
| **FFA** (mmol/l) | **0.75** (0.1) | **0.72** (0.1) | NS |

The metabolomic results revealed that the plasma concentration of total fatty acids and several individual fatty acid species were higher in the YT2 group than in the OT2 group (Table 6.6). Similarly, the values among YT2 subjects were significantly higher than those among non-T2DM subjects for total fatty acids ($p$=0.001), palmitic acid ($p$=0.001) and oleic acid ($p$=0.001). There was no difference in branched-chain amino acid (BCAA) concentration between the YT2 and the OT2 groups, but both groups had higher concentrations than was observed among the non-T2DM subjects for L-valine (261±10μM, $p$=0.029) and L-leucine / isoleucine (170±7μM, $p$=0.004). The concentration of the lipid related acylcarnitine: C10-OH/C8-DC, was higher in the YT2 than in the OT2 group, while the concentration of the amino acid related acylcarnitine: C3, was higher in the OT2 group. Due to limited sample stock accumulation at the time of testing apparatus availability, metabolomic examination was only possible at baseline. In the entire cohort at baseline, HOMA-IR was correlated with BCAA concentration ($r$= 0.6, p=0.001), as were total fatty acid and individual fatty acid species.

Table 6.6. Baseline metabolomic profile data. NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 (n = 22) | OT2 (n = 16) | YT2 v OT2 |
|---|---|---|---|
| **Total fatty acids** (μM) | **15614** (918) | **10808** (713) | **<0.001** |
| **Palmitic acid** (μM) | **2664** (172) | **1850** (170) | **<0.01** |
| **Oleic acid** (μM) | **4937** (412) | **3094** (213) | **<0.01** |
| **L-valine** (μM) | **305** (13) | **298** (14) | NS |
| **L-leucine / isoleucine** (μM) | **211** (10) | **202** (1) | NS |
| **C10** (μM) | **0.22** (0.013) | **0.22** (0.016) | NS |
| **C10 :1** (μM) | **0.11** (0.008) | **0.10** (0.011) | NS |
| **C8** (μM) | **0.12** (0.007) | **0.10** (0.010) | NS |
| **C10-OH/C8-DC** (μM) | **0.05** (0.003) | **0.04** (0.003) | **<0.001** |
| **C5's** (μM) | **0.12** (0.010) | **0.15** (0.012) | NS |
| **C3** (μM) | **0.37** (0.037) | **0.45** (0.027) | **<0.05** |
| **C4/Ci4** (μM) | **0.19** (0.020) | **0.21** (0.018) | NS |

At baseline, the OT2 group had a significantly higher adiponectin concentration than the YT2 group, and a tendency towards a higher leptin concentration (Table 6.7). Due to limited sample stock accumulation at the time of testing apparatus availability, adipokine testing was only possible at baseline. An examination for various potential correlations was performed in the entire cohort.

Leptin concentration was:

- positively correlated with:
    - BMI ($r$=0.69, $p$=0.001).
    - body fat percentage ($r$=0.68, $p$=0.001).
    - fat mass ($r$=0.61, $p$=0.001).
    - waist circumference ($r$=0.49, $p$=0.001).
    - weight ($r$=0.29, $p$=0.02).

- negatively correlated with:
    - $VO_2$max ($r$=-0.34, $p$=0.04).

Adiponectin concentration was:

- positively correlated with:
    - HDL-cholesterol ($r$= 0.56, p=0.001).
- negatively correlated with:
    - HOMA-IR ($r$=-0.29, $p$=0.046).

Table 6.7. Baseline adipokine profile data. NS = non-significant. Data expressed as Mean (Standard Error of Mean).

| | YT2 (n = 22) | OT2 (n = 40) | YT2 v OT2 |
|---|---|---|---|
| **Leptin** (ng/ml) | **31.5** (4.1) | **38.1** (2.7) | NS |
| **Adiponectin** (μg/ml) | **4.70** (0.2) | **5.11** (0.1) | **0.046** |

6.3.2.2 Six month effect of combined interventions

When standard lipid based tests were performed, there were no lipid related differences between groups at baseline among those who completed the six month intervention (Table 6.8). After the six month intervention, there was a reduction in triglyceride concentration in the OT2 group only (from 1.85±0.1 to 1.48±0.1mmol/l, $p$=0.01), with no trend towards improvement in the YT2 group. The improvement in lipid profile in the OT2 group was primarily attributable to those who completed the dietary intervention prior to the exercise intervention.

Table 6.8. Lipid profile data pre and post 6 month intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 15) | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| **HDL** (mmol/l) | **0.92** (0.07) | **0.90** (0.04) | **1.07** (0.06) | **1.13** (0.08) |
| **Trigs** (mmol/l) | **1.68** (0.1) | **1.65** (0.2) | **1.85** (0.1) | **1.48** (0.1)* |
| **FFA** (mmol/l) | **0.75** (0.07) | **0.67** (0.01) | **0.72** (0.10) | **0.62** (0.10) |

6.3.2.3 Exercise intervention

There were no standard lipid related differences between groups at baseline among those who completed the exercise intervention (Table 6.9). After the exercise intervention, there was an increase in mean HDL-cholesterol and a reduction in mean triglyceride concentration in each group, but these results did not reach statistical significance. However, among those

who completed the exercise intervention as their second intervention, there was a significant increase in HDL-cholesterol among both the YT2 (+0.07±0.02mmol/l, $p$=0.03) and the OT2 subjects (+0.15±0.05mmol/l, $p$=0.02).

Table 6.9. Lipid profile data pre and post exercise intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 16) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **HDL** (mmol/l) | **0.87** (0.03) | **0.93** (0.04) | **1.09** (0.06) | **1.13** (0.07) |
| **Trigs** (mmol/l) | **1.76** (0.26) | **1.56** (0.15) | **1.78** (0.16) | **1.48** (0.12) |
| **FFA** (mmol/l) | **0.73** (0.11) | **0.77** (0.10) | **0.67** (0.12) | **0.68** (0.10) |

6.3.2.4 Dietary intervention

There were no standard lipid related differences between groups at baseline among those who completed the dietary intervention (Table 6.10). After the dietary intervention, there were no significant changes in lipid profile, but there was a non-significant trend towards a reduction in triglyceride and FFA concentration in each group. There was no priming benefit associated with preceding the dietary intervention with exercise.

Changes in triglyceride concentration were:

- positively correlated with changes in:
    - weight ($r$=0.52, $p$=0.003).
    - glucose concentration ($r$=0.48, $p$=0.006).
    - BMI ($r$=0.46, $p$=0.009).
    - $HbA_{1c}$ ($r$=0.45, $p$=0.01).

- waist circumference ($r$=0.42, $p$=0.02).
- HOMA-IR ($r$=0.38, $p$=0.04).

- negatively correlated with a change in:
  - OGIS-2hr ($r$=-0.54, $p$=0.006).
  - QUICKI ($r$=-0.47, $p$=0.02).

Table 6.10. Lipid profile data pre and post dietary intervention. Data expressed as Mean (Standard Error of Mean). Significantly different from baseline values (within group): $P<0.05$ *. Significantly different from YT2 at baseline (between groups): $P<0.05$ †.

| | YT2 (n = 10) | | OT2 (n = 22) | |
|---|---|---|---|---|
| | **Pre** | **Post** | **Pre** | **Post** |
| **HDL** (mmol/l) | **0.94** (0.06) | **0.87** (0.03) | **1.01** (0.04) | **1.02** (0.05) |
| **Trigs** (mmol/l) | **1.82** (0.18) | **1.78** (0.25) | **1.74** (0.13) | **1.69** (0.14) |
| **FFA** (mmol/l) | **0.78** (0.08) | **0.66** (0.11) | **0.70** (0.11) | **0.62** (0.13) |

## 6.4 **Discussion**

The study results reveal that one of the main distinguishing features of patients with early-onset T2DM is their lipid profile. The T2DM subjects had higher triglyceride concentrations than the non-T2DM subjects, but the YT2 subjects also had higher circulating concentrations of total fatty acids and several individual fatty acid species: palmitic acid and oleic acid, than the OT2 group. The YT2 group also had a higher concentration of circulating lipid related acylcarnitine, suggesting that they may be subject to a worse lipid burden for oxidation. BCAA concentrations have previously been shown to be elevated in insulin resistant individuals (112), and elevated concentrations in healthy individuals have been shown to predict the future development of diabetes (122). T2DM subjects had elevated concentrations of BCAA compared to non-T2DM subjects, but there were no BCAA differences between the YT2 and the OT2 groups.

The YT2 subjects also had a different adipokine profile, with a significantly lower concentration of adiponectin. Higher adiponectin concentrations are thought to be protective against insulin resistance (80), with concentrations having been shown to be lower in obese subjects (81), and lower again in patients with T2DM (82). It was similarly shown in the current study that adiponectin concentration was negatively correlated with HOMA-IR. Leptin is thought to play a role in regulating satiety (83), with concentrations increasing with increasing levels of obesity (81). While there was no significant difference in leptin concentration (or calorie intake, or obesity level) between the T2DM groups, there was a tendency towards a lower leptin concentration among the YT2 subjects.

Early-onset T2DM has previously been described as an extreme subphenotype of diabetes, associated with increased risk of complications due to severe hyperglycaemia and insulin resistance. In the current study, the T2DM subjects had worse glycaemic control and insulin resistance than the non-T2DM subjects, but there was no difference in insulin resistance, β-cell function, $HbA_{1c}$ or fasting blood glucose concentration between the YT2 and OT2 groups at baseline. However, for the young group to be severely insulin resistant at such an early age is of great concern, as they will have greater time exposure to risk factors, increasing the risk of developing diabetes complications. Fasting insulin levels were higher than expected among the T2DM subjects, and there was no difference in the basal insulin/glucose ratio between T2DM subjects and non-T2DM controls, which may suggest that despite differences in dynamic β-cell function, a degree of fasting β-cell function is still intact. Previous studies have shown no difference in insulin concentrations between YT2 and

YOb subjects, but have demonstrated lower concentrations among OT2 subjects [MCQUAID]. In the current study, the lack of difference between groups may reflect the fact that the short duration of diabetes onset was quite similar among subjects, without significantly greater exposure among the OT2 subjects. There was a tendency towards an increase in insulin concentration in the OT2 group after lifestyle intervention, which may reflect the improvement in β-cell function which was observed in this group. A different pattern of adaptation appears to occur among YT2 subjects however, as a trend towards a reduction in insulin concentration was observed, which may reflect a lower insulin requirement as a result of a tendency towards lower insulin resistance.

The YT2 group obtained a significant reduction in $HbA_{1c}$ after the 6 month intervention, contrary to previous observations in a similar cohort of subjects. However, a significant reduction in triglyceride concentration after the 6 month intervention was observed only in the OT2 group, with no trend towards improvement detected in the YT2 group. There was an increase in insulin sensitivity in the YT2 group after the exercise intervention, but not after the 6 month intervention as was observed in the OT2 group. Similarly, the YT2 group did not obtain the improvement in $HbA_{1c}$ or β-cell function after the dietary intervention, or the improvement in β-cell function after the 6 month intervention that occurred in the OT2 group. However, this trend might not have been apparent, had more YT2 subjects participated in and completed the intervention. Lipid and OGTT related priming effects were obtained in each case by preceding the exercise intervention with diet. While insulin sensitivity was correlated with $VO_2max$ at baseline, changes in $VO_2max$ were not correlated with changes in insulin sensitivity after the exercise intervention.

## 6.5 **Conclusion**

The YT2 and the OT2 groups were similarly insulin resistant at baseline. The YT2 group had several distinguishing features including a significantly higher concentration of total fatty acids, palmitic acid and oleic acid, and a lower concentration of adiponectin. The acylcarnitine data also reflects differences in lipid and amino acid based metabolism between groups. Contrary to previously reported findings, the YT2 group responded to lifestyle intervention with an improvement in $HbA_{1c}$ after the 6 month intervention. However, there was a trend towards increased adaptation among the OT2 subjects (e.g. regarding triglyceride concentration). These findings further support the observation that early-onset T2DM has distinctive characteristics, and is metabolically different from later-onset T2DM.

# Chapter Seven

## Summary and Discussion

## 7.1 **Summary**

### 7.1.1 **Study design**

The current study was a longitudinal, randomised, controlled, crossover trial, which included testing that was carried out over a 3 year period. To determine if early-onset T2DM had distinctive characteristics and responded in a different manner to lifestyle intervention than later-onset T2DM, two groups of obese (BMI > 30 $kg/m^2$), sedentary subjects with T2DM were recruited: a YT2 group (<30 years of age) and an OT2 group (>50 years of age). Sixty-three subjects took part in baseline examination: 23 YT2 and 40 OT2. Metformin was the main diabetes medication taken by participants at baseline, and medication doses were not changed throughout the course of the study.

A subgroup of participants completed the 6 month lifestyle intervention (10 YT2 and 15 OT2 subjects), consisting of a 3 month exercise intervention and a separate 3 month dietary intervention, assigned in a random order. The energy expenditure per week during the exercise intervention was matched with the weekly dietary energy deficit (-2500 kcal per week). During the exercise intervention, participants trained 4 times per week at 70% $VO_2$max, expending 625kcal per session on a stationary bicycle ergometer. All training sessions were supervised, mean initial training time per session was 67±2.4 minutes, and training was progressive, with $VO_2$max reassessed on a monthly basis. During the dietary intervention, subjects per prescribed a low fat, reduced calorie diet, which reduced calorie intake by 357kcal per day. Participants filled out 3 day food diaries during the diet, and were met every 2 weeks to be weighed. The overall 6 month effect of both interventions was

examined (including the effect of intervention sequence), in addition to the effect of the individual interventions (and the priming effect of intervention sequence).

Testing was carried out over two days on each occasion (at baseline, after 3 months between interventions, and after 6 months at the end of the study). On one of the days a non-fasting $VO_2$max test was carried out, while on the other, fasting measurements were made, including: anthropometric measurements, blood sampling, an OGTT, and a muscle biopsy from the thigh. The test parameters examined included the following:

- Clinical: Height, weight, BMI, waist circumference, hip circumference, WHR, body fat percentage, fat mass, FFM, calories per day, carbohydrate, fat and protein consumption per day.
- Physical: Daily physical activity levels (MET–min/wk) assessed using the International Physical Activity Questionnaire, $VO_2$max (L/min and ml/kg/min), maximum heart rate, cycling load at $VO_2$max, $VO_2$ at anaerobic threshold, and respiratory exchange ratio.
- Metabolic: Intrinsic mitochondrial function measured using high resolution respirometry as oxygen flux under the influence of pyruvate and malate, succinate, rotenone, ADP (State 3 respiration: maximal physiological respiration), oligomycin, and FCCP (State 4 respiration: maximal uncoupled respiration).
- OGTT: Fasting glucose and insulin, $HbA_{1c}$, QUICKI, HOMA-IR, and OGIS-2hr.
- Biochemical: HDL-cholesterol, triglyceride, FFA, leptin, adiponectin, and metabolomic based variables (total fatty acids, individual fatty acid species, BCAA's, and acylcarnitines).

7.1.2 **Results**

7.1.2.1 Experiment 1: (Chapter Four)

Baseline: There were no differences in daily physical activity levels or daily energy intake, and no anthropometric differences between groups at baseline.

Six month: There was a similar significant reduction in weight, BMI and waist circumference in both groups after the 6 month intervention. There was a reduction in mean fat mass in both groups, but this only reached statistical significance in the OT2 group.

Exercise: After the exercise intervention, there was a similar significant reduction in fat mass and waist circumference in both groups. There was no priming effect associated with preceding the exercise intervention with the dietary intervention.

Diet: After the dietary intervention, there was a similar significant reduction in self-reported daily energy intake in each group, and a significant reduction in weight and BMI in both groups. There was no significant fat loss in either group. There was a similar reduction in mean waist circumference in both groups, but it only reached statistical significance in the OT2 group. There was no priming effect associated with preceding the dietary intervention with exercise.

7.1.2.2 Experiment 2: (Chapter Five)

Baseline: There were no differences in $VO_2$max, anaerobic threshold or intrinsic mitochondrial function between groups at baseline.

Six month: After the 6 month intervention, there was a similar significant increase in $VO_2$max in both groups. There was an increase in intrinsic mitochondrial function after the 6

month intervention in the YT2 group. Adaptation in intrinsic mitochondrial function was hindered by performing the dietary intervention first.

Exercise: After the exercise intervention there was a similar significant increase in $VO_2$max in both groups, and a significant increase in intrinsic mitochondrial function in the YT2 group. There was a tendency towards a slower rate of increase in $VO_2$max in the YT2 group. There was no additional priming benefit associated with performing the dietary intervention prior to the exercise intervention.

Diet: There was no increase in $VO_2$max or intrinsic mitochondrial function in either group after the dietary intervention. There was no priming effect associated with preceding the intervention with exercise.

7.1.2.3 Experiment 3: (Chapter Six)

Baseline: There were no significant differences in fasting blood glucose, $HbA_{1c}$, indices of insulin sensitivity (OGIS-2hr, QUICKI and HOMA-IR), β-cell function, HDL-cholesterol, triglyceride, FFA's, leptin, or BCAA's between groups at baseline. However, the YT2 group had a significantly higher concentration of total fatty acids, individual species of fatty acid (oleic and plamitic acid), and a lower concentration of adiponectin.

Six month: After the 6 month intervention, there was a similar significant reduction in $HbA_{1c}$ in both groups. There was a significant reduction in triglyceride in the OT2 group only. There was a significant increase in mean insulin sensitivity and β-cell function in the OT2 group only. Performing the dietary intervention before the exercise intervention was associated with a trend towards increased benefit.

Exercise: There were no significant changes in lipid based parameters in either group after the exercise intervention. However, performing the dietary intervention before the exercise intervention was associated with an increase in HDL-cholesterol in both groups. After the exercise intervention, there was no increase in β-cell function in either group, but there was a significant increase in QUICKI in the YT2 group.

Diet: There were no significant lipid based changes after the dietary intervention in either group. There was a similar reduction in mean $HbA_{1c}$ in both groups, but this only reached statistical significance in the OT2 group. There was an increase in β-cell function in the OT2 group only. There was no priming effect associated with preceding the dietary intervention with exercise.

## 7.2 **Discussion**

It has been previously reported that YT2 patients are severely insulin resistant (25), with extremely poor glycaemic control upon initial presentation (18). In the current study, there were no anthropometric differences between groups, and it was demonstrated that the YT2 group was as insulin resistant as the OT2 group, with similar β-cell function and a similar degree of hyperglycaemia. For the YT2 group to be hyperglycaemic and severely insulin resistant at such an early age increases their risk of developing complications as they will have greater time exposure to risk factors. Furthermore, diabetes complications have been reported in YT2 patients with poorly controlled T2DM by their mid thirties (15).

Impaired mitochondrial function has been implicated in the aetiology of insulin resistance and T2DM. The lipotoxicity hypothesis suggests that FFA's accumulate ectopically as

triglyceride in the cytosol of cells other than adipocytes, such as in skeletal muscle cells, ultimately inhibiting insulin signalling. The accumulation of ectopic lipid is consistent with excessive lipid supply (as a result of a high fat intake and obesity related dyslipidaemia) or inadequate lipid disposal (as a result of reduced physical activity or reduced mitochondrial oxidative capacity), or both. A mismatch between lipid supply and oxidative capacity leads to the partial processing of triglyceride, which results in the production of lipid intermediates (ceramide, diacylglycerol and long-chain fatty acyl-coenzyme A), the chronic accumulation of which are though to interfere with the cascade that results in the translocation of GLUT4. It was therefore hypothesised that among equally obese and sedentary subjects, that the YT2 group would have impaired mitochondrial function, that would explain the earlier presentation of T2DM. Interestingly however, there were no differences in intrinsic mitochondrial function between groups at baseline. However, a reduction in maximum whole body oxidative capacity and mitochondrial oxidative function has been previously shown to occur with age, and as there were no differences between groups, this may reflect a relative impairment in the YT2 group.

As the lipotoxic conditions contributing to T2DM are thought to relate to excessive lipid supply or inadequate lipid disposal, and as there were no detectable differences in intrinsic mitochondrial oxidative capacity between groups, the deficiency in the YT2 group appears to be related to lipid supply. The YT2 group had a higher concentration of the lipid related acylcarnitine, C10-OH/C8-DC, and as acylcarnitines are intermediates of lipid oxidation, their accumulation is consistent with a backlog of substrate for metabolism. The current study demonstrated that the YT2 group had a worse lipid profile, with higher circulating

levels of total and several individual species of fatty acid. These observations could reflect inherent differences between groups, or may reflect a difference in the rate at which patients with early-onset T2DM became obese. As fat intake, physical activity levels and mitochondrial function were the same between groups, the elevated lipid concentrations in the YT2 group appear to have originated from endogenous sources, which may be suggestive of excessive lipolysis. Alternatively, this may reflect inappropriate substrate selection for oxidation, and greater "metabolic inflexibility" in the YT2 group.

Another distinctive feature of the YT2 group relates to their adipokine profile, whereby the younger group had a lower adiponectin concentration. Adiponectin concentration has previously been shown to be inversely correlated with insulin resistance (80), as was also demonstrated in the current study, and this may represent another contributing factor to the earlier onset of disease in the YT2 group. BCAA's have recently been implicated in the pathogenesis of insulin resistance and have been shown to be predictive of the future development of T2DM (122), so it was hypothesised that these might also be candidate biomarkers of interest in the YT2 group. In the current study, concentrations of BCAA's were correlated with insulin resistance, but there was no difference between groups.

Lifestyle intervention is recognised as an important component of the management and treatment of diabetes, both before and after the introduction of medication, and so both diet and exercise were included in the current study. It has been previously shown that YT2 patients are not as responsive to lifestyle intervention, with no changes in anthropometric parameters, $VO_2$max, or glycaemic control after an aerobic exercise intervention (25). On

this basis, it was hypothesised that the YT2 group would not respond as well as the OT2 group to either intervention. In the current study, an individually prescribed, progressive exercise intervention was used, unlike in the previously described study, and in this case, the YT2 group responded with a reduction in fat mass and waist circumference, an increase in $VO_2$max, mitochondrial function and insulin sensitivity, and a reduction in $HbA_{1c}$. However, given these adaptations, further associated improvements in glycaemic control would have been expected. Furthermore, there were trends towards some additional lifestyle related health benefits that were only observed in the OT2 group, including:

- A reduction in fat mass and triglyceride concentration and an increase in QUICKI and β-cell function after the 6 month intervention.
- A reduction in waist circumference and $HbA_{1c}$, and an increase in β-cell function after the dietary intervention.
- A trend towards a faster rate of increase in $VO_2$max after the exercise intervention.

However, these additional benefits may also have been observed in the YT2 group, had a greater number of younger subjects taken part in, and completed the lifestyle intervention, as there were trends toward improvement in the YT2 group (with the exception of triglyceride concentration, where no changes were observed).

One of the goals of this study was to replicate components of the methodologies used in previous studies that examined lifestyle intervention in early-onset T2DM, and in each case, the numbers of subjects recruited per group in these studies were small [n=11 (17), n=7 (25), and n=7 (102)]. Although the prevalence of early-onset T2DM is increasing, the availability of YT2 subjects is still low, and there are still less young patients with T2DM than there are

older. Similarly, the number of subjects recruited in the current study was also quite low, but all of the YT2 subjects who attended clinics in St. James's Hospital during the study period were approached to take part. A power calculation was performed after the completion of the study to establish if a sufficient number of subjects had been recruited. As previous studies have shown no physiological benefits among patients with early-onset T2DM to exercise intervention (25), one of the primary variables of interest was $VO_2$max as an indicator of intervention effectiveness. The power calculation [$n = s(1- \beta)/(\alpha)(ES)$, where n = sample size, s = variation, $\beta$ = power, $\alpha$ = significance level, and ES = effect size], revealed that 15 subjects would have been sufficient to provide 80% power and 5% significance, based on a 20% effect size, as seen in the control group of a similarly designed study (25), and a standard deviation of 0.8L/min among subjects with early-onset T2DM. The mean dropout rate from lifestyle intervention studies is approximately 25%, and can be as high as 31% (242), so taking into account a potential 30% drop out rate, the target number of subjects for recruitment would have been 40 (20 YT2 and 20 OT2), and in fact 23 YT2 subjects were recruited, with another 4 screened but subsequently rejected due to the discovery of type 1 diabetes and polycystic ovary syndrome. It was predicted that with this number of subjects, a significant increase in $VO_2$max would be detectable in response to lifestyle intervention, and indeed, a statistically and clinically significant 15% increase was noted. While this was associated with a substantial mean improvement in many other variables, these did not reach statistical significance in many cases in the YT2 group, demonstrating what appears to be false negatives. It was subsequently calculated that if an additional 3 YT2 subjects with pre and post values similar to the mean within the group had completed the interventions, that the reduction in fat mass after the 6 month intervention, the reduction in waist circumference

after the dietary intervention, and the reduction in weight after the exercise intervention would all have reached statistical significance. Similarly, if an extra 5 YT2 subjects had completed the 6 month intervention, the improvement in OGIS-2hr would have reached statistical significance. Had an extra 5 YT2 subjects completed the 6 month intervention, it would have brought the total to 15, which is the number estimated from the power calculation. However, any increase in the number of subjects per group would not have changed the main findings of the study, which were that there were distinctive differences between groups at baseline, and that the YT2 group adapted to lifestyle intervention. Had more subjects been added, there would have been no further differences noted between groups at baseline, and no further differences in the pre-post delta comparison between groups after lifestyle intervention, assessed using ANOVA.

Only 40% of the subjects who completed baseline testing ended up completing both the 3 month dietary intervention and the 3 month exercise intervention, but it was not intended that all of the subjects take part in the lifestyle intervention. Among the total cohort, a number of subjects, particularly in the OT2 group, were recruited at the end of the study for baseline examination only to add to the strength of this examination, and some were recruited with only enough time to perform one intervention. When this cohort of subjects is excluded, the proportion of subjects who completed both interventions is increased to 60%. No subjects dropped out during the dietary intervention. They either dropped out after the dietary intervention prior to the transition to exercise, or during the exercise intervention. The drop out rate among those who started the exercise intervention was 25%, which is in keeping with most lifestyle interventions (242), and there were no anthropometric, metabolic or

biochemical differences, or differences in physical capacity between those who dropped out, and those who completed the intervention. Dropout from exercise was not related to exercise intensity, as exercise was performed at 70% $VO_2$max, while anaerobic threshold occurred at 76% $VO_2$max in the YT2 group, and 81% VO2max in the OT2 group. Patients dropped out for a number of reasons, including pregnancy, moving house and changing job. The time commitment that was required to complete the exercise intervention probably also had a contributory role however, as lack of time is the main reason given by subjects for not participating in regular exercise (130).

As it had been previously demonstrated that YT2 subjects were not responsive to exercise intervention, it was hypothesised that they would obtain greater benefit from the dietary intervention in the current study. Interestingly, there was a trend towards a greater number of health benefits (anthropometric, $VO_2$max and mitochondrial) associated with the exercise intervention, despite the greater drop out rate. This could reflect the different physiological impact of the exercise intervention, or instead, the greater degree of direct supervision during the intervention. Additional insulin sensitizing benefits were also obtained when the exercise intervention was preceded by the dietary intervention. The benefit of performing a dietary intervention prior to an exercise intervention, may be worth investigating further, particularly for patients who consider themselves to be too obese and deconditioned to begin exercise training straight away before losing some weight, or for those recovering from acute lower limb injuries which prevent them from exercising in the short term. While traditional diabetes management in clinical settings involves the prescription of medication and referral to a dietician, the current study findings highlight the importance of also making appropriate

referrals so that patients can obtain personalised exercise prescription. It would therefore appear that patients with early-onset T2DM would benefit from a low fat, reduced calorie diet, in addition to relatively high intensity, supervised, progressive aerobic exercise. As early-onset T2DM is considered to be a difficult condition to manage once established, the emphasis should always be on disease prevention and health promotion to keep healthy people healthy (as a form of primary prevention), to screen individuals for treatable risk factors, and then ultimately to treat those with established disease, to help manage the condition and prevent the development of complication (as a form of secondary prevention). Screening for risk factors (such as elevated BMI and waist circumference, and evidence of sedentary behaviour) and "brief intervention" can be used by all healthcare professionals in all settings as a starting point, with referral to physiotherapists or exercise physiologists as required (246). As obesity and diabetes are complex multifactorial issues, there is the need for a multifaceted approach and the inclusion of "real-life" interventions in schools, workplaces, communities, as well as in healthcare settings, that are cost effective, sustainable and scalable. Lifestyle intervention offered by public healthcare services will be increasingly based in Primary Care settings as they become more established and take over the management of chronic diseases from the acute hospitals (247). Intervention could take the form of a combination of one-on-one exercise consultations consisting of exercise counselling / coaching and exercise prescription for self-management, or referral to exercise classes incorporating a multidisciplinary educational component, similar to those of Phase 3 Cardiac Rehabilitation. The results of the current study demonstrate that this could be effective if performed as a 12 week intervention with 4 training sessions per week of just over an hour in duration. Patients could then progress on to a Phase 4 style maintenance

programme. The class setting would offer patients the opportunity to gain experience of exercising in a supervised setting, and an element of camaraderie. Exercise consults with patients should emphasise self-management (goal setting and problem solving skills) and self-monitoring (weight, food intake, physical activity and blood sugar levels) and recommendations should take into account personal preferences and daily routines. Walking would be a useful way to start, with progression to brisk walking, but cycling such as was used in the current study would be a good way to add increased intensity, as the patient improves. Cycling is also a form of non-weight bearing exercise that could be used effectively by T2DM patients with peripheral neuropathy without causing damage to the extremities. Resistance training could be added, either in isolation on certain days, or as part of a circuit, but the emphasis should be on high intensity aerobic exercise such as that used in this study. As part of the translation of the findings of the current study out of the lab, it would be recommended that the exercise related advice given to patients be individualised, with specific training times and intensities prescribed, which would be expected to yield greater benefits beyond those obtained from the use of generic exercise guidelines. The implementation of individualised exercise prescription such as this would require exercise testing, and necessitate the transition of procedures such as $VO_2$max testing out of laboratory settings and into clinical settings, so that it could be used for prescription and also as an outcome measure. At first glance, $VO_2$max testing can seem like a labour intensive process, but the protocol recommendations are that a test should be completed within 8 to 17 minutes, which would not be time prohibitive in a clinical setting. Although these tests can be performed quickly, the equipment is expensive and staff training is required. A low cost alternative would be the use of shuttle run testing, a 12 minute run or 6 minute walk test,

which can be performed with minimal equipment, but adequate space is required. However, a 3 minute step test could be effectively performed in an office setting if required, and exercise related parameters could be extrapolated from submaximal heart rate data (248). Target heart rates could then be given to patients, and using the intensity employed in the current study (70% $VO_2$max), the equivalent heart rate would be approximately 80% heart rate max (which patients could work up to over a number of weeks if necessary). While moderate intensity exercise is usually recommended to patients, the results of the current study show that high intensity exercise can be used effectively in the management of both younger and older patients with T2DM. This is of particular interest, as a lack of time is the main reason given for not participating in regular exercise (130), and high intensity training is a time efficient way to train and is associated with a greater rate of energy expenditure, and greater improvements in $VO_2$max and insulin sensitivity than lower intensity exercise. Another recommendation from the current study is the use of waist circumference measurements and bioelectrical impedance to measure body composition and fat distribution, both of which could be easily utilized in clinical settings. The results of the study showed that among these patients, weight loss does not always result in fat loss, and that even in the absence of weight loss, a reduction in fat mass can still occur.

## 7.3 **Limitations and Future Work**

- Most lifestyle interventions use both diet and exercise together, but as part of a study, this does not allow the individual contribution of either to be determined. Therefore, in the current study, the protocol was designed to facilitate the separate examination of diet and exercise, in addition to examining the priming effect of sequence. A large

number of subjects were recruited at baseline, but due to the dropout rate from the study, and the subgroups that the crossover design created, the overall interpretation of the results became challenging. While the crossover design allowed the effect of intervention sequence to be examined, it created complications when the individual interventions were compared. Had the primary objective been only to compare diet and exercise, a gap between interventions could have been included to allow participants to return to baseline before the transition to the next intervention, but no definitive "washout period" is possible for lifestyle intervention, in contrast to the situation for studies of drug therapy. Had a larger number of subjects been recruited, they could have been assigned solely to one intervention, but due to limited patient access and the time constraints of the current study, a crossover design was used to increase the data yield. Future studies could use a combination of various protocols to further examine different exercise intensities, durations, and frequencies for the optimal management of early-onset T2DM. Furthermore, it will also be necessary to develop "real-life" interventions that will allow for the "translation" of study findings out of the laboratory and into the community, to encourage greater compliance. This is currently being examined as part of the follow up DEXLIFE project (an FP7 funded study) by collaborators in Dublin City University.

- Due to limited sample stock accumulation at the time of testing apparatus availability, metabolomic examination was only possible at baseline, but it would also have been interesting to examine changes in total and individual fatty acid species, and BCAA's after lifestyle intervention. Based on the results of the current study, it is hypothesised

that further differences in lipid profile would be detected, with greater improvements occurring in the OT2 group. This issue is currently being examined by DEXLIFE collaborators from Metabolon, USA.

- Other studies use markers of mitochondrial function as a surrogate estimate of oxidative capacity, but in the current study, a direct measure of function was obtained using high resolution respirometry. Although this measured oxidative capacity per unit volume of mitochondrial protein, a true measure of muscle fibre mitochondrial content was not obtained. Although there was no difference in intrinsic mitochondrial oxidative function between groups, there could have been a difference in mitochondrial content, and therefore a difference in total mitochondrial capacity. However, it has previously been shown that there was no deficiency in porin (a mitochondrial membrane protein, used as a marker of mitochondrial volume), among YT2 subjects when compared to age and BMI matched non-diabetic subjects (102). Additional markers of mitochondrial density (cardiolipin, citrate synthase and mitochondrial DNA copy-number), size (examined in muscle biopsies using transmission electron microscopy), and morphology (Mitofusin-2), would also be of interest, including samples from non-T2DM control subjects, and some of these are currently being tested in the laboratory of Professor Antonio Zorzano in the Institut De Recerca Biomedica Barcelona as part of the follow up DEXLIFE project.

- The protocol used to examine intrinsic mitochondrial function was based on a standard protocol that utilizes sugar based substrates. Given the differences in lipid

profile between groups, it could be hypothesised that a deficiency in mitochondrial oxidative capacity could have been observed had lipid based substrates been used. However, studies that have included both types of protocol have tended to obtain similar results irrespective of the substrate used (85). To further examine the contribution of mitochondrial function to the pathogenesis of early-onset T2DM, future work could also include the use of phosphorous magnetic resonance spectroscopy ($^{31}$P-MRS) to non-invasively determine ATP turnover, testing for mitochondrial enzyme activity, mitochondrial biogenesis (PGC-1α), muscle fibre type distribution, intramyocellular lipid accumulation (using $^{1}$H-MRS imaging or by histological examination), and lipid intermediate concentration.

- The study could have benefited from the inclusion of an objective measure of physical activity as questionnaires are not particularly sensitive, and as there is a risk of recall bias (228), as it is known that patients with T2DM do not report their lifestyle related habits completely accurately (179). Objective measures of physical activity are recommended (249), but are not 100% dependable either, as the accuracy of pedometers is highest with regard to step counts and less accurate when extrapolated to estimate distance and energy expenditure, with the potential for overestimation to occur at low speeds on treadmill, and underestimation at the highest speeds (250). Accelerometers are considered reliable at most speeds (r=0.87 - 0.92) (251). The validity is predominantly based on laboratory based testing on treadmills however, and not in free living environments (251), and while the results of many accelerometers do not correlate well with doubly labelled water studies (252), they

can be used effectively to provide information regarding patterns of activity. Doubly labelled water provides an account of average energy expenditure over relatively extended periods (several days), while accelerometers make direct triaxial measurements of movement (in the form of acceleration, which is a change in speed with respect to time), and provide specific information regarding the intensity, frequency and duration of activity, in addition to data on inactivity. Over short periods of analysis, the act of wearing an accelerometer can prompt subjects to increase their physical activity levels, which can be even more of an issue when using pedometers with a visible display screen (253), and another concern is the risk that accelerometers can fail while in the possession of subjects when used over prolonged periods (228). Although no objective measure of physical activity was recorded during the current study, the validity of the IPAQ results at baseline was supported by the low $VO_2$max results observed in both groups, and by the obesity levels of subjects. It would have been interesting to record physical activity levels throughout the course of the intervention, as during exercise training, general physical activity levels outside of the programme can reduce initially due to fatigue (244). However, as subjects were sedentary to begin with in the current study, there would not have been much scope for further reduction. It is also suggested that general physical activity levels can increase towards the latter stages of exercise interventions as fitness levels rise (244), but the anthropometric data does not support this. Similarly, the $VO_2$max and weight data do not suggest that there was any change in physical activity levels during the dietary intervention. Any increase in daily physical activity levels would not have affected $VO_2$max however, as general activity is usually not of

sufficient intensity to increase $VO_2$max, and in the current study, there was no correlation between $VO_2$max and general daily physical activity levels.

- Use of the euglycaemic–hyperinsulinaemic clamp technique would have been preferable for testing insulin resistance, but it was not possible due to the labour intensive nature of the other testing procedures (especially high resolution respirometry, which had to be performed on fresh muscle samples), and the level of supervision that was required during the exercise intervention. However, the OGIS test has been shown to correlate well with the clamp technique (53, 54).

- Bioelectrical impedance was used to calculate fat mass and FFM, and while the use of DEXA may have provided a more true reflection of body composition, bioelectrical impedance provides a reproducible objective measure. The main factor that can confound bioelectrical impedance readings is hydration status, but to overcome this, subjects were tested under similar fasting conditions on each occasion.

- Lower socioeconomic status is correlated with a higher incidence of obesity and T2DM, and with a higher incidence of T2DM among adolescents. Socioeconomic status was not formally assessed in the current study, but both groups of participants were recruited from the same hospital which caters for inner city patients that live locally and come from the same catchment area. While it is not suspected that there were any socioeconomic differences between groups, the study would have benefited from the inclusion of a questionnaire to verify this, with questions to determine

employment status, income and education, such as those used in The Whitehall II study (254).

- Finally, another limitation of the study was the gender imbalance within the groups. However, this did not explain any differences between groups as there was a similar proportion of male and female subjects in each group. Had more subjects been included, a more comprehensive gender related examination may have been possible, including an examination of the effect of lifestyle intervention.

## 7.4 **Conclusion**

Subjects with early-onset T2DM have a distinctive "metabolic signature" which distinguishes them from later-onset T2DM. Lipotoxicity has previously been implicated in the pathogenesis of T2DM, and interestingly, the YT2 group in the current study had a distinctive lipid profile (relating to total and individual species of fatty acid), which correlated with insulin resistance. Furthermore, the YT2 group also had a distinct adipokine profile, with a lower adiponectin concentration, which was also negatively correlated with insulin resistance. Because impaired mitochondrial function has also previously been implicated in the development of insulin resistance, it was hypothesised that this would be a pronounced feature of early-onset T2DM, but there were no differences between groups. Although there was no difference in intrinsic mitochondrial function or $VO_2$max between groups, these variables have been shown to decline with age, and having values similar to those of the OT2 group could constitute a relative deficiency among the YT2 subjects.

Extreme insulin resistance in such a young group also exposes the YT2 subjects to an increased risk of developing diabetes complications.

Lifestyle intervention has previously been demonstrated to be effective in the management of later-onset T2DM, but it has also recently been shown to be less effective in the management of early-onset T2DM. However, the current study demonstrated that patients with early-onset T2DM do respond to lifestyle intervention which includes individually prescribed, supervised, progressive aerobic exercise training at 70% $VO_2$max, with anthropometric improvements, improvements in $HbA_{1c}$, and improvements in whole body and mitochondrial oxidative capacity.

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# Appendix I

International Physical Activity Questionnaire

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

# LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

**FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)**

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

**Background on IPAQ**

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

**Using IPAQ**

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

**Translation from English and Cultural Adaptation**

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

**More Information**

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

*PART 1: JOB-RELATED PHYSICAL ACTIVITY*

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

**Yes** ☐

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

## PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

*13.* **How much time did you usually spend on one of those days** walking **from place to place?**

_____ **hours per day**

_____ **minutes per day**

## *PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY*

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

*PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY*

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

*PART 5: TIME SPENT SITTING*

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

**This is the end of the questionnaire, thank you for participating.**

# Appendix II

## Innocor Calibration

Calibration of the metabolic analysis equipment took place prior to each test. A one-point autocalibration was performed on the oxygen sensor using ambient air, which was calibrated to match the figure 20.93%. Once a year, an additional two point calibration was performed by a representative of the manufacturer using air and pure oxygen. The flowmeter was calibrated prior to $VO_2$max testing using a syringe of known volume (3.0 litre volumeter, Series 5530, Hans Rudolf, Inc. USA). The syringe was filled and emptied 5 times at 3 different rates of flow and the figures were accepted if the new "gain values" were in the range of 0.9 to 1.1. If the values were outside this range, the nylon screen in the pneumotachometer was replaced. As the volume and flow measurements were detected closer to their source than the gas concentration measurements, they were detected more quickly, and for accurate $VO_2$ data to be obtained, it was necessary to ensure that the gas-flow delay did not vary excessively. This calibration was performed by taking eleven large, rapid breaths in followed by slow exhalations out through the mouthpiece, to ensure that the readings did not vary by more than 20 to 40ms from day to day, as per the manufacturers guidelines. Ambient room conditions were also taken into account and entered into the metabolic analyser. Room temperature and humidity were measured using an electronic thermometer / hygrometer (Hygro-Thermometer Clock L55AJ, Maplin Electronics, England), while atmospheric pressure was measured using a dial barometer (Temperature Compensated Precision Barometer, Diplex ltd. England).

# Appendix III

**Oral presentation at the Irish Society of Chartered Physiotherapists Annual Conference (ISCP), Croke Park, Dublin, 16th November 2012.**

**Associated publication:**
**O'Hanlon D, Wanic K, Pazderska A, Shah S, Cooper D, Collura N, O'Gorman D, Nolan JJ. Changes in fat mass and waist circumference are the best anthropometric indicators of adaptation to lifestyle intervention in patients with type 2 diabetes. Innovation: Ideas into Action Physiotherapy in a challenging environment. Book of Abstracts, p 7.**

**Title:** Exercise management of type II diabetes in primary care

**Authors:** D McCarthy[1], C Blake[1],
[1]UCD School of Public Health, Physiotherapy and Population Science, Belfield, Dublin 4,, Ireland

**Objective -** The aim of this study was to assess the effects of a combined supervised resistance and self managed pedometer walking programme in type two diabetes mellitus patients with a follow up at four months post programme. Compliance with the programme was also assessed. This was a pilot study to test the feasibility of a larger study and to provide data for sample size calculation for this study.

**Research Design and Methods –** A total of 13 participants were randomly assigned to an intervention (n=7) or control group (n=6). Both groups were well matched for HbA1c, age, height, weight and physical activity. The intervention group took part in an 8 week exercise programme that included twice weekly supervised resistance training and three times weekly pedometer (provided free of charge) based walking programme which they carried out unsupervised at home. Pre and post outcome measures included glycated haemoglobin (HbA1c), fasting blood glucose, BMI, strength, physical activity levels and quality of life using the SF-36. Follow up occurred at four months post programme. Compliance was also assessed. Ethical approval was given by University College Dublin Ethics Committee.

**Results –** HbA1c decreased by 0.27% ($p<0.05$), BMI decreased ($p<0.05$), strength increased, measured by 1RM, ($p<0.05$) and physical activity levels increased ($p<0.05$). There was no significant change in quality of life. Only one participant did not complete the programme. At follow up four months post programme 85.7% ($p<0.05$) were still exercising in the intervention group.

**Conclusions –** Combined resistance and self managed pedometer based walking programme lead to an improvement in glycaemic control in patients with diabetes type two. Pedometers and putting the patient in control of part of their programme lead to high compliance and adherence rates post study. A larger study needs to be carried out to confirm the above results. This is the first study to assess the effects of this type of programme in patients with type two diabetes.

**Title:** Changes in fat mass and waist circumference are the best anthropometric indicators of adaptation to lifestyle intervention in patients with type 2 diabetes.

**Authors:** D O'Hanlon[1,2], K Wanic[2], A Pazderska[2], S Shah[2], D Cooper[1], N Collura[2], D O'Gorman[1], JJ Nolan[3],
[1]DCU, Dublin, Ireland, [2]MRU, SJH, Dublin, Ireland, [3]Steno, Gentofte, Denmark

**Purpose:** The aim was to examine the relationship between weight loss, anthropometric measurements, and food diary outcomes after a 12 week hospital based lifestyle intervention among young (age <30years) patients with type 2 diabetes (T2DM).

**Relevance:** Approximately 15% of weight lost through dieting is thought to be fat-free mass, making it is difficult to determine the true effect of lifestyle intervention.

**Participants:** 10 obese (body mass index > 30) patients with T2DM were randomised to an exercise intervention, while 14 matched patients were randomised to a dietary intervention matched for energy restriction.

**Methods:** During the exercise intervention, patients performed supervised aerobic exercise 4 days per week, while during the dietary intervention, patients filled out food diaries and met with a dietician every two weeks. Anthropometric measurements were made before and after, with bioelectrical impedance used to examine body composition.

**Analysis:** Data analysis was performed using *t*-tests (SPSS version 16), and expressed as Mean ± SEM.

**Results:** There was no change in weight after the exercise intervention, however, there was a reduction in fat mass (-2.6±0.9kg, $p=0.02$), and waist circumference (-4.2±1.5cm, $p=0.02$). There was a significant self-reported reduction in energy intake during the dietary intervention (-812±258kcal/day, $p=0.01$), after which there was a reduction in weight (-2.5±1.0kg, $p=0.03$), but no reduction in fat mass, or waist circumference.

**Conclusion:** Patients with T2DM underreport their calorie intake, as the reported reduction in energy intake should have resulted in greater fat loss. Food diaries and changes in weight are therefore not the best indicators of progress during lifestyle intervention. Exercise is better than dietary restriction at fat mass reduction, which is the most important aspect of weight reduction, even though total body

# Appendix IV

**Audio poster presentation at the 48th Annual Meeting of the European Association for the Study of Diabetes (EASD), Berlin, Germany, 3rd October 2012.**

**Associated published abstract:**
**O'Hanlon D, Wanic K, Pazderska A, Shah S, Cooper D, Collura N, O'Gorman D, Nolan JJ. Altered Response to Diet and Exercise Intervention in Early-Onset Type 2 Diabetes. Diabetologia, 2012, vol 55, pS248.**

## 600

**The effects and physiological mechanisms of free-living interval-walking training on glycaemic control in type 2 diabetes patients: a randomised, controlled trial**

**K. Karstoft**[1], K. Winding[1], S.H. Knudsen[1], B.K. Pedersen[1], J.S. Nielsen[2], T.P. Solomon[1];
[1]Faculty of Health Sciences, University of Copenhagen, Rigshospitalet, [2]Department of Endocrinology, Diabetes Research Centre, Odense University Hospital, Denmark.

**Background and aims:** In type 2 diabetes patients, free-living walking training is feasible but shows limited effect upon glycaemic control variables. On the other hand, interval training methods have shown huge improvements in glycaemic control but suffer from lower adherence rates. In this study, we first evaluated the feasibility of free-living walking training in type 2 diabetes patients; secondly, we investigated the effects of interval-walking versus continuous-walking training upon glycaemic control; and thirdly, we assessed the underlying physiological mechanisms of changes in glycaemic control.
**Materials and methods:** Subjects with type 2 diabetes (58.7 ± 1.4 years, 29.5 ± 0.9 kg/m2) were randomized to a control group (n=8), a continuous-walking training group (n=12), or an interval-walking training group (n=12). Training groups were instructed to train 5 sessions per week, 60 minutes per session and were controlled with an accelerometer and a heart rate monitor. Before and after the 4 month intervention, maximal oxygen consumption (VO2max) was assessed, glycaemic control was measured using continuous glucose monitoring (CGM), and insulin secretion/sensitivity was measured using a hyperglycaemic clamp (5.4 mmol/l above fasting glucose concentration).
**Results:** Training groups demonstrated high and equal training adherence (89 ± 4%), and training energy-expenditure and mean training intensity were comparable. VO2max was unchanged in the control group and continuous-walking group, but increased in the interval-walking group (16 ± 4 %, P<0.05). Glycaemic control (mean CGM glucose levels) worsened in the control group (delta mean CGM glucose = 1.2 ± 0.4 mmol/l, P<0.05), whereas mean and maximum CGM glucose levels decreased in the interval-walking training group (delta mean CGM glucose = -0.8 ± 0.3 mmol/l, P=0.05, delta maximum CGM glucose = -2.8 ± 0.8 mmol/l, P<0.05). The continuous-walking training group showed no changes in glycaemic control. In the interval walking training group, the insulin sensitivity (57 ± 17 %, P<0.05) increased, whereas the insulin secretion did not change (3 ± 6 %, P>0.05). The disposition index increased comparable to the insulin sensitivity (60 ± 16 %, P<0.05). In the continuous-walking and control group, no changes were seen in any of these parameters.
**Conclusion:** Free-living walking training is feasible in type 2 diabetes patients and interval-walking training is superior to energy-expenditure matched continuous-walking training upon improving glycaemic control. Furthermore, interval-walking induced improvements in glycaemic control seem to be dependent on improvements in insulin sensitivity and increased disposition.
*Clinical Trial Registration Number: NCT01234155*
*Supported by: DD2, Danish Agency for Science, Trygfonden, EFSD/Amylin grant*

## 601

**Effects of high-intensity interval training on glucose and fat metabolism in healthy, sedentary middle aged men**

**A.M. Savolainen**[1], K.K. Kalliokoski[1], J.J. Eskelinen[1], V. Lepomäki[1], I. Heinonen[1], K. Virtanen[1], R. Parkkola[1], J. Kapanen[2], J. Knuuti[1], P. Nuutila[1], J.C. Hannukainen[1];
[1]Turku PET Centre, University of Turku, Finland, [2]Paavo Nurmi Centre, Turku, Finland.

**Background and aims:** Lifestyle interventions have been shown to improve insulin sensitivity in liver and abdominal adipose tissues in obese patients and in patients with prediabetes or type 2 diabetes mellitus. Recently, two weeks of low-volume, high-intensity interval training (HIT) has been shown to increase whole body insulin sensitivity and glucose metabolism in skeletal muscle. The aim of this study was to investigate whether HIT also affects glucose and fatty acid metabolism in internal organs.
**Materials and methods:** Eight healthy, sedentary, middle aged men (mean ± SD, age: 47 ± 5 years; BMI: 26 ± 2.9 kg·m$^{-2}$; $VO_{2max}$: 34 ± 4 ml·kg$^{-1}$·min$^{-1}$) were studied before and after two weeks and six sessions of HIT (4-6 x 30 s all out sprints on a cycle ergometer with 4 minutes of recovery). Skeletal muscle, liver, pancreas, abdominal subcutaneous and visceral fat tissue insulin stimulated glucose uptake and fasting free fatty acid uptake were measured using FDG and FTHA PET -methods. In addition, muscle, liver and pancreas fat content was assessed with magnetic resonance spectroscopy.
**Results:** Following HIT intervention, $VO_{2max}$ increased by 4.7 % (from 34 ± 4 to 35.5 ± 4 ml·kg$^{-1}$·min$^{-1}$, student paired t-test, p=0.019). Fasting serum free fatty acid concentration (from 0.46 ± 0.14 to 0.33 ± 0.09 mmol·l$^{-1}$, p=0.054) and plasma total cholesterol level (from 5.4 ± 0.7 to 4.5 ± 0.6 mmol·l$^{-1}$, p<0.001) decreased. Whole body insulin sensitivity increased by 12 % but without statistical significance (39.1 ± 11.4 *vs.* 43.4 ± 16.3 µmol·kg$^{-1}$·min$^{-1}$, p = 0.22). Although glucose uptake in m. quadriceps femoris increased by 38% (from 44 ± 11 to 60 ± 18 µmol·kg$^{-1}$·min$^{-1}$, p=0.004), two weeks of HIT had no influence on glucose uptake in liver, pancreas and abdominal adipose tissues. The results of free fatty acid uptake and MRS studies will be presented in the congress.
**Conclusion:** Two weeks of low-volume high-intensity interval training seems to be an effective method to improve insulin sensitivity in skeletal muscle, but has no effect on glucose metabolism in internal organs in healthy middle aged men. Further studies are needed in patients with prediabetes and type 2 diabetes to understand the role of exercise training in the metabolism of internal organs.
*Clinical Trial Registration Number: NCT01344928*
*Supported by: EFSD/ Novo Nordisk, Ministry of Education, Academy of Finland, Orion Farmos*

## 602

**Altered response to diet and exercise intervention in early-onset type 2 diabetes**

**D.J. O'Hanlon**[1], K. Wanic[2], A. Pazderska[2], S. Shah[2], D.E. Cooper[1], N. Collura[2], D.J. O'Gorman[1], J.J. Nolan[3];
[1]School of Health and Human Performance, Dublin City University, Ireland, [2]Metabolic Research Unit, St James's Hospital, Trinity College Dublin, Ireland, [3]Steno Diabetes Centre, Gentofte, Denmark.

**Background and aims:** We have reported that patients with early-onset type 2 diabetes (YT2) are much more insulin resistant, and less responsive to lifestyle and medical interventions than patients with later onset diabetes (OT2). We have found (unpublished data) that YT2 has a different metabolic signature, with higher fasting concentrations of total and several individual fatty acid species than OT2. The aim of this study was to further examine and compare the adaptive responses to lifestyle intervention in YT2 and OT2 subjects.
**Materials and methods:** YT2 and OT2 subjects were recruited for baseline examination, and to participate in a 6 month lifestyle intervention including a reduced-calorie diet (-2500kcal/week) and exercise training at 70% $VO_2$max. Testing included a $VO_2$max test to exhaustion, fasting anthropometric measurements (weight, waist circumference, and fat mass using bioelectrical impedance), a muscle biopsy from the vastus lateralis (to examine intrinsic mitochondrial function: state 3 respiration measured by respirometry) blood sampling to examine lipid profile, and an OGTT to measure insulin sensitivity (based on a 2 hour OGTT).
**Results:** We recruited 69 patients for evaluation: 23 YT2 (27.9±0.9 years) and 46 BMI-matched OT2 patients (55.3±1.2 years). At baseline, there were no differences between groups in waist circumference, $VO_2$max, intrinsic mitochondrial function, or indices of insulin sensitivity. There was no difference in fasting triglyceride concentration, but despite a similar diet, the YT2 patients had higher total cholesterol (4.7±0.2 vs 4.2±0.1mmol/l, $p$=0.02), and LDL-cholesterol (2.8±0.2 vs 2.2±0.2mmol/l, $p$=0.01). A subgroup of 25 subjects (10 YT2 and 15 OT2) completed the lifestyle intervention, after which only the OT2 group had a significant reduction in fat mass (Δ 3.1±0.9kg, $p$=0.01). Both groups had comparable improvements in $VO_2$max. However, a reduction in fasting triglyceride concentration (Δ 0.37±0.1mmol/l, $p$=0.01), and an increase in insulin sensitivity (OGIS-2hr: Δ 47.5±18.3ml/min/m$^2$, $p$=0.02), occurred only in the OT2 group.
**Conclusion:** YT2 responds differently to lifestyle intervention than OT2. Despite equal compliance, only OT2 subjects exhibited a reduction in fat mass, with improvements in fasting triglyceride concentration and insulin sensitivity. These observations suggest that metabolic factors contribute to treatment resistance in YT2 patients.
*Supported by: EFSD/Novo Nordisk grant*

# Appendix V

**Audio poster presentation at the American Diabetes Association (ADA) Annual Meeting, 72nd Scientific Sessions, Philadelphia, Pennsylvania, 11th June 2012.**

**Associated published abstract:**
**O'Hanlon D, Wanic K, Pazderska A, Shah S, Cooper D, Collura N, O'Gorman D, Nolan JJ. Altered Response to Diet and Exercise Intervention in Early-Onset Type 2 Diabetes. Diabetes, 2012, vol 61, pS182.**

sidered among clinical approaches to risk reduction in East Asians with diabetes, who have different profiles for macrovascular complications.

*Supported by: The Ministry of Health, Labor and Welfare, Japan*

### 716-P

**Self-Reported Physical Activity is Associated With Beta-Cell Function in Mexican Americans**

ZANGHUA CHEN, MARYHELEN BLACK, RICHARD M. WATANABE, ENRIQUE TRIGO, MIWA TAKAYANAGI, THOMAS A. BUCHANAN, ANNY H. XIANG, *Los Angeles, CA, Pasadena, CA*

Intensive exercise training has been shown to improve insulin sensitivity and prevent type 2 diabetes. We assessed whether daily-living physical activity (PA) is associated with insulin sensitivity and other type 2 diabetes-related traits. Subjects were participants of BetaGene, a study of obesity, insulin resistance and beta-cell function in Mexican Americans. PA was self-reported and categorized into three groups according to HHS physical activity guidelines for Americans: "*Low*" (vigorous < 75 mins/wk and moderate < 150 mins/wk), "*Moderate*" (vigorous ≥ 75 mins/wk or moderate ≥ 150 mins/wk), and "*High*" (vigorous ≥ 75 mins/wk and moderate ≥ 150 mins/wk). Trend in PA was tested for association with metabolic traits measured by OGTTs, IVGTTs, BMI, DEXA and history of gestational diabetes (GDM) in women. Results were from 1,152 subjects with complete data ("Low"=501, "Moderate"=448, and "High"=203) with mean age 34.7 ± 8.0 years, mean BMI 29.6 kg/m$^2$, and 73% female. After adjustment for age and sex, higher level of PA was significantly associated with lower 2-hr glucose, fasting and 2-hr insulin, and higher beta-cell function index (p=0.01, 0.001, 0.008, and 0.009, respectively). Greater PA was marginally associated with lower fasting glucose and higher insulin sensitivity (p=0.10 and 0.08, respectively), but was not associated with BMI or body fat percentage (BFP) (p>0.38 for each). Age- and sex-adjusted mean beta-cell function index for "*Low*", "*Moderate*" and "*High*" PA were 8265, 8526, and 9601, respectively. Women in the "*High*" and "*Moderate*" PA groups were 0.6 (95% CI: 0.3-0.98) and 0.8 (95% CI: 0.6-1.1) times likely to have had GDM compared to women in the "*Low*" PA group. Results were similar after further adjustment for BMI or BFP. We conclude that greater daily-living PA is associated with an improved glucose and insulin profile and better beta-cell function that were not explained by differences in body fat. Physical activity may have direct effect to protect beta cell function.

*Supported by: NIDDK*

### 717-P

**Altered Response to Diet and Exercise Intervention in Early-Onset Type 2 Diabetes**

DECLAN O'HANLON, KRZYSZTOF WANIC, AGNIESZKA PAZDERSKA, SYED SHAH, NOELLE COLLURA, DIANE COOPER, DONAL O'GORMAN, JOHN J. NOLAN, *Dublin, Ireland, Gentofte, Denmark*

We have reported that patients with early-onset type 2 diabetes (YT2) are much more insulin resistant, and less responsive to lifestyle and medical interventions than patients with later onset diabetes (OT2). We have found (unpublished data) that YT2 has a different metabolic signature, with higher fasting concentrations of total and several individual fatty acid species than OT2.We recruited 69 patients for evaluation: 23 YT2 (27.9±0.9 years) and 46 OT2 (55.3±1.2 years), BMI-matched. At baseline, there were no differences between groups in waist circumference, $VO_2$max, intrinsic mitochondrial function (state 3 respiration measured by respirometry), or indices of insulin sensitivity. There was no difference in fasting triglyceride concentration, but despite a similar diet, the YT2 patients had higher total cholesterol (4.7±0.2 vs 4.2±0.1mmol/l, $p$=0.02), and LDL-cholesterol (2.8±0.2 vs 2.2±0.2mmol/l, $p$<0.01).A subgroup of 25 subjects (10 YT2 and 15 OT2) completed a 6 month lifestyle intervention including a reduced-calorie diet (-2500kcal/week) and exercise training at 70% $VO_2$max, after which only the OT2 group had a significant reduction in fat mass (Δ 3.1±0.9kg, $p$<0.01). Both groups had comparable improvements in $VO_2$max. However, a reduction in fasting triglyceride concentration (Δ 0.37±0.1mmol/l, $p$=0.01), and an increase in insulin sensitivity (Δ 47.5±18.3ml/min/m$^2$, $p$=0.02), estimated by OGIS (based on a 2 hour OGTT), occurred only in the OT2 group.YT2 responds differently to lifestyle intervention than OT2. Despite equal compliance, only OT2 subjects exhibited a reduction in fat mass, with improvements in fasting triglyceride concentration and insulin sensitivity. These observations suggest that metabolic factors contribute to treatment resistance in YT2 patients.

*Supported by: EFSD*

### 718-P

**Active Adults With Type 1 Diabetes Using CSII Should Reduce Basal Insulin Infusion by 80% not 50% to Avoid Hypoglycemia during Aerobic Exercise**

ALISTAIR N. LUMB, JACQUI CARR, GARY PETERS, FREDRIK KARPE, IAN W. GALLEN, *High Wycombe, United Kingdom, Oxford, United Kingdom*

Rates of hypoglycaemia associated with exercise can be reduced in children and adolescents with Type 1 Diabetes using CSII by the reduction of basal insulin infusion by 50% at the start of exercise. However, it is not clear whether this strategy is also useful in adults. We performed a study of the effect of basal insulin reduction on blood glucose during exercise in middle-aged patients with Type 1 diabetes treated with CSII.Twelve adults (6 men and 6 women) with mean age 46 years (range 34-65 years) and mean HbA1c of 51 mmol/mol (range 42-62) were enrolled. All participants exercised regularly for at least 1 hour per week and had no significant complications of diabetes. $VO_2$ MAX (mean 35.0 ml/kg/min, range 24.3-44.5) was measured at an initial visit. At subsequent visits baseline samples were taken 2 hours after a standardised meal containing 60g carbohydrate which was accompanied by the usual bolus insulin. Exercise commenced 90 minutes later. Exercise sessions lasted for 60 minutes at 50% $VO_2$ MAX and were successfully completed by all participants. One group of 6 participants (M50) applied a 50% basal insulin infusion reduction during exercise, the other group of 6 an 80% reduction (M80). A series of studies were performed with reductions made 0, 30, 60 or 90 minutes before exercise. There were no differences between the groups in age, glycemic control and $VO_2$ MAX.Mean baseline blood glucose levels were not different between the groups (8.7 mmol/l (M80) v 8.5 mmol/l (M50), p = 0.50). There was no clear relationship between the timing of basal insulin reduction and hypoglycemia in either of the groups, but overall there were significantly more episodes of hypoglyaemia (blood glucose < 3.6 mmol/l) in the M50 group than in the M80 group (86% v 12%, p <0.001).These data suggest that a basal insulin infusion reduction of 80% rather than 50% is preferable to avoid hypoglycemia in adults during aerobic exercise, and this should be reflected in clinical advice.

*Supported by: Life Scan, Inc.*

### 719-P

**Longitudinal Association between TV Watching and Diabetes Risk Markers in SEARCH for Diabetes in Youth Study**

CHAO LI, BETTINA BEECH, TESSA CRUME, RALPH B. D'AGOSTINO, JR., DANA DABELEA, LAWRENCE DOLAN, JILL LANDSBAUGH, ANGELA D. LIESE, ELIZABETH MAYER-DAVIS, RUSSELL PATE, DAVID J. PETTITT, ANWAR T. MERCHANT, *Columbia, SC, Winston-Salem, NC, Aurora, CO, Cincinnati, OH, Chapel Hill, NC, Santa Barbara, CA*

Studies have shown that TV watching is associated with adverse health outcomes. To date, few studies have examined the effect of TV watching longitudinally in youth. To address this issue in diabetes, we assessed the effect of TV watching over 60 months on A1C and serum lipids among 1389 youth (>10 yr. old) with newly diagnosed type 1 (T1D) and type 2 diabetes (T2D) participating in the SEARCH for Diabetes in Youth Study. We used mixed models to assess associations between TV watching (time varying) and change in A1C and serum lipids over time, adjusting for age, sex, race, parental education, household income, insurance type, BMI z-score, family composition, and treatment for diabetes, dyslipidemia, and hypertension. A1C increased over time among T1D (p<0.01) and T2D (P<0.01) cases. At baseline A1C was higher if participants watched TV for ≥3 hrs./ day on weekdays compared with those who watched less for both T1D and T2D (estimateT1D=0.20, p<0.01; estimateT2D=0.39, p=0.01). When considering time varying TV watching practices, A1C was higher by 0.16 in T1D (p<0.01) and 0.45 in T2D (p<0.01) if participants watched TV for ≥3 hrs./day on weekdays compared with those who watched less during follow up adjusted for baseline TV watching. Triglyceride levels were higher by 7.31 mg/dL (p<0.01) among youth who watched TV for ≥3 hrs./day on weekdays during follow-up (but not at baseline) than those who watched less in T1D cases. Other lipids were not associated with TV watching (p>0.05 in all models). Decreasing TV watching may contribute to lower A1C and improved lipid profile decreasing cardiovascular risk in youth with diabetes.

For author disclosure information, see page 797.

Guided Audio Tour poster  ADA-Funded Research

# Appendix VI

**Oral presentation at the Irish Society of Chartered Physiotherapists Annual Conference (ISCP), Mullingar, 11th November 2011.**

**Associated published abstract:**
**O'Hanlon D, Wanic K, Pazderska A, O'Gorman D, Cooper D, Collura N, Lithander F, Nolan JJ. Differential effects of exercise and diet on metabolic parameters and physical fitness in early onset type 2 diabetes. Physiotherapy Ireland, 2012, vol 33, no. 1, p 54.**

# Differential effects of exercise and diet on metabolic parameters and physical fitness in early onset type 2 diabetes.

**Authors:** O'Hanlon D [1,2], K Wanic [3], Pazderska A [3], O'Gorman D [4], Cooper D[4], Collura N [5], Lithander F [6], Nolan JJ [7].

**Affiliations:** [1]Physiotherapy Department, St James's Hospital, Dublin. [2]Department of Clinical Medicine, Trinity College Dublin. [3]Metabolic Research Unit, St James's Hospital, Dublin. [4]School of Health and Human Performance, DCU. [5]Department of Clinical Nutrition, St James's Hospital, Dublin. [6]Unit of Nutrition and Dietetics, Trinity College Dublin. [7]Steno Diabetes Centre, Denmark.

**Contact Details:** dohanlo@stjames.ie

**Purpose:** The aims were to characterise the clinical and metabolic differences between early onset type 2 diabetes (ET2DM) and later onset T2DM (LT2DM), and to investigate the effectiveness of lifestyle intervention.

**Relevance:** Diet and exercise are first line treatments for diabetes, but we have previously shown that patients with ET2DM are less responsive to exercise training than matched control subjects.

**Participants:** 19 patients with ET2DM, and 30 BMI and fitness matched LT2DM patients were recruited.

**Methods:** 10 ET2DM and 16 LT2DM patients were randomly assigned to a 12 week supervised exercise intervention at 70% $VO_2$max, while 14 ET2DM and 22 LT2DM patients were randomly allocated to a 12 week dietary intervention matched for caloric deficit. Anthropometric measurements, blood sampling and $VO_2$max testing (progressive incremental exercise testing to exhaustion using open-circuit indirect calorimetry), were performed before and after the interventions. Ethical approval was obtained for all procedures.

**Analysis:** Data analysis was performed using *t*-tests, and expressed as Mean ± SEM.

**Results:** Patients with ET2DM had significantly higher concentrations of total cholesterol and LDL cholesterol at baseline. There was similar weight loss (2.8kg±0.6) between groups after the diet, but also a 1.5kg (±1.0, $p$=0.01) reduction in fat free mass (FFM). There was a 15.8% (±1.9, $p$=0.01) increase in $VO_2$max in both groups after the exercise intervention, and the preservation of FFM. Weight loss (1.02kg±0.4, $p$=0.02) occurred after exercise in the LT2DM group only. Female subjects were more obese, had a lower $VO_2$max, and did not respond as well to lifestyle intervention as male subjects.

**Conclusion:** ET2DM has some unique metabolic characteristics. Patients with ET2DM and LT2DM respond to lifestyle intervention, but have a different pattern of adaptation.

**Implications:** Physiotherapists are ideally positioned to use lifestyle intervention, and in particular exercise prescription to effectively contribute to the management of patients with diabetes.

**Key words:** Diabetes, Exercise, Diet.

**Funding acknowledgement:** EFSD.

# Appendix VII

**Oral presentation at World Physical Therapy Conference (WCPT), Amsterdam, 22nd June 2011.**

**Associated published abstract:**
**O'Hanlon D, Wanic K, Pazderska A, O'Gorman D, Cooper D, Collura N, Lithander F, Nolan JJ. Graded aerobic exercise intervention improves VO2max in early onset type 2 diabetes. Physiotherapy. 2011, vol. 97, p eS927.**

**Participants:** The examination subjects were the right knee joints of 15 healthy volunteers who provided informed consent (eight males, seven females; mean age: 24.3-year-old).
**Methods:** Traction at 100 N and 200 N was applied to the right lower thighs at seven knee joint angles (the fully extended position, 25°, 35°, 45°, 55°, 70°, and 90°), and the joint space widths obtained from ultrasonographic images (B mode) of the knee joint before traction and 10 seconds after the beginning of traction.
**Analysis:** In each experimental conditions, the separation distance was defined as the difference between the joint space width before traction and that during traction.
**Results:** The mean separation distances [mm] (standard deviation) with traction of 200 N were, in increasing order of the angle, 0.3 (0.4), 1.3 (0.8), 1.3 (0.7), 1.7 (1.0), 1.6 (0.9), 1.1 (0.6), and 0.7 (0.8), respectively. The mean separation distances for 25°, 35°, 45°, and 55° were significantly greater than that in the fully extended position ($p < 0.05$), and the mean separation distances for 45° and 55° were significantly larger than that for 90° ($p < 0.05$). The angle that was estimated to maximize the separation distance from the regression formula, which shows the relation between the joint angle and the separation distance, $y = -0.0005x^2 + 0.0502x + 0.3484$, was approximately 50°. In cases of traction at 100 N, no significant differences were found between any of the angles ($p > 0.05$).
**Conclusions:** Collagen fibers being the main component of ligaments and articular capsules tend to be gradually stretched under continued addition of an external force. Taking these characteristics into consideration, the separation distance of the present study obtained with short duration of traction is considered not to indicate the anti-stretch force of tissues stretched by the traction but to mainly reflect the loose level of periarticular tissues removed by the addition of the traction force. Accordingly, it was demonstrated that the MLPP of the normal knee is the flexed position at approximately 50°, which shows the largest separation distance, and with larger angles than experts have previously indicated.
**Implications:** The findings of this study will contribute to effective conducting of joint mobilization to the impaired knees.
**Keywords:** Knee joint; Maximally loose-packed position; Joint traction
**Funding acknowledgements:** None.
**Ethics approval:** This study was approved by the ethics committee of Tokyo Metropolitan University.

**Research Report Platform Presentation**
**Number: RR-PL-3524** **Wednesday 22 June 16:30**
**RAI: Auditorium**

## GRADED AEROBIC EXERCISE INTERVENTION IMPROVES VO2MAX IN EARLY ONSET TYPE 2 DIABETES

O'Hanlon D.[1], Wanic K.[2], Pazderska A.[3], O'Gorman D.[4], Cooper D.[4], Collura N.[5], Lithander F.[6], Nolan J.[7]
*[1]Trinity College Dublin, Clinical Medicine, Dublin, Ireland, [2]St. James's Hospital, Metabolic Research Unit, Dublin, Ireland, [3]St. James Hospital, Metabolic Research Unit, Dublin, Ireland, [4]Dublin City University, Health & Human Performance, Dublin, Ireland, [5]St. James's Hospital, Clinical Nutrition, Dublin, Ireland, [6]Trinity College Dublin, Nutrition and Dietetics, Dublin, Ireland, [7]St James Hospital, Metabolic Research Unit, Department of Endocrinology, Dublin, Ireland*

**Purpose:** The prevalence of type 2 diabetes (T2DM) has increased dramatically and it is predicted that more than 50 million Europeans will be diagnosed by 2030. At the same time the age of diagnosis has decreased and the fastest growing demographic are those aged between 15 and 40 years. Earlier onset increases the patient exposure to the disease, and therefore increases the risk of complications, particularly cardiovascular disease. We recently reported that patients with early onset T2DM have a blunted or absent metabolic response to aerobic exercise, associated with abnormalities in skeletal muscle mitochondrial proteins. The aim of this study was to investigate the effectiveness of a graded 12-week exercise intervention.
**Relevance:** Exercise has been shown to be the key intervention in the prevention and early treatment of T2DM.
**Participants:** Twenty-one subjects were recruited; 6 patients with early onset T2DM (28.5 ± 1.4 years, BMI 37.5 ± 5.4 kg/meter$^2$), 7 patients with later onset T2DM (53.3 ± 1.9 years, BMI 33.5 ± 2.5 kg/meter$^2$), and 8 young obese non-diabetic subjects (22.9 ± 2.2 years, BMI 35.6 ± 1.7 kg/meter$^2$). Subjects were sedentary and weight stable prior to the study. The patients with diabetes were recruited from outpatient clinics in St. James's Hospital Dublin, and the non-diabetic subjects were recruited using local advertisements in Dublin City University.
**Methods:** The study was a longitudinal, controlled study. Baseline testing included anthropometric measurements and a VO2max fitness test, after which subjects began a 12-week supervised progressive aerobic exercise intervention training four days per week at 70% VO2max, and expending 2500 kcal per week. Supplementary VO2max tests were performed at week 4 and week 8 of the exercise intervention, and in the case of any increase in VO2max, the training intensity was increased to 70% of the new VO2max. Dietary intake was kept constant throughout and monitored using food diaries. Testing was repeated after the intervention.

**Analysis:** Statistical analysis was performed using SPSS statistical analysis software (version 16). The data were expressed as Mean ± the Standard Error of the Mean. Statistical significance was set at $p < 0.05$.

**Results:** All groups were matched for height, weight, VO2max and BMI at baseline. VO2max increased significantly after exercise training in the early onset T2DM group (from 2.82 ± 0.16 l/minutes to 3.17 ± 0.15 l/minutes, $p = 0.01$), the later onset T2DM group (from 2.34 ± 0.21 l/minutes to 2.62 ± 0.22 l/minutes, $p = 0.04$), and the young obese group (from 2.78 ± 0.17 l/minutes to 3.05 ± 0.17 l/minutes, $p = 0.03$), by 12.4%, 11.97%, 9.7% respectively, with no significant differences between groups. There were no changes in body weight.

**Conclusions:** A graded aerobic exercise intervention, with increased intensity at 4 and 8 weeks led to improvements in VO2max in these very insulin resistant subjects in spite of the absence of weight loss.

**Implications:** Physical fitness in severely obese subjects with early onset T2DM, in spite of severe insulin resistance and tissue resistance to exercise, can be improved through the use of graded and personalised exercise prescription. These findings can form the basis for effective early interventions in this very high risk patient group.

**Keywords:** Type 2 diabetes; Lifestyle intervention; Exercise
**Funding acknowledgements:** European Foundation for the Study of Diabetes.

**Ethics approval:** From the ethics committee of St. James's Hospital Dublin, and The Adelaide and Meath Hospital, Incorporating The National Children's Hospital.

**Research Report Poster Display**
**Number: RR-PO-306-17-Wed Wednesday 22 June 12:00**
**RAI: Exhibit Halls 2 & 3**

## MUSCLE THICKNESS AS AN INDICATOR TO ESTIMATE CAPACITY FOR DAILY ACTIVITY IN CHILDREN WITH CEREBRAL PALSY

Ohata K.[1], Tsuboyama T.[1], Haruta T.[2], Ichihashi N.[1], Nakamura T.[3]
[1]*Graduate School of Medicine, Kyoto University, Department of Human Health Science, Kyoto, Japan,* [2]*Mukougaoka Special Support School, Department of Programs to Promote Independents, Mukou, Japan,* [3]*Department of Orthopedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

**Purpose:** Many previous studies have examined muscle thickness in the context of activity measures in children and adolescents with cerebral palsy (CP). The present study aim was to determine the clinical relevance of a muscle thickness assessment for the quadriceps femoris muscle (MTQ) and to use this to estimate capacity for daily activity in children with CP.

**Relevance:** We established how assessing capacity for daily activity can contribute to motor function management in children with CP.

**Participants:** We examined 55 children and adolescents (29 females, 26 males; mean age 11y 1mo, SD 3y 6mo) with spastic (quadriplegia, hemiplegia, or diplegia), athetotic, or hypotonic CP. Our subjects, who ranged from levels I to V on the Gross Motor Function Classification System (GMFCS) participated in at least one measurement session.

**Methods:** We measured body height, weight, MTQ, and activity limitations in all subjects. We measured MTQ from B-mode ultrasound images, and used the Pediatric Evaluation of Disability Inventory Mobility score (PEDI-M) to evaluate activity limitations. These scores were calculated at annual measurement sessions spanning three years. Thirty-four children participated all three years.

**Analysis:** We used stepwise multiple regression analysis to develop an equation to estimate the predicted PEDI-M. The first year, we used age, sex, body weight, and MTQ to predict the actual PEDI-M scores in the 55 participants. The 34 children who were measured annually over three years' time were divided into two groups based on their PEDI-M score relative to the actual PEDI-M score. The Friedman test analyzed longitudinal change in each group.

**Results:** The stepwise multiple regression analysis verified that MTQ was an independent determinant of PEDI-M scores (70%). Children who have low predicted PEDI-M scores relative to the actual PEDI-M scores showed a significant decrease in actual PEDI-M score during the three-year time period ($p < 0.05$). In contrast, those with a relatively high predicted PEDI-M score showed a significant increase in the actual PEDI-M score ($p < 0.05$).

**Conclusions:** MTQ is a good predictor of daily activity in children with CP. Sufficient MTQ is required to improve and maintain daily function.

**Implications:** MTQ assessments may help estimate capacity for daily activity in children with CP.

**Keywords:** Cerebral palsy; Muscle thickness; Activity
**Funding acknowledgements:** None.

**Ethics approval:** Ethical approval for this study was given by the ethics committee of Kyoto University Graduate School and Faculty of Medicine.

# Appendix VIII

**Oral presentation at the Rehabilitation and Therapy Research Society Conference (RTRS), Limerick, 13th May 2011.**

**This presentation won best presentation of the conference.**

**Associated published abstract:**
**O'Hanlon D, Wanic K, Pazderska A, O'Gorman D, Cooper D, Collura N, Lithander F, Nolan JJ. Progressive aerobic exercise training improves VO2max and mitochondrial function in early onset type 2 diabetes. Physical Therapy Reviews. 2011, vol. 16, no. 4, p 285.**

Abstracts

# Rehabilitation and Therapy Research Society Seventh Annual Conference

## Fostering Clinical Research Partnerships

*13 May 2011 at the Faculty of Health Sciences, University of Limerick, Ireland*


### Progressive aerobic exercise training improves $VO_2$max and mitochondrial function in early onset type 2 diabetes

**Declan O'Hanlon[1,2], Krzysztof Wanic[1,2], Agnieszka Pazderska[2], Donal O'Gorman[3], Diane Cooper[3], Noelle Collura[2], Fiona Lithander[1], John Nolan[4]**

[1]Trinity College, Dublin, Ireland, [2]St. James's Hospital, Dublin, Ireland, [3]Dublin City University, Dublin, Ireland, [4]Steno Diabetes Centre, Copenhagen, Denmark

**Background:** The prevalence of type 2 diabetes (T2DM) is increasing dramatically and the age of diagnosis continues to fall. Earlier onset increases the patient exposure to the disease, increasing the risk of complications. We recently reported that early onset T2DM (EOT2DM) is an extreme subphenotype of T2DM, demonstrating a blunted metabolic response to aerobic exercise and abnormalities in skeletal muscle mitochondrial proteins. Obesity, lipotoxicity and impaired mitochondrial function are all thought to contribute to the development of T2DM. The aim of this study was to investigate the effect of a graded 12-week exercise intervention.

**Methods:** Twenty-seven subjects were recruited; six young (<30 years) obese (BMI>30 kg/m$^2$) patients with EOT2DM, 10 older (>50 years) obese patients with later onset T2DM and 11 young obese non-diabetic subjects. Baseline testing included measurements of weight, $VO_2$max, and a biopsy of the vastus lateralis muscle for examination of intrinsic mitochondrial oxidative function by respirometry. After testing, subjects began a 12-week supervised progressive aerobic exercise intervention training, 4 days per week, at 70% $VO_2$max, expending 625 kcal per session. Testing was repeated after the intervention.

**Results:** All groups were matched for height, weight, BMI, $VO_2$max and mitochondrial function at baseline. $VO_2$max increased significantly (13.9$\pm$2.5%) in each group after exercise training, with no significant difference between groups. Intrinsic mitochondrial function increased significantly in each group with no differences between groups. Weight loss (2.4$\pm$0.86 kg) was observed in the young obese group only.

**Conclusions:** Both groups with T2DM were more resistant to weight loss than the young obese group. Personalised exercise prescription at 70% $VO_2$max improves aerobic capacity and intrinsic mitochondrial function in these very insulin resistant patients with EOT2DM in spite of the absence of weight loss. These findings can form the basis for effective early intervention in this high risk patient group.

# Appendix IX

**Published abstract:**

**A. Pazderska, K. Wanic, D. O'Hanlon, D. Cooper, N. Collura, K.J. Clarke, D.J. O'Gorman, R.K. Porter, A. Zorzano, J.J. Nolan. Increased skeletal muscle mitochondrial respiration in patients with type 2 diabetes following dietary and exercise interventions. Diabetologia. 2011, vol. 54, p S248.**

bances associated with sustained exercise may lead to worsening control unless great care is taken to adjust carbohydrate intake and insulin dosage. Metabolomic is becoming widely spread used as a new and powerful tool for discerning significant changes at metabolic level. In this study we wanted to identify: 1) the effects of regular exercise practice on phenotypic characteristics, and 2) the impact of acute exercise on the serum metabolome of patients with T1D compared to controls.

**Material and methods:** A total of 45 type 1 diabetic patients without chronic complications and 45 controls were included. From each group individuals were classified as non competitive athletes (≥3 sessions of exercise per week) or sedentary. We obtained data of fitness levels (maximal test on a cycle-ergometer), body composition (DEXA), glycemic control and dietary intake. Further, 10 male with T1D (35.1±2.7 years old) and 11 controls (32.7±2.8 years old) with similar cardio-respiratory capacity ($VO_2$max 33.4±7.1mL. $kg^{-1}.min^{-1}$ vs. 33.9±9.1, respect.) were selected to perform an acute test of exercise (30 min of cycle-ergometer at 80% of $VO_2$max). Fasting serum samples were withdrawn prior and at the end of the exercise and were analysed using two different platforms: $^1$H NMR and gas chromatography- mass spectrometry.

**Results:** In the table below are summarized the phenotypic characteristics of the total population. Athletes (T1D and controls) showed better fitness capacity and lower total and abdominal fat, comparing to sedentary groups. In type 1 diabetes group, athletes elicit a tendency to better HbA1c (7.3±1.2 vs7.7±1.3) higher total energy intake (2096±434 vs 1832±466 kcal/day) and a significant increase in carbohydrate consume (200±52 vs 161±56g/day, p=0.02) in comparison to sedentary counterparts. In the acute exercise test, T1D presented elevated levels of insulinemia before (18.6±14.6 UI/L) and after 30 min (25.1±21.5). In the untargeted metabolomic analysis we identified significant increments of indicators of tricarboxylic acid cycle (malate, fumarate, succinate, citrate, α-ketoglutarate), lypolisis (glycerol) and fatty acid oxidation (oleic acid, palmitoleic acid, linoleic acid) in both groups, greater in control than in diabetes.

**Conclusions:** Subjects with T1D and controls presented similar metabolic characteristics, and the blunted response to exercise in T1D group is probably consequence of hiperinsulinemia due to insulin treatment.

| | Type 1 diabetes | | Controls | |
|---|---|---|---|---|
| | Athletes n=23 | Sedentary n=22 | Athletes n=24 | Sedentary n=21 |
| Sex | 17/6 | 15/7 | | |
| Age (years) | 35.0±10.8 | 41.5±12.1 | 32.5±8.1 | 35.2±8.3 |
| Diabetes Evolutions (years) | 16.5±12.0 | 19.1±8.7 | -- | -- |
| BMI (kg/m²) | 24.0±2.8 | 25.5±3.6 | 24.3±2.6 | 24.8±2.8 |
| **Fitness level** | | | | |
| $VO_2$max (ml$O_2$/kg/min) | 39.8±7.2 | 22.8±5.8* | 41.9±10.1 | 26.1±6.5*# |
| PAQ questionnaire (MET/min/week) | 3071.3±1737.0 | 1398.3±961.5* | 2698.3±1547.8 | 1345.5±1987.8*# |
| **Body composition** | | | | |
| Total body fat (%) | 22.9±7.1 | 32.2±8.0* | 23.3±5.6 | 34.2±8.1*# |
| Abdominal fat (%) | 25.2±10.4 | 36.6±9.7* | 26.9±9.9 | 39.8±10.1*# |

Data expressed as mean±SD. Level of significance p<0.05. a: p<0.05 vs Type 1 diabetes athletes; b: p<0.05 vs controls athletes; c: p<0.05 vs Type 1 diabetes athletes

*Supported by: CIBERDEM-12-12-2009*

## 604

### Increased skeletal muscle mitochondrial respiration in patients with type 2 diabetes following dietary and exercise interventions

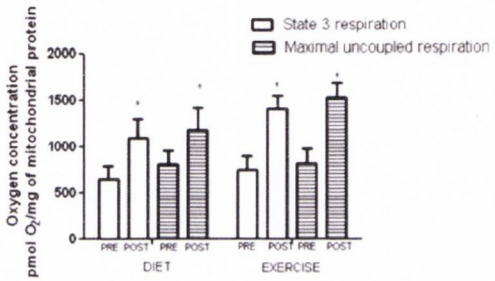
**A. Pazderska**[1], K. Wanic[1], D. O'Hanlon[1], D. Cooper[2], N. Collura[1], K.J. Clarke[3], D.J. O'Gorman[4], R.K. Porter[3], A. Zorzano[5,6], J.J. Nolan[1];
[1]Metabolic Research Unit, St James's Hospital,Trinity College Dublin, [2]School of Health and Human Performance, Dublin City University, [3]School of Biochemistry and Immunology, Trinity College Dublin, [4]School of Health and Human Performence, Dublin City University, Ireland, [5]Institute for Research in Biomedicine, Barcelona, [6]Departament de Bioquinica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Spain.

**Background and aims:** Type 2 diabetes is characterized by insulin resistance, associated with mitochondrial dysfunction in skeletal muscle. Diet-induced weight loss and aerobic exercise intervention lead to improvements in insulin sensitivity. We compared mitochondrial respiration in muscle biopsies from sedentary patients with type 2 diabetes following either diet or exercise intervention.

**Materials and methods:** 12 patients with type 2 diabetes (mean age 44.4+/-3.15 years; BMI 36.4 +/-1.8 kg/m$^2$; weight 108.1+/-6.6 kg) participated in either intervention, four of whom completed both. Both diet and exercise were designed to cause a 2,500 calorie deficit per week. High resolution respirometry was used to measure oxygen flux capacity in isolated mitochondria from vastus lateralis muscle biopsies taken pre and post interventions. Results represent mean +/- SEM. Non-parametric tests were used for statistical analysis.

**Results:** Aerobic training significantly increased maximal oxygen consumption (2.6 vs. 3.0 L/min; p=0.008) and reduced fat mass (44.9 vs. 42.6 kilograms; p=0.011). Aerobic training resulted in increased mitochondrial state 3 respiration (749.1 vs. 1408.5 pmol $O_2$/mg protein; p=0.036) and maximal uncoupled respiration (818.4 vs. 1531.5 pmol $O_2$/mg protein; p=0.036). Following the dietary intervention, subjects lost weight (111.8 vs.106.8 kg; p= 0.028) and fat mass (50.0 vs. 46.8 kg; p=0.046), while $VO_2$ max was unchanged (2.26 vs. 2.34 L/min; p=0.39). Dietary intervention resulted in increased mitochondrial state 3 respiration (642.2 vs. 1085.9 pmol $O_2$/mg protein; p=0.018) and maximal uncoupled respiration (803.1 vs. 1172.7 pmol $O_2$/mg protein; p=0.028). Insulin sensitivity measured by OGIS was 303.0 and 352.7 pre and post exercise, 290 and 318 pre and post diet, not reaching statistical significance in either group.

**Conclusion:** Skeletal muscle mitochondrial respiration is substantially improved in obese sedentary patients with type 2 diabetes by both diet and aerobic exercise, and to a greater extent with exercise.




*Supported by: EFSD*

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### Resistance alone or combined resistance plus aerobic exercise training increases IRS-1 expression in muscle of type 2 diabetic subjects

**M.M.P. Jorge**[1], V.N. Oliveira[1], A.L.D. Diniz[1], E.R. Ropelle[2], J.B. Carvalheira[2], F.S. Espindola[1], P.T. Jorge[1], B. Geloneze[2];
[1]Internal Medicine, Federal University of Uberlandia, Minas Gerais, Uberlandia, [2]Internal Medicine, State University of Campinas, São Paulo, Brazil.

**Background and aims:** Exercise training is known to improve insulin sensitivity and recent research has reported that combined exercise can be the most effective modality. The purpose of this study was to compare the effects of different exercise training on IRS-1, GLUT-4, JNK2, NFkB tissue expression and IKK fosforilation on skeletal muscle of type 2 subjects.

**Materials and methods:** Forty eight type 2 diabetics were randomly assigned to three groups of training (3 times/week, 60 min/session) designated as aerobic group (n=12), resistance group (n=12), combined group (n=12) and a control group (n=12). Inclusion criteria was being type 2 diabetes according ADA diagnostic criteria, age between 30 and 70 years old and BMI ranging from 25 and 40 kg/m2. Exclusion criteria include current insulin therapy, conditions that could preclude physical activity and corticosteroid use. Muscle microbiopsies were performed before and after training (between 60 and 96 hours after the last bout of exerciser) to quantify IRS -1, GLUT-4, JNK2, NFkB expression and IKK fosforilation on skeletal muscle.

**Results:** After training GLUT-4, JNK2, NFkB expression and IKK fosforilation did not change but IRS-1 expression increased by 65% in the resistance ($p < 0.05$) and by 89,7% in the combined group ($p<0.01$). We used the analysis of variance (Two-way ANOVA) to assess significant differences between the groups and bonferroni post-tests to compare the mean before and after the training.

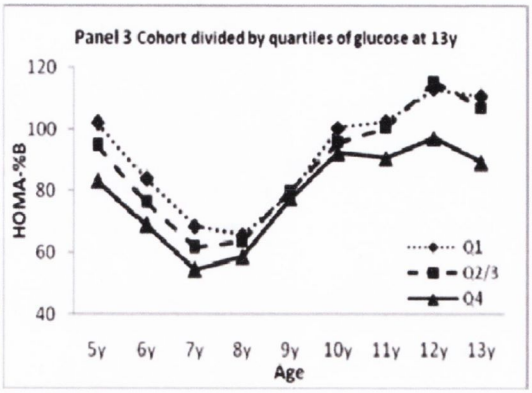
**Conclusion:** The increased IRS-1 expression on skeletal muscle of type 2 diabetes persists longer than sixty hours after training on the resistance and combined group despite a normal GLUT-4 muscle expression. The inhibitory effect of exercise on inflammatory pathways like JNK2, NFkB expression and IKK fosforilation was not seen 60 to 96 hours after the last bout of exercise. This persistent effect of resistance and combined exercise training on IRS-1 expression could be responsible for the best effect related on improvements of insulin signaling transduction in these modalities of exercise.

*Supported by: FAPEMIG*

# Appendix X

**Published abstract:**

**K. Wanic, A. Pazderska, S. Shah, D. O'Hanlon, J.R. Bain, R.D. Stevens, C.B. Newgard, J.J. Nolan. Distinctive metabolic signature in subjects with early onset type 2 diabetes. Diabetologia. 2010, vol. 53, p S249.**




*Supported by: Bright Futures Trust/CGF*

## 618

**Insulin resistance and increased PAI-1 as factors of non-alcoholic fat liver disease in children, adolescents and youth metabolic syndrome**

V.S. Dimitrijevic-Sreckovic[1], B.M. Sreckovic[2], P.B. Djordjevic[1], D.M. Gostiljac[1], M. Civcic[1], I. Soldatovic[3], H.S. Petrovic[1];
[1]Diabetology, Institute of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, [2]Cardiology, Clinical Center Bezanijska Kosa, [3]Statistics, Institute for Medical Statistics and Informatics, Clinical Center of Serbia, Belgrade, Serbia.

**Background and aims:** In metabolic syndrome (MS) patients, abdominal obesity accompanied with hyperinsulinism and insulin resistance is related to hypertension and lipid status disturbance where thrombotic and inflammatory factors and low antioxidant status and tendency to early atherosclerosis are present. Hepatic fat accumulation in childhood obesity is associated with increased visceral fat and insulin resistance (IR). IR results in fat deposition in the liver and occurrence of non-alcoholic fat liver disease (NAFLD). The study was aiming at determining NAFLD and its most important provoking factors in MS and pre-metabolic (pre-MS) syndrome patients.
**Materials and methods:** The study included 173 obese individuals aged 7 to 30 classified into 3 groups: I-children (7-15), II-adolescents (16-20) and III-youth (20-30). Three of the following five criteria were used for metabolic syndrome (MS) diagnosis in adolescents: waist circumference >90Pct; triglycerides >1.7mmol/l; HDL-cholesterol<1.0mmol/l; hypertension>90Pct, glycemia >6.0mmol/L. ATP III classification was applied for youth. Patients with less than three afore mentioned criteria were considered patients with pre-MS. OGTT was used to evaluate the extent of disorder. Insulin sensitivity was determined by HOMA IR. PAI-1 was determined by plasminogen substrate assay. SGOT, SGPT and γ-GT were considered liver function parameters. Liver ultrasonography was used to diagnose NAFLD.
**Results:** NAFLD, increasing considerably with age, was found in 7.3% children, 18.9% adolescents and 29.0% youth ($p<0.05$). NAFLD existed in 17.5% pre-MS and 29.0% MS patients. NAFLD found by groups: pre-MS patients - I-11.5%, II-17.7%, III-20.4%; MS patients - II-20.4%, III-40.0%. Logistic regression analysis indicated the most important NAFLD factors: body weight - odds ratio (OR) 1.039, $p<0.001$; LDL-cholesterol OR 1.55, $p<0.05$; creatinine clearance OR 1.01, $p<0.05$; uric acid OR 1.00, $p<0.05$; insulins - 0min OR 1.012, $p<0.002$, 120min OR 1.008, $p<0.001$; HOMA IR OR 1.059, $p<0.001$; PAI-1 OR 2.79, $p<0.001$; SGPT OR 1.27, $p<0.001$. Patients with NAFLD had increased WC (110.7±11.9cm), LDL-cholesterol (3.3±1.0mmol/l), triglycerides (1.81±1.15mmol/l), uric acid (383.8±86.3), insulins 0min (61.1±81.3U/l) and 120min (93.1±108.4U/l), HOMA IR (14.7±4.4±19.3μmol/mU/ml), PAI-1 (7.3±0.6U/ml), SGPT (56.7±20.9U/l), γ-GT (44.1±22.8U/l). Patients without NAFLD had normal SGPT, γ-GT, uric acid and increased WC (98.6±16.7cm), insulins 0min (21.6±31.3U/l) and 120 min (44.3±49.5U/l), HOMA IR (6.2±3.4μmol/mU/ml), triglycerides (1.74±1,63mmo/l), PAI-1 (6.0±1.4U/ml) but lower than NAFLD patients.
**Conclusion:** Obesity, hyperinsulinemia with IR (characterized by increased uric acid and PAI-1), SGOT and LDL-cholesterol are the most frequent risk factors for NAFLD. NAFDL may be the liver sign of pre-MS and MS children, adolescents and youth associated with visceral obesity, IR, lipid status disturbance, thrombotic and inflammatory factors.

## 619

**Steatohepatitis is closely associated with insulin resistance and the metabolic syndrome from early stages of their development**

Y. Mori[1], K. Ura[2], K. Matsuura[1], Y. Itoh[1], J. Yokoyama[1], N. Tajima[1];
[1]Department of Internal Medicine, Jikei University School of Medicine, Tokyo, [2]Department of Internal Medicine, Utsunomiya Memorial Hospital, Utsunomiya, Japan.

**Background and aims:** Non-alcoholic fatty liver disease (NFALD), insulin resistance, and the metabolic syndrome were examined for correlation in individuals undergoing elaborate health checkup programs with the influence of abdominal obesity being ruled out.
**Materials and methods:** Of the 909 subjects undergoing the health checkups, 626 individuals who underwent a 75 g OGTT and were evaluated by abdominal ultrasound for fatty liver and the metabolic syndrome were enrolled in the study, and 130 individuals each with fatty liver (fatty liver group; FLG) and without fatty liver (non-fatty liver group; NFLG), who were matched for gender, age, BMI, and waist circumference, were compared for relevant biochemical parameters, insulin resistance, number of risk factors implicated per individual, and frequency of the metabolic syndrome detected.
**Results:** There was no significant difference between the FLG and the NFLG in the male to female ratio (%), age, BMI (24.8 ± 2.9 and 24.2 ± 1.8, respectively), and waist circumference (85.8 ± 6.6 and 84.5 ± 5.8, respectively). In contrast, significantly higher values were noted in the FLG than in the NFLG with regard to the area under the glucose curve at 75 g OGTT (363.0 ± 81.3 versus 319.0 ± 70.6; $P < 0.001$), area under the insulin curve (109.0 ± 80.4 versus 76.1 ± 45.1; $P < 0.001$), HOMA-R index (1.73 ± 1.24 versus 1.17 ± 0.56; $P < 0.001$), HbA1c, AST, ALT, TG, and LDL-C, while HDL-C was significantly lower in the FLG than in the NFLG. Additionally, significantly higher values were noted in the FLG than in the NFLG with regard to the number of risk factors implicated per individual (1.83 ± 1.15 versus 1.37 ± 1.09; $P < 0.001$), frequency of the metabolic syndrome detected (30/130, 23.1% versus 14/130, 10.8%; $P < 0.05$).
**Conclusion:** Study results suggested that NAFLD is closely associated with insulin resistance and the metabolic syndrome even when the influence of abdominal obesity is excluded. It was further suggested that, given the BMI of < 25 kg/m$^2$ and the waist circumference of no more than 85 cm in the subjects, NAFLD appears to be implicated in the pathogenesis of insulin resistance and the metabolic syndrome from quite early stages of their development.

## 620

**Distinctive metabolic signature in subjects with early onset type 2 diabetes**

K. Wanic[1], A. Pazderska[1], S. Shah[1], D. O'Hanlon[1], J.R. Bain[2], R.D. Stevens[2], C.B. Newgard[2], J.J. Nolan[1];
[1]St. James's Hospital, Trinity College Dublin, Metabolic Research Unit, Ireland, [2]Duke University, Durham, USA.

Subjects with early onset type 2 diabetes have severe insulin resistance, reduced $VO_2$ max response to exercise, and abnormal mitochondrial function relative to equally obese insulin resistant control subjects. Having previously used a metabolomics approach to demonstrate that obese insulin resistant subjects have a distinct metabolic profile compared to lean controls, we have now studied subjects with early-onset type 2 diabetes. We used targeted MS/MS and GC/MS-based metabolomics to measure fasting plasma concentrations of amino acids and total and free fatty acids in 24 subjects with early onset type 2 diabetes (mean age 26.1, BMI 35.6 kg/m2), 17 obese controls (mean age 22.8, BMI 34.2 kg/m2) and 28 lean controls (mean age 24.7, BMI 22.4 kg/m2). Confirming previous studies, the obese subjects had increased levels of branched-chain and other amino acids, total non-esterified fatty acids (NEFA), and several individual fatty acid species compared to lean controls. Interestingly, subjects with type 2 diabetes exhibited additional increases in levels of valine, leucine/isoleucine, glutamate/glutamine, and aspartate/asparagine, as well as NEFA and individual fatty acids compared to obese controls. Insulin resistance, measured by HOMA-IR correlated with concentrations of valine, leucine, histidine and glutamate.

| Parameter studied (μM) | Early onset type 2 | Obese controls | Lean controls | P value (1 vs. 2) | P value (1 vs. 3) |
|---|---|---|---|---|---|
| L-valine | 298 | 261 | 214 | 0.031 | <0.001 |
| L-leucine/isoleucine | 206 | 170 | 151 | 0.004 | <0.001 |
| L-aspartic acid/aspargine | 103 | 78 | 70 | 0.009 | <0.001 |
| L-glutamic acid/glutamine | 101 | 87 | 71 | 0.04 | <0.001 |
| Histidine | 80 | 92 | 72 | 0.02 | 0.01 |
| Total fatty acids | 15424 | 13004 | 9520 | 0.039 | <0.001 |
| Palmitic acid (C16:0) | 2629 | 2125 | 1442 | 0.021 | <0.001 |
| Oleic acid (C18:1) | 4881 | 3703 | 2385 | 0.025 | <0.001 |
| Stearic acid (C 18:0) | 41 | 31 | 25 | 0.012 | <0.001 |

We conclude that subjects with early-onset type 2 diabetes have a metabolomic profile distinguishing them from BMI-matched insulin resistant individuals with normal glucose tolerance. Further studies are needed to assess whether these changes are a reflection of altered mitochondrial function in these subjects.

*Supported by: an EFSD/Novo Nordisk grant*

## 621

**Insulin resistance is associated with metabolic syndrome but not with angiographically determined coronary artery disease in female patients**

**A. Vonbank**[1,2], C.H. Saely[1,2], P. Rein[1,2], S. Beer[1,2], C. Boehnel[1,2], V. Jankovic[1,2], H. Drexel[1,2];
[1]Vorarlberg Institute for Vascular Investigation and Treatment, Feldkirch, Austria, [2]Private University of the Principality of Liechtenstein, Triesen, Liechtenstein.

**Background and aims:** Insulin resistance (IR) is the key feature of the metabolic syndrome (MetS) and in prospective studies predicts atherothrombotic events. Its association with directly visualised coronary atherosclerosis, especially in female patients, is unclear. We hypothesised that IR is associated with both angiographically determined coronary artery disease (CAD) and with the MetS.
**Material and methods:** We enrolled 354 consecutive female patients undergoing coronary angiography for the evaluation of suspected or established stable CAD; significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥50%. IR was determined by the HOMA index; the MetS was defined according to ATPIII criteria.
**Results:** HOMA-IR scores were significantly higher in MetS female patients than in female subjects without the MetS (4.9 ± 4.7 vs. 1.9 ± 1.1; p <0.001). In contrast HOMA-IR did not differ significantly between patients with significant CAD and those who did not have significant CAD 3.3 ± 3 vs. 3.1 ± 3; p = 0.823). When both, the presence of MetS and of significant CAD were considered, HOMA-IR was significantly higher in patients with the MetS both among those who had significant CAD (4.9 ± 4.8 vs. 1.9 ± 1.1; p <0.001) and among those who did not have significant CAD (5.0 ± 4.7 vs. 1.9 ± 1.1; p <0.001) whereas it did not differ significantly between patients with significant CAD and subjects without significant CAD in patients with the MetS (5.0 ± 4.7 vs. 4.9 ± 4.8; p = 0.383) nor in those without MetS (1.9 ± 1.1 vs. 1.9 ± 1.0; p = 0.860). Similar results were obtained with the IDF definition of the metabolic syndrome.
**Conclusion:** In female patients IR is significantly associated with the MetS but not with angiographically determined coronary atherosclerosis.

## 622

**Obstructive sleep apnoea and metabolic abnormalities in type 2 diabetes**

**S.K. Wangnoo**[1], M.A. Siddiqui[1], M. Gupta[1], M.S. Kanwar[2];
[1]Endocrinology, [2]Pulmonary Medicine, Apollo Hospital, New Delhi, India.

**Background and aims:** The burgeoning load of type 2 diabetes is a major public health concern in our part of the world with high morbidity, mortality, and health-care costs. Recent reports have indicated that the majority of patients with type 2 diabetes also have obstructive sleep apnea (OSA). There is compelling evidence that OSA is a significant risk factor for cardiovascular disease and mortality. Because both diabetes and OSA are associated with increased cardiovascular morbidity and mortality, it is possible that the presence of both conditions results in additive or even synergistic health risks. The aim of this study was to evaluate the prevalence of OSA in the study population and its effect on the metabolic profile.
**Materials and methods:** After taking the informed consent of the subjects, we performed polysomnography studies in 30 consecutive patients with diabetes and obesity according to the Asian-Indian criteria recruited from outpatient clinics between July 2009 and January 2010. Apnoea- hypopnoea index (AHI) > or = 10/hour was considered relevant for OSA diagnosis. Subjects with AHI< 10 were considered as controls. We assessed AHI, Epworth sleepiness scale (ESS), body mass index (BMI, kg/m$^2$), glycosylated haemoglobin (HbA1c, %), fasting serum total cholesterol (mg%), HDL-(mg%), LDL-cholesterol(mg%), triglycerides (TG) (mg%), HOMA index and highly sensitive C-reactive protein (hsCRP, mg/l).
**Results:** Data are presented as mean +/- SD or median (interquartile range) for parametric and nonparametric data respectively. 22 out of 30 subjects (73%) of with diabetes had OSA (AHI > or =10). AHI in the OSA group was 21 (16-30) and 5 (3-8) in controls (p < 0.001). BMI was higher in OSA (33.8 +/- 5.8) vs. controls (29.4 +/- 3.1) (p = NS). Patients with OSA had higher HbA1c (9.72 +/- 0.9) vs. (8.94 +/- 0.8)( p = 0.03), TG (210 +/- 55.2) vs. (140.2 +/- 41.9) (p = 0.046), HOMA-IR (2.35 +/- 1.6) vs. (1.93 +/- 1.5)( p = 0.046) and hsCRP (4.2 +/- 0.9) vs. (2.89 +/- 1.4)( p = 0.01). HDL-cholesterol was lower in OSA group compared to control (30.8 +/- 6.1 vs. 40.3 +/- 11.4) (p = 0.02). HbA1c correlated best with AHI(p < 0.001, r = 0.39).
**Conclusion:** Identifying the possibility of previously unrecognized OSA amongst patients with diabetes is important for treating physicians even in the absence of specific symptoms. The high prevalence of OSA in obese patients with type 2 diabetes is also associated with more severe metabolic derangements and its treatment along with adjustment of antidiabetic therapy may ameliorate some of the associated morbidity and mortality.

# Appendix XI

**Published abstract:**

**Pazderska A, Wanic K, O'Hanlon D, Clarke K, Croghan S, Porter R, Nolan JJ. 12 weeks aerobic exercise improves intrinsic mitochondrial function in males with type 2 diabetes. The Irish Journal of Medical Science. 2010 vol. 179, p S509.**

attend for scheduled appointment. 641 (18.6%) of those who scored 1 (most affluent) did not attend, 385 (20.9%) in score 2, 353 (20.2%) in score 3, 782 (23.5%) in score 4 and 509 (26.7%) in the score 5 (most deprived) group did not attend, $p = 0.0001$. This shows a clear decrease in attendance levels in those who are deemed to be more disadvantaged. The most disadvantaged women overall were 40% less likely to attend than their most affluent counterparts (OR 0.6, 95% CI (0.55–0.71), $p = 0.001$).

Women of lower socio-economic status and those who live further from an antenatal centre are less likely to attend for screening for Diabetes in pregnancy.

## OC10 12 weeks aerobic exercise improves intrinsic muscle mitochondrial function in patients with type 2 diabetes

A. Pazderska[1], K. Wanic[1], D. O'Hanlon[1], K. Clarke[2], S. Croghan[1], R. Porter[2], J.J. Nolan[1]

*Metabolic Research Unit, St James's Hospital, Trinity College Dublin, Dublin[1], School of Biochemistry and Immunology, Trinity College Dublin, Dublin[2]*

Exercise is known to increase maximal oxygen uptake ($VO_2$ max) and improve insulin resistance in patients with type 2 diabetes. Several recent studies have shown that exercise enhances in vivo mitochondrial function. However, it remains unclear whether this improvement is due to an increase in skeletal muscle mitochondrial mass or in intrinsic mitochondrial function. The aim of this study was to assess changes in electron transport capacity in mitochondria isolated from muscle biopsies from patients after an exercise programme.

Six sedentary men with type 2 diabetes (age 41 ±11.5; HbA1c 8.3 ±1.7%; BMI 34.4 ±6.7) participated in a 12 weeks aerobic exercise programme consisting of four supervised sessions/week at 70% $VO_2$ max. Muscle biopsies from vastus lateralis were obtained before and after the intervention. High resolution respirometry was used to measure oxygen flux capacity in isolated skeletal muscle mitochondria. *T* test was used for statistical analysis.

$VO_2$ max improved following the intervention (baseline $VO_2$ max: 2.65 L/min, post exercise: 2.99 L/min; $p = 0.012$). Following training, significant increases were observed in oxygen fluxes, expressed per milligram of mitochondrial protein (pmol$O_2$/s/mg protein), stimulated by parallel electron input from complexes I and II in the presence of pyruvate + malate and succinate (188.21 vs. 420.96; $p = 0.024$). Similarly, significant increases were observed in oxygen fluxes in the presence of ADP (506.61 vs. 1527.15; $p = 0.007$), as well as in response to uncoupling by FCCP (572.40 protein vs. 1645.18; $p = 0.013$).

12 weeks aerobic exercise training leads to improvements in several components of intrinsic mitochondrial function in patients with type 2 diabetes.

## OC11 The role of MRP8/14 in *in stent* restenosis in the diabetic rat

A. Stocca[1], A. Duffy[2], D. O'Toole[3], Tim O'Brien[1]

*REMEDI, NUI Galway, Ireland[1], Medtronic, Galway, Ireland[2], Lung Biology Group, Department of Anesthesia, NUI Galway, Ireland[3]*

**Objective:** The most common cause of death in diabetes mellitus is cardiovascular disease. Patients frequently undergo vascular intervention such as stenting. The occurrence of *in stent* restenosis (ISR) has been reduced by the use of drug eluting stents in non-diabetic patients but the incidence of restenosis and stent thrombosis remains higher in diabetic patients. Using a type 2 diabetes mellitus model we investigated the pathogenesis of *in stent* restenosis.

**Research design and methods:** Stents were placed in Zucker Fatty Rat (ZFR) and wildtype rat carotid arteries, and tissues were harvested 14 days post surgery for morphometric analysis. Un-stented carotid arteries were harvested for microarray analysis. In vitro apoptosis, proliferation and migration assays were performed on Rat and human aortic endothelial cells (EC).

**Results:** ZFRs developed an exaggerated intimal response to stent placement compared to wildtype controls 14 days post stent placement. MRP8 and MRP14 were up-regulated in unstented ZFR carotid arteries in comparison to controls. MRP8 was also elevated in EC exposed to high glucose conditions. EC function was impaired by high glucose concentrations, and this effect could be mimicked by MRP8 over-expression. MRP8 knockdown by siRNA significantly restored EC function. MRP8 inhibition was also achieved using pharmacological blockers of glucose-induced pathways.

**Conclusions:** ZFRs developed an exaggerated intimal response after stent placement above that observed in controls. MRP8 was elevated in diabetic animals unstented carotid arteries and in high glucose treated EC. The EC function impairment caused by elevated glucose levels could be mimicked by MRP8 over-expression and reversed/prevented by MRP8 knockdown. Thus MRP8 likely plays a role in exaggerated ISR in diabetes mellitus, and MRP8 inhibition may be useful in improving stenting outcome.

## OC12 Association between poor sleep quality and cardiovascular risk factors in diabetes

W.A. Mahmood, M.S. Draman, L.A. Behan, J. McDermott, S. Sreenan

*Department of Endocrinology and Diabetes, Connolly Hospital, Blanchardstown, Dublin 15*

Sleep restriction has been shown to contribute to reduced glucose tolerance and insulin sensitivity in non-diabetic subjects and to impact on A1c in African Americans with type 2 diabetes (T2DM). We used the Pittsburgh Sleep Quality Index questionnaire (PTSQI score) to assess sleep quality in 241 patients with diabetes. Blood pressure, fasting lipids and HbA1c were also measured. Looking at the whole group, 133 (55%) had good sleep quality (GSQ, score ≤5) and 108 (45%) had poor sleep quality (PSQ, score >5). Females had poorer sleep quality than males (7.1 ± 4.6 vs. 5.0 ± 3.9, $p < 0.0001$). PSQ patients were more likely to have hypertension (69.4 vs. 47.4%, $p = 0.001$). Log A1c (1.93 ± 0.2 vs. 1.87 ± 0.2, $p = 0.019$), total cholesterol (4.76 ± 0.91 vs. 4.52 ± 0.83, $p = 0.04$) and log triglycerides (0.47 ± 0.52 vs. 0.33 ± 0.54, $p = 0.04$) were higher in PSQ patients but use of anti-diabetic medications was not significantly different. Type 1 diabetes (T1DM) patients [36 (14.9%)] were more likely to have hypertension if they had PSQ [(37 vs. 5%, $p = 0.014$)]. Patients with pain had higher PTSQI score (8.7 ± 4.7 vs. 5.6 ± 4.2, $p = 0.001$) and tended to have a higher HbA1c (7.07 ± 1.5 vs. 6.79 ± 1.43, $p = 0.34$). Excluding patients with pain, there was a significant correlation between HbA1c and PTSQI score in T1DM ($r = 0.369$, $p = 0.034$) but not in T2DM ($r = 0.1$, $p = 0.188$). In T2DM patients, LogHbA1c was significantly correlated with PTSQI score ($r = 0.158$, $p = 0.036$). In summary, these data support the suggestion that poor sleep quality is associated with and may contribute to poorer glycaemic, lipid and blood pressure control in patients with diabetes.

# Appendix XII

**Published abstract:**

**K. Wanic, A. Pazderska, S. Shah, D. O'Hanlon, J.R. Bain, R.D. Stevens, C.B. Newgard, J.J. Nolan. Distinctive Metabolic Signature in Subjects with Early Onset Type 2 Diabetes. Diabetes. 2010, vol. 59, (suppl 1) A1-A708.**

**Title: Distinctive Metabolic Signature in Subjects with Early Onset Type 2 Diabetes.**

**Results**: Subjects with early onset type 2 diabetes have severe insulin resistance, reduced VO2 max response to exercise, and abnormal mitochondrial function relative to equally obese insulin resistant control subjects. Having previously used a metabolomics approach to demonstrate that obese insulin resistant subjects have a distinct metabolic profile compared to lean controls, we have now studied subjects with early-onset type 2 diabetes.

We used targeted MS/MS and GC/MS-based metabolomics to measure fasting plasma concentrations of amino acids and total and free fatty acids in 24 subjects with early onset type 2 diabetes (mean age 26.1, BMI 35.6 kg/m2), 17 obese controls (mean age 22.8, BMI 34.2 kg/m2) and 28 lean controls (mean age 24.7, BMI 22.4 kg/m2).

Confirming previous studies, the obese subjects had increased levels of branched-chain and other amino acids, total non-esterified fatty acids (NEFA), and several individual fatty acid species compared to lean controls. Interestingly, subjects with type 2 diabetes exhibited additional increases in levels of valine, leucine/isoleucine, glutamate/glutamine, and aspartate/asparagine, as well as NEFA and individual fatty acids compared to obese controls. Insulin resistance, measured by HOMA-IR correlated with concentrations of valine, leucine, histidine and glutamate.

| Parameter studied (μM) | Early onset type 2 | Obese controls | Lean controls | P value (1 vs. 2) | P value (1 vs. 3) |
|---|---|---|---|---|---|
| L-valine | 298 | 261 | 214 | 0.031 | <0.001 |
| L-leucine/isoleucine | 206 | 170 | 151 | 0.004 | <0.001 |
| L-aspartic acid/aspargine | 103 | 78 | 70 | 0.009 | <0.001 |
| L-glutamic acid/glutamine | 101 | 87 | 71 | 0.04 | <0.001 |
| Histidine | 80 | 92 | 72 | 0.02 | 0.01 |
| Total fatty acids | 15424 | 13004 | 9520 | 0.039 | <0.001 |
| Palmitic acid (C16:0) | 2629 | 2125 | 1442 | 0.021 | <0.001 |
| Oleic acid (C18:1) | 4881 | 3703 | 2385 | 0.025 | <0.001 |
| Stearic acid (C 18:0) | 41 | 31 | 25 | 0.012 | <0.001 |

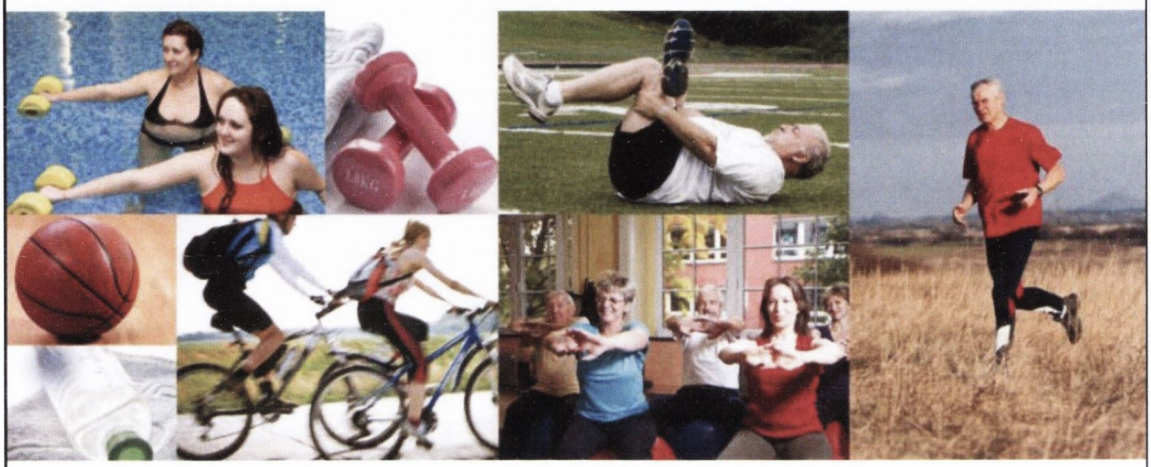
We conclude that subjects with early-onset type 2 diabetes have a metabolomic profile distinguishing them from BMI-matched insulin resistant individuals with normal glucose tolerance. Further studies are needed to assess whether these changes are a reflection of altered mitochondrial function in these subjects.

# Appendix XIII

**Magazine Publication:**

**Declan O'Hanlon, Diane Cooper, Donal O'Gorman. Benefits of exercise and physical activity. Exercise – in one form or other – is an essential component of successful diabetes management. Diabetes Professional. 2011, vol. 7, no. 2, p 15.**

Exercise – in one form or other – is an essential component of successful diabetes management



Declan O'Hanlon, Diane Cooper and Donal O'Gorman

# Benefits of exercise and physical activity

**LIFESTYLE INTERVENTION**, including diet and exercise, remains the cornerstone of type 2 diabetes management, reducing the incidence and slowing the progression of impaired glucose tolerance to type 2 diabetes. Lifestyle modification that incorporates an increase in physical activity of at least 150 minutes per week reduces the risk of type 2 diabetes by 58%.[1]

Regular physical activity improves glucose control along with positively affecting lipids, blood pressure, cardiovascular events, mortality and quality of life. Exercise also increases total daily energy expenditure, contributing to weight loss and weight maintenance. Weight loss improves insulin sensitivity, but exercise improves insulin sensitivity even in the absence of weight loss.

**Current recommendations**

The American Diabetes Association and The American College of Sports Medicine recommend a minimum of 150 minutes of moderate intensity aerobic exercise per week for patients with diabetes to maintain general health[2] *(see Table 1, column A)*.

This can be used as a starting point, but for those who are obese, greater volumes of exercise will be required to bring about meaningful weight loss. Longer training sessions are as effective if split into several shorter sessions throughout the day. The acute increase in insulin sensitivity after exercise can last for between 24 and 72 hours, depending on the volume of exercise performed, so to this end, it is recommended that aerobic exercise is performed at least every second day.

Resistance training should be performed two or three times per week in addition to aerobic exercise, including up to three or four sets of 10-15 repetitions per exercise and progressing to 8-10 repetitions with heavier weights *(see Table 1, column B)*.

It is necessary to include variety as part of an exercise routine to keep participants interested and to avoid repetitive strain. Circuit training is an excellent way to achieve this. It can be performed individually or with groups of patients, with the latter adding a social element that facilitates motivation. Simple circuits can consist of 5-10 exercises *(see Table 1, Column C)*.

**Special considerations for diabetes**

Moderate intensity exercise is safe for most patients, but stress test screening should be performed on high-risk sedentary patients who wish to participate in high intensity physical activity.

A number of pre-screening tools exist, such as the PARmed-X Physical Activity Readiness Medical Examination (which can be accessed on http://uwfitness.uwaterloo.ca/PDF/parmedx.pdf). This can be used to stratify patients and to assess

Table 1

## Examples of aerobic, resistance, circuit training and low-impact exercises

| A) Aerobic exercise | B) Resistance training | C) Circuit training | D) Low-impact exercise |
|---|---|---|---|
| Walking<br>Jogging<br>Swimming<br>Cycling<br>Rowing<br>Dancing<br>Skating<br>Cross trainer<br>Stepper machine | Leg press<br>Leg curl<br>Knee extension<br>Bench press<br>Shoulder press<br>Lateral pull down | Squats<br>Lunges<br>Knee raises<br>Jumping jacks<br>Sit ups<br>Press ups<br>Shoulder press<br>Bicep curls<br>Tricep dips | Cycling<br>Swimming<br>Rowing<br>Cross trainer<br>Resistance training |
| 150min to seven hours per week<br>At least every second day<br>Moderate intensity | Two to three times per week<br>Three to four sets<br>10-15 reps then 8-10 reps with heavier weights | 30sec – 3min/exercise<br>30-60min/session | |

suitability for exercise.

Resistance training is not recommended for patients with proliferative retinopathy because of the risk of retinal detachment or haemorrhage. The same precautions apply to other forms of high-intensity exercise. Additional safety considerations relate to hydration status, the inclusion of a warm-up and medication requirements. Patients who require insulin or insulin secretagogue medications should always check their blood glucose concentration before and after exercise, reducing their medication before exercise or consuming additional carbohydrate as appropriate.

### Practical guidelines

Before beginning exercise, a short (10-minute) low-intensity warm-up should be performed, especially if high intensity exercise is planned. This can consist of light aerobic exercise. Upon cessation of exercise, a similar intensity cool down should be performed. Stretching should be performed for 15-20 seconds in a controlled manner after a warm-up and cool-down, taking care not to overstretch. This will maintain joint flexibility, ensuring sufficient flexibility to perform the exercises in question, and is needed as the muscles can become tight as a result of training.

Exercise should not induce joint pain, and if it does, modification is required. Exercise specialists, exercise physiologists and physiotherapists are in an excellent position to advise patients in this regard. Low-impact or non weight-bearing exercise can be used in the case of lower limb joint pain, back pain or peripheral neuropathies. This can include cycling, swimming, rowing, use of cross-trainer or resistance training *(see Table 1, column D)*.

The effect of impact can also be reduced by wearing a pair of supportive shock absorbing runners (replacing them at regular intervals before they are worn out), exercising on a soft surface or losing weight through dieting.

General lifestyle-related physical activity (gardening, hoovering, taking the stairs, etc) provides a means of increasing daily energy expenditure, but the majority of patients will require some supplemental formal aerobic exercise. Walking is the easiest form of exercise with patients advised to accumulate 10,000 steps per day.

The brisker the pace, the greater the rate of energy expenditure. Moderate intensity exercise should be challenging but comfortable, should increase the heart rate and respiratory rate, and generate a mild degree of sweating, but with patients sufficiently in control to be able to speak.

Resistance training can be undertaken in the home using simple household items such as bottles of water to provide resistance for exercise. The rest period between sets may vary between 30-120 seconds.

Exercise routines should be started slowly by setting realistic goals, especially for those who have been sedentary, but should be progressed continuously. Progression can include increasing the exercise intensity, duration or frequency, and in the case of resistance training, increasing the weight or the number of repetitions. The initial goal is a 5-10% reduction in body weight, at a rate of between 1-2lb per week, using both diet and exercise. Exercise prescription should be specific to encourage compliance, it needs to be tailored to meet the patient's individual requirements, emphasising exercise for life, and should be enjoyable!

There are numerous options available and all that is required is a little creativity on the part of the patient and the healthcare professional. Gyms offer a wide range of exercise options, but effective training can be performed with minimal equipment at home.

### Benefits

Exercise is central to the prevention and treatment of diabetes. Both aerobic and resistance training improve insulin sensitivity. As well as treating the glucose and lipid abnormalities associated with diabetes, exercise also has positive effects with regard to blood pressure, cardiovascular risk, weight loss and weight maintenance, quality of life and mortality.

Exercising is safe for most patients with type 2 diabetes, but the presence of certain complications must be considered. Circuit training provides a convenient combination of both aerobic exercise and resistance training, allowing participants to train effectively at home without the need for expensive equipment.

For a continuous insulin sensitising effect, it should be performed at least every second day, with more prolonged and regular sessions required for weight loss. Exercise must be included to optimise health in patients with type 2 diabetes.

**Declan O'Hanlon** is a physiotherapist, exercise physiologist and a PhD candidate at TCD; **Diane Cooper** is a sport and exercise scientist and a PhD candidate at DCU; and **Donal O'Gorman** is director, Centre for Preventive Medicine and lecturer in the School of Health and Human Performance, DCU. The authors presented a workshop on exercise at the recent Innovation in Diabetes meeting held in the Convention Centre, Dublin

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# Appendix XIV

**Paper submitted for review to Diabetes Care.**

**Wanic K, Pazderska A, O'Hanlon D, Shah S, Stevens RD, Bain JR, Newgard CB, Nolan JJ. Distinctive Metabolomic Signatures in Subjects with Type 2 Diabetes: Comparison of early and late onset Type 2 Diabetes.**

**Distinctive Metabolomic Signatures in Subjects with Type 2 Diabetes: Comparison of early and late onset Type 2 Diabetes.**

**Wanic K [1, 4], Pazderska A [1, 4], O'Hanlon D [1], Shah S [1], Stevens RD [2], Bain JR [2], Newgard CB [2], Nolan JJ [1,3]**

[1] Metabolic Research Unit, St. James's Hospital, Trinity College, Dublin 8, Ireland

[2] Duke University, Sarah W Stedman Nutrition and Metabolism Center, Durham, US

[3]Steno Diabetes Center, Gentofte, Denmark

[4] These authors contributed equally to this work.

Corresponding author:
John J Nolan MD,
Steno Diabetes Center A/S
Niels Steensens Vej 2-4
DK-2820 Gentofte
Denmark
Phone: +45 4443 5003 Fax +45 3968 1048
jjnl@steno.dk

**Abstract:**

Objective: Young adults with type 2 diabetes have been shown to be severely insulin resistant with impaired mitochondrial and V02 max responses to exercise. We investigated underlying metabolomic profiles in young type 2 patients as well as a range of control and comparator groups.

Research Design and Methods: We used targeted mass spectrometry to measure fasting plasma concentrations of amino acids, acyl carnitines and fatty acids in 22 subjects with early onset type 2 diabetes, 16 BMI-matched individuals with later-onset type 2 diabetes, 17 obese and 28 lean controls with normal glucose metabolism.

Results: Subjects with early – onset type 2 diabetes had greater circulating concentrations of valine, leucine/isoleucine, glutamate/glutamine, and aspartate/asparagine, as well as total and individual fatty acids when compared to obese controls. Insulin resistance, correlated with concentrations of valine, leucine/isoleucine and glutamate (Spearman Correlation Coefficients 0.6 – 0.7; $p < 0.001$). Concentrations of branched-chain amino acids were similar in the diabetes subjects with early and late onset. Early-onset subjects had higher concentrations of total and several species of individual fatty acids than later onset type 2 diabetes subjects. Principal component analysis confirmed different metabolic signatures between young and typical onset type 2 diabetes.

Conclusions: Subjects with early-onset type 2 diabetes have a metabolomic profile distinguishing them from young BMI-matched individuals with normal glucose tolerance. We confirmed previously described metabolomic differences between obese and lean individuals. We have shown for the first time a difference between the metabolomics profile in subjects with early or late onset diabetes.

**Introduction:**

Type 2 diabetes continues to increase in prevalence in all age groups including children and adolescents. The diagnosis of this chronic disease in much younger individuals than previously recognised has become an increasingly important health and socio-economic problem. (1-5).

Younger Type 2 diabetes patients possess a more adverse cardiovascular risk profile including more atherogenic lipid configuration compared with age and BMI- matched counterparts. (6,7). We have demonstrated that young subjects with type 2 diabetes have a reduced $VO_2$ max response to exercise and compromised mitochondrial function relative to equally obese insulin resistant controls (8,9). It has been reported that young type 2 diabetes patients are prone to develop microalbuminuria and hypertension despite equivalent or better glycaemic control than age-matched Type 1 diabetes individuals (10). Furthermore a recent study provided preliminary evidence for both structural and functional brain abnormalities in adolescents with Type 2 diabetes compared with obese normoglycaemic teenagers (11). In light of expected longer disease duration in these young subjects with diabetes and their increased risk of end-organ complications, a better understanding of their clinical and metabolic phenotype is an urgent priority in order to improve clinical care.

Metabolomics describes biochemical phenotypes that integrate upstream transcriptional, translational and post-translational processes. Therefore, metabolomic profiling may provide the most complete picture of the biological status contributing to the phenotype of insulin resistance and Type 2 diabetes (12).

There is a growing body of evidence that obese and lean humans have different circulating metabolomic profiles. Based on new research in this field, it has been hypothesized that altered catabolism of branched chain amino acids (BCCA) contributes to the development of obesity-associated insulin resistance (13). We hypothesized that there may be a difference or gradation in metabolomic profiles across the spectrum of phenotypes of glucose metabolism ranging from normoglycaemia through to established Type 2 Diabetes. In addition, we hypothesized that there may be metabolomic distinctions between subjects with early and late onset Type 2 Diabetes.

**Study Groups and Methods:**

*Study Subjects*

Patients aged between 15 and 30 years with either obesity or Type 2 diabetes were recruited from the endocrinology and diabetes out-patient departments. The study was approved by the institution's ethics committee. All participants provided written informed consent. We recruited 22 subjects with early onset type 2 diabetes, 16 individuals with late-onset type 2 diabetes, 17 obese and 28 lean controls with normal glucose metabolism. Lean subjects were participants in an independent cardiovascular risk factor screening study among construction industry workers. We collected fasting serum samples for metabolomic profiling from all study participants. All subjects with type 2 diabetes and obese controls completed a 75 gram oral glucose tolerance test. Lean control subjects had their fasting glucose measured and underwent glucose tolerance test if fasting glucose was greater than 5.6 mmol/L. Individuals with active cardiovascular conditions, liver or kidney disease, malignancy, secondary forms of diabetes and/or receiving treatment with corticosteroids were excluded from the study. Clinical characteristics of the study groups are shown in Table 1.

*Anthropometrics and physiological measurements*

Weight, height and blood pressure were measured using standard methods.

Insulin sensitivity and beta-cell function were estimated by HOMA_IR and HOMA_B, respectively. The following formulas were used: HOMA_IR = (fasting insulin in mU/mL x fasting glucose in mM) / 22.5. HOMA_B = 20 × fasting insulin (mU/ml)/fasting glucose (mmol/ml) − 3.5 (14).

*Laboratory analyses*

All blood samples were taken after an overnight fast. We used targeted tandem mass spectrometry (MS/MS) and gas chromatography/mass spectrometry (GC/MS) - based metabolomics to measure fasting plasma concentrations of 15 amino acids, a panel of acyl carnitines and fatty acids in all subjects, using previously described methods (summarized in 13).

Conventional metabolites were measured at the hospital laboratory using standard methods. Plasma total cholesterol and triglycerides were measured using enzymatic methods (Human liquicolor kits; Hitachi Modular; Roche Diagnostics, Basel Switzerland). Plasma HDL-

cholesterol was measured directly by enzymatic methods (Randox direct kits; Hitachi Modular). Plasma glucose was measured using a glucose oxidase method (bio Merieux kit; Hitachi Modular) and HbA1c was measured using cation-exchange and reversed-phase chromatography Hi- Auto A1c analyzer system; HA 8140; Menarini, Florence Italy).

*Statistical analysis*

SPSS for Windows statistical software (version 16.0, 2001, SPSS Inc., Chicago, IL, USA) was used in statistical analysis. Data are expressed as means / standard error means (SEM). T-test or Mann-Whitney U tests were used where appropriate. In order to reduce multidimensionality of metabolite levels obtained by targeted MS we performed Principal Component Analysis (PCA) assuming approximation of normal distribution of amino acids and acyl carnitines in our cohort (15). The individual metabolites were clustered into 11 principal components with eigenvalues higher than 1 as per Kaiser criterion (16) that explained more than 79% of the total variance. The components were analyzed for differences between the study groups with the Mann-Whitney U test.

The concentrations of 13 out of 45 acylcarnitines were unmeasurable in at least 10% of samples and hence they were excluded from the PCA (full list of attached in the online supplement).

Statistical significance was set at $p<0.05$. No corrections were made for multiple comparisons.

**Results:**

Confirming previous studies (13), obese subjects had increased fasting plasma concentrations of branched-chain and other amino acids, total and non-esterified fatty acids (NEFA), and several individual fatty acid species, when compared to lean controls.

Interestingly, subjects with early – onset type 2 diabetes exhibited additional and signficant increases in concentrations of valine, leucine/isoleucine, glutamate/glutamine, and aspartate/asparagine, as well as total and individual fatty acids compared to obese controls. In the entire cohort, insulin resistance, measured by HOMA-IR, correlated with concentrations of valine, leucine/isoleucine and glutamate (Spearman Correlation Coefficients 0.6 – 0.7; $p < 0.001$) (Table 2).

In further analysis we compared BMI-matched subjects with different ages of onset of diabetes but similar glycemic control. Triglyceride concentrations were higher among younger Type 2 diabetes patients compared with older Type 2 diabetes patients. Concentrations of BCAA were similar in both groups. Interestingly, subjects with early-onset type 2 diabetes had higher concentrations of histidine and lower concentrations of serine compared to the older group. In addition, early-onset subjects had significantly higher concentrations of total fatty acids as well as several species of individual fatty acids compared with later onset type 2 diabetes subjects (Table 3). Of note, total and free fatty acids and several species of both esterified and non-esterified individual fatty acids correlated significantly with HOMA-IR levels (data not shown). Several differences were also observed in circulating concentrations of acyl carnitines (Table 4).

In order to reduce multidimensionality of our data we performed Principal Component Analysis (PCA) assuming approximation of normal distribution of amino acids and acyl carnitines in our cohort.

Our PCA finding regarding early-onset type 2 diabetes subjects compared with their young obese but normoglycaemic counterparts was consistent with our single amino acid data. One of the components shown to be significantly different ($p = 0.034$) between the groups consisted of branched-chain amino acids and C5 acylcarnitines (Isovaleryl carnitine, 3-methylbutyryl carnitine or 2-Methylbutyryl carnitine). The constituents of this component are considered to be downstream metabolites of the BCAA supporting the association of altered catabolism of BCAA and insulin resistance (13,17).

Two principal components were found to differ between young and later onset type 2 diabetes. However, each of these was responsible for only a small part of the overall variance in the data set. The first of the above-mentioned components included Ci4-DC/C4-DC (Methylmalonyl carnitine or Succinyl carnitine), C3 (Propionyl carnitine), C4/Ci4 (Butyryl carnitine or Isobutyryl carnitine), and C5's (Isovaleryl carnitine, 3-methylbutyryl carnitine or 2-Methylbutyryl carnitine) ($p = 0.042$). The same component was also different between typical-onset type 2 diabetes and young obese normoglycaemic subjects ($p = 0.045$).

The second of the above-mentioned components was mostly driven by C5:1 (Tiglyl carnitine) but also contained Histidine ($p < 0.001$). This component differed between the older type 2 diabetes group and young normoglycaemic obese individuals ($p < 0.001$) and between young people either obese or lean ($p < 0.001$).

Of note, C5's (Isovaleryl carnitine, 3-methylbutyryl carnitine or 2-Methylbutyryl carnitine) correlated significantly with insulin resistance measured by HOMA_IR (Spearman 0.28 with $p = 0.01$).

**Discussion**

Early onset type 2 diabetes subjects have an adverse metabolic and clinical phenotype when compared with later-onset type 2 diabetes subjects (6-8). They are at risk of developing complications of the disease, not only because of the cumulative duration of diabetes but also due to an adverse cardiovascular risk profile with dyslipidaemia and hypertension (18-21). In our clinical experience, type 2 diabetes in young subjects is more difficult to manage by behavioural interventions. This may be explained in part by severe insulin resistance and possibly underlying metabolic defects within skeletal muscle, including mitochondrial dysfunction (6-10). It has been previously shown by metabolomic profiling that obese and lean human subjects have different profiles for BCAA (13). Obese subjects have elevated levels of BCAA and other metabolites that are generated from their catabolism, including C3 and C5 acylcarnitines (13,17). Also, elevated levels of BCAA and aromatic amino acids have been shown to be prognostic for incident type 2 diabetes in two separate human studies (22). Very recent work has also shown that BCAA are highly responsive to our most efficacious therapy for type 2 diabetes, bariatric surgery (23), and that baseline levels of BCAA and related metabolites are prognostic for outcomes of therapeutic intervention (24). All of these findings suggest that BCAA can contribute to development of insulin resistance. In animal studies, insulin resistance triggered by a fat-rich diet or such a diet supplemented with BCAA is mediated by the accumulation of certain acyl carnitines in muscle and chronic phosphrorylation of mTOR, JNK, and IRS1Ser307 (13; 17).

There is a growing body of evidence to support the hypothesis that BCAA contribute to the development of diabetes mellitus (17, 25). These findings provide a valuable insight into the pathogenesis of type 2 diabetes and underscore the role of circulating amino acids in this process. [This brief paragraph seems repetitive of material in the preceding paragraph].

Recently, Fiehn and colleagues (26) carried out metabolomic profiling of more than 350 metabolites in plasma from obese type 2 diabetes versus obese non-diabetic African-American women. The authors found several metabolites to be elevated in Type 2 diabetes including: leucine, 2-ketoisocaproate, valine, cystine, histidine, 2-hydroxybutanoate, long-chain fatty acids (oleic, palmitoleic, palmitic), and other metabolites (fructose, glucuronate

etc) indicating concurrent impact of diabetes on intermediary metabolism of all classes of macronutrients. They also reported a positive association between HbA1c and leucine and valine concentrations in these subjects.

Acylcarnitine metabolism in skeletal muscle is associated with insulin resistance in both rodents and humans (27, 28). The nature of that relationship remains a focus of ongoing research. Type 2 diabetes subjects have been shown to have increased plasma concentrations of certain acylcarnitines (summed C10-C14) compared with non-diabetic individuals (29). The concentrations of acylcarnitines correlated with HbA1c levels. This may be explained by inefficient fatty acid beta-oxidation. Accumulation of acylcarnitines has also been shown to stimulate inflammatory pathways such as NFkB (29).

Acylcarnitine concentrations tend to be higher in older, mostly overweight men with lower levels of physical activity. Rodent models with lipid-induced mitochondrial dysfunction strengthen this observation and illustrate the role of medium- and long-chain acylcarnitines (30).

Mihalik et al. (31) examined plasma acylcarnitine profiles in lean, obese, and Type 2 diabetes individuals. They found similar increases in concentrations of long-chain acylcarnitines in both obese and type 2 diabetes individuals. In addition, they demonstrated an increase in both short- and medium-chain acyl carnitines as well as increased C4-dicarboxylcarnitine that correlated with HbA1c levels. This suggests generalized complex oxidation defects in type 2 diabetes individuals. However, more recently the same group (32) compared acylcarnitine species, common amino acids and fat oxidation byproducts and plasma amino acids in type 2 diabetes adolescents with normal weight and obese normoglycaemic youths. Their results showed lower concentrations of amino acids in young type 2 diabetes subjects compared with normal weight controls. Medium to short chain acylcarnitines were also lower compared with both normal weight and obese subjects. The reason for the apparent discordance between the elevated levels of amino acids and acylcarnitines observed in the current study in early-onset diabetes and the decline in these metabolites in adolescents is currently unknown and will require further study.

Ha et al (33) measured circulating metabolic intermediates associated with inflammation, oxidative stress, and arterial stiffness in men with newly-diagnosed type 2 diabetes. Among other findings they reported eight acylcarnitines that were increased in men with type 2

diabetes. Their finding suggested that decanoyl carnitine and lysoPC (C14:0) are most predictive of the risk of developing diabetes.

In the present study we focused particularly on the metabolic characteristics of two groups of type 2 diabetes patients, those with early onset and those with later onset of the disease, by applying a metabolomic analysis. We measured an extensive panel of plasma amino acids, fatty acids and acyl carnitines. Based on our findings, we conclude that subjects with early-onset type 2 diabetes have a metabolomic profile distinguishing them from young BMI-matched insulin resistant individuals with normal glucose tolerance. The profile components that mark this distinction correlate in these subjects with measures of insulin resistance. We confirm obesity-driven differences in amino acids, acyl carnitines and fatty acids between obese and lean individuals. In direct analysis we observed no differences in BCAA between subjects with various ages of onset of Type 2 diabetes. However, principal component analysis was highly suggestive of some differences in metabolic signatures between these two groups. The differences seemed to be driven by acyl carnitines involved in catabolism of BCAA. Furthermore, subjects with early-onset Type 2 diabetes exhibited additional increases in the concentrations of total and free fatty acids and several individual fatty acids compared to late-onset Type 2 diabetes individuals. Increased concentration of histidine observed in the population of early onset type 2 diabetes may have some relation to the compromised beta-cell function present in this population when compared with young obese normoglycaemic controls. The evidence to support an association comes from in vitro studies which demonstrated that L-histidine-induced interactions within voltage dependent calcium channels can regulate glucose-induced insulin secretion by beta-cells (34). However, concentrations of histidine were neither correlated with HOMA-B nor fasting insulin levels in our cohort. Further studies are needed to confirm whether the distinct metabolomic profiles we have observed in these patients are a reflection of altered mitochondrial function in type 2 diabetes patient diagnosed early in their life.

**Author Contributions:**

KW researched data, wrote manuscript, AP researched data, reviewed and edited manuscript, DOH researched data, SS researched data, RDS researched data, JB researched data, CN

researched data, reviewed and edited manuscript, JN researched data, wrote manuscript, reviewed and edited manuscript.

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**Duality of interest**

The authors declare that there is no duality of interest associated with this manuscript.

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Table 1 Clinical characteristics of the study groups

| Parameter studied | Nondiabetic lean controls (n=28) (1) | | Nondiabetic obese controls (n=17) (2) | | Early-onset type 2 (n=22) (3) | | Later-onset type 2 (n=16) (4) | | P value 1 vs 2 | P value 2 vs 3 | P value 3 vs 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | | | |
| Age (years) | 24.8 | 0.2 | 22.8 | 0.9 | 26.4 | 0.9 | 55.7 | 1.4 | 0.037 | 0.008 | <0.001 |
| BMI ($kg/m^2$) | 22.4 | 1.1 | 34.3 | 1 | 35.4 | 1.8 | 31.9 | 1.2 | <0.001 | NS (0.97) | NS (0.1) |
| SBP | 124 | 2.4 | 116 | 3.3 | 127 | 2.9 | 131 | 5 | NS | 0.02 | NS |
| DBP | 73 | 1.7 | 72 | 9.3 | 73 | 1.8 | 75 | 3 | NS | NS | NS |
| HDL_cholesterol (mmol/l) | 1.4 | 0.05 | 1.1 | 0.1 | 1.0 | 0.05 | 1.0 | 0.05 | <0.001 | NS | NS |
| Triglycerides (mmol/l) | 0.5 | 0.1 | 1.6 | 0.2 | 2.5 | 0.3 | 1.4 | 0.1 | 0.001 | 0.02 | 0.003 |
| Fasting glucose (mmol/l) | 4.7 | 0.05 | 4.9 | 0.09 | 9.7 | 0.7 | 8.7 | 0.6 | 0.049 | <0.001 | NS |
| Fasting insulin (mmol/l) | 5.2 | 0.5 | 20.2 | 9.0 | 33.1 | 11.4 | 35.3 | 12.1 | <0.001 | NS | NS |
| HOMA-IR | 1.1 | 0.1 | 4.6 | 2.2 | 14.9 | 5.1 | 14.5 | 5.8 | <0.001 | <0.001 | NS (0.7) |
| HOMA-B | 88.0 | 6.8 | 280.6 | 95.5 | 113.2 | 37.3 | 130.5 | 37.9 | 0.001 | 0.001 | NS (0.1) |
| HbA1c (%) | N/A | N/A | N/A | N/A | 8.4 | 0.4 | 7.3 | 0.3 | N/A | N/A | NS (0.07) |

Table 2 Metabolomic profiles in early onset type 2 diabetic subjects, obese and lean controls.

| Parameter studied (μM) | Early onset type 2 | Obese controls | Lean controls | P value (1 vs. 2) | P value (1 vs. 3) |
|---|---|---|---|---|---|
| L-valine | 305 ± 1 | 261 ± 10 | 215 ± 5 | 0.029 | <0.001 |
| L-leucine/isoleucine | 211 ± 10 | 170 ± 7 | 152 ± 4 | 0.004 | <0.001 |
| L-aspartic acid/aspargine | 106 ± 8 | 78 ± 6 | 69 ± 4 | 0.008 | <0.001 |
| L-glutamic acid/glutamine | 104 ± 5 | 87 ± 5 | 71 ± 2 | 0.025 | <0.001 |
| Histidine | 80 ± 3 | 92 ± 3 | 72 ± 2 | 0.004 | 0.013 |
| Total fatty acids | 15614 ± 918 | 13004 ± 599 | 9520 ± 474 | 0.027 | <0.001 |
| Palmitic acid (C16:0) | 2664 ± 172 | 2125 ± 107 | 1442 ± 78 | 0.013 | <0.001 |
| Oleic acid (C18:1) | 4937 ± 412 | 3703 ± 332 | 2385 ± 169 | 0.014 | <0.001 |

Table 3 Selected amino acids and fatty acid in early versus later onset type 2 diabetes subjects.

| Parameter studied (μM) | Early-onset type 2 (n=22) | Later-onset type 2 (n=16) | P value |
|---|---|---|---|
| L-valine | 305 ± 13 | 298 ± 14 | NS |
| L-leucine /isoleucine | 211± 10 | 202 ± 1 | NS |
| Histidine | 80 ± 3 | 60 ± 3 | < 0.001 |
| Serine | 89 ± 4 | 102 ± 4 | <0.05 |
| Total fatty acids | 15614 ± 918 | 10808 ± 713 | <0.001 |
| Palmitic acid (C16:0) | 2664 ± 172 | 1850 ± 170 | <0.01 |
| Oleic acid (C18:1) | 4937 ± 412 | 3094 ± 213 | <0.01 |

Table 4 Concentrations of selected acyl carnitines.

| Parameter studied (μM) | Nondiabetic lean controls (1) | Nondiabetic obese controls (n=17) (2) | Early-onset type 2 (n=22) (3) | Later-onset type 2 (n=16) (4) | P value 1 vs. 2 | P value 2 vs. 3 | P value 3 vs. 4 |
|---|---|---|---|---|---|---|---|
| C5 :1 | 0.115 ± 0.005 | 0.142 ± 0.005 | 0.150 ± 0.006 | 0.103 ± 0.006 | <0.001 | N.S. | <0.001 |
| Ci4-DC/C4-DC | 0.035 ± 0.003 | 0.040± 0.003 | 0.037± 0.003 | 0.017 ± 0.004 | N.S. | N.S. | N.S. |
| C5's | 0.105 ± 0.007 | 0.114 ± 0.009 | 0.121 ± 0.010 | 0.148 ± 0.012 | N.S. | N.S. | N.S. |
| C3 | 0.346 ± 0.015 | 0.332 ± 0.031 | 0.365 ± 0.037 | 0.452 ± 0.027 | N.S. | N.S. | <0.05 |
| C4/Ci4 | 0.170 ± 0.010 | 0.161 ± 0.013 | 0.190 ± 0.020 | 0.206 ± 0.018 | N.S. | N.S. | N.S. |