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The Effect of Adiposity on Triglyceride Metabolism in Men and Women With and Without Type 2 Diabetes Mellitus

by

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ABSTRACT

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DM

THE EFFECT OF ADIPOSITY ON TRIGLYCERIDE METABOLISM IN MEN AND WOMEN WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

by Anna Jane Stears

Despite the known association between adverse cardiovascular outcomes and obesity, it remains unknown whether increasing adiposity affects cardiovascular outcomes in patients with type 2 diabetes. The aims of this study were to investigate the effects of adiposity on triglyceride metabolism, a known cardiovascular risk factor, in patients with type 2 diabetes compared to control participants.

45 participants with type 2 diabetes (DM) and 45 age-matched controls (CON) with a body mass index (BMI) between 18.0-50.0 kg/m^2 were studied. Fasting blood and breath samples were taken, followed by a standard breakfast containing the stable isotope ^{13}C-tripalmitin. Following the meal, ^{13}C-palmitic acid (^{13}C-PA) in the triglyceride (TAG) and non-esterified fatty acid (NEFA) plasma fractions and breath $^{13}CO_2$ were measured for 6 hours.

Fasting TAG correlated positively with BMI and waist circumference (WC) in both groups [DM (BMI: r=0.338, p=0.028, WC: r=0.339, p=0.043) and CON (BMI: r=0.340, p=0.022, WC: r=0.461, p=0.001)]. Fasting NEFA did not correlate with BMI or WC in either group [DM (BMI: r=0.252, p=0.099, WC: r=0.278, p=0.096) or CON (BMI: r=0.166, p=0.288, WC: r=0.095, p=0.544)]. In contrast to this, ^{13}C-PA TAG area under the curve (AUC) did not correlate with BMI or WC in DM (BMI; r=-0.210, p=0.172, WC: r=-0.102, p=0.543), but correlated positively with BMI and WC in CON (BMI: r=0.288, p=0.055, WC: r=0.296, p=0.048). There was no difference in ^{13}C-PA TAG AUC between the total DM and CON cohorts [60.05 (34.40-100.59) vs 44.04 (29.43-76.43) μg/ml/6h, p=0.118], but there was a significant difference between DM and CON in the lowest quartile of BMI [77.1 (38.6-104.3) vs 34.2 (22.6-44.5)] μg/ml/6h, p=0.01). ^{13}C-PA NEFA AUC correlated *negatively* with both BMI and WC in DM (BMI; r=-0.352, p=0.018, WC: r=-0.486, p=0.002), and *negatively* with only WC in CON (r=-0.311, p=0.04). In DM, there was a *negative* correlation between ^{13}C-PA NEFA AUC and 30-minute insulin (r=-0.424, p=0.004), but there was no correlation in CON. Whole body fat oxidation by calorimetry correlated positively with BMI in both DM (r=0.322, p=0.043) and CON (r=0.314, p=0.04). Breath $^{13}CO_2$ did not correlate with BMI in either group (DM: r=-0.14, p=0.928, CON: r=0.127, p=0.405).

In DM, metabolism of dietary triglyceride was impaired at all levels of adiposity. Elevated diet-derived ^{13}C-PA NEFA is likely to reflect increased adipose tissue 'spillover.' In DM, deficiency of postprandial insulin is likely to contribute to this. Excess circulating dietary triglyceride may accumulate in tissues outside adipose tissue and exacerbate insulin resistance and beta cell failure. It is important therefore, that health policy makers understand that by limiting accessibility of therapies, such as GLP-1 agonists, to patients with a BMI>35 kg/m^2, non-obese patients with diabetes are denied access to potentially beneficial treatment. It is also important that health care professionals encourage a low fat diet in all patients with diabetes, regardless of BMI or WC.

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DECLARATION OF AUTHORSHIP

I, Anna Jane Stears declare that the thesis entitled 'The Effect of Adiposity on Triglyceride Metabolism in Men and Women With and Without Type 2 Diabetes Mellitus' and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly while in candidature for a research degree at this University;

- where any part of this thesis has been previously submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

- where I have consulted the published work of others, this is always clearly attributed;

- I have acknowledged all main sources of help;

- where the thesis is based on work done jointly by others, I have made it clear exactly what was done by others and what was I have contributed myself;

- parts of this work have been presented as oral presentations and published in abstract form at Diabetes UK 2004, The European Association for the Study of Diabetes 2004, and Heart UK 2005.

Signed:

Date:

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LIST OF ABBREVIATIONS

% body fat	Percentage body fat
^{13}C-PA NEFA	^{13}C - palmitic acid in the non-esterified fatty acid fraction
^{13}C–PA TAG	^{13}C - palmitic acid in the triglyceride fraction
ACCORD	Action to Control Cardiovascular Risk in Diabetes Study
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
Apo B	Apolipoprotein B
AUC	Area under the curve
BMI	Body mass index
BMR	Basal metabolic rate
CE	Cholesteryl esters
CHO	Carbohydrate
CO_2	Carbon dioxide
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DPP-IV	Dipeptidyl peptidase IV
DXA	Dual-emission X-ray absorptiometry
$FeCO_2$	Expired CO_2 content
FeO_2	Expired O_2 content
$FiCO_2$	Inspired CO_2 content
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes Trial
FiO_2	Inspired O_2 content
GAD	Glutamic acid decarboxylase
GC-C-IRMS	Gas chromatography-combustion isotope ratio mass spectrometry
GLP-1	Glucagon like peptide-1
h	Hour
HbA_{1c}	Glycated haemoglobin
HDL	High density lipoprotein
HOMA	Homeostatic model assessment
HOMA-%B	Homeostatic model assessment-%beta cell function
HOMA-IR	Homeostatic model assessment-insulin resistance
HSL	Hormone sensitive lipase
IDL	Intermediate density lipoprotein
INC AUC	Incremental area under the curve
INC AUC TAG	Incremental area under the curve triglyceride
IV	Intravenous
LADA	Late onset autoimmune diabetes
LDL	Low density lipoprotein
LPL	Lipoprotein lipase

m	Metre
MMOL/L	Millimole per litre
MODY	Maturity onset diabetes of the young
MRI	Magnetic resonance imaging
MTP	Microsomal triacylglycerol transfer protein
µg/ml	Microgram per millilitre
µU/ml	Microunit per millilitre
NEFA	Non-esterified fatty acids
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NIH	National Institute for Health
O_2	Oxygen
PC	Phosphatidylcholine
PCT	Primary Care Trust
PPAR	Peroxisome proliferator-activated receptor
PPD	Postprandial dyslipidaemia
RER	Respiratory exchange ratio
RQ	Respiratory Quotient
SCOUT	Sibutramine Cardiovascular Outcome Trial
SOS	Swedish Obesity Study
TAG	Triglycerides
TNF alpha	Tumour necrosis factor alpha
TZD	Thiazolidenedione
UKPDS	United Kingdom Prospective Diabetes Study
VA-HIT	Veterans Affairs HDL Intervention Trial
VCO_2	Amount of CO_2 produced
VLDL	Very low density lipoprotein
VO_2	Amount of O_2 extracted
WHO	World Health Organization
WHR	Waist to hip ratio
WTCRF	Wellcome Trust Clinical Research Facility

Chapter 1 Introduction

1.1 Clinical Context of Study

Type 2 diabetes and obesity are common problems affecting the health of populations worldwide. The prevalence of both is increasing in both developed countries and urbanised populations within developing countries (1). One of the most important consequences of both is premature death from cardiovascular disease (2-4). Surprisingly, although obesity is known to be an independent risk factor for the development of type 2 diabetes (5;6), it is not known if obesity is an independent risk factor for cardiovascular disease in patients once they have developed diabetes and recent studies have suggested that in adults with type 2 diabetes with normal or low body weight the risk may be greater (7-10). There is also a paucity of research investigating the metabolic effects of different levels of adiposity in patients with type 2 diabetes.

The aim of this study was to investigate the effects of adiposity on triglyceride metabolism in patients with and without type 2 diabetes, focusing on postprandial triglyceride metabolism. Abnormalities in postprandial triglyceride metabolism are already known to contribute to cardiovascular risk (11;12).

The study was designed to include a large enough population of participants to give sufficient power to examine metabolic variables across a range of easily available clinical measures of adiposity and also to use a technique where the metabolism of recently ingested fat can be examined in detail and separately from that of endogenously produced triglyceride. This enables some insight into the pathophysiology of disturbances in postprandial triglyceride metabolism and allows examination of which, if any, abnormalities are associated with diabetes per se, and which, if any, are associated with level of adiposity.

The results of this study may help clinicians better tailor treatment in patients with type 2 diabetes depending on the phenotype of the patient, and therefore avoid a 'one size fits all' approach. For example, currently newer drugs for type 2 diabetes such as the glucagon-like peptide-1 analogues are limited to patients with diabetes with a body mass index (BMI) of >35.0kg/m^2, but leaner patients, or centrally obese patients may also benefit from these agents. Also efforts regarding dietary

intervention may be concentrated on obese patients with diabetes, whereas lean patients may equally benefit. The study may also provide initial evidence to update guidelines which currently restrict the use of potentially useful medications due the body mass index of a patient but where the restrictions are not based on outcome data.

1.2 Detailed Aims of Study

The aims of this study were to describe the relationship between body mass index (BMI), waist circumference and percentage (%) body fat with fasting and postprandial triglyceride metabolism by examining exogenous and endogenous triglyceride metabolism following a mixed meal containing a labelled stable isotope (1,1,1 ^{13}C tripalmitin) in a population of patients with and without type 2 diabetes mellitus.

1.3 Null Hypothesis

There is no difference in the relationship between adiposity and fasting and postprandial triglyceride metabolism in participants with and without type 2 diabetes mellitus.

1.4 Primary study measure

The relationship between BMI and ^{13}C - palmitic acid in the triglyceride (TAG) fraction area under the curve (^{13}C–PA TAG AUC) in participants with type 2 diabetes vs the relationship between BMI and ^{13}C - palmitic acid in the triglyceride (TAG) fraction area under the curve (^{13}C–PA TAG AUC) in control participants.

1.5 Secondary study measures

The relationship between BMI and incremental area under the curve triglyceride (INC AUC TAG) in participants with type 2 diabetes vs the relationship between BMI and INC AUC TAG in control participants.

The relationship between BMI and other metabolic variables including fasting TAG, fasting non-esterified fatty acids (NEFA), AUC postprandial TAG, AUC postprandial NEFA, AUC dietary derived ^{13}C-palmitic acid in the NEFA fraction (^{13}C-PA NEFA AUC) and measures of substrate oxidation and energy expenditure in participants with diabetes vs control participants in the fasting and postprandial states.

The relationship between waist circumference and % body fat and the above metabolic variables in participants with diabetes vs control participants.

Differences in fasting and postprandial triglyceride metabolism between the whole cohort of participants with type 2 diabetes and control participants after controlling for BMI, waist circumference and % body fat.

1.6 Reason for choice of primary study measure

^{13}C-PA TAG AUC was used as the primary measure of triglyceride metabolism as this reflects metabolism of dietary derived lipid as distinguished from endogenous circulating lipid and therefore potentially adds more mechanistic information. BMI was used as the primary measure of adiposity as this is the measure used most frequently in clinical practice and the measure which is used in clinical guidelines to stratify therapeutic interventions. Other measures of adiposity were also used so that they could be compared to the effect of BMI as BMI can have limitations as a measure of adiposity in some patients/ patient populations. This is discussed further later in the thesis.

1.7 Structure of thesis

In order to put the primary and secondary research questions and results into context, this thesis has been structured in chapters to answer the following questions:

1. Are there differences in lipid metabolism, substrate oxidation and energy expenditure between participants with diabetes and control participants?

2. Do differences in lipid metabolism, substrate oxidation and energy expenditure still exist between participants with diabetes and control participants, after adjustment for differences in BMI, waist circumference and % body fat?

3. Are there any relationships between lipid metabolism, substrate oxidation and energy expenditure and BMI, waist circumference and % body fat in participants with diabetes and control participants?

4. Do the relationships between lipid metabolism, substrate oxidation and energy expenditure and BMI, waist circumference and % body fat differ between participants with diabetes and control participants?

5. What are the possible mechanisms underlying differences in the relationship between lipid metabolism, substrate oxidation and energy expenditure and BMI, waist circumference and % body fat in participants with diabetes and control participants?

6. Do different measures of adiposity give different results and which is the best predictor of metabolic phenotype?

7. Are there any changes in the routine management of patients with type 2 diabetes which are recommended as a consequence of the study findings?

1.8 Literature review

1.8.1 Introduction to literature review

The literature review sets the scene for the study by defining the terms type 2 diabetes and obesity, and summarising what is known and existing controversies regarding the relationships between obesity, type 2 diabetes and cardiovascular

disease. It also summarises the published literature on postprandial triglyceride metabolism in patients with obesity and diabetes. The background to the methodology used in the study is described and finally there are suggestions of what this study may add to the published literature.

The main source of literature used is Pub Med along with use of respected textbooks in the fields of diabetes and obesity. A formal meta-analysis using statistical techniques was not performed. The search terms obesity, adiposity, BMI, waist circumference, % body fat, diabetes, fasting lipids and postprandial lipids were used.

1.8.2 Definition and classification of diabetes mellitus

Diabetes mellitus is the name given to a group of disorders which are defined by an elevated plasma glucose concentration. The WHO has published guidelines for the diagnosis and classification of diabetes since 1965. The criteria are mainly derived from data which suggest that individuals with plasma glucose concentrations above a certain level are at increased risk from participants with diabetes retinopathy; the WHO also state that these individuals are also at risk from premature mortality and of both microvascular and cardiovascular complications (13). The current World Health Organization (WHO) diagnostic criteria for diabetes are a fasting plasma glucose $\geq$ 7.0 mmol/l (126 mg/dl) or a 2 hour plasma glucose $\geq$ 11.1 mmol/l (200 mg/dl) during a 75 g oral glucose tolerance test. More recently in 2011, an expert committee of the American Diabetes Association and the European Association for the Study of Diabetes recommended a move to the use of HbA_{1c} (glycated haemoglobin, a measure of chronic glycaemia) of > or = 6.5% (48.0mmol/mol) to diagnose diabetes mellitus and have also included a random venous plasma glucose concentration $\geq$ 11.1 mmol/l with osmotic symptoms as a diagnostic criteria for diabetes (14). Measuring HbA_{1c} is easier to perform as this does not require a fasting blood sample or a glucose tolerance test, but may be of limited accuracy in some circumstances such as pregnancy, co-existent haemoglobinopathy, haemolytic disease, renal failure and anaemia.

There are five main types of diabetes i) Type 1 diabetes is due to autoimmune destruction of pancreatic beta cells with subsequent failure of insulin secretion, ii) Type 2 diabetes is due to relative tissue insensitivity to insulin and progressive beta cell failure, iii) secondary diabetes is beta cell failure secondary to pancreatic damage for example from pancreatitis, or diabetes secondary to drugs such as steroids, anti-viral/anti-psychotic therapy, where the mechanism is not fully understood, iv) monogenic diabetes previously known as 'maturity onset diabetes of the young'(MODY) and v) gestational diabetes (15). This is a simplistic classification and in reality type 2 diabetes is a heterogeneous group of disorders. There are other rare causes of diabetes such as mitochondrial diseases, lipodystrophy and other rare severe insulin resistance syndromes including insulin receptor mutations and insulin antibodies (Figure 1.1).

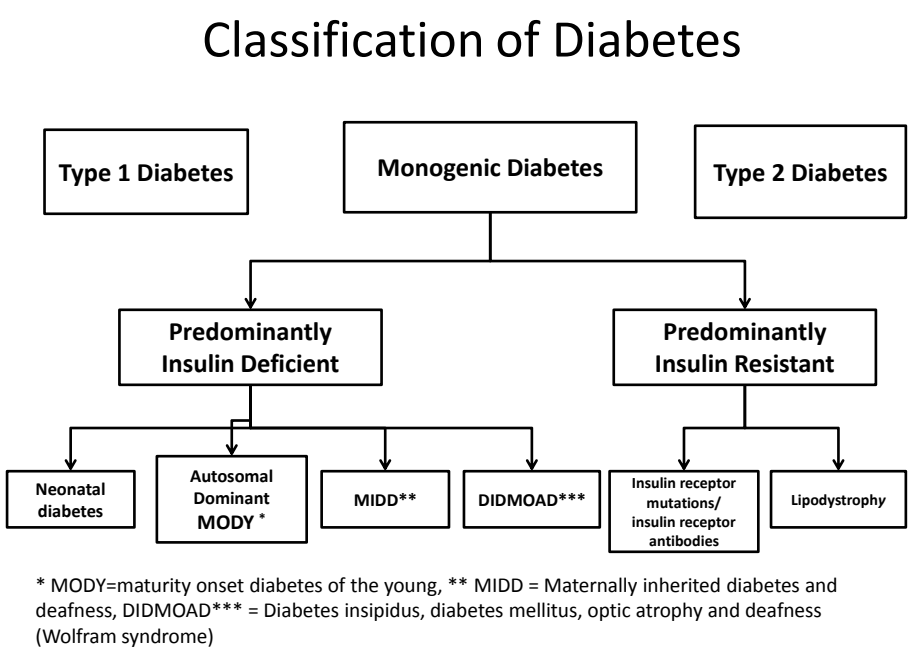


Figure 1-1: Classification of Diabetes (figure courtesy of Dr Victoria Parker)

The WHO do not provide specific definitions for type 2 diabetes as distinct from type 1 diabetes and in clinical practice it is not always easy to classify patients as having a specific type of diabetes.

Diabetes mellitus causes microvascular and macrovascular complications and there is a greatly increased cardiovascular risk, especially in people with type 2 diabetes

(4). In 2011 there were 366 million people with diabetes, and this is expected to rise to 552 million by 2030 (16).

There is a strong association between obesity and the onset of type 2 diabetes and increasing prevalence of obesity is thought to be the main factor for the increasing prevalence of type 2 diabetes (5;6). Management of patients with type 2 diabetes and obesity therefore both pose a huge economic burden on healthcare systems (17;18).

1.8.3 Definition and classification of obesity

The term obesity originates from the Latin word 'obesitas' which means 'fatness'. Adolphe Quetelet (1796-1874) first introduced the 'Quetelet Index' or 'Body Mass Index' (BMI) as a measure of fatness corrected for height. BMI is calculated as the ratio of the weight of the individual in kg to their height squared (kg/m^2). BMI has since been found to correlate with the amount of body fat in an individual (19), and is also able to predict risk of morbidity/mortality associated with excess body fatness (20). Life insurance data in the early twentieth century described the association between excess bodyweight and a reduced life expectancy, and this was confirmed in later studies in different populations and led to the WHO classification of obesity in 1995 (Table 1.1).

The WHO has categorised obesity as a worldwide epidemic (21). The 1998 Health Survey for England showed that 17% of men and 20% of women in the UK were obese, and the Joint Health Surveys Unit document 'Forecasting Obesity to 2010' estimated the prevalence of overweight and obesity in the UK in 2003 to be 38% and 22% respectively (22).

The measure of BMI has shortcomings as it does not predict cardiovascular risk equally effectively in different populations (23). Also, a patient with a high BMI may have a relatively low fat mass if they are very muscular, whereas a patient with a low BMI may have a high percentage body fat, or may have a high proportion of centrally located fat which is metabolically less healthy Other measures of 'body fatness' are also used in clinical practice which better measure the distribution of body fat. These measures include waist circumference, waist to hip ratio (WHR) and

skinfold thickness, the former of which have been found in for example the INTERHEART study to be better predictors of risk than BMI (24). Also more technical measures of body composition are possible using bioimpedance, dual-emission X-ray absorptiometry (DXA) scans and magnetic resonance imaging (MRI) scans (1). However in most healthcare settings it is still the BMI which is the primary estimate of body fat content.

Category	BMI (kg/m^2)
Underweight	16.5 to 18.5
Normal	18.5 to 25
Overweight	25 to 30
Obese Class I	30 to 35
Obese Class II	35 to 40
Obese Class III	over 40

Table 1-1: WHO international classification of obesity according to body mass index (adapted from Williams G and Frubeck G. Obesity, Science to Practice. Wiley-Blackwell; 2009. (1))

1.8.4 Cardiovascular health risks of obesity

Being overweight or obese is associated with significant excess morbidity and mortality. A prospective study of more than 1 million adults in the US showed that a

high BMI is associated with increased risk of death from all causes especially cardiovascular disease (CVD) (25). The mechanisms by which increasing BMI predisposes to CVD are not fully understood and are likely to be multifactorial. These include metabolic (dyslipidaemia, insulin resistance, hyperglycaemia), haematological (eg procoagulant changes) and other factors such as chronic inflammation and activation of the renin angiotensin system) (1). The site of excess body fat may have a more important effect on cardiovascular risk than the total amount of fat per se. A growing body of evidence suggests the importance of central (abdominal, visceral) obesity in increasing cardiovascular risk (24;26;27). As already mentioned, there is a strong association between obesity and the risk of developing type 2 diabetes which is itself a strong risk factor for CVD (5;6).

1.8.5 The 'obesity paradox'

There has been recent controversy in the association between BMI and outcomes with a U-shaped association between BMI and mortality in patients with heart failure and CVD (28). Obesity is known to predispose patients to heart failure by causing cardiac muscle dysfunction, possibly secondary to intramyocardial lipid accumulation (29), but obese patients with heart failure paradoxically seem to have a more favourable prognosis. Lavie et al. studied 209 patients with heart failure and found that higher BMI and higher percentage body fat were associated with better event-free survival during a 2 year follow-up period. In multivariate analysis, a higher percentage body fat was the strongest independent predictor of event-free survival. Lavie et al. describe this as an 'obesity paradox'(30). Recent studies have suggested that an obesity paradox may also exist in patients with type 2 diabetes, for example in a recent pooled analysis of 5 longitudinal cohort studies, adults who were normal weight at the time of incident diabetes had higher mortality than adults who were overweight or obese (8-10).

1.8.6 Relationship between obesity and cardiovascular risk in type 2 diabetes

Both obesity and type 2 diabetes are associated with an increased relative risk of CVD (2-4). It could therefore be anticipated that overweight or obesity would

increase the risk of CVD in people with type 2 diabetes. However the contribution of BMI to cardiovascular risk in people with type 2 diabetes remains controversial (7;8;11;31-33). There is some recent data suggesting an increased cardiovascular risk with increasing obesity in patients with type 2 diabetes, (34), another author suggests a U shaped curve (35) and another suggests an inverse relationship (8). The United Kingdom Prospective Diabetes Study (UKPDS) diabetes cardiovascular risk engine does not use obesity to calculate cardiovascular risk. The Diabetes Trials Unit, Oxford, UK, examined measures of obesity and found that they did not contribute independently to the estimated cardiovascular risk in the presence of the other risk factors used currently by the UKPDS risk engine. These factors include age, sex, duration of diabetes, fasting lipids (total and high density lipoprotein (HDL)-cholesterol) and blood pressure.

The measure of adiposity used in cardiovascular outcome studies is usually BMI as this is measured routinely in clinical practice. However other measures of adiposity may also be important in risk prediction in individuals with diabetes. Sluik et al (36) studied associations between BMI, waist circumference, waist/hip ratio, and waist/height ratio and mortality in 5,435 individuals with diabetes mellitus. BMI was not associated with higher mortality, whereas all measurements of abdominal obesity showed a positive association. The strongest association was observed for waist/height ratio. Another cross sectional study in 4,828 participants (37) assessed the presence of impaired glucose tolerance or impaired fasting glucose or type 2 diabetes in relation to the criteria used for the diagnosis of obesity using BMI compared to body fat percentage. The authors found a higher than expected number of subjects with prediabetes or type 2 diabetes in the obese category according to body fat percentage and that body fat percentage was significantly higher in lean (by BMI) women with prediabetes or type 2 diabetes as compared to those with normoglycemia. They concluded that assessing body fat percentage may help to diagnose disturbed glucose tolerance beyond information provided by BMI and waist circumference. This study did not provide any data on cardiovascular outcomes.

Finally, an interesting recent genetic study has identified a body-fat *reducing* allele (rs2943650 SNP near *IRS1*) which was associated with a 0.16% lower body fat percentage per copy of the major allele. The near *IRS-1* allele was associated with a

metabolically unhealthy phenotype including an increased visceral to subcutaneous fat ratio, insulin resistance, dyslipidemia, risk of diabetes and coronary artery disease (38).

1.8.7 Weight loss and cardiovascular risk

There has been a paradoxical observation that weight loss, either incidental or intentional, is associated with an increased mortality risk (28). It is unknown if weight loss has any long-term advantages in patients with type 2 diabetes. The Action for Health in Diabetes (LookAHEAD) study has been designed to answer this question. Participants in the Look AHEAD trial were randomly assigned to intensive lifestyle intervention (with an intensive behavioural treatment to increase physical activity and reduce caloric intake) or diabetes support and education (with less intense educational intervention). Nilsson recently reviewed the implications of the paradoxical observation that weight loss, either incidental or intentional, might be associated with an increased mortality risk in the management of patients with type 2 diabetes (7). Nilsson concluded that randomized controlled trials such as LookAHEAD (39) and CRESCENDO (40) would hopefully contribute to our understanding of the longer term effects of intentional weight loss and thereby resolve the current controversy. Unfortunately the CRESCENDO trial, which was investigating the cardiovascular outcomes associated with the weight loss medication rimonabant, was prematurely discontinued because of concerns by health regulatory authorities in three countries about suicide in individuals receiving rimonabant. Outcome data from LookAHEAD are still awaited.

1.8.8 Relationship between type 2 diabetes and obesity

Increasing BMI is associated with increased risk of type 2 diabetes (5;6). Daousi et al. showed that of a total of 2721 patients with type 2 diabetes attending a UK diabetes clinic, 86% were overweight or obese (41). However, although the association between type 2 diabetes and obesity is strong, 80% of obese people do not develop type 2 diabetes, and 20% of people with type 2 diabetes are not obese (6). The different phenotypes of non-obese patients with type 2 diabetes has been reviewed by Vaag (42) and illustrate the heterogeneity of the underlying

pathophysiology in patients with type 2 diabetes (Figure 1). The principal metabolic defect in some non-obese patients with type 2 diabetes may be beta cell dysfunction and not insulin resistance. Examples of non-obese patients labelled with type 2 diabetes include patients with monogenic diabetes (MODY) (43;44). Patients with late onset autoimmune diabetes (LADA) may also be wrongly classified as having type 2 diabetes and again these patients are not generally obese. Another example is patients with lipodystrophy (congenital or acquired reduction in subcutaneous fat stores) who may have type 2 diabetes and an unfavourable lipid profile (45). Finally, patients with poorly controlled type 2 diabetes may become catabolic and lose weight.

1.8.9 Mechanisms underlying the relationship between type 2 diabetes/insulin resistance and obesity

The exact mechanism by which increasing BMI predisposes to type 2 diabetes is unknown. There are differing viewpoints on this relationship. Some authors hypothesise that adipose tissue produces a factor which predisposes the individual to develop type 2 diabetes for example NEFA (46) or tumour necrosis factor alpha (TNF alpha) or other adipokines (47). Others hypothesise that there is a 'common soil hypothesis' where the association between obesity and type 2 diabetes is not causal and that a common abnormality predisposes both to obesity and insulin resistance/type 2 diabetes. For example, a primary abnormality in the incretin system may cause reduced pancreatic insulin secretion and also a reduction in satiety signalling. It may also be that an individual has a genetic predisposition to develop diabetes, but requires an environmental 'second hit', such as low levels of physical activity or a high fat diet to stress the metabolic system and cause decompensation, leading to high glucose concentrations and dyslipidaemia (48).

Common genetic or environmental factors have been shown to predispose to both type 2 diabetes and obesity (48). There is a well-established relationship between excess fat in tissues outside the adipose tissue depots, for example in skeletal muscle, liver and the pancreas which have an association, possibly causative, with the development of insulin resistance, fatty liver disease, beta cell dysfuction and type 2

diabetes. There is also an association between liver fat content and postprandial dyslipidaemia (49;50).

Studies of patients with lipodystrophy (congenital or acquired reduction in subcutaneous fat stores) have helped identify mechanisms behind the relationship between adipose tissue and diabetes and have led to the hypothesis that adipose tissue may be protective against metabolic risk factors and that 'adipose tissue failure' of which lipodystrophy is an extreme example, pre-disposes individuals to increased metabolic risk (51-54).

Studies of weight loss and the associated changes in metabolism such as the ongoing LookAHEAD study may help cast light on the underlying mechanisms (39;55).

1.8.10 Effects of drug therapy for glycaemic control on body weight in patients with type 2 diabetes mellitus

One of the difficulties associated with establishing the relationship of BMI and cardiovascular risk in type 2 diabetes is that many treatments that improve glycaemic control in type 2 diabetes also cause weight gain. This includes sulphonylureas, glitazones, and insulin therapy. More recently licensed medicines such as glucagon like peptide-1 (GLP-1) analogues, exenatide and liraglutide are associated with weight loss, and the DPP-IV inhibitors (eg sitagliptin and vildagliptin) are weight neutral (56). The effect of these drugs on cardiovascular outcomes and mortality is unknown, but prediction models suggest potential for reduction in cardiovascular outcomes. The potential for cardiovascular risk reduction with DPP-IV inhibition has been reviewed recently (57). A recent meta-analysis provides evidence that DPP-IV inhibitors are safe from a cardiovascular standpoint and may possibly decrease risk of adverse cardiovascular events (58). The meta-analysis included eighteen randomized trials, with 4,998 patients randomized to DPP-IV inhibitors and 3,546 to a comparator, with a median duration of therapy of 46.4 weeks. In the pooled analysis, the relative risk of any adverse cardiovascular event with a DPP-IV inhibitor was 0.48 (0.31 to 0.75, $p = 0.001$). Longer term prospective cardiovascular

outcome studies with both GLP-1 agonists and DPP-IV inhibitors are currently underway (59;60).

1.8.11 HbA_{1c}, CVD outcomes and relationship to BMI

There has been controversy recently regarding the HbA_{1c} targets in patients with type 2 diabetes. Recent studies have shown worse outcomes with very tight glycaemic control which may be secondary to hypoglycaemia (61). It is unclear whether CVD outcomes differ with different drug interventions and whether there is any relationship between CVD outcomes and to drug related changes in BMI.

1.8.12 Relationships between type 2 diabetes, obesity, and lipid metabolism

Disturbances in lipid metabolism are known to play a major role in CVD risk in people with and without type 2 diabetes (33;62). Reduction in cardiovascular risk in patients with and without type 2 diabetes by lowering LDL-cholesterol is well established (63-65).

Several studies have shown that obesity, especially central obesity (66;67) and type 2 diabetes (68;69) are associated with abnormalities of lipid metabolism. The most common lipid abnormalities found in both obesity and in type 2 diabetes are elevated fasting TAG concentrations, low HDL-cholesterol concentrations and changes in LDL particles (small dense LDL). This so called 'atherogenic lipoprotein profile' is an important cardiovascular risk factor (70;71). The mechanisms and relationship with insulin resistant states are described by Ginsberg (72). These lipid abnormalities have also been termed 'diabetic dyslipidaemia' (73). Many observational and prospective studies have shown that TAG and HDL-cholesterol concentration have greater predictive powers for CVD in participants with diabetes than total cholesterol or low density lipoprotein (LDL) -cholesterol concentration (68;74). Whether there is an independent cardiovascular risk associated with elevated triglyceride concentrations per se remains controversial. There is a strong inverse correlation between HDL-cholesterol and plasma TAG and therefore hypertriglyceridaemia may be an epiphenomenon associated with a reduction in HDL-cholesterol, and/or an

increase in triglyceride rich lipoproteins, which may be the causative factor in increasing cardiovascular risk. A meta-analysis published in 1997 showed plasma TAG concentration to be an independent risk factor for cardiovascular disease (33), but this is still not widely accepted. The American Heart Association has recently published a very comprehensive scientific statement regarding TAG metabolism in different cohorts of individuals, such as those with the Metabolic Syndrome, type 2 diabetes and lipodystrophy, and discusses the possible mechanisms which may explain the associated cardiovascular risk for example the association of hypertriglyceridaemia with atherogenic remnant particles. The document also suggests treatment guidelines for individuals with hypertriglyceridaemia. The American Heart scientific statement concludes that 'This scientific statement reaffirms that triglyceride is not directly atherogenic but represents an important biomarker of CVD risk' (75).

1.8.13 Postprandial triglyceride dyslipidaemia as an independent cardiovascular risk factor

Most of the large epidemiological and prospective studies of CVD risk have used fasting lipid concentrations as these are easier to measure in clinical practice and offer consistency in large studies. Most of the time, however, humans in 'westernised' populations are in a postprandial state. Postprandial triglyceride metabolism is described in more detail below. Postprandial dyslipidaemia (PPD) refers to an abnormal increase in the magnitude and/or duration of response of TAG rich lipoproteins following fat ingestion (76). PPD is thought to contribute to increased CVD risk independently of fasting TAG concentrations, although this remains controversial in patients with diabetes (11;12;77). The mechanism of increased CVD risk in PPD is uncertain but is likely to be related to the excess production and/or reduced clearance of atherogenic TAG rich lipoprotein particles such as chylomicron remnants and very low density lipoprotein (VLDL) remnants and an increased production of atherogenic lipoproteins including small dense LDL and an associated reduction in HDL-cholesterol concentration (the atherogenic lipid phenotype as described above) (78;79). The effects of disordered postprandial

triglyceride metabolism on coronary artery disease and carotid artery atherosclerosis have been reviewed by Lopez-Miranda et al. (80); the authors summarize numerous studies which show a relationship between postprandial dyslipidaemia and coronary artery disease.

1.8.14 Overview of lipid metabolism in the fasting and postprandial states

In 'healthy' participants plasma TAG concentration increases from a fasting concentration of about 1.0 mmol/l to a maximum of about 2.0 mmol/l between 2-4 hours after a meal (76). The postprandial changes in lipoprotein metabolism usually last for 6-8 hours after a meal. Following a meal containing fat, circulating triglyceride rich lipoproteins include both VLDL, produced by the liver, and chylomicrons containing dietary derived triglyceride, and also their respective hydrolysis products VLDL remnants and chylomicron remnants. There is evidence that VLDL and chylomicrons compete for a common saturable TAG removal mechanism via lipoprotein lipase (LPL) (81).

In insulin-resistant states such as obesity and type 2 diabetes, the production of VLDL by the liver is inappropriately high (82). There is also evidence for a reduction in LPL activity in insulin resistant states. This causes high triglyceride concentrations, in the fasting postprandial states. The high concentration of triglyceride rich lipoproteins and their prolonged residence time in the circulation may lead to activation of cholesterol ester transfer protein (CETP) and increased exchange of lipoprotein core lipid cholesterol ester for triglycerides between the triglyceride rich lipoproteins and LDL and HDL particles (83). This enrichment of LDL and HDL with triglyceride renders these lipoproteins more readily hydrolysed by hepatic lipase (Figure 1.2). This results in smaller, denser LDL particles and lower concentrations of HDL, a combination which is widely recognised to be atherogenic (69).

1.8.15 Lipid metabolism in the fasting state

Figure 1.2 illustrates the metabolism of fat in the fasting state such as during an overnight fast. During fasting the principal source of energy is from NEFA. NEFA

are derived from the breakdown of stored TAG in adipose tissue. Stored TAG is hydrolysed by the enzyme hormone sensitive lipase (HSL). Circulating NEFA are oxidised by the peripheral tissues to provide energy. Carbon dioxide is the metabolic end-product of beta oxidation.

1.8.16 Postprandial triglyceride metabolism

Figure 1.3 illustrates the metabolism of exogenous fat after eating a mixed meal. TAG from the meal is packaged into chylomicrons in the intestinal epithelial cell. Chylomicrons are TAG-rich apolipoprotein B (Apo B) containing lipoproteins. This is regulated by microsomal triacylglycerol transfer protein (MTP) which transfers dietary TAG to Apo B in the formation of chylomicrons. (84) Chylomicrons enter the circulation via the thoracic duct. At the peripheral tissues TAG in the chylomicrons is hydrolysed by lipoprotein lipase (LPL) to NEFA and glycerol. LPL activity is insulin sensitive. NEFA released from chylomicron hydrolysis are taken up into adipose tissue for storage or taken up by muscle cells for oxidation. Some NEFA also escape into the circulation. The remaining particles (chylomicron remnants) bind to the LDL receptors at the liver via apo E and are removed from the circulation. Adipose tissue HSL is inhibited by the postprandial rise in insulin concentration. Lipolysis of stored triglyceride in adipose tissue decreases and circulating NEFA concentrations therefore fall in the postprandial period.

1.8.17 Endogenous fat metabolism

NEFA is also used in the liver to synthesise VLDL which is a source of endogenous TAG. In healthy individuals this is suppressed by insulin in the postprandial period.

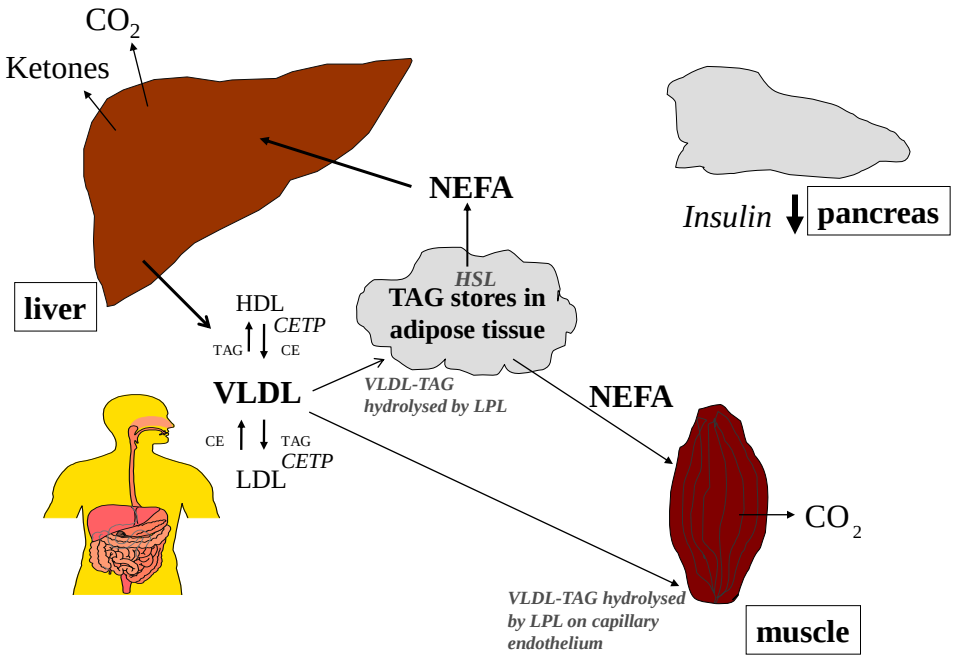


Figure 1-2: Lipid metabolism in the fasting state

Figure 1.2 illustrates the metabolism of fat in the fasting state such as during an overnight fast. During fasting the principal source of energy is from non-esterified fatty acids (NEFA). NEFA are derived from the breakdown of stored triglyceride (TAG) in adipose tissue. Stored TAG is hydrolysed by the enzyme hormone sensitive lipase (HSL). Circulating NEFA are oxidised by the peripheral tissues to provide energy. Carbon dioxide (CO_2) is the metabolic end-product of beta oxidation. The principle circulating TAG in the fasting state is within very low density lipoprotein (VLDL) particles. VLDL are TAG-rich particles, synthesised by the liver. VLDL triglycerides are hydrolysed by lipoprotein (LPL) located on the capillary endothelium. VLDL remnants or intermediate density lipoproteins (IDL) are taken up by liver receptors via apoE or converted to LDL. Cholesterol-ester transfer protein (CETP) catalyses the exchange of esterified cholesterol (CE) from high density lipoprotein (HDL) and LDL to triglyceride rich lipoproteins with the transfer in return of TAG to LDL and HDL particles. Triglyceride enrichment of LDL particles increases the action of hepatic lipase (HL), which hydrolyses triglycerides from the core of LDL and turns them into smaller and denser particles. Small dense LDL are thought to be more atherogenic as they can enter the subendothelial space and become oxidised.

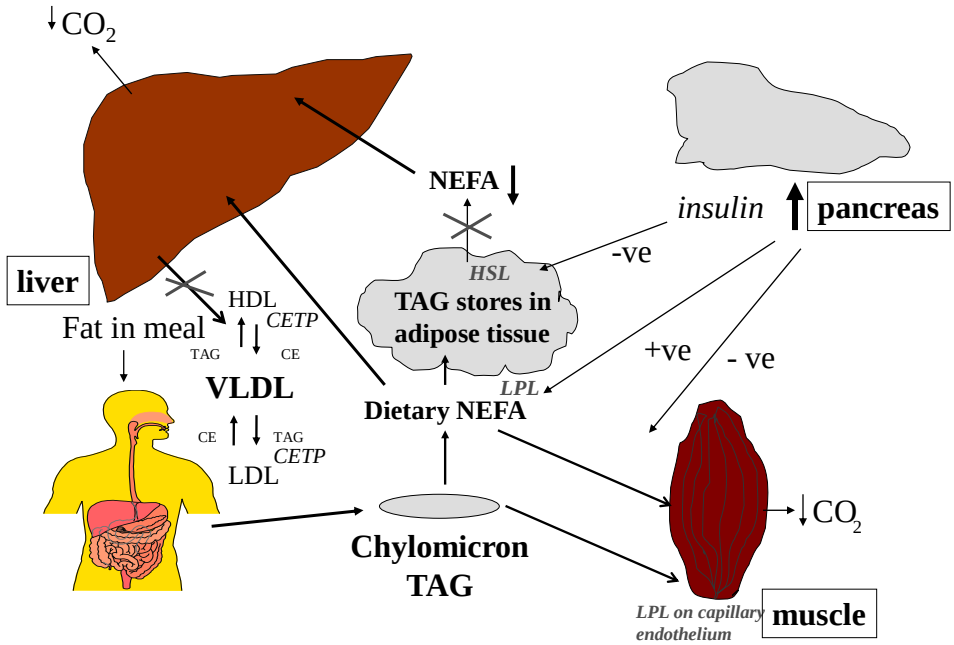


Figure 1-3: Lipid metabolism in the postprandial state

Figure 1.3 illustrates lipid metabolism in the postprandial state. Dietary triglyceride (TAG) is incorporated into chylomicrons in the intestinal epithelial cells. Chylomicrons enter the plasma via the intestinal lymph. Lipoprotein lipase (LPL) hydrolyses the triglyceride in chylomicrons to fatty acids (NEFA), which are taken up by muscle cells for oxidation or adipocytes for storage. The remaining particles, (chylomicron remnants), are removed from the circulation through binding of their surface apoE to the low density lipoprotein (LDL) receptor in the liver. Very low density lipoprotein (VLDL) particles are TAG-rich particles, synthesised by the liver. VLDL triglycerides are also hydrolysed by LPL. VLDL remnants or intermediate density lipoproteins (IDL) are taken up by liver receptors via apoE or converted to LDL. After eating, insulin concentrations normally rise and suppress TAG lipolysis from adipose tissue and hepatic VLDL production. Insulin also increase chylomicron triglyceride hydrolysis by LPL. In insulin resistance states such as central obesity and type 2 diabetes insulin action and/or concentrations are sub-optimal, resulting in high circulating TAG concentrations. Elevated TAG activates cholesterol ester transfer protein (CETP) and increases reverse lipid transport of TAG and esterified cholesterol (CE) which contributes to the formation of small dense LDL and a reduction in normal HDL particles, the so called 'atherogenic lipid phenotype'.

1.8.18 Assessment of postprandial triglyceride metabolism

One of the challenges in the design of studies exploring postprandial triglyceride metabolism is distinguishing between exogenous and endogenous fat metabolism. There is no standard way of measuring postprandial triglyceride metabolism, and normal values have not been defined. Many studies to date have used a high fat meal containing retinyl palmitate as a marker of exogenous lipid metabolism. This method has been questioned as a marker of intestinally derived TAG, as some studies have demonstrated a late appearance in plasma of retinyl palmitate compared with chylomicrons after a test meal which may be due to delayed absorption of vitamin A caused by polyunsaturated fats in the test meal (85;86).

1.8.19 Stable isotope techniques

Stable isotope techniques can be used to assess how long dietary fat remains in the circulation and allow a distinction to be made between the metabolism of endogenous lipid and exogenous (dietary) lipid (Figure 1.4).

This study uses a relatively novel approach of investigating postprandial triglyceride metabolism by measuring ingested lipid metabolism in the different lipid fractions using a stable isotope tracer (1,1,1-^{13}C tripalmitin) within the test meal (87-90). The oxidation of dietary-derived lipid can be specifically assessed by measuring the levels of label excreted in the breath as CO_2 production, and by using a study meal containing ^{13}C-labelled fatty acids - the amount of $^{13}CO_2$ in the breath enables calculation of how much dietary lipid has been oxidized.

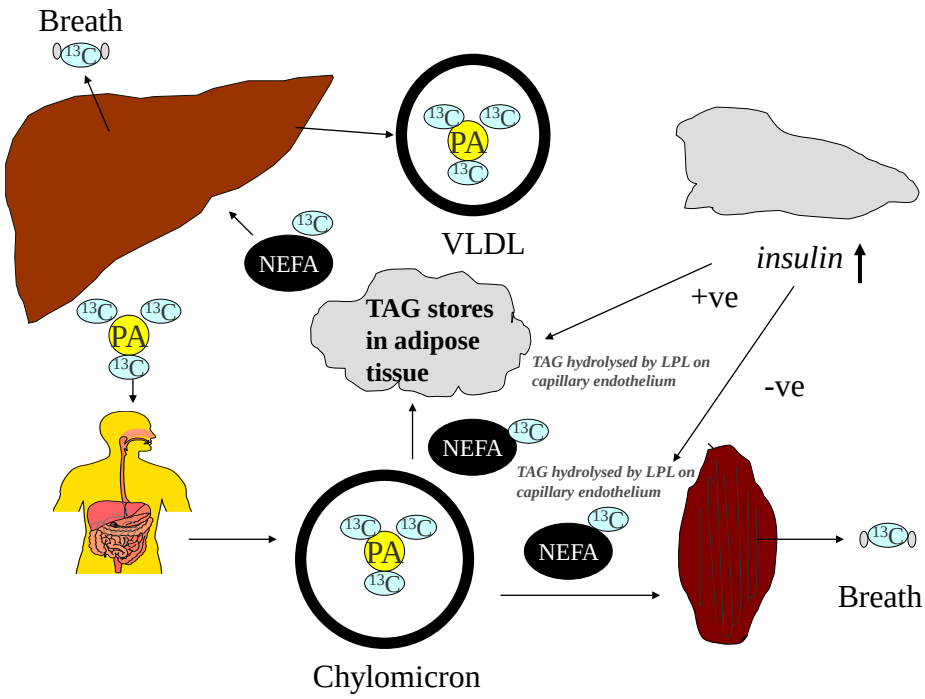


Figure 1-4: Stable isotope techniques

Figure 1.4 illustrates stable isotope methodology in postprandial triglyceride studies. Using a study meal containing (1,1,1-^{13}C) tripalmitin, the fatty acid ^{13}C-palmitic acid (^{13}C-PA) is incorporated into the chylomicron particles. Peripheral tissue lipoprotein lipase on the capillary endothelium (LPL) hydrolyses the chylomicron triglyceride (TAG) and ^{13}C-PA NEFA is released. ^{13}C-PA in NEFA is then either taken up and stored by the tissues, or remains in the circulation. The latter is sometimes referred to as 'overspill'. ^{13}C-PA NEFA removed from the circulation by the liver can reappear as ^{13}C-PA in very low density lipoproteins (VLDL) and then compete for removal from the circulation with ^{13}C-PA-TAG in chylomicrons. ' In this study ^{13}C-PA concentrations were measured in the TAG and non-esterified fatty acid (NEFA) plasma fractions, but not specifically in the different lipoprotein classes.

1.8.20 Factors affecting postprandial triglyceride metabolism

Type 2 diabetes, insulin resistance and obesity, are among the many factors known to affect the postprandial triglyceride response. Other factors include habitual high fat and/or high carbohydrate diet, meal composition and meal size, physical activity, alcohol consumption, smoking, age, gender, menopausal status, fasting hypertriglyceridaemia and genetic polymorphisms. In a recent review of postprandial triglyceride metabolism (80) the authors conclude that the most important factors are the meal components, obesity, type 2 diabetes, exercise, smoking status, gender and pre-existing hypertriglyceridaemia (Table 1.2, adapted from from Lopez-Miranda J et al, (80)). The effects of type 2 diabetes and obesity are discussed in more detail below.

Factors	Extent of change in postprandial triglyceride concentration
Dietary	
Amount of fat (meal)	+++
Type of fat (meal)	+/-
Type of fat (habitual diet)	+/-
Carbohydrates	++
Protein (meal)	No/-
Alcohol	++
Fibre	No/-
Lifestyle	
Physical exercise	--
Tobacco use	++
Physiological factors	
Gender	+ (males)
Age	+
Menopausal status	+ (postmenopausal status)
Pathophysiology	
Fasting hypertriglyceridaemia	+++
Central obesity	++
Insulin resistance	++
Type 2 diabetes	++

+++, ++, +, very important, important or moderate increase; --, -, important or moderate reduction; 'No', no noticeable change.

Table 1-2: Factors affecting postprandial triglyceride metabolism

Table 1.2 is adapted from Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial triglyceride metabolism. Br J Nutr 2007 Sep;98(3):458-73) (69)

1.8.21 Effects of type 2 diabetes on postprandial triglyceride metabolism

Relatively few studies have examined postprandial triglyceride metabolism in participants with type 2 diabetes. No studies have investigated the effect of adiposity on postprandial triglyceride metabolism in diabetes across a wide range of BMI as most studies have been designed to reduce variability in BMI in order to facilitate close matching of body habitus in comparison groups. A large recent study in 539 participants with type 2 diabetes showed only a weak correlation between TAG at 90 minutes after a standardised liquid meal and waist circumference (r=0.123), and found a similar association for BMI (r=0.108). The associations between postprandial TAG and waist circumference/BMI were stronger in a matched group with impaired fasting glucose (r=0.246 and r=0.246 respectively). Postprandial TAG was not independently associated with either BMI or waist circumference in a multiple linear regression analysis (91). Other studies have had conflicting results, one study found an association between postprandial TAG and waist circumference (92) and another did not, and one study found an association between postprandial TAG and BMI but another did not (93;94).

Fasting TAG has consistently been found to be an important determinant of postprandial TAG in patients with diabetes (91;95). Two studies (96;97) show that participants with diabetes with fasting hypertriglyceridaemia have an exaggerated postprandial triglyceride response but participants with diabetes with normal fasting TAG have similar responses to control participants except for clearance of chylomicron remnants, which was impaired in both groups of participants with diabetes. This is probably due to impairment of clearance of postprandial triglyceride due to the increased overall pool size caused by fasting hypertriglyceridaemia. It is not clear if postprandial dyslipidaemia (except for chylomicron remnant clearance) was related to diabetes per se in these studies, or to the fasting hypertriglyceridaemia in the patients with type 2 diabetes as neither study included hypertriglyceridaemic control participants. In one study (97) all participants were obese, and in the other (96) no participants were obese. Cooper et al. suggest that postprandial

dyslipidaemia in type 2 diabetes is associated with beta cell output of insulin precursors (96).

Chen et al. found an elevation of meal-derived TAG (retinyl palmitate) in VLDL-TAG but not chylomicron-TAG in 10 participants with type 2 diabetes compared with 10 control participants matched for BMI (98).

Van Wijk et al. measured capillary TAG during three days at six fixed time-points each day in an out-of-hospital situation. They included 19 participants with type 2 diabetes (mean BMI 30.6 kg/m^2), 45 overweight and obese non-participants with diabetes (mean BMI 29.5 kg/m^2) and 78 lean participants (mean BMI 23.7 kg/m^2). They did not include any lean participants with diabetes patients. Fasting TAG and AUC TAG were both higher in participants with diabetes and obese non-participants with diabetes compared with lean participants. Fasting TAG and waist circumference best associated with TAG AUC. They concluded that daylong triglyceridaemia was similarly increased in participants with diabetes and obese non-participants with diabetes compared with lean participants and that fasting TAG and central obesity largely determined daylong triglyceridaemia, independent of the presence of type 2 diabetes (92).

Madhu et al. (93) studied postprandial lipids in 20 male type 2 participants with diabetes and 20 age and sex matched healthy controls. BMI was similar in both groups with (mean of about 25.8 kg/m^2). Fasting serum lipids were not significantly different between the two groups. Postprandial TAG AUC, TAG area under incremental curve, and peak TAG were all higher in the participants with diabetes. TAG AUC correlated significantly with fasting serum TAG ($r=0.62$) and BMI ($r=0.7$), but not with waist hip ratio or fasting serum insulin levels. Postprandial lipaemia did not correlate with fasting blood glucose or HbA1c.

Annuzzi et al. (99) compared ten participants with obesity and type 2 diabetes, 11 with obesity alone and 11 normal-weight controls (with fasting normo-triglyceridaemia) before and after a fat-rich meal. LPL activity was determined in abdominal subcutaneous adipose tissue biopsy samples. Insulin sensitivity was

measured by hyperinsulinaemic euglycaemic clamp. They showed that obese control and obese participants with diabetes showed a similarly higher postprandial increase in large VLDL than normal weight controls and that obese participants with diabetes had an increased chylomicron response compared to obese controls. Obese participants with diabetes also had significantly lower fasting and postprandial adipose tissue heparin-releasable LPL activity than obese and normal weight controls

Erikkson et al. (100) also investigated postprandial regulation of adipose tissue lipoprotein lipase. Eight participants with type 2 diabetes and eight age, sex and BMI matched control participants underwent subcutaneous abdominal adipose tissue biopsies in the fasting state and 3.5 hours following a standardized lipid-enriched meal. Postprandial, but not fasting, TAG were significantly higher in the participants with diabetes than in the control participants. Fasting and postprandial adipose tissue LPL activity as well as post-heparin plasma LPL activity was non-significantly lower among the diabetes patients, but they concluded that after food intake adipose tissue LPL activity is enhanced to a similar degree in patients with type 2 diabetes and in healthy control participants.

Madhu et al.(94) compared postprandial triglyceride responses to a standard oral fat challenge in participants with impaired fasting glucose and impaired glucose tolerance, newly detected diabetes mellitus and normal glucose tolerance in forty four participants. There was a significantly higher TAG area under curve and peak TAG in patients with newly detected diabetes mellitus but not with impaired fasting glucose and impaired glucose tolerance when compared with normal glucose tolerance. TAG responses correlated significantly with fasting plasma glucose and 2 hour plasma glucose on the oral glucose tolerance test but not with age, sex, BMI, waist, or insulin resistance.

Finally, Tan et al. examined postprandial NEFA metabolism in a patient with the peroxisome proliferator-activated receptor (PPAR) gamma mutation P467L. The patient had partial lipodystrophy and type 2 diabetes (101). A mixed meal was used which included 600 mg (1,1,1-13C) tripalmitin. Two control groups were used, healthy volunteers, and patients with type 2 diabetes. The P467L patient had elevated fasting and postprandial NEFA concentrations, and impaired postprandial adipose

fatty acid trapping of ^{13}C-palmitic acid. No formal data comparison was presented on the differences between the healthy and participants with diabetes control participants.

1.8.22 Triglyceride metabolism and glycaemic control in type 2 diabetes

Patients with poorly controlled or newly presenting diabetes have high plasma glucose concentrations. There is often a co-existing hypertriglyceridaemia and elevated NEFA, although the latter are not routinely measured in clinical practice. The cause of these changes is insulin deficiency which causes impaired glucose uptake into tissues, impaired suppression of NEFA release from adipose tissue in the fasting state and also the failure of adipose tissue uptake of postprandial NEFA which causes increased NEFA uptake by the liver and increased production of VLDL. There is also the phenomenon of 'glucolipotoxicity' on the pancreatic beta cell, where excess NEFA and glucose further depletes insulin production from the beta cell and causes a further deterioration to metabolic control (102). Treatment with medication such as insulin, metformin or sulphonylurea therapy will cause a reduction in all elevated metabolic measurementss including glucose, TAG and NEFA concentrations (103).

1.8.23 Effects of obesity on postprandial triglyceride metabolism

Studies of postprandial triglyceride metabolism in obese participants suggest that obesity (especially when in a central distribution) appears to be associated with postprandial dyslipidaemia. Obese participants have up to three times higher postprandial TAG concentrations than non-obese controls (104-111). An abnormality in chylomicron metabolism in obese participants has been described in some of these studies (104;106;108). Mekki et al. showed that participants with android obesity showed exaggerated postprandial TAG responses and impaired chylomicron clearance despite normal fasting TAG (108).

1.8.24 Effects of insulin resistance on postprandial triglyceride metabolism

Studies have shown that insulin sensitivity is a determinant of postprandial triglyceride responses in healthy adults independent of BMI, WHR, and blood glucose (112;113). Postprandial lipaemia has been correlated with fasting insulin concentrations (105). The mechanism is not known but may be due to impaired insulin-mediated suppression of hepatic VLDL production and fatty acid release from adipose tissue (114;115).

Studies have been performed to examine the effect of the insulin resistance syndrome on the postprandial response in non participants with diabetes (115-118). Bickerton et al. (118) investigated adipose tissue fatty acid metabolism in insulin resistant overweight men compared to a control group. The men were given a mixed meal incorporating a stable isotope tracer 100 mg (U-^{13}C) palmitic acid. Fasting and postprandial TAG concentrations were significantly higher in the insulin resistant men. The authors suggest that the elevated TAG is due to reduced oxidation and increased esterification of fatty acids in the liver. There was no difference in fasting or postprandial NEFA. Systemic NEFA production and release of NEFA from subcutaneous adipose tissue (per unit of fat mass) were reduced in insulin resistant men compared with controls. The authors suggested that this was due to high fasting insulin concentrations.

1.8.25 Possible mechanisms causing postprandial dyslipidaemia in type 2 diabetes, insulin resistant states and obesity

Pathophysiological mechanisms affecting normal postprandial triglyceride metabolism can occur at any point in the normal process of lipid metabolism which have been described above. These processes have been reviewed recently by Tomkin (119) and also by Paglialunga (120). Examples of mechanisms possibly involved include impaired regulation of hydrolysis of dietary fat by pancreatic lipase and defects in regulation of intestinal absorption and chylomicron formation. Effects of diet, including intake of specific fatty acids, carbohydrate and alcohol, defects in action of lipoprotein lipase, changes in blood flow to adipose tissue, skeletal muscle

and liver, failure of suppression of VLDL production by the liver in the postprandial period which causes competition for LPL, differences in apoprotein E genotype and expression. Abnormalities in many of these processes have been found in participants with type 2 diabetes, obesity and insulin resistance and it is extremely difficult to tease out if specific defects are unique to each of these conditions. This is especially difficult as type 2 diabetes and insulin resistance are heterogenous conditions.

Many of these processes are regulated by insulin. It would be interesting to see if the abnormalities in postprandial triglyceride metabolism exist in patients with type 2 diabetes, after controlling for once other factors, for example the effects of obesity and insulin resistance, as this would suggest that relative insulin deficiency is important. Some evidence of benefit of insulin therapy in postprandial dyslipidaemia has been found in studies treating patients with type 2 diabetes (121). There are also benefits from glucose and lipid lowering drugs (122;123). It is hoped that this study will add to the current evidence as to whether diabetes per se or the related obesity often co-existing with diabetes is the principal defect in causing postprandial dyslipidaemia.

1.8.26 Metabolic flexibility

'Metabolic flexibility' has been defined as 'the capacity for the organism to adapt fuel oxidation to fuel availability' (124) or put another way, 'metabolic inflexibility' has been defined as 'the impaired capacity to increase fat oxidation upon increased fatty acid availability, and to switch between fat and glucose as the primary fuel source after a meal' (125). Metabolic inflexibility has been postulated as a mechanism for the development of insulin resistance. It has been hypothesised that an impaired capacity to upregulate muscle lipid oxidation in the face of high lipid supply may lead to increased muscle fat accumulation and the development of insulin resistance. However it is unclear if the associations between insulin resistance, obesity and type 2 diabetes and so called 'metabolic inflexibility' are cause or effect. There are a number of metabolic pathways in a number of different tissues, which may be involved. These include the loss of the first phase insulin

response after eating, failure of skeletal muscle to switch between metabolism of lipid in the fasting state to glucose in the fed state, and failure of a change from NEFA efflux to NEFA storage in adipose tissue after eating (126). In their review, Galgani et al. (124) examined the roles of glucose disposal rate, adipose tissue lipid storage, and mitochondrial function in metabolic flexibility. They concluded that from current evidence, it cannot be assumed that impaired metabolic flexibility is responsible for the accumulation of intramyocellular lipid and insulin resistance, and that after controlling for insulin-stimulated glucose disposal rate, metabolic flexibility is not altered in obesity regardless of the presence of type 2 diabetes. They add that the assessment of metabolic flexibility to high-fat diets is more relevant than metabolic flexibility during a hyperinsulinemic clamp and that studies examining metabolic flexibility using high fat diets are needed.

1.8.27 Effects of therapeutic interventions on postprandial dyslipidaemia in patients with type 2 diabetes

Therapeutic interventions which affect postprandial triglyceride metabolism may help identify the mechanisms causing postprandial dyslipidaemia. Therapeutic interventions tested in previous studies have included lifestyle interventions such as exercise and dietary modification and the use of medication. The effects of primarily glucose and lipid lowering medication respectively on postprandial dyslipidaemia in patients with type 2 diabetes have been reviewed recently (122;123). The test meals used varied in the different studies.

1.8.28 Exercise

Several studies have shown that aerobic exercise acutely prior to fat ingestion reduces postprandial triglyceride concentrations. For example one study examined the effects of moderate-intensity cycling on postprandial TAG concentrations. Twelve male subjects consumed a meal of moderate-fat content (45 % of total energy) on two occasions at least 1 week apart. On day 1, subjects either cycled for 30 min at 65 % of maximum heart rate in the afternoon or rested. On day 2 of both

study episodes, subjects consumed the test meal for breakfast. The total and incremental areas under the serum TAG concentration were 30% ($p = 0.039$) and 33% ($p = 0.012$) lower on the exercise days compared with the control (no-exercise) days (127). Another study by the same author (128) investigated the role of long term physical activity status in 26 active and inactive older adults on postprandial lipaemia. After an overnight fast, participants consumed a test meal of moderate fat content (35%). Capillary blood samples were collected in the fasted state and then at 2, 4, and 6 hours postprandially. After adjusting for fasting TAG concentrations, BMI and waist circumference, postprandial capillary TAG concentrations were significantly lower in the active than inactive group ($p=0.046$). These studies therefore demonstrate that both regular physical activity and acute physical activity lowers postprandial lipaemia in adults.

1.8.29 Diet

Habitual dietary of either fat or carbohydrate type and quantity has been shown to affect postprandial triglyceride metabolism. In one study, metabolic responses to fat and carbohydrate ingestion were investigated in twenty four lean male individuals known to consume a habitual diet high or low in fat. High fat consumers had a significantly higher resting metabolic rate and higher resting and postprandial heart rate than low fat consumers. Fat oxidation was significantly higher in high fat consumers than in low fat consumers following the fat load (129). However in another study (130) the effects of a high-fat breakfast on postprandial fat and carbohydrate metabolism were investigated in 28 lean, male subjects with habitual dietary fat intakes between 21 and 44% of daily energy intake, demonstrated that the fat level of the habitual diet did not affect the baseline or the postprandial values in the respiratory quotient or the plasma levels of triglycerides. In this study only the area under the curve for insulin was higher in the high fat consumers, suggesting that a habitual high fat intake may pre-dispose to insulin resistance.

Dietary carbohydrate content and glycaemic index (GI) also affects postprandial triglyceride metabolism .Wolever et al (131) explored long-term changes in postprandial responses on low- GI or low-carbohydrate diets in patients with type 2 diabetes. Changes in postprandial triglycerides differed among the groups

($p < 0.001$). After 12 months postprandial triglycerides were significantly higher than at baseline in those participants with a low-carbohydrate/high-monounsaturated-fat diet with a high-carbohydrate/high-GI meal ($p = 0.028$). This study illustrates that carbohydrate content and type has both acute and chronic effects on postprandial triglycerides metabolism in patients with type 2 diabetes. Habitual and acute meal content is therefore important to consider when performing studies of postprandial triglyceride metabolism.

1.8.30 Glucose-lowering medication

The effect of anti-participants with diabetes medication on postprandial triglyceride metabolism is reviewed in (122) and is discussed below.

1.8.31 Insulin

Insulin treatment, especially with the short acting insulin analogues in addition to long acting insulin, has been found to have favourable effects on postprandial triglyceride metabolism in patients with type 2 diabetes mellitus in several studies (121). Insulin is not usually the primary treatment of type 2 diabetes, as oral medication is generally tried first, due to the inconvenience of needing to inject insulin and the increased risk of hypoglycaemia.

1.8.32 Sulphonylureas

A study examined the effect of one dose of 5 mg glibenclamide administration on postprandial lipaemia in eight patients with type 2 diabetes. There was a significant decrease in postprandial glycaemia and increase in AUC insulin after glibenclamide administration compared to placebo. The AUC values of total plasma TAG and of chylomicron TAG were significantly lower compared to placebo. The AUC postprandial TAG in VLDL-1, VLDL-2 and intermediate density lipoprotein (IDL) were not different compared to placebo. No significant differences were noted in NEFA concentrations (132).

1.8.33 Metformin vs repaglinide

Lund et al. compared the effect of metformin versus repaglinide on postprandial metabolism in non-obese type 2 diabetes patients. Fasting levels and AUC plasma glucose, TAG and NEFA reduced equally between treatments. Insulin concentrations were lower with metformin treatment (133).

1.8.34 PPAR gamma agonists (eg rosigliazone, pioglitazone)

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear receptor that is encoded by the PPAR-γ gene. PPAR-**γ** regulates fatty acid storage and glucose metabolism. The genes activated by PPAR-**γ** stimulate lipid uptake into adipocytes and adipocyte differentiation (134).

The 'glitazone'or 'thiazolidenedione' class of drugs including rosiglitazone and pioglitazone activate the PPAR-γ receptor and act as 'insulin sensitisers' ie they lower serum glucose without increasing pancreatic insulin secretion. A recent study examined the effects of 12 weeks treatment with rosiglitazone, a PPAR-γ agonist on fasting and postprandial triglyceride metabolism in patients with type 2 diabetes (135). The study did not have a healthy control comparator group. A mixed meal was used which included 600 mg (1,1,1-^{13}C) tripalmitin. In the patients treated with rosiglitazone, they found no change in fasting NEFA, but a reduction in postprandial NEFA concentration, and a reduction in the postprandial rise in the ^{13}C-palmitic acid in the NEFA fraction. The rate of LPL action was unchanged in adipose tissue and skeletal muscle. The authors suggest that the postprandial reduction in NEFA concentration may represent decreased postprandial spillover of NEFA from visceral adipose tissue depots. Fasting TAG was not changed, postprandial TAG was decreased and ^{13}C-palmitic acid TAG was not changed with rosiglitazone treatment. Rosiglitazone has recently been withdrawn from use in Europe due to regulatory concerns regarding increased cardiovascular risk. Pioglitazone remains available for use, but in restricted patient groups due to an increased risk of bladder cancer associated with the drug.

1.8.35 GLP-1 analogues

GLP-1 is a gastrointestinal peptide (incretin hormone), which enhances glucose-induced insulin secretion and lowers glucagon release. A recent study in rodents has shown that GLP-1 receptor activation is also involved in the regulation of intestinal lipoprotein biosynthesis and secretion (136). GLP-1 is inactivated in vivo by the enzyme dipeptidyl peptidase IV (DPP-IV). Recently new drugs which are GLP-1 analogues (eg exenatide) and DPP-IV inhibitors (eg. sitagliptin, vildagliptin) have been brought successfully to the market for the treatment of type 2 diabetes. They are currently used primarily for their glucose lowering effects, and have the added advantage that GLP-analogues also cause weight reduction, whereas DPP-IV inhibitors are weight neutral. In the UK, use of exenatide has been generally limited to patients with type 2 diabetes over a BMI of >35 kg/m^2 (Figure 1.5). Recent studies investigating the effects of these drugs on lipid metabolism are described below.

Exenatide

A recent study in human participants examining the effect of the GLP-1 analogue, exenatide showed reduced postprandial elevation of TAG, apolipoproteins B-48 and CIII, remnant lipoprotein-cholesterol and remnant lipoprotein-TAG (all $p<0.001$). Postprandial declines in NEFA were less pronounced but persisted longer with exenatide compared to placebo ($p<0.05$). These effects of exenatide were not influenced either by glucose tolerance status or by treatment with statins (137). Another study comparing exenatide with insulin glargine over a 12 month period showed beneficial effects of exenatide compared to insulin glargine on postprandial glycaemia and lipidaemia. The authors suggest that exenatide may offer additional cardiovascular risk reduction by inhibiting postprandial excursions of proatherogenic lipids and lipoproteins (138).

Dipeptidyl-Peptidase-4 Inhibitors

A study examining the effects of vildagliptin, a novel DPP-IV inhibitor, on postprandial triglyceride and lipoprotein metabolism in patients with type 2 diabetes showed improved postprandial plasma TAG and apolipoprotein B-48-containing TAG-rich lipoprotein particle metabolism after a fat-rich meal (139).

1.8.36 Lipid lowering medication

Currently available drugs for lipid lowering include fibrates, statins, ezetimibe, fish oils, nicotinic acid derivatives and bile acid sequestrants. Drugs still in development include the CETP inhibitors. The effect of lipid lowering therapy on postprandial triglyceride metabolism has been the subject of a recent systematic review (123) and has also been comprehensively reviewed by Packard (140).

Fibrates

The most widely used drugs for treatment of fasting hypertriglyceridaemia are the fibrate class of drugs (eg fenofibrate, gemfibrozil). Fibrates decrease fasting TAG concentrations by 50-70% and increase the HDL- cholesterol concentration by 10-30% (140). Clinical studies have also shown an improvement in postprandial TAG concentrations which is likely to be partly due to the reduction in TAG pool size secondary to reducing fasting TAG. Fibrates decrease the production of and enhance the clearance of TAG-rich lipoproteins, including VLDL, chylomicrons and TAG rich remnant lipoproteins through the activation of the nuclear hormone receptor peroxisome proliferator-activated receptor-alpha (PPARα). Clinical studies with fibrates have also shown an increase in lipoprotein lipase (LPL) activity, decreased apolipoprotein CIII production, and an increase in fatty acid oxidation by the liver(141). Fibrates can also affect the postprandial lipid profile by reducing cholesterol ester transfer protein (CETP) activity and therefore reducing CETP mediated lipid exchange. This is the likely mechanism by which fibrates increase HDL-cholesterol concentrations (140-142).

Statins

Statins are usually prescribed to treat hypercholesterolaemia. They inhibit cholesterol synthesis by inhibiting the enzyme hydroxmethylglutural (HMG) CoA reductase. Statins such as simvastatin and pravastatin at high doses lower plasma TAG concentrations by 10-20%, although the effect may be greater in pataients with combined hyperlipidaemia (140;143). Statins have been shown in several studies to increase chylomicron clearance and reduce postprandial lipaemia (144). The

mechanism by which statins reduce postprandial TAG is uncertain and may be by increasing TAG remnant clearance by the liver by increasing LDL receptor activity and/or by reducing VLDL concentrations and therefore reducing competition for TAG hydrolysis by lipoprotein lipase. The mechanism by which statins reduce VLDL is also unclear, but may be by the inhibition of VLDL production by the liver (123;140).

Ezetimibe

Ezetimibe is prescribed for the treatment of hypercholesterolaemia. Ezetimibe where it inhibits the absorption of cholesterol from the intestine and is thought to bind to the Niemann-Pick C1-Like 1 (NPC1L1) protein on the gastrointestinal tract epithelial cells which is an important mediator of cholesterol absorption. A recent 6 week study showed that treatment with ezetimibe and simvastatin, compared to simvastatin alone significantly decreased fasting and postprandial chylomicron cholesterol and TAG content and significantly decreased chylomicron postprandial apoB-48 concentrations. There is currently no data suggesting improved cardiovascular outcomes with ezetimibe however.

Nicotinic acid derivatives

Nicotinic acid derivatives such as extended release niacin are usually used second line to treat elevated HDL-cholesterol. Recent studies have shown that they reduce postprandial TAG concentrations (145), but there use has not shown increased cardiovascular benefit when used in combination with intensive simvastatin therapy (146).

Omega 3 fatty acids

Omega 3 fatty acids are used second line to treat hypertriglyceridaema and have been shown to reduce postprandial TAG concentrations, however their effect on cardiovascular outcomes is currently unknown and they appear to increase insulin resistance and may affect pancreatic beta cell responsiveness to elevated plasma glucose(147) (148).

Cholestyramine

Cholestyramine is a bile acid sequestratant which is used second line to treat hypercholesterolaemia. This drug has been shown to raise fasting and postprandial TAG concentrations, possibly by stimulating hepatic TAG synthesis and therefore increasing competition for lipolysis in the postprandial state (140).

Cholesterol ester transfer protein inhibitors

There are currently no licensed cholesterol ester transfer protein (CETP) inhibitors in clinical use, but several are in development. CETP plays a significant role in catalysing HDL metabolism and reverse cholesterol transport. Reverse cholesterol transport leads to the formation of small dense LDL and small dense HDL, both of which are involved in the progression of atherosclerosis. CETP is highly expressed in fasting and postprandial hypertriglyceridemic states, and a reduction in CETP activity is associated with an increase in HDL-cholesterol levels, therefore CETP is considered as a good candidate target for drug therapy for cardiovascular risk reduction. However, the relationship between reduced CETP function and atherosclerosis is complex and a phase 3 trial with the CETP inhibitor Torcetrapib was closed prematurely due to an unexpected increase in cardiovascular events. Torcetrapib administration was associated with an 'off target' increase in blood pressure. It is currently unclear whether this effect is a class effect of the CETP inhibitors and other studies with CETP inhibitors are ongoing (142).

1.8.37 Cardiovascular outcomes after reducing triglycerides in type 2 diabetes

The Veterans Affairs HDL Intervention Trial (VA-HIT) (149) showed that fibrate therapy with gemfibrozil reduced the rate of coronary heart disease events in patients with type 2 diabetes. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial did not show the same benefit, although post hoc analysis of data from the FIELD study suggested a benefit for patients with both elevated triglyceride concentrations and low HDL cholesterol concentrations. (150) The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study examined combination treatment with a fibrate (fenofibrate) and a statin (simvastatin) compared with treatment with a

statin alone in high-risk patients with type 2 diabetes (151). This study failed to show a reduction in cardiovascular events in the combination therapy arm compared to the statin monotherapy arm. The ACCORD study subgroup analysis showed an advantage in patients with the highest TAG concentration at the start of the study (>2.3mmol/l).

Statins, which have variable TAG lowering effect have been shown in a number of trials to reduce cardiovascular events in patients both with and without type 2 diabetes, although this principally through lowering of LDL-cholesterol and not due to changes in TAG metabolism (64).

1.8.38 Weight loss medication and postprandial lipids

Orlistat (a pancreatic lipase inhibitor) has a favourable effect on postprandial triglyceride metabolism (122). Sibutramine (an inhibitor of noradrenaline, serotonin and dopamine reuptake) increases satiety and produces weight loss. There is no postprandial triglyceride data currently available for sibutramine, although fasting profiles have been shown to improve in obese patients with type 2 diabetes (152). Sibutramine has recently been removed from the market due to possible increased cardiovascular risk in the Sibutramine Cardiovascular Outcome Trial (SCOUT) (153).

1.8.39 Long-term outcomes of weight loss treatment in type 2 diabetes

Several studies have shown that in the short term weight loss improves metabolic risk factors in patients with type 2 diabetes. ‘Anti-obesity’ drug treatment eg with orlistat leads to improvement in glycaemic control and lower incidence of new onset type 2 diabetes (154). Bariatric surgery is also effective in improving glycaemic control, in the Swedish Obesity Study (SOS) 67% of patients had remission of type 2 diabetes at 10 years (155). The mechanism of improvement in metabolic control following bariatric surgery is still under investigation. There are acute improvements in metabolic status immediately following surgery and before weight loss occurs, which may be secondary to changes in gut hormone status, this has recently been

reviewed (156). Patients with type 2 diabetes have been shown to maintain sustained improvement of metabolic control by maintaining weight loss (155).

The benefits of weight loss on longer-term morbidity and mortality in type 2 diabetes remain unknown. The National Institute for Health (NIH) funded LookAHEAD trial 1-year data shows that intensive lifestyle intervention resulted in clinically significant weight loss in people with type 2 diabetes. This was associated with improved diabetes control and CVD risk factors. Continued intervention and follow-up in LookAHEAD and its sub-studies should show whether these changes are maintained and will reduce CVD outcomes (55).

A cautionary note regarding weight loss treatments is the recent removal of the marketing authorisation for the appetite suppressant sibutramine due to excess cardiovascular morbidity despite weight loss in the post-marketing SCOUT trial (157;158). This illustrates that weight loss does not confer outcome advantages in all cases and that the mechanism of weight loss is important, not just the weight loss itself. Also, some drugs have 'off target' effects which may increase adverse effects such as cardiovascular disease.

1.8.40 Postprandial dyslipidaemia and the pathogenesis of type 2 diabetes

It has been suggested that elevated NEFA in the fasting and postprandial state may contribute to the pathogenesis of insulin resistance, beta cell failure and type 2 diabetes (159-162). Possible mechanisms leading to insulin resistance have been reviewed by Savage et al. (163). More than 40 years ago Randle and co-workers (162) suggested that fatty acids compete with glucose for metabolism in muscle. Randle proposed that in people with type 2 diabetes, increased fatty acid availability to the tissues through elevated circulating NEFA causes impaired whole body glucose uptake and oxidation. He described a series of studies, which demonstrate that the oxidation of fatty acids in muscle reduces the uptake and oxidation of glucose. He suggested that this was by inhibition of key enzymes in the glycolytic pathway by elevated concentrations of acetyl CoA and citrate and accumulation of glucose-6-phosphate. This mechanism has now been challenged by results of other

studies where NEFA concentrations were held at high or low levels for 5 hours during hyperinsulinaemic-euglycaemic clamps. In these studies, although skeletal muscle insulin sensitivity was reduced by high NEFA concentrations, the intracellular glucose-6-phosphate concentration was decreased not increased as suggested by Randle (164). Subsequent lipid infusion studies have shown that in skeletal muscle, glucose transport is the rate controlling step due to reduction in insulin receptor substrate 1-associated phosphoinositol 3-kinase activity (165). Other studies (166) suggest that competition also influences substrate metabolism in the liver where elevated NEFA switch liver metabolism to glucose production rather than glucose oxidation.

It remains unclear whether the relationship between elevated NEFA and insulin resistance is cause or effect. Bickerton et al. found no difference in fasting or postprandial NEFA concentrations between insulin resistant and insulin sensitive men in either their cohort study of 636 men or their metabolic study of 20 men. In fact the insulin resistant men had lower NEFA concentrations, and a negative correlation between fasting insulin and fasting NEFA concentrations (118). It may be that elevated NEFA concentrations occur only when there is beta cell failure and pancreatic insulin production begins to fall.

1.8.41 Current treatment priorities for patients with type 2 diabetes

The focus of treatment for type 2 diabetes has historically been primarily glycaemic (blood glucose) control, and also control of specific cardiovascular risk factors including cholesterol, blood pressure and TAG. The reason for this is that there is a good body of evidence from the Diabetes Control and Complications Trial (DCCT) and UKPDS showing that tight blood glucose control reduces the risk of microvacular complications of diabetes (167;168) and to a lesser extent the macrovascular complications of diabetes, although the latter is more controversial (169). The approach to weight loss management in type 2 diabetes has tended to be less well structured in the UK and less well supported by Primary Care Trusts (PCTs), with patchy provision of specialised weight management clinics and access to bariatric surgery. This is partly because there is no long term outcome data

regarding weight loss in patients with diabetes. Clinicians are guided by recommendations from NICE which also consider health economic factors. Currently use of some, especially newer, medications is often restricted by NICE after their economic evaluation of the medication. The restrictions may depend on the age and BMI of the patient (170). For example the GLP-1 agonists such as exenatide and liraglutide are restricted for use in most patients with diabetes who have a BMI of >35kg/m^2. These restrictions are not based on long term outcome data as this is not yet available and therefore may be amended as new evidence of efficacy is published.

Until further evidence is available, clinicians have to decide where best to channel their energy in patients with type 2 diabetes, on weight reduction or glycaemic control and control of other cardiovascular risk factors such as LDL-cholesterol. Intuitively, it would be best to concentrate on all of the above, but weight reduction is difficult to achieve within the setting of a traditional diabetes clinic. Specialist weight management services including bariatric surgery are more successful (155). However, provision of specialist weight loss services vary across regions of the UK and diabetes and obesity services are not often combined although this is gradually changing. Realistically however, it is unlikely that all obese patients with type 2 diabetes will be offered bariatric surgery due to the financial constraints facing the National Health Service (NHS).

In the future, treatment for patients with type 2 diabetes may be more tailored to the individual patient's phenotype, genotype and metabolism.

1.8.42 Aims of study

The current assumption in the management of patients with type 2 diabetes is that obesity (measured by BMI) is unhealthy and that patients should be encouraged to lose weight. It is probably also assumed by most clinicians that obese patients with type 2 diabetes have a worse prognosis and worse metabolic risk factors (no evidence for this –personal assumption). Efforts to encourage a weight reducing diet are generally greater in obese patients with diabetes and some medical therapy such

as GLP-1 agonists are restricted for use in patients with diabetes with a BMI >35kg/m^2, but there is no current evidence that the long term cardiovascular outcomes with strict dietary intervention and/or GLP-1 agonists in obese type 2 diabetes patients are better than outcomes in lean type 2 diabetes patients. The contribution of obesity or body fat distribution to fasting and postprandial triglyceride metabolism in patients with type 2 diabetes is currently unknown. There are inconsistencies in the literature regarding the contribution of obesity to circulating fatty acid concentrations in the presence and absence of type 2 diabetes. The aim of this study is to describe the relationship between BMI, waist circumference and percentage body fat with triglyceride metabolism in participants with and without type 2 diabetes mellitus, by examining exogenous and endogenous triglyceride metabolism following a mixed meal containing a labelled stable isotope (1,1,1 ^{13}C tripalmitin).

This study is the first to use ^{13}C stable isotope methodology to investigate fasting and postprandial triglyceride and glucose metabolism in volunteers of varying BMI with and without type 2 diabetes. This methodology helps distinguish between exogenous and endogenous fat metabolism. The study has a relatively large number of participants (45 participants with diabetes and 45 control participants), and benefits from having a healthy reference population.

If the methodology is found to be useful, then further studies could be performed using therapeutic interventions to further explore the mechanisms underlying postprandial dyslipidaemia and perhaps to help clinicians better tailor treatment to individual patients.

Chapter 2 Study design and methods

2.1 Subject selection

45 participants with type 2 diabetes and 45 participants without diabetes with a range of body mass indices (BMI) between 18-50 kg/m^2 were recruited following local Ethics Committee approval.

2.1.1 Recruitment of participants

The aim of the recruitment strategy was to recruit participants over a wide range of BMI, with the overall groups of control participants and patients with type 2 diabetes being matched for mean BMI. It was also proposed that the diabetes and control groups would be matched for gender. The participants with diabetes were recruited in person and by invitation letter from the diabetes clinic at the Royal South Hants Hospital and by advertisements in the local press. Participants without diabetes were recruited from a large cohort of healthy volunteers who had previously participated in a study to determine reference ranges for DXA scanning by a postal invite letter and also by advertisements in the local press.

2.1.2 Inclusion and exclusion criteria

Participants were included if they were Caucasian, aged between 18-75 years, and were generally healthy and self caring and able to provide informed consent to participate in the study. Duration of diabetes, use of oral hypoglycaemic therapy or glycaemic control were not used as inclusion or exclusion criteria. All participants with diabetes were asked if they would be willing to omit their oral hypoglycaemic medication on the morning of the study. Although it is recognised that the duration of action of some oral hypoglycaemic medication (for example PPAR gamma agonists) is longer than 12 hours, it was felt that withdrawal of oral hypoglycaemic medication for a longer period was not in the best interest of the participants and would not be ethical. Also it was felt that uncontrolled glycaemia would have an adverse effect on triglyceride metabolism.

Potential participants were excluded if they had end-stage renal or liver impairment, or other serious, life-threatening co-morbidities such as advanced cancer or if they were acutely unwell or had very poor mobility. Potential participants were also

excluded if they were non-Caucasian, were aged over 75 years, or if they were unable to give informed consent. Potential participants were excluded if they were taking insulin or lipid lowering therapy, but there were no restrictions on oral hypoglycaemic agents or other medication.

2.2 Study day procedures

The study was performed in the Wellcome Trust Clinical Research Facility (WTCRF) at Southampton General Hospital. After informed consent was obtained the volunteers were entered into the study. For a week prior to the study day, the volunteers were asked to avoid foods naturally enriched with ^{13}C for example maize products, pineapple and cane sugar as this may have made measures of study meal derived ^{13}C inaccurate. They were also advised to avoid alcohol and strenuous exercise for two days prior to the study day as these factors can affect triglyceride metabolism. They were provided with a standard meal the evening prior to admission to the WTCRF in a fasting state the following morning. The evening meal consisted of chicken pasta bake, mixed salad and lemon cheesecake and a sugar free lemonade drink. The evening meal was not standardised to the bodyweight of the subjects. The volunteers were asked not to take any prescribed oral hypoglycaemic medication on the morning of the study and to take any other prescribed medication with a sip of water. On the morning of the study the volunteers arrived fasting at 08.00am and were asked to sit quietly and relax before the start of the study.

2.2.1 Anthropometric measures

Anthropometric measures were all performed by myself or Dr Masding using a standardised procedure. Height was measured in centimetres using a calibrated stadiometer and weight in kilograms using Seca alpha digital scales. Percentage (%) body fat was measured using bioelectrical impedance (Bodystat 1500, Isle of Man, UK). This is a lightweight, hand-held, battery operated Bioimpedance Analyser which is easy to use and requires no specialist skills. It is a non-invasive device. The Bodystat 1500 works by passing a battery generated signal through the whole body and measures the bioelectrical impedance at a fixed frequency of 50 kHz. The model used had two main cable leads and each lead has two crocodile/alligator clips. These

clips were attached to tabs on the sticky electrodes which were attached on the skin of the participant's right hand and foot. The subject's gender, age, height and weight were then entered into the device. A complete body composition analysis was displayed on the screen within three seconds with readings for percentage body fat, lean body mass and total body water.

Waist circumference in centimetres was determined with the participant wearing their underwear at the mid-point between the bottom rib and the anterior superior iliac crest using a material tape measure. Hip circumference was measured at the widest point around the pelvis of the participant. These measurements were difficult in some of the participants at the upper extremes of BMI. Testing for precision, accuracy and inter-observer error of these measurements were not made.

2.2.2 Indirect calorimetry

The participants underwent indirect calorimetry using a flow-through ventilated canopy system to measure resting energy expenditure and substrate oxidation (Gas Exchange Monitor, Europa Scientific, Crewe, UK). This was performed after a period of 30 minutes supine rest, before and then hourly after consuming the study meal.

2.2.3 Breath specimen collection

A baseline specimen of expired breath was collected using the Quintron (EF Brewer, Wisconsin, USA) breath sampling system. The participants were asked to fill the whole collection bag by exhaling into the bag until it was full. This was a simple procedure and all participants were instructed how to do this by myself or Dr Masding so that they could perform this procedure indecently at home that evening and the following morning for the final breath collections. The participants were provided with written instructions of the times that the samples were required (8. 14, 14 and 24 hours after consumption of the study meal).

2.2.4 Intravenous access

A cannula (B-D Insyte-W, 18 gauge, Becton Dickinson, UK) was placed in a forearm vein by me or Dr Masding and a baseline blood sample was taken and then

samples were taken every 30 minutes for the first three hours and then hourly until 6 hours thereafter.

2.3 Preparation of study meal and emulsion

The volunteers received a lipid:glucose:casein emulsion containing 700 mg of a stable isotope (1,1,1- ^{13} C tripalmitin 99 atom percent excess; Masstrace, Woburn, MA) followed by a standardised mixed meal (Rice Krispies, full fat milk and a cheese sandwich). The meal provided an overall total of 45 g lipid, 93 g carbohydrate and 33 g protein (3,720-kJ) (Figure 2.1). The emulsion was made fresh before consumption. The glucose powder, cane sugar and casein powder were dissolved in hot water. The 1,1,1-^{13}C-tripalmitin was melted with the double cream, olive oil and sunflower oil. The constituents were then blended together in a beaker suspended in hot water for 5 minutes, and Nesquik^{TM} powder was added. The emulsion was blended for a further 5 minutes, and then served to the participants who were asked to consume it immediately prior to the rest of the studymeal. The study meal was carefully prepared in a nutrition kitchen with all ingredients weighed and measured carefully by myself or Dr Masding. The participants were encouraged to consume all of the emulsion drink and the whole study meal.

Figure 2-1: Study meal and emulsion

2.3.1 Post meal study procedures

During the study the volunteers were asked to rest and only water was consumed for the remainder of the study period. Venous blood samples were collected at half hourly intervals for the first three hours and then at hourly intervals until 6 hours after ingestion of the test meal. Samples of expired breath were taken and indirect calorimetry was performed at hourly intervals for 6 hours. After this time the volunteers were provided with an optional non-standardised meal (pizza and chips) and a drink of tea or coffee, and were then discharged home. The volunteers then continued to collect expired breath samples at home at 8, 10, 14 and 24 hours after consumption of the test meal.

2.4 Sample analysis

The blood samples were put into heparinised blood tubes. These were centrifuged for 15 minutes in a Beckman GS-15R centrifuge at 2000 rpm and 4^{o}C. Plasma was aliquoted after centrifugation and these aliquots were immediately frozen at -20 ^{o}C. If samples were to be stored for more than 28 days then they were moved to a -70 ^{o}C freezer.

2.4.1 Plasma glucose and insulin analysis

Samples for plasma glucose and insulin analysis were taken to the Chemical Pathology Laboratory, Southampton General Hospital. Plasma glucose concentration was determined using an automated glucose analyser (AU600, Olympic Diagnostics, Southall, UK). Plasma insulin concentration was measured using an automated enzyme linked immunosorbent assay (ES700:Roche Diagnostics, Lewes, UK).

2.4.2 Plasma triglyceride and non-esterified fatty acid (NEFA) analysis

The concentrations of plasma triglyceride (TAG) and non-esterified fatty acids (NEFA) were determined at each timepoint. Plasma TAG and NEFA concentrations

were calculated from the gas chromatography-combustion isotope ratio mass spectrometry (GC-IRMS) chromatograms using the peak area for the fatty acids and standards within each triglyceride and NEFA fraction. The plasma concentration of TAG and NEFA over the 6 hour study period were then plotted and the area under the curve (AUC) and incremental area (INC AUC) under the curve was calculated using the trapezoidal method using GraphPad PRISM 3 software.

2.4.3 HbA_{1c}

Blood samples to measure HbA_{1c} were not taken routinely as part of the study measurements, but were retrospectively obtained from the biochemistry laboratory at Southampton General Hospital where an HbA_{1c} measure was available within the 2 months prior to the study participation. This was available only in the participants with diabetes.

2.4.4 Measurement of ^{13}C enrichment in the breath and plasma lipid fraction

Isotopes are atoms of the same element that have the same number of protons and electrons but different numbers of neutrons. This means that the various isotopes have similar charges but different masses. The superscript number to the left of the element designation indicates the number of protons plus neutrons in the isotope. ^{13}C (Carbon -13) is a natural, stable isotope of carbon and makes up about 1.1% of all natural carbon on Earth. Compounds enriched in ^{13}C can be used in human metabolism studies as these compounds are safe to ingest as they are non-radioactive. The ingestion of a ^{13}C-enriched food can be used to provide a marker of an exogenously derived metabolite. Mass spectrometry can be used to measure the concentration of ^{13}C-labelled metabolite compared to the predominant naturally occurring ^{12}C (Carbon-12) and can then be assumed to be derived from an exogenous source. In this study 1,1,1-^{13}C-tripalmitin was used to provide a marker of exogenously derived triglyceride, which could then be measured in different lipid fractions (ie TAG and NEFA) and excreted on the breath after oxidation as $^{13}CO_2$. Other studies have used algal lipid which is naturally rich in ^{13}C. The ratio of ^{13}C to ^{12}C is slightly higher in plants employing C4 carbon fixation than in plants employing C3 carbon fixation and so foods naturally enriched in ^{13}C such as

pineapple and maize should be avoided prior or during studies using ^{13}C as a marker of exogenously derived metabolites eg triglycerides.

In this study lipid extraction, solid phase extraction and methylation were used to separate and purify the lipid (triglyceride and NEFA) fractions from the plasma samples, prior to analysis using GC-C-IRMS. TAG and NEFA concentrations were then calculated from the fatty acid composition of the plasma (171). GC-C-IRMS was used to determine ^{13}C enrichment of in palmitic acid fatty acid methyl esters isolated from plasma TAG and NEFA and continuous flow-isotope ratio mass spectrometry was used to measure ^{13}C–enrichment in CO_2 in the breath samples (see below for more details on the methods used).

(i) Lipid extraction

Neutral lipid was extracted from the plasma sample by a modification of the method described by Folch (172). The plasma sample was thawed and recovery standards (triheptadecanoin and heneicosanoic acid, 100 μl C17:0 for TAG, 30 μl of C21:0 for NEFA) were added to 1 ml of the plasma sample. 5 ml of chloroform: methanol 2:1 solution was then added and was shaken for 15 minutes. Then 1 ml of 1 M NaCl solution was added. This mixture was centrifuged for 10 minutes at 2000 rpm, 14^{o}C. The aqueous layer was removed and discarded, and the solvent layer (containing the lipid) was retained. The remaining interfacial protein disc was re-dissolved in 1 ml 0.9% w/v NaCl, and the remaining lipid was extracted from it using the same process.

(ii) Solid phase extraction

Triglyceride and NEFA were purified by solid phase extraction. The total plasma lipid extracts were dissolved in 1 ml of chloroform and applied to an aminopropyl silica column under gravity (BondElut cartridge, Varian, USA). Residual solvent was removed under vacuum, and the column was washed twice with 1 ml chloroform under vacuum. The resulting solution was dried under nitrogen at 40^{o}c and retained for extraction of TAG. Phosphatidylcholine (PC) was eluted with chloroform: methanol 3:2 solution, and was discarded. 1 ml of methanol was then

drawn through the column under vacuum and discarded in order to remove any residual phospholipid. NEFA was eluted with 2mls of chloroform: methanol: acetic acid 100:2:2 solution under vacuum.

The solution from the first stage of the solid phase extraction process was then dissolved in 1 ml of hexane and a fresh aminopropyl silica column was preconditioned by 4 washes with 1 ml of hexane, care was taken not to let the column dry out. Cholesteryl esters (CE) were eluted from the solution and discarded. TAG was eluted with 1 ml washes with hexane: chloroform: ethylacetate 100:5:5 solution under vacuum. This procedure was then repeated. Solvent fractions containing the eluted TAG and NEFA were dried under nitrogen at 40^{o}C.

(iii) Methylation

1 ml of toluene was added to the TAG and NEFA solvent fractions. Then 2 ml of 2% sulphuric acid in methanol solution was added. The resulting mixture was heated overnight for 18 hours at 50^{o}C. After cooling, the mixture was neutralised with 2 ml of a solution of 0.25 M $KHCO_3$ and 0.5 M K_2CO_3. 2 ml of hexane was added. The resulting mixture was shaken for 15 minutes and then centrifuged for 10 minutes at 2000 rpm to separate organic and aqueous phases. The organic solvent was transferred to vials suitable for a gas chromatography autosampler, then dried under nitrogen at 50^{o}C and dissolved in dry hexane. The fractions were washed 4 times with hexane. The reference standard C23:0 methyl ester (1 mg/ml) was added, in the same amount as the recovery standard for TAG (100 μl C17:0) and NEFA (30 μl C21:0). The lipid fractions were then frozen at -20^{o}C.

(iii) GC-C-IRMS

GC-C-IRMS was used to determine ^{13}C enrichment of in palmitic acid fatty acid methyl esters isolated from plasma TAG and NEFA. Fatty acid methyl esters were resolved using Hewlett Packard 6890 gas chromatography equipped with a 50 m x 0.25 um x 0.32 mm BPX-70 fused silica capillary column (SGE Europe Limited, Milton Keynes, UK). Fatty acid methyl esters were converted to CO_2 by heating at

860^{o}C in the presence of PtCuO using an Orchid combustion interface (PDZ-Europa). The $^{13}CO_2$:$^{12}CO_2$ ratio was calculated by a 20/20 Stable Isotope Analyser (PDZ-Europa). Plasma TAG and NEFA concentrations were calculated from the GC-C-IRMS chromatograms using the peak area for the fatty acids and standards within each TAG and NEFA fraction respectively. The results were expressed as µg ^{13}C-palmitic acid/ml plasma.

2.4.5 Measurement of ^{13}C enrichment in breath samples

The proportion of ^{13}C label excreted on the breath as a proportion of the total dose of administered ^{13}C allowed determination of the extent of oxidation of fat in the test meal (exogenous fat) over the study period.

^{13}C –enrichment in the breath samples was determined by continuous flow-isotope ratio mass spectrometry using a 20-20 stable isotope analyser which has a gas/solid/liquid interface (Europa Scientific Ltd, Crewe, UK)(171). The enrichment of ^{13}C in each sample was calculated from the increase in the ratio of $^{13}CO_2$ to $^{12}CO_2$ compared with that obtained from a working reference standard (5% $CO_{2)}$. The proportion of ^{13}C-labelled palmitic acid excreted in the breath as $^{13}CO_2$ was expressed as a percent of the administered ^{13}C-label per hour and as the cumulative percentage dose excreted over 6 hours and 24 hours.

2.5 Energy expenditure, fat and carbohydrate oxidation

The amount of energy used for the basic requirements for life, such as breathing and circulation of blood and is known as the basal metabolic rate (BMR) (173). The BMR is usually measured after an overnight fast, with the subject awake and comfortable at a set temperature. Energy expenditure is increased by exercise and also by feeding. This latter process is known as diet-induced thermogenesis, and represents the energy requirements for the activity of the gastrointestinal tract and the metabolic requirements for substrate storage (173).

Energy for these processes is provided by the oxidation of fuels (fat, carbohydrate and protein) and requires oxygen (O_2), and produces carbon dioxide (CO_2) and water. Heat is also released (174). The energy expenditure of an individual can be assessed through the measurement of heat loss and gas exchange.

Energy expenditure, and net fat and carbohydrate oxidation at each timepoint was calculated using data obtained from indirect calorimetry (175).

2.6 Indirect calorimetry

Indirect calorimetry measures the heat released by oxidative processes (176) and can be used to assess changes in energy expenditure and substrate oxidation in the fasting state and after a meal. It is based on the principle that for each litre of O_2 consumed, there should be a known amount of heat released by oxidation (174). By measuring the amount of oxygen consumed by the subject, a measurement of energy expenditure can be made as well as a determination of the proportion of different nutrients being oxidized. To measure energy expenditure, measurements of inspired and expired O_2 content (FiO_2 and FeO_2 respectively) and the inspired and expired CO_2 content ($FiCO_2$ and $FeCO_2$) are made. By subtracting FeO_2 from FiO_2, the amount of O_2 extracted can be calculated (VO_2).

In this study, indirect calorimetry was performed using a flow-through ventilated canopy system. A clear plastic canopy was placed over the head of the subject, air was drawn through this by a pump, the expired air was collected and the O_2 and CO_2 content was measured by on-stream analysers (173) (Figure 2.2).

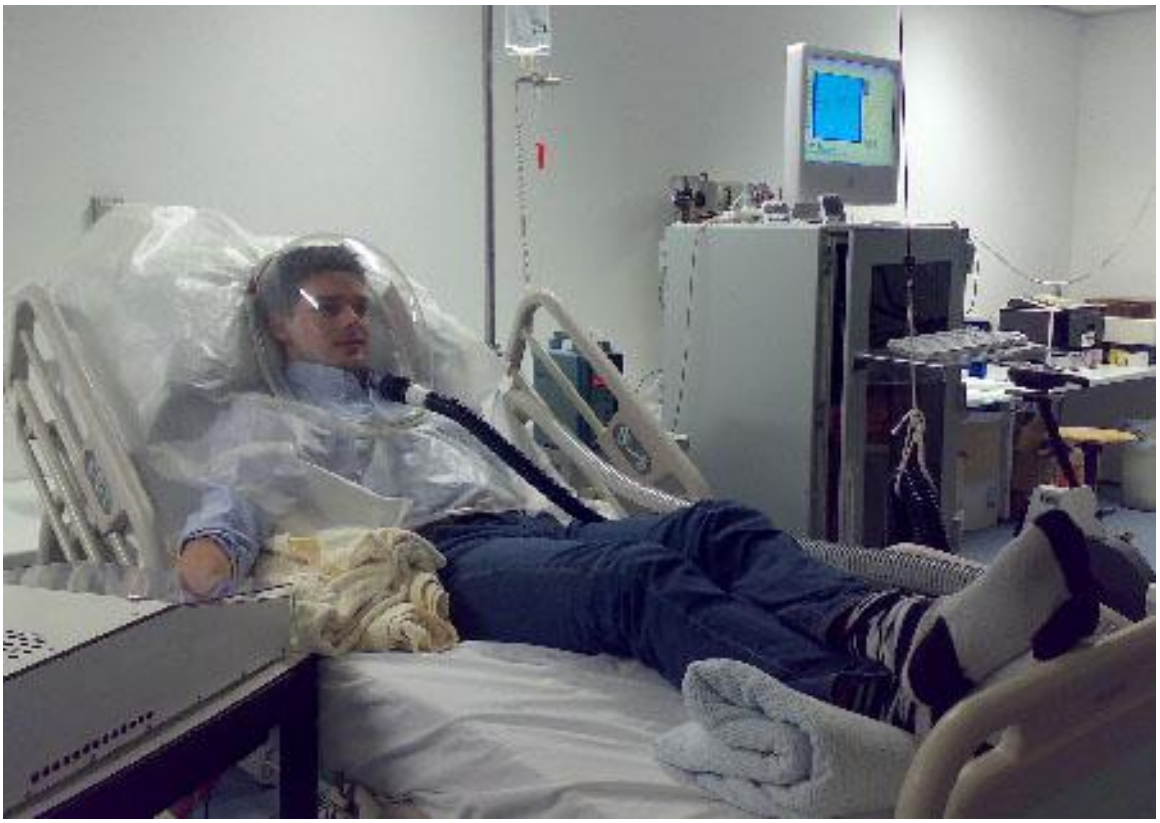


Figure 2-2: Indirect calorimetry

(Picture courtesy of MuscleMetabolism Maastricht (Permission obtained 20.08.09))

2.6.1 Calibration of system

Indirect calorimetry results can be affected by errors in the measurement of VO_2 and VCO_2. Small errors affect the calculation of energy expenditure therefore indirect calorimetry equipment must be constantly calibrated. A known amount of a mixture of inert gases is pumped into the system and the amount coming out of the other end is measured. This allows adjustment for gas that may be lost in the system, and to adjust VO_2 and VCO_2 measurements for these errors (176).

2.6.2 Accounting for different substrates in calculating energy expenditure

Different substrates have different calorific values per unit mass. The oxidation of 1 g of carbohydrate liberates 15.6 kJ or 3.7 kcal, whilst oxidizing 1g of fat liberates 39.4 kJ or 9.4 kcal (173). Protein may also be oxidized, causing the release of nitrogen, liberating 20.1 kJ or 4.8 kcal of energy per gram. The respiratory exchange ratio (RER) is used to decide which substrate is being oxidized – this is calculated by dividing VCO_2 by VO_2 (173). The human body does not oxidise just one substrate at a time, so the RER is used to estimate how much energy is being expended at that time. Thus equations have been calculated to adjust for RER, such as the widely used Weir formula (177).

Energy expenditure (kJ/min) = $3.941(VO_2) + 1.106(VCO_2) – 2.17$(urinary nitrogen excretion)

2.6.3 Measuring substrate oxidation

Indirect calorimetry is best suited to assessing net substrate oxidation, i.e. changes in substrate oxidation from a baseline measurement, rather than absolute substrate oxidation, which can be measured using other techniques, such as stable isotope infusion methods, or the double-labelled water techniques (173). Stoichiometric principles are used to calculate from the gas exchange measurements obtained by the indirect calorimeter how much carbohydrate and fat are being oxidised. This is based upon the amount of O_2 being consumed and CO_2 being produced for each of the major energy substrates, fat, carbohydrate and protein. The mostly widely used equations for assessing relative substrate oxidation are those described by Frayn (175).

Fat oxidation (g/min) = $1.67\ VO_2 – 1.67\ VCO_2 – 1.92$ n (where n denotes the urinary excretion of nitrogen)

Carbohydrate oxidation (g/min) = $4.55\ VCO_2 – 3.21\ VO_2 – 2.87$ n

The usual amount in g/min of urinary nitrogen from protein oxidation is minimal, so that errors in measurement will only have minimal effects on the accuracy of estimates of carbohydrate and fat oxidation (176). Therefore, a urinary nitrogen excretion rate of 0.01 g/min was assumed for this study.

2.6.4 Respiratory quotient (RQ)

Respiratory Quotient (RQ) is the ratio of CO_2 production to O_2 consumption for a macronutrient type. The RQ for 1 mole of glucose is 1.0 and for 1 mole of TAG is 0.70, ie carbohydrate oxidation generates more energy than fat oxidation.

2.7 Oxidation of exogenous fat

The oxidation of dietary-derived lipid can be specifically assessed by measuring the levels of label excreted in the breath as CO_2 production as a by-product of lipid oxidation. By using ^{13}C-labelled fatty acids, the amount of $^{13}CO_2$ in the breath facilitates calculation of how much dietary lipid has been oxidized. The proportion of ^{13}C label excreted on the breath as a proportion of the total dose of administered ^{13}C allows determination of the extent of oxidation of fat in the test meal (exogenous fat) over the study period.

2.8 Measurement of insulin resistance and beta cell function

2.8.1 Homeostatic model assessment

The homeostatic model assessment (HOMA) is used to give an estimate of insulin sensitivity and β-cell function in the fasting state, using fasting plasma insulin and glucose concentrations (178). The relationship between glucose and insulin in the fasting state reflects the balance between hepatic glucose output and pancreatic insulin secretion and the feedback loop between the liver and β-cells. The predictions used in the model were derived from experimental data in humans and animals. The model does not distinguish between hepatic and peripheral insulin sensitivity. It is

likely to represent principally liver insulin resistance as it is determined in the fasting state. The liver is principally responsible for glucose homeostasis in the fasting state, whereas skeletal muscle insulin resistance is more likely to affect glucose concentrations in the postprandial state. This study used the original HOMA model which contains a mathematical approximation of the original non-linear solution for the calculation of insulin resistance and β-cell function (see below). HOMA2, an updated computer model is now also sometimes used (179).

It is important to note that β-cell function from the HOMA model must always be viewed in the context of the insulin resistance result. For example if a subject is highly insulin sensitive (ie low insulin resistance), β-cell function may appear be reduced, not because the beta cells are failing, but because less insulin production is required due to the high insulin sensitivity.

Equations

Insulin resistance

HOMA-(IR) = (fasting plasma insulin (mU/l) x fasting plasma glucose (mmol/l))/22.5
(Normal IR is defined as 1.0)

2.8.2 Beta cell function

HOMA-(%B) = (20 × fasting plasma insulin(mU/l))/(fasting plasma glucose(mmol/l) − 3.5)
(Normal β-cell function is defined as 100%)

2.8.3 First phase insulin response

The first phase insulin response is the initial rise in plasma insulin concentration that is detected after intravenous or oral glucose administration. This is also sometimes known as the ‘early insulin response’ or the ‘insulinogenic index’. One of the

defining features of type 2 diabetes is a gradual reduction in beta-cell function and beta cell volume (180). In type 2 diabetes the first phase insulin response to both intravenous (IV) and oral glucose is reduced (181). Both oral and IV techniques have been used as a surrogate measure of beta cell function. In studies using the oral glucose tolerance test it has been shown that an early deficiency in insulin release is associated with a greater rise in plasma glucose later in the oral glucose tolerance test, which then results in increased plasma insulin concentrations at later time points.

In this study we have not used the standard experimental methods for measuring the first phase insulin response which is to use an intravenous glucose challenge or an oral glucose challenge, but have measured the glucose and insulin concentrations at 30 minutes after ingestion of the mixed meal and named this the 'first phase insulin response'. This is also a surrogate measure for beta cell function in the postprandial state.

2.9 Overall metabolic model and data analysis

The glucose, TAG and NEFA excursions (both labelled and total) following the test meal have been described using the area under the curve (AUC) using the trapezoidal method using GraphPad PRISM 3 software (182) for the 6 hour study period.

Prolonged retention of ^{13}C labelled palmitic acid in TAG fraction in the circulation (^{13}C-PA TAG) was assumed to reflect impaired chylomicron clearance from the circulation by peripheral tissues via hydrolysis to NEFA by lipoprotein lipase. Elevation of ^{13}C-PA in the NEFA fraction reflected hydrolysis of chylomicron-TAG and impaired entrapment of the resultant fatty acids by peripheral tissues. The amount of recovery of ^{13}C in breath as $^{13}CO_2$ reflects oxidation of dietary lipid by peripheral tissues.

2.10 Statistical methods

2.10.1 Power calculations

A previous study performed by Dr Masding, myself and colleagues (90), used the same protocol to examine differences in postprandial ^{13}C-PA AUC TAG and TAG AUC in pre and post-menopausal women with and without type 2 diabetes. In this study there were eight participants per group. Assuming an alpha value of 5% 8 participants in each group gave a power of 97.3% for ^{13}C-PA AUC TAG and a power of 99.6% for AUC TAG. The data used in these calculations are summarised in Table 2.1 below. The results of this previous study were used to estimate the sample size required to reach a power of above 90% for the primary outcome variable (^{13}C-PA TAG AUC) and a secondary outcome variable (AUC TAG) in the current study. Assumptions were made that the group size to reach a power of 90% would need to be larger than the group size of 6 shown above, as the current study included a combination of male and female participants and included participants with a wide range of BMI which was likely to increase the variance of the outcome variables. Also, duration of diabetes was not recorded and this may have had an effect on triglyceride metabolism, this may also have increased the variance of the outcome variables. As the groups were to be split into quartiles of BMI for comparison of different quartiles it was assumed that a sample size of approximately 8 participants per BMI quartile (ie 32 participants with diabetes and 32 control participants) would provide sufficient power. We successfully recruited more than this number of participants (45 in each group), but decided to include all participants in the study to further increase the power of the study. No adjustments for multiple comparisons were made in this study. This was because this is primarily an exploratory study, however this needs to be taken into consideration when the study results are significant.

Variable	Mean (sd) in DM participants	Mean (sd) in CON participants	Power (alpha 5%)	
			6 participants per group	8 participants per group
^{13}C-PA in TAG AUC (ug/ml/6h)	49 (15)	25 (9)	91.9%	97.3%
TAG AUC (mmol/l/6h)	21(8)	7 (3)	98.0%	99.6%

Table 2-1: Power calculations

2.10.2 Data analysis

All data has been entered into a computerised database (SPSS v17.0). The data for each metabolic variable and each demographic and anthropometric measure was analysed to determine whether the distribution of the data was normal. This was done using a frequency histogram with a normal distribution curve fitted, and where the appearance of the histogram was equivocal, calculating the Kolmogorov-Smirnov statistic, where a non-significant result (>0.05) suggests normality. Normally distributed data was described using mean and standard deviation. Where the distribution was not normally distributed, the data was described using median and interquartile values.

2.10.3 Comparing differences between groups

Where data was normally distributed, non paired t-tests were used to detect differences between groups. Where data was not normally distributed, the Mann-Whitney U test was used to detect differences between groups.

2.10.4 Significance of findings

Throughout the study, statistical significance is assumed where $p < 0.05$. Adjustment such as the Bonferroni correction was not made for repeated analyses. This was because the study was exploratory in nature and it was felt that using the Bonferroni correction may cause important physiological trends to be missed. However it must be kept in mind that the likelyhood of false positive findings ie a difference or association having being found by chance is increased because of this approach.

2.10.5 Adjusting for co-variates

Analysis of co-variance (ANCOVA) was used to adjust for the effects of body composition on the dependent variables. ANCOVA was used to examine the differences between the participants with diabetes and control groups while controlling for an additional variable, for example BMI. ANCOVA was also used to adjust postprandial values for the effect of the fasting concentration, for example the effect of fasting TAG on the area under the curve (AUC) TAG. There is no non-parametric alternative to ANCOVA, so the results in the ANCOVA tables are presented as mean (standard deviation).

2.10.6 Testing for associations between variables

Associations between variables, for example the association between increasing BMI and AUC TAG, were examined using Spearman Rank Order Correlation. This was performed in participants with diabetes and control participants separately. Spearman correlations were used in preference to Pearson correlations due to the relatively small sample size and because many of the variables were not normally distributed.

2.10.7 Testing for interactions

Multiple linear regression (adjusted ANOVA) was also used to determine any an interactions (ie difference) between the effects of adiposity on metabolic variables between participants with diabetes and control participants, for example to determine if there was any difference in the effect of BMI on postprandial AUC TAG in participants with diabetes compared to control participants.

2.10.8 Creating groups

In Chapter 5 the participants were split into groups by quartile of BMI. The quartiles were created by splitting the whole participant cohort into quartiles of BMI using the combined groups containing both the participants with diabetes and the control participants. This is a statistically valid approach as it allows the BMI cut offs to be the same in both groups so that the groups can be directly compared with each other. This 'whole group' approach has the disadvantage of producing different numbers of participants in the groups in the diabetes and control quartiles (Table 2.2).

Quartile	BMI quartiles (kg/m^2)	Controls (n) (%)	Participants with diabetes (n) (%)
1	18.0-25.3	14 (31.1)	9 (20.0)
2	25.3-29.7	12 (26.7)	10 (22.2)
3	29.71-34.2	11 (24.4)	12 (26.7)
4	34.21-49.2	8 (17.8)	14 (31.1)

Table 2-2: Distribution of participants by BMI quartile in participants with diabetes and control groups

Chapter 3 Comparisons of triglyceride metabolism, substrate oxidation and energy expenditure between participants with diabetes and control participants with adjustments for adiposity

3.1 Introduction

This chapter first describes the baseline characteristics of the study participants in the participants with diabetes and control groups respectively. The participants in the groups were intentionally recruited to have an overall similar mean body mass index (BMI), median age and gender between the diabetes and control groups. The relative estimated insulin resistance and beta cell function between the groups is then described. There is then a comparison of study meal related lipid, glucose and insulin excursions in the blood between participants with diabetes and controls before and after adjustment for BMI, waist circumference and percentage body fat. Finally there is a comparison of fasting and postprandial fat and carbohydrate oxidation between participants with diabetes and controls before and after with adjustment for BMI, waist circumference and percentage body fat. This chapter uses analysis of covariance (ANCOVA) to see if the differences in metabolism which were identified between the participants with diabetes and control groups remained after controlling for the effect of different measures of adiposity. The measures of adiposity chosen were BMI, waist circumference and % body fat as it was felt that these measures each represent different aspects of adiposity.

3.2 Baseline characteristics of participants

There was no significant difference in age, gender, BMI, height, weight, waist-to-hip ratio, percentage (%) body fat, fat mass and fat free mass between the participants with diabetes and control participants, although numerically the participants with diabetes had a higher BMI and weight. The participants with diabetes also had a greater waist circumference ($p=0.019$) (Table 3.1).

All regular medication being taken by the participants is summarised in Table 3.3 (participants with diabetes) and Table 3.4 (control participants). 20 participants with diabetes patients were taking a sulphonylurea, 19 were taking metformin and 3 were taking a glitazone. No patients were taking Glucagon like Peptide-1 (GLP-1) analogues or Dipeptidyl peptidase IV (DPPIV) inhibitors at the time of the study.

3.3 Insulin resistance and beta cell function by homeostatic model assessment analysis

The patients with diabetes were significantly more insulin resistant by homeostatic model assessment (HOMA-IR) (6.37 (3.77-7.70) vs 2.11 (1.14-3.03)), p <0.0001), and had lower beta cell function (HOMA-%beta) (35.64 (22.33-49.00) % vs 81.05 (63.20-133.08)) %, p <0.0001), than the control participants (Table 3.2).

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Number of participants	45 (28M/17F)	45 (24M/21F)	0.52†
Age (years)	58.00 (48.50-64.00)	58.00 (46.50-65.50)	0.95*
Weight (kg)	86.33±20.07	89.73±17.07	0.39
Height (m)	1.72 (1.61-1.77)	1.70 (1.63-1.75)	0.41*
BMI (kg/m^2)	29.53±5.99	31.36±5.97	0.15
Waist (cm)	100.27±14.94	107.74±13.06	0.02
Waist-to-Hip ratio	0.93±0.07	0.95±0.064	0.16
Body fat (%)	32.43±8.59	34.67±9.00	0.23
Fat mass (kg)	28.38±11.85	31.90±12.10	0.17
Lean mass (kg)	57.95±13.33	57.84±9.94	0.96

Table 3-1: Baseline characteristics of participants with diabetes and control participants

The data in Table 3.1 are shown as mean±sd, except for those marked *median (interquartile values), M=male, F=female. All statistical tests for differences between groups are non-paired t tests except for those marked * which are Mann-Whitney U tests and † which is using the chi-squared test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
HOMA-IR	2.11 (1.14-3.03)	6.37 (3.77-7.70)	<0.0001
HOMA-%B	81.05 (63.20-133.08)	35.64 (22.33-49.00)	<0.0001

Table 3-2: HOMA model calculations of insulin resistance and beta cell function in participants with diabetes vs control participants.

The data in Table 3.2 are shown as median (interquartile values). All statistical tests for differences between groups are Mann-Whitney U tests.

Participants with diabetes	Sulphonylurea	Metformin	Glitazone	ACEI	Beta blocker	Other
1	N	Metformin	N	N	N	Seroxat
2	Glibenese	Metformin	N	N	N	Thyroxine
3	Gliclazide	Metformin	N	N	Atenolol	Prozac
4	Tolbutamide	N	N	Ramipril	N	N
5	Glibenese	Metformin	N	N	N	N
6	Glipizide	Metformin	N	N	N	N
7	N	N	N	N	N	N
8	N	N	N	N	N	N
9	N	N	N	N	N	N
10	N	Metformin	N	N	Atenolol	N
11	N	N	N	N	N	N
12	N	N	N	N	N	N
13	N	N	N	N	N	N
14	N	N	N	N	N	N
15	Tolbutamide	Metformin	N	N	Atenolol	BFZ
16	N	N	N	N	N	N
17	N	Metformin	N	Ramipril	N	Prozac
18	N	N	N	N	N	N
19	Glibenclamide	N	Rosiglitazone	N	Atenolol	BFZ
20	U	U	U	U	U	U
21	N	Metformin	N	N	N	N
22	Gliclazide	N	N	N	N	N
23	Gliclazide	Metformin	N	Lisinopril	N	Diltiazem
24	Gliclazide	Metformin	N	Enalapril	N	Lansoprazole
25	N	N	N	N	N	N
26	N	N	N	N	N	N
27	Gliclazide	N	N	Losartan	N	N
28	Gliclazide	Metformin	N	N	N	N
29	Glimepiride	N	N	N	N	N
30	Gliclizide	Metformin	N	Ramipril	N	Amlodipine
31	Glibenclamide	Metformin	Rosiglitazone	N	N	N
32	Tolbutamide	Metformin	N	N	N	N
33	N	Metformin	Rosiglitazone	N	N	Allopurinol
34	N	N	N	N	N	N
35	N	N	N	N	N	N
36	Gliclazide	N	N	N	N	Omeprazole
37	N	Metformin	N	N	N	N
38	Glipizide	N	N	N	N	N
39	N	N	N	N	N	N
40	Glipizide	N	N	N	N	Aspirin
41	N	Metformin	N	U	U	U
42	N	N	N	N	N	N
43	N	N	N	N	N	N
44	Gliclazide	Metformin	N	N	N	N
45	N	N	N	N	N	N

Table 3-3: Regular medication taken by participants with diabetes

N; none of this type, U; unknown, BFZ; bendroflumethiazide:
ACEI;angiotensin converting enzyme inhibitor

Control participants	ACEI	Beta blocker	Other
1	N	N	N
2	N	N	N
3	N	N	N
4	N	N	Amitryptiline
5	N	N	N
6	N	N	N
7	N	N	N
8	N	N	N
9	N	N	N
10	N	N	N
11	N	N	Thyroxine
12	N	N	N
13	N	N	N
14	N	N	N
15	N	N	Frusemide, pyridoxine
16	N	Atenolol	N
17	N	N	N
18	N	N	Thyroxine
19	N	N	N
20	N	N	Ibuprofen, cod liver oil
21	N	N	zoton
22	N	N	N
23	Ramipril	N	BFZ, nifedipine,
24	N	N	N
25	N	N	N
26	N	N	N
27	Enalapril	Bisoprolol	Amlodipine
28	N	N	N
29	N	N	N
30	N	N	N
31	N	N	N
32	N	Atenolol	N
33	Enalapril	N	N
34	N	N	N
35	N	N	Omeprazole
36	N	Atenolol	N
37	N	N	N
38	N	N	N
39	N	N	N
40	N	N	N
41	N	Propranolol	Omeprazole
42	N	N	N
43	N	N	N
44	N	N	Lansoprazole
45	N	N	N

Table 3-4: Regular medication taken by control participants

N; none of this type; BFZ; bendroflumethiazide: ACEI;angiotensin converting enzyme inhibitor

3.4 Fasting triglycerides

Fasting triglycerides (TAG) were significantly higher in the participants with diabetes group (2.10 (1.60-2.85) vs 1.20 (0.80-2.35) mmol/l, p=0.001) (Table 3.5). There was a large variance in the results between participants. The difference between participants with diabetes and controls remained significant when adjusted for BMI (p=0.011) waist circumference (p=0.03) and percentage (%) body fat (p=0.007) (Tables 3.13 to 3.18).

3.5 Postprandial triglycerides

3.5.1 Total postprandial TAG

Following ingestion of the study meal, plasma TAG rose in both groups (Figure 3.1). The TAG area under the curve (AUC) was significantly greater in the participants with diabetes (17.34 (12.01-25.95) vs 9.00 (7.50-18.74) mmol/l, p=0.001) (Table 3.5). There was a large variance between the study participants. The difference in area under the curve (AUC) TAG between participants with diabetes and control participants remained significant when adjusted for BMI (p=0.006), waist circumference (p=0.025) and % body fat (p=0.004). (Tables 3.13 to 3.18).

3.5.2 Incremental postprandial AUC TAG

The incremental rise in postprandial AUC TAG is the AUC TAG excluding the AUC that is contributed to by the fasting TAG concentration. There was no difference in the incremental rise in AUC TAG between participants with diabetes and control participants (4.00 (1.53-7.37) vs 2.50 (1.61-4.35) mmol/l, p=0.175 (Figure 3.1, Table 3.5). There was no effect on the results after adjusting for adiposity. This suggests that the fasting TAG contributed significantly to the AUC TAG (see below).

3.5.3 Contribution of fasting TAG to postprandial AUC TAG

AUC TAG was adjusted for the effect of the fasting TAG using analysis of co-variance (ANCOVA). The difference between AUC TAG seen between the participants with diabetes and control participants was not statistically significant once the result was been adjusted for fasting TAG (p=0.572) confirming the difference is due to fasting TAG. This is likely to be due to the increased TAG pool size in the presence of fasting hypertriglyceridaemia .

3.5.4 Postprandial ^{13}C-PA labelled triglyceride

^{13}C - palmitic acid in the triglyceride fraction AUC (^{13}C–PA TAG AUC) represents the absorption and clearance of ^{13}C-PA labelled TAG within chylomicrons from the study meal, and then the reappearance of ^{13}C-PA labelled TAG in the very low density lipoprotein (VLDL) particles from the liver. ^{13}C-PA labelled TAG may also reappear in HDL and LDL lipoprotein particles in exchange for cholesterol esters secondary to reverse lipid transport by cholesterol ester transfer protein (CETP).

The ^{13}C–PA TAG rose in the postprandial period in both participants with diabetes and control groups and reached a peak at about 2.5 hours after study meal consumption in both groups. The ^{13}C-PA labelled TAG was still detectable in the plasma in both groups at the end of the sampling period at 6 hours post meal consumption (Figure 3.2).

^{13}C–PA TAG AUC was higher in the participants with diabetes group but this did not reach statistical significance (60.05 (34.40-100.59) vs 44.04 (29.43-76.43) μg/ml/6h, p=0.107). This data suggests that mean chylomicron clearance was similar in both groups overall. This result was not changed after adjusting for BMI, waist circumference or % body fat (Tables 3.13 to 3.18).

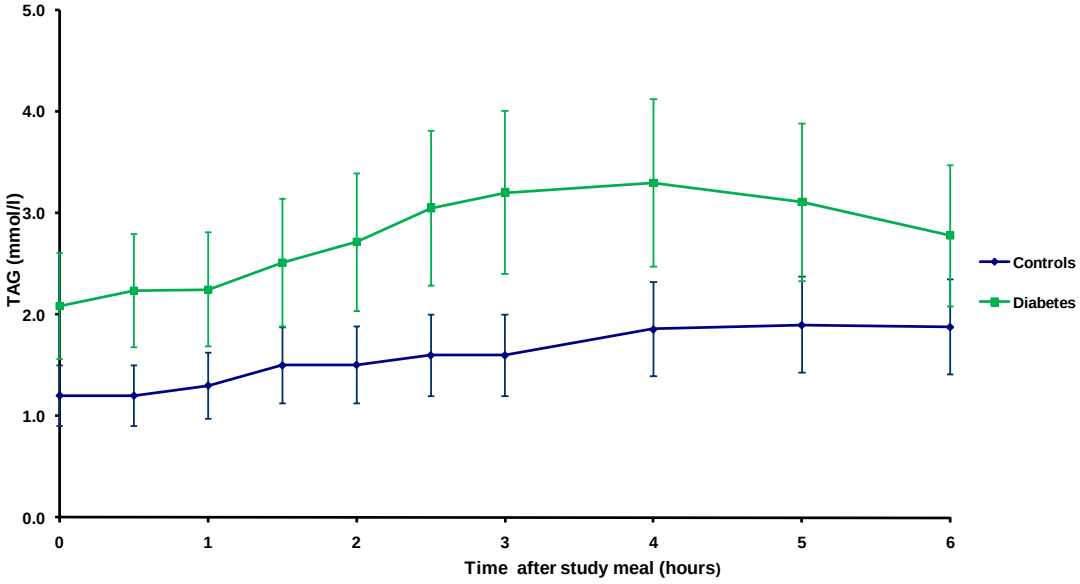


Figure 3-1: Concentration of triglyceride (TAG) in plasma before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). Fasting TAG DM vs CON, p=0.001, area under the curve (AUC) TAG DM vs CON, p=0.001, incremental (INC) AUC TAG DM vs CON, p=0.175. Statistical tests for differences between groups are Mann-Whitney U tests.

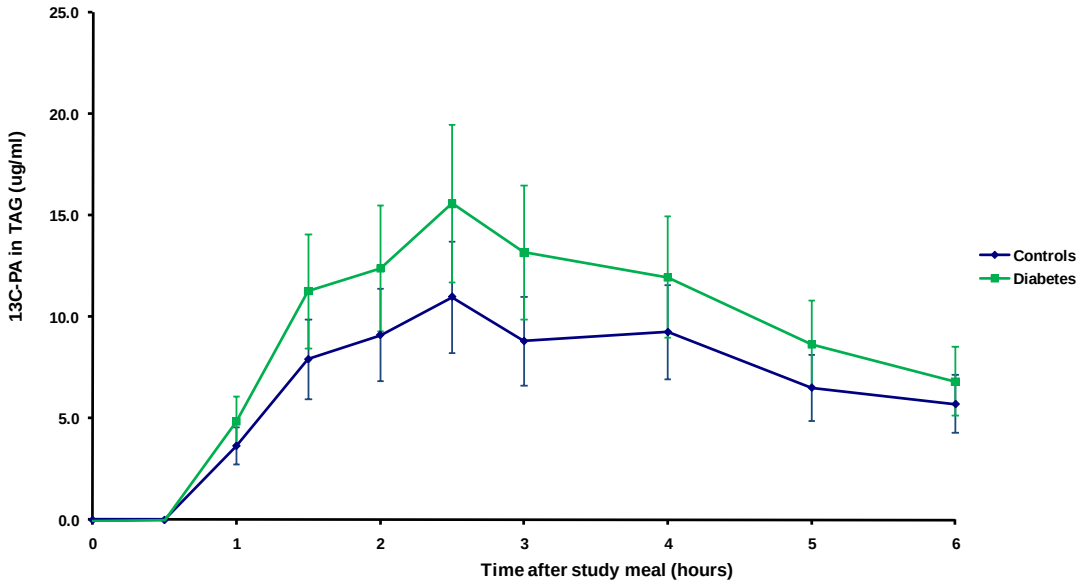


Figure 3-2: Concentration of ^{13}C-palmitic acid in plasma triglyceride (^{13}C-PA AUC TAG) before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). ^{13}C-PA AUC TAG DM vs CON, p=0.107. Statistical tests for differences between groups are Mann-Whitney U tests.

3.6 Non-esterified fatty acids

Fasting non-esterified fatty acids (NEFA) were higher in participants with diabetes than controls (178.30 (151.28-217.37) vs 126.60 (99.26-163.00) umol/l, p<0.0001)) (Table 3.5). The difference in fasting NEFA between participants with diabetes and control participants remained significant when adjusted for BMI (p = 0.001), waist circumference (p-0.002) and % body fat (p=0.001) (Tables 3.13-3.15).

Following ingestion of the study meal, plasma NEFA concentration fell rapidly in both groups (Figure 3.3). The NEFA concentration reached a nadir at about 1.5 hours post meal in the control group and at 2 hours in the participants with diabetes group and then after a 1 hour plateau rose to approximately equal concentrations in both groups by the end of the study period.

NEFA concentrations remained higher throughout the postprandial period in the participants with diabetes, and the AUC NEFA was significantly higher in the participants with diabetes group (574.90 (444.74-747.65) vs 379.32 (326.07-501.93) umol/l, p<0.0001) (Table 3.5). There was a large variance between the study participants. The difference in AUC NEFA between diabetes and control participants remained significant when adjusted for BMI (p<0.0001), waist circumference (p<0.0001) and % body fat (p<0.0001) (Tables 3.13-3.15).

The difference in AUC NEFA between the participants with diabetes and control groups remained significantly different after adjustment for fasting NEFA (p=0.002).

3.6.1 ^{13}C-PA labelled non-esterified fatty acids

^{13}C - palmitic acid in the non-esterified fatty acid fraction (^{13}C-PA NEFA) is derived from lipoprotein lipase (LPL) hydrolysis of dietary derived chylomicron ^{13}C-PA TAG providing circulating ^{13}C-PA NEFA and glycerol.

The concentration of ^{13}C-PA NEFA rose in the postprandial period in both participants with diabetes and control groups, and reached a peak at about 1.5 hours after study meal consumption in both groups (Figure 3.4). The ^{13}C-PA NEFA was still detectable in the plasma in both groups at the end of the sampling period at 6 hours post meal consumption. The ^{13}C-PA NEFA AUC was significantly higher in the participants with diabetes group (2.60 (1.95-3.26) vs 2.14 (1.40-2.43))μg/ml/6h, p=0.003) (Table 3.5). The difference in ^{13}C-PA NEFA AUC between participants with diabetes and control participants remained significant when adjusted for BMI (p=0.001) waist circumference (p=<0.0001) and % body fat (p=0.004). (Tables 3.13-3.15).

3.6.2 Postprandial NEFA suppression

The normal switch of lipid metabolism from the fasting to the fed state is marked by a reduction in NEFA production from adipose tissue, and a reduction of VLDL-TAG production from the liver.

There was no significant difference between the participants with diabetes and control groups for *absolute* reduction in total NEFA concentrations at either 30 or 60 minutes (p=0.38 and p=0.68 respectively). The control group had a significantly greater reduction in *percentage* NEFA reduction at 60 minutes compared to the participants with diabetes group (58.9% vs 38.2%, p<0.0001) but there was no difference in percentage reduction at 30 minutes (p=0.18) (Table 3.6). There was no significant difference in increase in ^{13}C-PA NEFA at 30 minutes between the groups (p=0.70), but at 60 minutes there was numerically more ^{13}C-PA labelled NEFA detected in the participants with diabetes 0.26 vs 0.39 ng/ml/hr (p=0.06). Therefore it is likely that the ingested fat is making a significant contribution to the measured total NEFA at 60 minutes (Table 3.7).

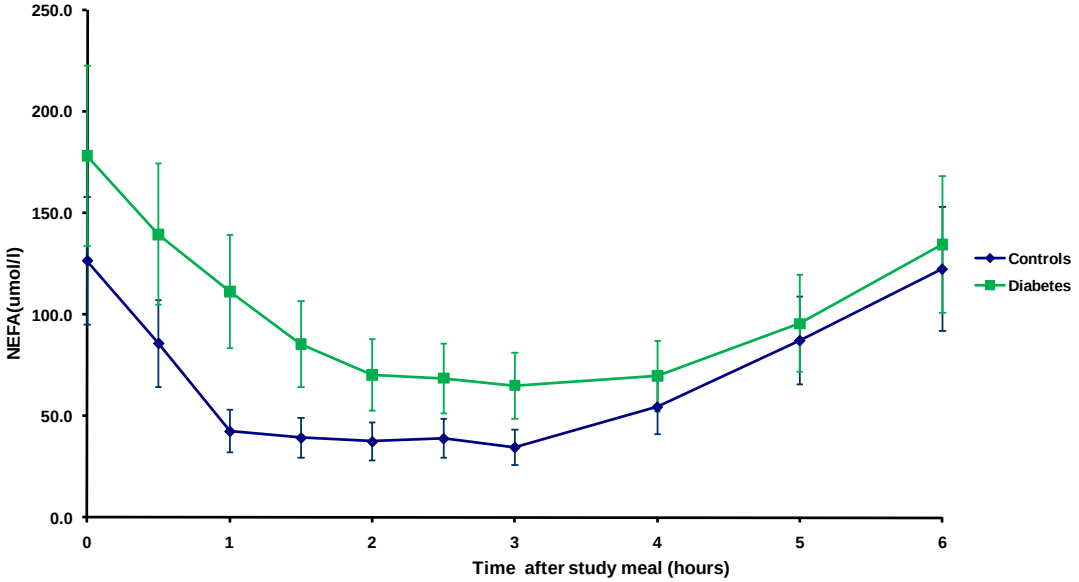


Figure 3-3: Concentration of non-esterified fatty acid (NEFA) in plasma before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). Fasting NEFA in DM vs CON, p=<0.0001. Area under the curve (AUC) NEFA in DM vs CON, p<0.0001. Differences between the groups tested using the Mann Whitney U test.

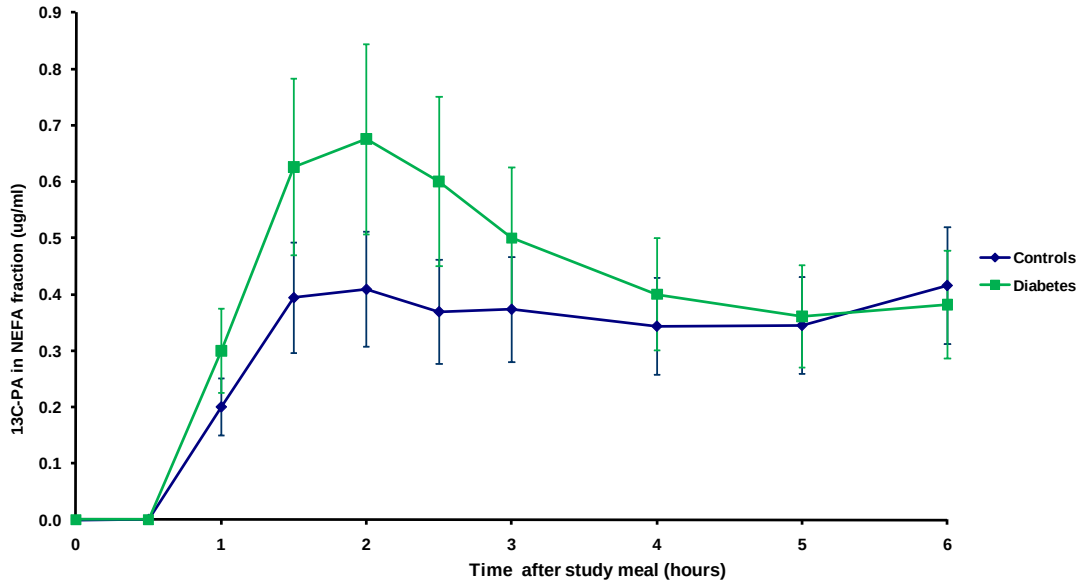


Figure 3-4: Concentration of ^{13}C-palmitic acid in non-esterified fatty acid (NEFA) fraction of plasma before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). ^{13}C-PA in NEFA AUC in DM vs CON, p=0.003. Differences between the groups tested using the Mann Whitney U test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Fasting TAG (mmol/l)	1.20 (0.80-2.35)	2.10 (1.60-2.85)	0.001
AUC TAG (mmol/l/6h)	9.00 (7.50-18.74)	17.34 (12.01-25.95)	0.001
Incremental AUC TAG (mmol/l/6h)	2.50 (1.61-4.35)	4.00 (1.53-7.37)	0.175
^{13}C-PA in TAG fraction AUC (µg/ml/6h)	44.04 (29.43-76.43)	60.05 (34.40-100.59)	0.107
Fasting NEFA (umol/l)	126.60 (99.26-163.00)	178.30 (151.28-217.37)	<0.0001
AUC NEFA (umol/l/6h)	379.32 (326.07-501.93)	574.90 (444.74-747.65)	<0.0001
^{13}C-PA in NEFA fraction AUC (µg/ml/6h)	2.14 (1.40-2.43)	2.60 (1.95-3.26)	0.003

Table 3-5: Fasting and postprandial (AUC) plasma triglyceride and non-esterified fatty acid concentrations in participants with diabetes and control groups.

Data are median (interquartile values). Differences between the groups were tested using the Mann Whitney U test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Absolute reduction in NEFA (0-30 minutes) (umol/l)	41.71±34.40	56.56±105.47	0.38
Percentage reduction in NEFA (0-30 minutes) %	29.54±22.67	20.33±39.34	0.18
Absolute reduction in NEFA (0-60 minutes) (umol/l)	81.47±46.00	89.06±110.14	0.68
Percentage reduction in NEFA (0-60 minutes) %	58.88±20.00	38.17±24.68	<0.0001

Table 3-6: Postprandial NEFA suppression in participants with diabetes and control participants.

Data are mean±sd. Differences between the groups tested using non-paired t-test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Absolute increase in ^{13}C-PA in NEFA (0-30 minutes) (ng/ml/hr)	0.03±0.10	0.04±0.18	0.70
Absolute increase in ^{13}C-PA in NEFA (0-60 minutes) (ng/ml/hr)	0.26±0.23	0.39±0.37	0.06

Table 3-7 : ^{13}C-PA in NEFA fraction-absolute increase from baseline at 30 and 60 minutes in participants with diabetes and control participants.

Data are mean±sd. Differences between the groups tested using non-paired t-test.

3.7 Glucose

The participants with diabetes had higher fasting glucose concentration (10.00 (8.38-11.38) vs 5.50 (5.30-5.88) mmol/l, p<0.0001)) (Table 3.8). The difference between participants with diabetes and control participants which remained significant when adjusted for BMI (p<0.0001), waist circumference (p<0.0001) and % body fat (p<0.0001). (Tables 3.13-3.15).

In the postprandial period the plasma glucose concentration rose significantly in the participants with diabetes group but remained relatively stable in the control group (Figure 3.5). As expected, glucose AUC was significantly higher in the participants with diabetes (80.66 (64.15-94.21) vs 36.24 (34.00-39.33) mmol/l, p<0.0001)). The difference in AUC glucose between participants with diabetes and control participants remained significant when adjusted for BMI (p= p<0.0001), waist circumference (p= p<0.0001) and % body fat (p= p<0.0001). (Tables 3.13-3.15).

AUC glucose remained significantly higher in the participants with diabetes group after adjustment for fasting glucose using ANCOVA (p=0.023).

3.8 Insulin

The participants with diabetes had higher fasting insulin (14.00 (9.33-18.43) vs 8.45 (5.60-12.78) μU/ml, p=0.001)) (Table 3.8). The difference remained statistically significant when adjusted for the effect of BMI (p=0.007) waist circumference (p=0.011) and % body fat (p=0.005), (Tables 3.13-3.15).

After consumption of the study meal, the insulin concentration rose sharply in the control group, reaching a peak at approximately 60 minutes after ingestion of the study meal, and then fell to baseline concentrations (Figure 3.6). The participants with diabetes showed a loss of this ‘first phase’ insulin response following meal ingestion and insulin concentrations remained lower than in control participants until 150 minutes post meal consumption, with a delayed peak at about 2 hours after the meal.

There was a statistically significant difference between the 30-minute insulin concentration which was *lower* in participants with diabetes compared to control participants (37.80 (24.75-51.75) vs 71.10 (42.70-110.15) μU/ml, p<0.0001)) (Table 3.8). The difference remained statistically significant when adjusted for the effect of BMI (p<0.0001), waist circumference (p<0.0001) and % body fat (p<0.0001), (Tables 3.13-3.15).

There was no difference in the overall insulin AUC over 6 hours between the participants with diabetes and control participants (246.63 (180.19-296.39) vs 216.13 (148.83-317.69) μU/ml/6h, p=0.50)) (Table 3.8). This was not affected by adjusting for BMI (p=0.83), waist circumference (p=0.87) and % body fat (p=0.69), (Tables 3.13-3.15).

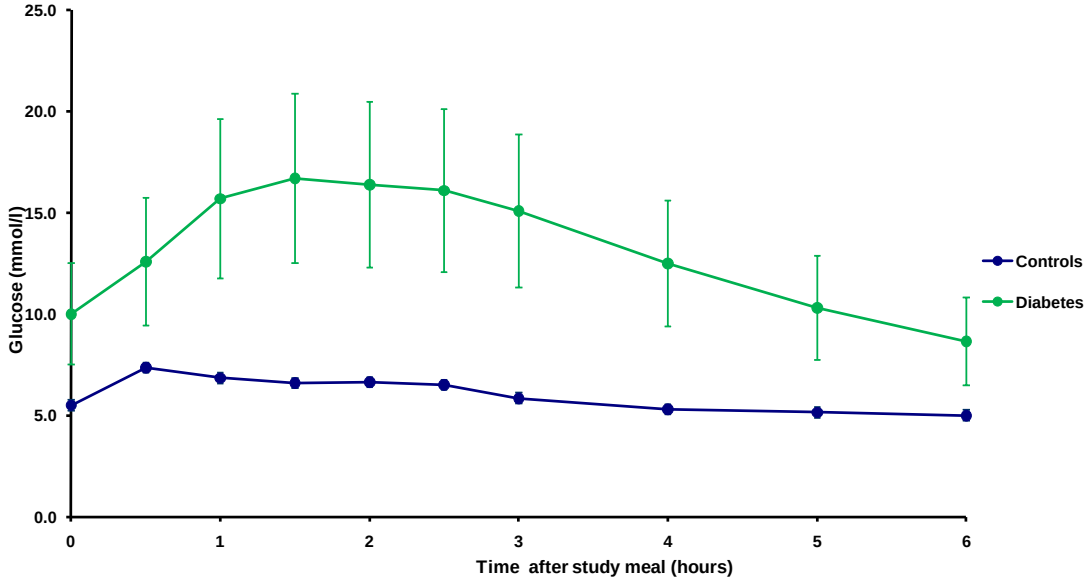


Figure 3-5: Concentration of glucose in plasma before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). Fasting glucose, DM vs CON, p<0.0001. Area under the curve (AUC) glucose, DM vs CON, p<0.0001. Differences between the groups were tested using the Mann Whitney U test.

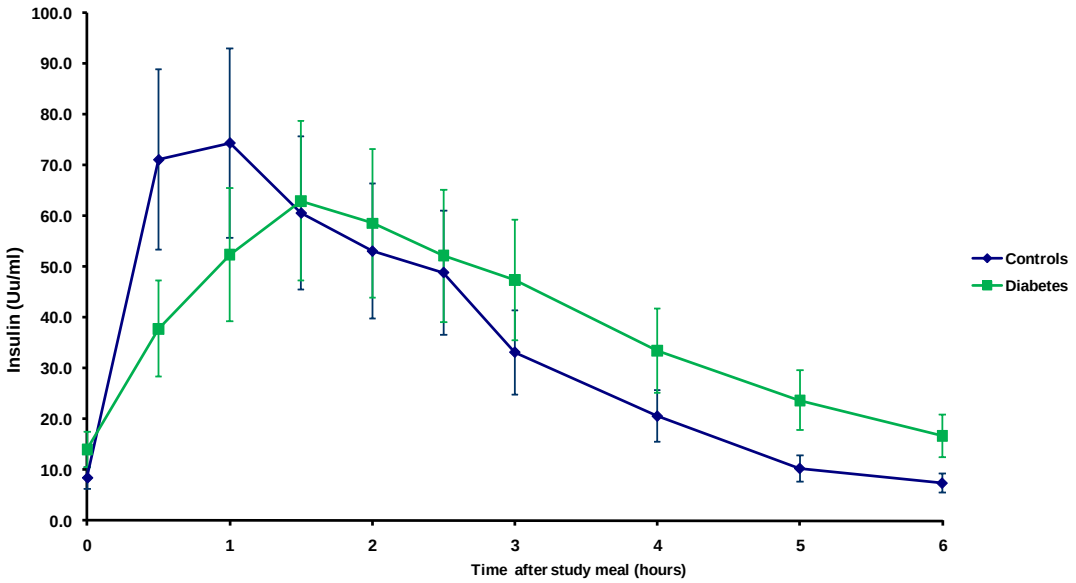


Figure 3-6: Concentration of insulin in plasma before and after the standard meal in participants with diabetes and control participants.

Participants with diabetes (DM) (green squares) and control participants (CON) (blue diamonds). Data are shown as (median (interquartile range)). Fasting insulin DM vs CON, p=0.001. 30 minute insulin DM vs CON, p<0.0001, area under the curve (AUC) insulin DM vs CON p=0.50. Differences between the groups were tested using the Mann Whitney U test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Fasting glucose (mmol/l)	5.50 (5.30-5.88)	10.00 (8.38-11.38)	<0.0001
AUC glucose (mmol/l/6h)	36.24 (34.00-39.33)	80.66 (64.15-94.21)	<0.0001
Fasting insulin (µU/ml)	8.45 (5.60-12.78)	14.00 (9.33-18.43)	0.001
30 minute insulin (µU/ml)	71.10 (42.70-110.15)	37.80 (24.75-51.75)	<0.0001
AUC insulin (µU/ml/6h)	216.13 (148.83-317.69)	246.63 (180.19-296.39)	0.50

Table 3-8: Fasting and postprandial (AUC) glucose and insulin in participants with diabetes vs control participants.

Data are median (interquartile values). Differences between the groups were tested using the Mann Whitney U test.

3.9 Energy expenditure measured by indirect calorimetry

There was no significant difference between the groups for fasting energy expenditure (296.95±55.61 vs 274.13±67.45 kJ/h, p=0.095) (Table 3.9) which was not affected after adjusting for BMI (p=0.22), waist circumference (p =0.23) or % body fat (p=0.095), (Tables 3.13-3.15).

There was also no significant difference between the groups for total energy expenditure AUC (1941.33±324.97 vs 1839.20±331.53 kJ/6h, p=0.155 between participants with diabetes and control participants, which was not affected after adjusting for BMI (p=0.38), waist circumference (p =0.25) or % body fat (p=0.16).

Fasting energy expenditure *per kg fat free mass* was higher in participants with diabetes (5.17±0.63 vs 4.78±0.61 kJ/h/kg, p=0.005), and also total energy expenditure per kg fat free mass over 6 hours was higher in participants with diabetes and this approached statistical significance (33.9±3.66 vs 32.4±4.10 kJ/6h/kg, p=0.08) (Table 3.9).

3.10 Fat and carbohydrate oxidation measured by indirect calorimetry

3.10.1 Fasting fat oxidation

Fasting fat oxidation was higher in the participants with diabetes group (2.81±1.62 vs 2.00±1.66 g/h, p=0.009) (Table 3.10). This remained statistically significant (although less so) when adjusted for measures of adiposity (p=0.05 adjusted for BMI, p=0.013 adjusted for waist circumference, and p=0.031when adjusted for % body fat). (Tables 3.13-3.15).

3.10.2 Postprandial fat oxidation

Fat oxidation rose slightly in the first 2 hours postprandially in both groups and then in the participants with diabetes group fell to fasting levels whilst in the control

group continued to rise gradually (Figure 3.7). The postprandial fat oxidation AUC was higher in the participants with diabetes but this did not quite reach statistical significance (19.75±9.39 vs 16.15±8.22 g/6h, p=0.06). When the difference in AUC fat oxidation between participants with diabetes and controls was adjusted for fasting fat oxidation rates the borderline significance was lost (p=0.698).

The borderline difference between participants with diabetes and controls was also not significant after adjusting for BMI (p=0.1), but remained borderline after adjustment for waist circumference (p=0.07) and % body fat (p=0.07) (Tables 3.13-3.15).

3.11 Carbohydrate oxidation

3.11.1 Fasting carbohydrate oxidation

There was no difference in carbohydrate (CHO) oxidation between participants with diabetes and controls in the fasting state (7.08±3.58 vs 7.75±4.40 g/h, p=0.45) (Table 3.10). This result was not affected after adjusting for BMI (p=0.32), waist circumference (p =0.35) or % body fat (p=0.49), (Tables 3.13-3.15).

3.11.2 Postprandial carbohydrate oxidation

CHO oxidation also rose in both groups in the first hour following the meal and then fell to approximately baseline values in both groups. CHO oxidation appears higher in the controls in the first three hours postprandially, but the absolute differences were small (Figure 3.8).

No difference was detected in the CHO oxidation AUC between the two groups (51.66±20.67 vs 54.98±19.55 g/6h, p=0.45). This result was not affected after adjusting for BMI (p=0.30), waist circumference (p =0.33) or % body fat (p=0.54), (Tables 3.13-3.15).

3.12 Respiratory Quotient

There was no statistically significanct difference in fasting respiratory quotient (RQ) in the participants with diabetes compared to the control paticipants (0.84±0.06 vs 0.86±0.06, p=0.09) (Table 3.10, Figure 3.9). This result was not affected after adjusting for BMI (p=0.11), waist circumference (p=0.13) or % body fat (p=0.10), (Tables 3.13-3.15).

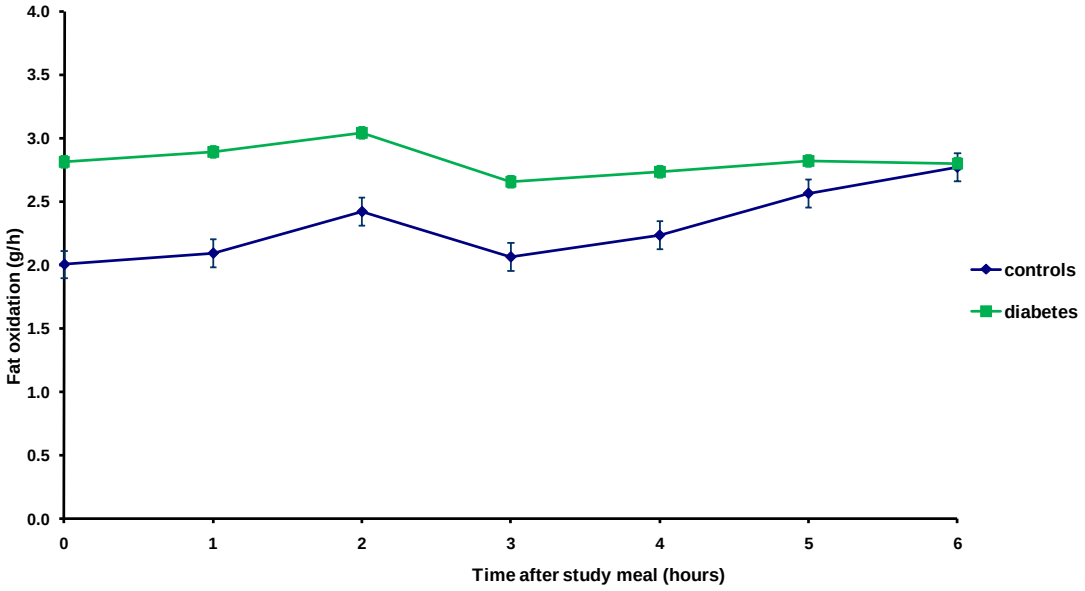


Figure 3-7: Fat oxidation measured by indirect calorimetry before and after the standard meal in participants with diabetes and control participants.

The data are mean ± SE. Participants with diabetes (green squares) and control participants (blue diamonds). DM vs CON, p= 0.009 fasting, p= 0.06 over 6 hours. Differences between the groups examined using non-paired t-tests.

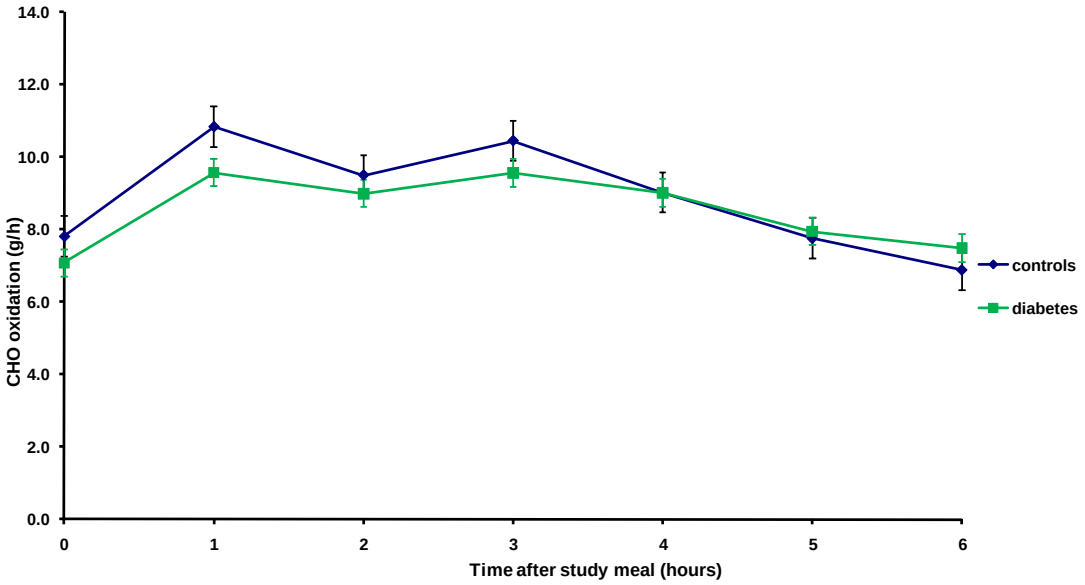


Figure 3-8: Carbohydrate oxidation measured by indirect calorimetry before and after the standard meal in participants with diabetes and control participants.

The data are mean ± SE. Participants with diabetes (green squares) and control participants (blue diamonds). DM vs CON, p= 0.45 fasting, p= 0.45 over 6 hours. Differences between the groups examined using non-paired t-tests.

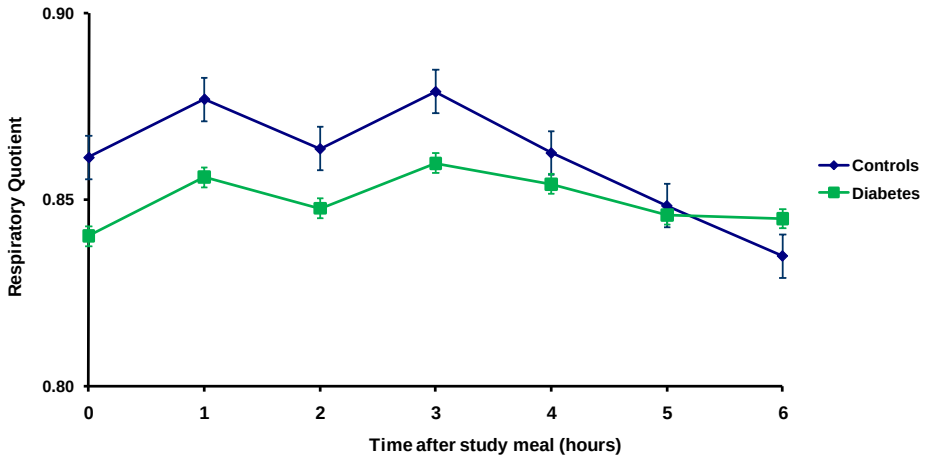


Figure 3-9: Respiratory quotient calculated from measured by indirect calorimetry measurements of fat and carbohydrate oxidation before and after the standard meal in participants with diabetes and control participants.

The data are mean ± SE. Participants with diabetes (green squares) and control participants (blue diamonds). DM vs CON p=0.09. Differences between the groups examined using non-paired t-tests.

3.13 Postprandial oxidation of ^{13}C-labelled dietary triglyceride

The results are expressed as $^{13}CO_2$ excretion in the expired breath as a percentage of administered dose of ^{13}C-PA label. This reflects beta oxidation of dietary derived ^{13}C-PA labelled triglyceride by the peripheral tissues.

Breath $^{13}CO_2$ rose steadily after the study meal in both participants with diabetes and control participants, reaching a peak at approximately 4 hours in both groups. $^{13}CO_2$ was still detectable in small amounts 24 hours after ingestion of the study meal. The main difference in $^{13}CO_2$ appearance on the breath between participants with diabetes and control participants was in the first eight hours (Figure 3.10).

Breath $^{13}CO_2$ was significantly higher in the participants with diabetes group over the 6 hour study period (9.81±3.34 vs 7.98±2.60 %dose/6h, p=0.003), this remained significant when adjusted for BMI (p = 0.007), waist circumference (p=0.007) and % body fat (p=0.004). Breath $^{13}CO_2$ was also significantly higher in the participants with diabetes group over the 24 hour study period (24.60±6.21 vs 20.96±5.36 %dose/24h, p=0.005) (Table 3.10). This also remained significant when adjusted for BMI (p=0.006) waist circumference (p<0.0001) and % body fat (p=0.003), (Tables 3.13-3.15).

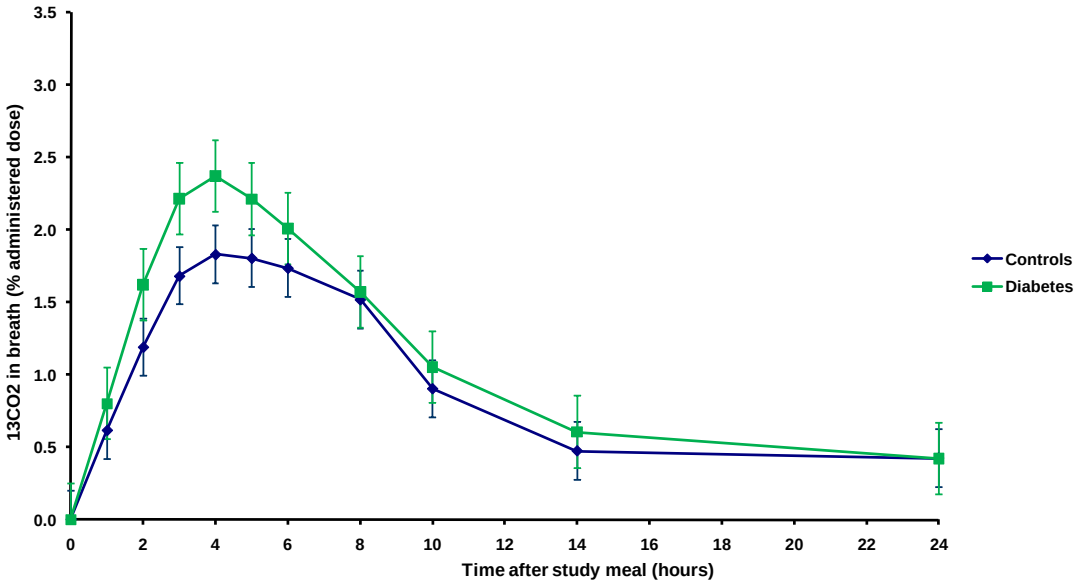


Figure 3-10 Oxidation of ingested dietary fat estimated by appearance of ingested ^{13}C-labelled lipid measured in expired breath for 24 hours after the standard meal in participants with diabetes and control participants.

The data are mean ± SE. Participants with diabetes (green squares) and control participants (blue diamonds). DM vs CON over first 6 hours p=0.003, over 24 hours p=0.005. Differences between the groups examined using non-paired t-tests.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
Fasting energy expenditure (kJ/hour)	274.13±67.45	296.95±55.61	0.095
Fasting energy expenditure/kg FFM(kJ/hour/kg)	4.78±0.61	5.17±0.63	0.005
AUC energy expenditure (kJ/6hours)	1839.20±331.53	1941.33±324.97	0.155
AUC energy expenditure/kg FFM (kJ/6hours/kg)	32.4±4.10	33.9±3.66	0.08

Table 3-9: Fasting and postprandial (AUC) energy expenditure in participants with diabetes and control participants

Data are mean±sd. Differences between the groups examined using non-paired t-test.

	CONTROLS	PARTICIPANTS WITH DIABETES	p value
$^{13}CO_2$ in breath over 6 hours (% dose/6h)	7.98±2.60	9.81±3.34	0.003
$^{13}CO_2$ in breath over 24 hours (% dose/24h)	20.96±5.36	24.60±6.21	0.005
Fasting fat oxidation (g/h)	2.00±1.66	2.81±1.62	0.009
AUC fat oxidation following standard meal (g/6h)	16.15±8.22	19.75±9.39	0.06
Fasting CHO oxidation (g/h)	7.75±4.40	7.08±3.58	0.447
AUC CHO oxidation following standard meal (g/6h)	54.98±19.55	51.66±20.67	0.451
Fasting RQ	0.86±0.06	0.84±0.06	0.086

Table 3-10: Fasting and postprandial (AUC) substrate oxidation in participants with diabetes and control participants

The data are mean±sd. Differences between the groups examined using non-paired t-test.

3.14 Relationship between fat oxidation and NEFA concentration

There were positive correlations between fasting fat oxidation and fasting NEFA concentration for the combined participants with diabetes and control groups ($r=0.502$, $p<0.0001$), control participants alone ($r=0.527$, $p<0.0001$), and participants with diabetes alone ($r=0.360$, $p=0.024$) (Table 3.11 and Table 3.12, Figure 3.11).

There were positive correlations between ^{13}C-PA fat oxidation and ^{13}C-PA in NEFA for the combined participants with diabetes and control groups ($r=0.531$, $p<0.0001$), control participants alone ($r=0.468$, $p=0.001$), and participants with diabetes alone ($r=0.469$, $p=0.001$).

Fasting fat oxidation was no longer statistically significant between participants with diabetes and control participants when adjusted for fasting NEFA ($p=0.247$), with a significant effect of fasting NEFA as a co-variable ($p=0.003$). A similar effect was found for the difference between participants with diabetes and controls for AUC fat oxidation as when adjusted for AUC NEFA ($p=0.362$), with a significant effect of fasting NEFA as a co-variable ($p<0.0001$). This data suggests that lipid oxidation is driven by substrate (ie NEFA) concentration.

		Fasting NEFA	NEFA AUC	^{13}C-PA in NEFA AUC
$^{13}CO_2$ in breath over 6 hours	Correlation coefficient	0.245	0.443	0.468
	p value	0.114	0.003	0.001
Fasting fat oxidation	Correlation coefficient	0.527	0.275	-0.080
	p value	<0.0001	0.078	0.612
AUC fat oxidation	Correlation coefficient	0.447	0.534	0.107
	p value	0.003	<0.0001	0.498

Table 3-11: Control participants: correlations between substrate concentration and rates of oxidation

		Fasting NEFA	NEFA AUC	^{13}C-PA in NEFA AUC
$^{13}CO_2$ in breath over 6 hours	Correlation coefficient	0.167	0.235	0.469
	p value	0.289	0.134	0.001
Fasting fat oxidation	Correlation coefficient	0.360	0.543	0.024
	p value	0.024	<0.0001	0.882
AUC fat oxidation	Correlation coefficient	0.512	0.692	0.185
	p value	<0.0001	<0.0001	0.247

Table 3-12: Participants with diabetes: correlations between substrate concentration and rates of oxidation

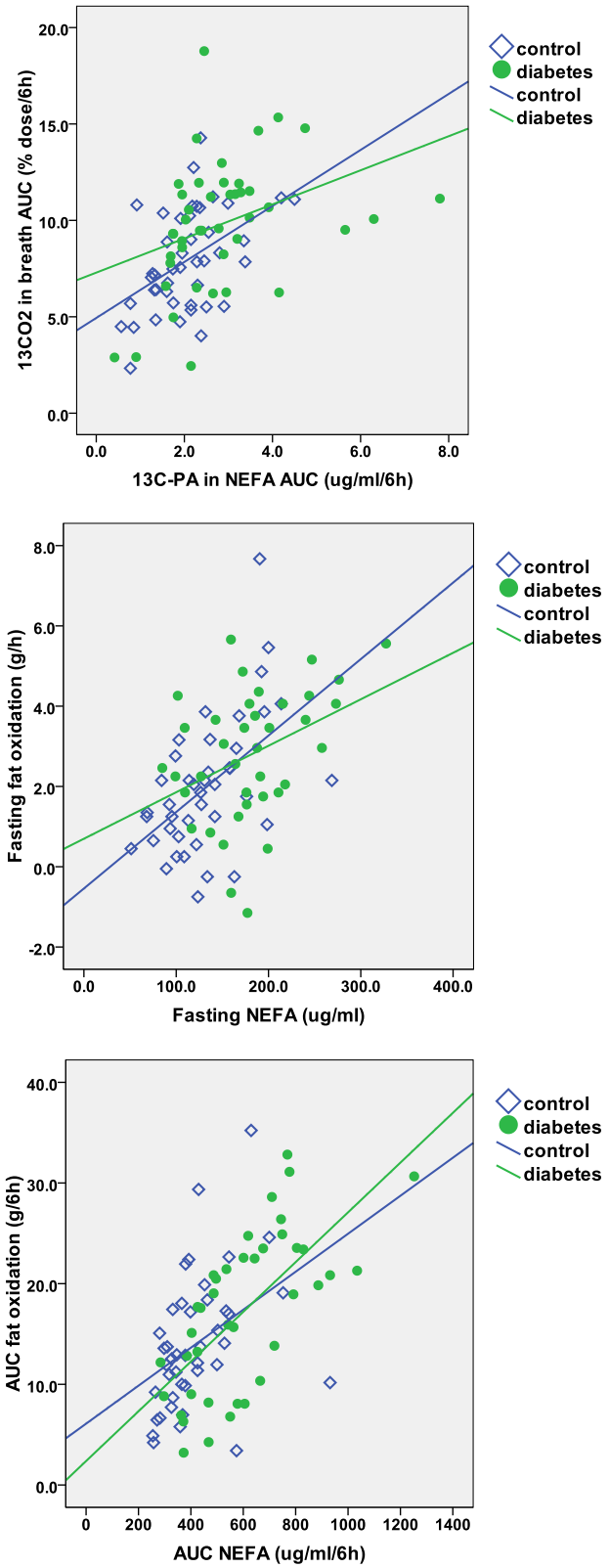


Figure 3-11: Correlations between substrate concentration and rates of oxidation in participants with diabetes and control participants.

See Tables 3.11 and 3.12 above for correlation coefficients and p values.

BMI	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (BMI)
	CON	DM		CON	DM		
Fasting glucose (mmol/l)	5.55±0.44	10.39±2.68	<0.0001	5.54±0.29	10.39±0.29	<0.0001	0.86
AUC glucose (mmol/l/6h)	36.55±3.68	80.21±20.40	<0.0001	36.38±2.22	80.37±2.22	<0.0001	0.48
Fasting insulin (µU/ml)	10.16±6.47	14.90±7.85	0.003	10.56±1.00	14.50±1.00	0.007	<0.0001
30 min insulin (µU/ml)	81.51±57.19	43.18±25.86	<0.0001	83.51±6.39	43.18±6.39	<0.0001	0.005
AUC insulin (µU/ml/6h)	258.83±148.84	282.0±173.59	0.50	266.83±23.1	274.10±23.10	0.83	0.002
HOMA-IR	2.51±1.65	6.75±3.53	<0.0001	2.60±0.41	6.61±0.41	<0.0001	0.014
HOMA-%B	101.47±69.1	46.6±39.45	<0.0001	103.72±8.22	44.37±8.22	<0.0001	0.003
Fasting triglyceride (mmol/l)	1.70±1.28	2.65±1.82	0.005	1.73±0.23	2.60±0.24	0.011	0.08
Postprandial (AUC) triglyceride (mmol/l/6h)	13.56±9.20	20.60±12.26	0.003	13.86±1.6	20.30±1.62	0.006	0.08
Postprandial incremental (AUC) TAG (mmol/l/6h)	3.79±3.62	5.00±4.09	0.145	3.80±0.58	4.98±0.60	0.16	0.76
^{13}C-PA in triglyceride fraction (µg/ml/6h)	54.16±35.32	66.45±38.18	0.118	53.93±5.54	66.63±5.60	0.113	0.76
Fasting NEFA (mmol/l)	132.24±45.38	200.19±113.67	<0.0001	134.55±13.28	197.93±13.12	0.001	0.16
Postprandial (AUC) NEFA (mmol/l/6h)	422.59±140.92	607.07±205.43	<0.0001	427.95±26.32	601.71±26.32	<0.0001	0.063
^{13}C-PA in NEFA fraction (µg/ml/6h)	2.05±0.84	2.80±1.38	0.003	2.01±0.17	2.85±0.17	0.001	0.02

Table 3-13: Results summary for plasma metabolic data with means corrected for BMI using ANCOVA

BMI	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (BMI)
	CON	DM		CON	DM		
Breath $^{13}CO2$ (% dose/6h)	7.98±2.60	9.81±3.34	0.003	7.99±0.45	9.78±0.46	0.007	0.68
Breath $^{13}CO2$ (% dose/24h)	20.96±5.36	24.60±6.21	0.005	20.96±0.87	24.60±0.92	0.006	0.99
Fasting energy expenditure (KJ/hour)	274.13±67.45	296.95±55.61	0.10	278.13±8.10	292.60±8.10	0.22	<0.0001
Fasting energy expenditure /kg FFM (KJ/hour/kg)	4.78±0.61	5.17±0.63	0.005	4.81±0.09	5.15±0.609	0.01	0.03
Postprandial (AUC) energy expenditure (KJ/6hours)	1839.20±331.53	1941.33±324.97	0.155	1861.82±42.54	1918.33±43.10	0.38	<0.0001
Postprandial (AUC) energy expenditure/kg/ FFM (KJ/6hours /kg)	32.4±4.10	33.9±3.66	0.08	32.5±0.60	33.9±0.60	0.11	0.29
Fasting RQ	0.86±0.06	0.84±0.06	0.086	0.86±0.009	0.84±0.009	0.11	0.38
Fasting net fat oxidation (g/h)	2.00±1.66	2.81±1.62	0.009	2.01±0.24	2.75±0.25	0.05	0.007
Postprandial (AUC) net fat oxidation (g/6h)	16.15±8.22	19.75±9.39	0.06	14.33±1.00	16.84±1.1	0.10	0.001
Fasting CHO oxidation (g/h)	7.75±4.40	7.08±3.58	0.447	7.84±0.61	6.97±0.62	0.32	0.07
Postprandial (AUC) CHO oxidation (g/6h)	54.98±19.55	51.66±20.67	0.451	55.58±3.00	51.03±3.07	0.30	0.025

Table 3-14: Results summary for oxidation data with means corrected for BMI using ANCOVA

Waist	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (waist)
	CON	DM		CON	DM		
Fasting glucose (mmol/l)	5.55±0.44	10.55±2.75	<0.0001	5.59±0.29	10.50±0.32	<0.0001	0.42
AUC glucose (mmol/l/6h)	36.55±3.68	81.76±20.20	<0.0001	36.52±2.13	81.78±2.33	<0.0001	0.95
Fasting insulin (µU/ml)	10.16±6.47	15.46±7.77	0.003	10.80±1.00	14.71±1.01	0.011	<0.0001
30 min insulin (µU/ml)	81.51±57.19	42.84±24.4	<0.0001	84.56±6.62	39.22±7.23	<0.0001	0.011
AUC insulin (µU/ml/6h)	258.83±148.84	277.25±157.36	0.50	269.86±22.23	264.19±24.27	0.87	0.007
HOMA-IR	2.51±1.65	7.04±3.58	<0.0001	2.71±0.39	6.81±0.43	<0.0001	0.001
HOMA-%B	101.47±69.1	47.5±38.38	<0.0001	104.92±8.49	43.43±9.30	<0.0001	0.009
Fasting triglyceride (mmol/l)	1.68±1.28	2.76±1.91	0.005	1.81±0.23	2.60±0.26	0.03	0.003
Postprandial (AUC) triglyceride (mmol/l/6h)	13.56±9.20	20.89±12.68	0.003	14.45±1.60	19.84±1.71	0.025	0.002
Postprandial incremental (AUC) TAG (mmol/l/6h)	3.79±3.62	4.69±3.97	0.145	3.92±0.57	4.52±0.64	0.493	0.21
^{13}C-PA in triglyceride fraction (µg/ml/6h)	54.12±35.32	63.21±36.86	0.118	54.89±5.47	62.29±5.97	0.372	0.43
Fasting NEFA (mmol/l)	132.24±45.38	205.37±122.60	<0.0001	134.53±13.98	202.70±15.12	0.002	0.41
Postprandial (AUC) NEFA (mmol/l/6h)	422.59±140.92	631.63±202.22	<0.0001	428.81±26.22	624.25±28.69	<0.0001	0.21
^{13}C-PA in NEFA fraction (µg/ml/6h)	2.05±0.84	2.82±1.33	0.003	1.96±0.57	2.94±0.17	<0.0001	0.01

Table 3-15: Results summary for plasma metabolic data with means corrected for waist circumference using ANCOVA

Waist	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (waist)
	CON	DM		CON	DM		
Breath $^{13}CO2$ (% dose/6h)	7.98±2.60	9.81±3.34	0.003	7.99±0.45	9.78±0.46	0.007	0.68
Breath $^{13}CO2$ (% dose/24h)	20.96±5.36	25.49±5.33	0.005	20.84±0.82	25.65±0.96	<0.0001	0.48
Fasting energy expenditure (KJ/hour)	274.13±67.45	306.80±53.02	0.095	282.78±7.55	296.42±8.29	0.23	<0.0001
Fasting energy expenditure /kg/ FFM (KJ/hour/kg)	4.78±0.61	5.19±0.62	0.005	4.80±0.10	5.16±0.10	0.02	0.11
Postprandial (AUC) energy exp. (KJ/6hours)	1839.20±331.53	2002.3±300.4	0.155	1880.93±42.54	1951.32±43.10	0.25	<0.0001
Postprandial (AUC) energy exp/kg FFM (KJ/6hours /kg)	32.4±4.10	33.9±3.30	0.08	32.31±0.58	34.10±0.65	0.05	0.27
Fasting RQ	0.86±0.06	0.84±0.06	0.086	0.86±0.009	0.84±0.010	0.13	0.66
Fasting net fat oxidation (g/h)	2.00±1.66	2.95±1.66	0.009	2.01±0.24	2.83±0.28	0.06	0.013
Postprandial (AUC) net fat oxidation (g/6h)	14.03±6.63	18.06±7.74	0.06	14.50±1.05	17.46±1.19	0.07	0.004
Fasting CHO oxidation (g/h)	7.75±4.40	7.33±3.81	0.447	7.96±0.62	7.10±0.70	0.35	0.03
Postprandial (AUC) CHO oxidation (g/6h)	54.98±19.55	53.14±21.51	0.451	56.19±3.03	51.61±3.42	0.33	0.011

Table 3-16: Results summary for oxidation data with means corrected for waist circumference using ANCOVA

% body fat	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (% body fat)
	CON	DM		CON	DM		
Fasting glucose (mmol/l)	5.55±0.44	10.39±2.68	<0.0001	5.56±0.29	10.38±0.29	<0.0001	0.72
AUC glucose (mmol/l/6h)	36.55±3.68	80.21±20.39	<0.0001	36.61±2.23	80.15±2.23	<0.0001	0.75
Fasting insulin (µU/ml)	10.16±6.47	14.90±7.85	0.003	10.40±1.04	14.66±1.04	0.005	0.004
30 min insulin (µU/ml)	81.51±57.19	43.18±25.86	<0.0001	81.88±6.66	42.80±6.66	<0.0001	0.54
AUC insulin (µU/ml/6h)	258.83±148.84	282.10±148.84	0.50	263.61±23.67	277.32±23.69	0.69	0.03
HOMA-IR	2.51±1.65	6.70±3.53	<0.0001	2.58±0.41	6.64±0.41	<0.0001	0.039
HOMA-%B	101.47±69.1	46.6±39.45	<0.0001	103.04±8.36	45.04±8.36	<0.0001	0.017
Fasting TAG (mmol/l)	1.68±1.28	2.65±1.82	0.005	1.70±0.24	2.64±0.24	0.007	0.60
Postprandial (AUC) TAG (mmol/l/6h)	13.56±9.20	20.60±12.26	0.003	13.61±1.62	20.56±1.64	0.004	0.76
Postprandial incremental (AUC) TAG (mmol/l/6h)	3.79±3.62	5.00±4.09	0.145	3.74±0.57	5.05±0.60	0.12	0.48
^{13}C-PA in TAG fraction (µg/ml/6h)	54.12±35.32	66.45±38.17	0.118	53.91±5.47	66.66±5.97	0.11	0.65
Fasting NEFA (mmol/l)	132.24±45.38	200.19±113.67	<0.0001	133.22±13.32	199.24±13.16	0.001	0.38
Postprandial (AUC) NEFA (mmol/l/6h)	422.59±140.92	607.07±205.43	<0.0001	426.81±26.24	602.86±26.24	<0.0001	0.06
^{13}C-PA in NEFA fraction (µg/ml/6h)	2.05±0.84	2.80±1.38	0.003	2.06±0.17	2.79±0.17	0.004	0.67

Table 3-17: Results summary for plasma metabolic data with means corrected for % body fat using ANCOVA

% body fat	Mean±SD		P value For DM vs CON	Adjusted Mean±SE		Adjusted p value for DM vs CON	P value for covariate (% body fat)
	CON	DM		CON	DM		
Breath $^{13}CO2$ (% dose/6h)	7.98±2.60	9.81±3.34	0.003	7.93±0.45	9.96±0.46	0.004	0.36
Breath $^{13}CO2$ (% dose/24h)	20.96±5.36	24.60±6.20	0.005	20.85±0.87	24.72±0.93	0.003	0.39
Fasting EE (KJ/hour)	274.13±67.45	296.94±55.61	0.095	274.01±9.61	297.06±9.62	0.095	0.85
Fasting EE /kg/ FFM (KJ/hour/kg)	4.78±0.61	5.17±0.63	0.005	4.80±0.09	5.15±0.09	0.006	<0.0001
Postprandial (AUC) EE (KJ/6hours)	1839.20±331.53	1941.3±324.0	0.155	1838.77±50.49	1941.77±51.09	0.16	0.90
Postprandial (AUC) EE/kg FFM (KJ/6hours /kg)	32.4±4.10	33.9±3.65	0.08	32.61±0.51	33.71±0.51	0.13	<0.0001
Fasting RQ	0.86±0.06	0.84±0.06	0.086	0.86±0.010	0.84±0.010	0.10	0.54
Fasting net fat oxidation (g/h)	2.00±1.66	2.80±1.61	0.009	2.01±0.25	2.80±0.26	0.031	0.71
Postprandial (AUC) net fat oxidation (g/6h)	14.03±6.63	17.16±7.81	0.06	14.13±1.10	17.05±1.12	0.07	0.15
Fasting CHO oxidation (g/h)	7.75±4.40	7.07±3.58	0.447	7.72±0.62	7.10±0.63	0.49	0.48
Postprandial (AUC) CHO oxidation (g/6h)	54.98±19.55	51.66±20.66	0.451	54.69±3.05	51.97±3.12	0.54	0.14

Table 3-18: Results summary for oxidation data with means corrected for % body fat using ANCOVA

3.15 Summary and discussion: Differences in triglyceride metabolism, substrate oxidation and energy expenditure between participants with diabetes and control participants matched for BMI

3.15.1 Lipids

TAG

Participants with diabetes had a higher fasting TAG compared with control participants matched for BMI. The AUC TAG following a standard meal was also higher in participants with diabetes, but this latter did not remain statistically significant when corrected for fasting TAG. In the current study dietary derived ^{13}C-PA TAG AUC was numerically, but not statistically significantly higher in the participants with diabetes. These data suggest that raised fasting TAG is a key contributor to the higher postprandial AUC TAG found in the participants with diabetes patients. This is likely to be secondary to the greater pool size of TAG requiring clearance from the circulation postprandially if fasting TAG are higher at baseline. In previous studies, fasting TAG has also been found to be an important determinant of postprandial TAG in patients with diabetes (91;95). Other studies however have shown that postprandial, but not fasting TAG is higher in participants with diabetes. Erikkson et al. (100) showed in eight participants with type 2 diabetes and eight age, sex and BMI matched control participants consuming a standardized lipid-enriched meal, that postprandial, but not fasting, TAG were significantly higher in the participants with diabetes than in the control participants. Madhu et al. (93) studied postprandial lipids in 20 male type 2 participants with diabetes and 20 age, BMI and sex matched controls and found that postprandial TAG AUC, TAG area under incremental curve, and peak TAG, but not fasting TAG were significantly higher in the participants with diabetes. In the latter two studies participants were selected who were not taking lipid lowering therapy and so this may explain why fasting TAG was normal due to selection bias. There are few studies using labelled TAG as a marker of ingested TAG in participants with diabetes compared to control participants. Chen et al. (98) found an elevation of meal-derived TAG identified by retinyl palmitate in VLDL-TAG but not chylomicron-TAG in 10 participants with type 2 diabetes compared with 10 control participants matched for BMI (98). From

this previous literature, it is unexpected that we did not find a significantly higher ^{13}C-PA TAG AUC or INC AUC TAG in the participants with diabetes. There was a wide variance between the participants, and this may have caused a reduction in power to detect a difference between the participants with diabetes and control participants. In future studies this large variance should be noted in power calculations, although we aimed to have sufficient power in this study as we based our calculations on differences in^{13}C-PA TAG AUC found in previous similar studies (see methods Chapter 2).

NEFA

Participants with diabetes had higher fasting and AUC NEFA with an apparently delayed NEFA nadir compared with BMI matched control participants, although this was not analysed statistically. There was a significantly higher ^{13}C-PA NEFA AUC (diet-derived NEFA) in the participants with diabetes. The explanation for the higher ^{13}C-PA NEFA AUC in the participants with diabetes could either be increased supply of ^{13}C-PA NEFA from increased hydrolysis of ^{13}C-PA labelled TAG and/or a decreased clearance of ^{13}C-PA NEFA from the circulation into tissues (principally adipose tissue and liver). As there was no significant difference in ^{13}C-PA TAG AUC between participants with diabetes and control participants, this suggests that reduced clearance of ^{13}C-PA NEFA in participants with diabetes is probably important. It is likely that there is impaired entrapment (increased 'spillover') of dietary derived NEFA into peripheral tissues (principally adipose tissue) in patients with type 2 diabetes compared to control participants matched for BMI. The mechanism for this may be relative insulin deficiency post meal due to reduced first phase beta cell production of insulin in participants with diabetes. The 30 minute insulin was significantly *lower* in the participants with diabetes. This is likely to contribute to reduced clearance of dietary lipid from the circulation in the postprandial period as LPL action and adipose tissue uptake of NEFA are insulin sensitive. There are few previous studies of isotope labelled meal derived NEFA metabolism. Tan et al. examined postprandial NEFA metabolism in a patient with the peroxisome proliferator-activated receptor (PPAR) gamma mutation P467L. The patient had partial lipodystrophy and type 2 diabetes. A mixed meal containing 600 mg (1,1,1-13C) tripalmitin was consumed by the participants. Two control groups

were used, healthy volunteers, and patients with type 2 diabetes. The P467L patient had elevated fasting and postprandial NEFA concentrations, and impaired postprandial adipose fatty acid trapping of ^{13}C-palmitic acid but no data was presented on the differences between the healthy and participants with diabetes control participants (88).

Fat oxidation

An alternative explanation for higher ^{13}C-PA NEFA AUC in participants with diabetes could be lower dietary NEFA disposal rates due to reduced oxidation of ^{13}C-PA NEFA in participants with diabetes, however in this study fat oxidation rates were *elevated* in the fasting and postprandial state in participants with diabetes compared to control participants. This was likely as a consequence of the increased availability of substrate. These findings supports Randle's hypothesis that there is uptake and metabolism of fat possibly preferentially to glucose metabolism in patients with diabetes (162) and refutes the theory that elevated lipid concentrations and excess adiposity in patients with type 2 diabetes is due to reduced fat oxidation (183). Previous experiments in isolated muscle have shown *reduced* lipid oxidation in muscle in type 2 diabetes (184), however the findings in the current study were measures of whole body lipid oxidation and are therefore not directly comparable to the opposite findings in isolated muscle in the in vitro studies.

Postprandial NEFA suppression

The participants with diabetes and control groups show similar rates of NEFA suppression over the first 30 minutes postprandially despite loss of the 'first phase' insulin response (ie 30 minute insulin) in the participants with diabetes group. The 30-minute NEFA is likely to be the best proxy for inhibition of lipolysis immediately after eating, as at 60 minutes post study meal it is likely that a significant proportion of the fat from the test meal has been absorbed. This NEFA suppression data suggests that inhibition of adipose tissue lipolysis is very insulin sensitive in the early postprandial period in both participants with diabetes and control participants, and that reduction of the first phase insulin response in participants with diabetes does not affect inhibition of adipose tissue lipolysis.

3.16 Summary and discussion: effects of adiposity on metabolic variables and differences between participants with diabetes and controls

3.16.1 Results adjusted for adiposity

Differences between participants with diabetes and controls in plasma lipids, glucose and insulin persisted when adjusted for measures of adiposity. This suggests that the diabetes per se has a greater effect on lipid, glucose and insulin metabolism than adiposity. This is in agreement with some (93;98) but not all (92), previous studies of fasting and postprandial triglyceride metabolism which have used subject groups matched for BMI. The current study is not in agreement with a previous study where capillary TAG was measured at six fixed time-points each day for three days in participants with type 2 diabetes, overweight and obese non-participants with diabetes and lean participants. No lean participants with diabetes were included. The authors found that fasting TAG and AUC TAG were both higher in participants with diabetes and obese control participants compared with lean control participants and concluded that daylong triglyceridaemia was similarly increased in participants with diabetes and obese control participants compared with lean control participants and that fasting TAG and central obesity largely determined daylong triglyceridaemia, *independent* of the presence of type 2 diabetes (92).

3.17 Conclusion and hypothesis

In the fasting state TAG, NEFA and glucose were elevated in participants with diabetes compared to control participants. This reflects elevated production of TAG and glucose by the liver overnight and inappropriately high adipose tissue TAG lipolysis. This is likely to be due to a combination of insulin resistance in the liver and adipose tissue and relative insulin insufficiency to overcome this.

In the postprandial state, beta cell failure, exemplified by reduction of the first phase insulin response after eating, in participants with diabetes contributes to the failure of postprandial insulin release leading to a high postprandial glucose concentration (185;186). After meal ingestion, suppression of NEFA appears normal initially, but there is failure of adipose tissue to store diet-derived NEFA postprandially in participants with diabetes compared with control participants which may be

secondary to relative insulin deficiency postprandially in participants with diabetes. This causes elevated circulating dietary ^{13}C-PA NEFA which can then be incorporated into circulating ^{13}C-PA VLDL-TAG by the liver. It is unclear why ^{13}C-PA TAG AUC concentrations was not significantly different between participants with diabetes and control participants, but the large inter-individual variance in adiposity between individuals may have contributed to increased variance of ^{13}C-PA TAG AUC and reduced the power to detect a difference between participants with diabetes and control participants. The participants in this study were purposefully chosen with a wide range of BMI, and although the groups were matched for mean BMI, it is possible that the wide range of BMI may have affected the variance of some of the metabolic variables. Chapters 4 and 5 explore the relationship between adiposity and triglyceride metabolism and explore whether these relationships are different in participants with diabetes and control participants and whether this may have affected the detection of differences in the groups as a whole.

Chapter 4 Comparison of the relationships between triglyceride metabolism and adiposity in participants with diabetes vs control participants.

4.1 Introduction

In Chapter 3 differences in triglyceride and glucose metabolism between participants with diabetes and controls were adjusted for BMI, waist circumference and percentage (%) body fat.

This chapter explores the relationships between the metabolic variables and BMI, waist circumference and % body fat in the control and participants with diabetes *separately* using Spearman's correlation analysis (Tables 4.1 and 4.2).

The data was then analysed using multiple linear regression (adjusted ANOVA) in order to detect differences in the relationship between the metabolic variables and adiposity measures between participants with diabetes and control participants.

4.2 Explanation of multiple linear regression model

4.2.1 Factors used in model

The factors used in the multiple linear regression model were:

i) metabolic variable of interest (eg AUC TAG).
ii) adiposity measure (eg BMI).
iii) Participant status ie diabetes or control.

4.2.2 Explanation of interaction effect

Multiple linear regression (adjusted ANOVA) analysis enables detection of a statistically significant difference in the relationship between the metabolic variables and measure of adiposity in participants with diabetes compared with control participants. This is also called an 'interaction effect.' If the p value for the interaction effect is <0.05 then there is a statistically significant difference in the relationship between the metabolic variable and adiposity measure in participants with diabetes compared with control participants. Figures 4.1-4.23 show the relationships using a best fit regression line for each metabolic variable with BMI, waist circumference and percentage body fat in participants with diabetes and

control participants. Tables 4.5 and 4.6 summarise the results for the interaction effects.

4.3 Effect of glycaemic control on triglyceride metabolism

Poor glycaemic control is independently associated with abnormal TAG metabolism (173), therefore if BMI or other measures of adiposity are associated with HbA1c or glucose concentrations, this could be a possible confounder in the relationship between TAG metabolism and adiposity. The next section explores the relationships between adiposity and glucose metabolism prior to the relationships with TAG metabolism being investigated.

4.4 Glucose

4.4.1 Fasting glucose

Controls

In control participants there was no correlation between fasting glucose and BMI (r=0.214, p=0.164), there was a significant positive correlation of fasting glucose with waist circumference (r=0.315, p=0.037). There was no correlation between fasting glucose and % body fat (r=-*0.119*, p=0.443) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no correlations between fasting glucose and measures of adiposity: BMI (r=-*0.020*, p=0.899, waist circumference (r=0.040, p=0.813), % body fat (r=0.095, p=0.541) (Table 4.1).

Relationship between fasting glucose and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between fasting glucose with BMI (p=0.584) or fasting glucose with waist circumference (p=0.538) or fasting glucose and % body fat (p=0.658). (Figure 4.1).

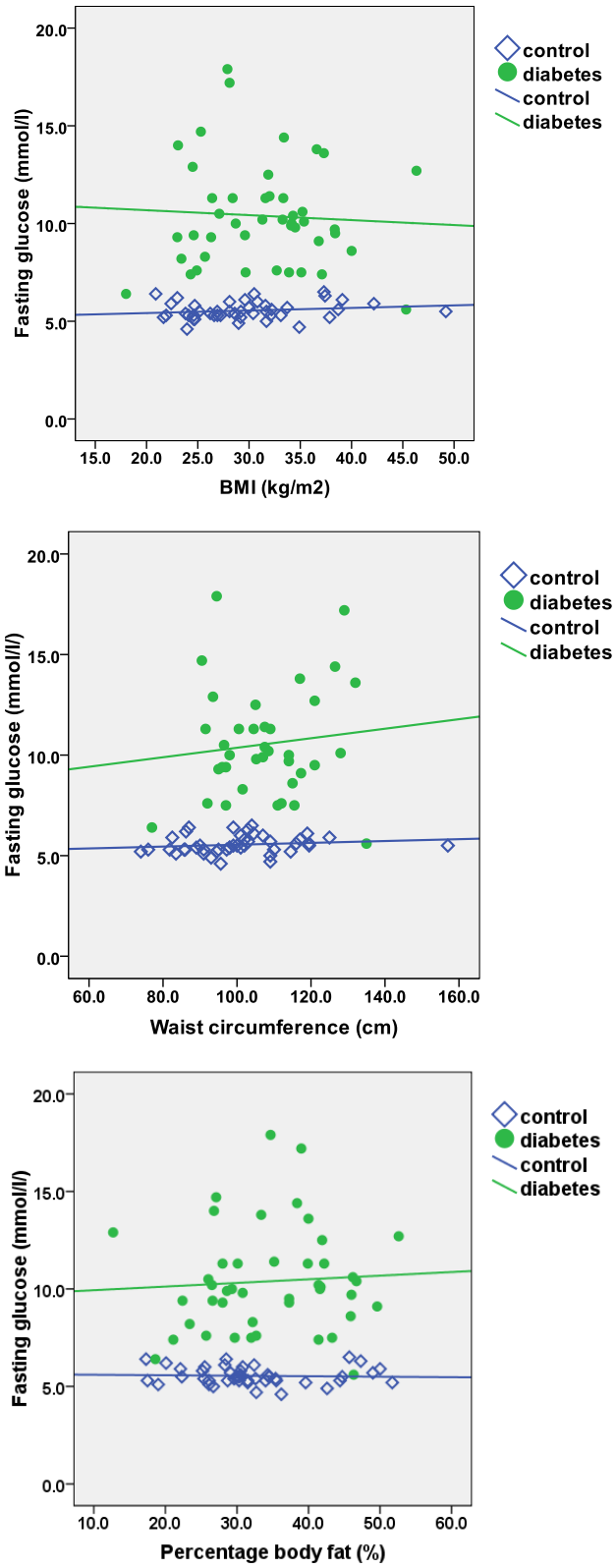


Figure 4-1: Scatterplots showing the relationship between fasting glucose and adiposity in participants with diabetes and control participants.

BMI (CON, r=0.214, ns; DM, r=*-0.020*, ns), waist circumference (CON r=0.315, p=0.037; DM r=0.040, ns) and % body fat (CON r= *-0.119*, ns; DM r=*-0.095*, ns). There were no interactions on multiple linear regression analysis.

4.4.2 AUC glucose

Controls

In control participants there was no correlation between AUC glucose and BMI (r=0.151, p=0.328), there was a significant positive correlation of AUC glucose with waist circumference (r=0.323, p=0.032).There was no correlation between AUC glucose and % body fat (r=-*0.037*, p=0.809) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no correlations between AUC glucose and measures of adiposity: BMI (r=-*0.164*, p=0.287), waist circumference (r=-*0.162*, p=0.339), % body fat (r=0.091, p=0.558) (Table 4.1).

Relationship between AUC glucose and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between AUC glucose and BMI (p=0.271) or waist circumference (p=0.370) or % body fat (p=0.759) (Figure 4.2).

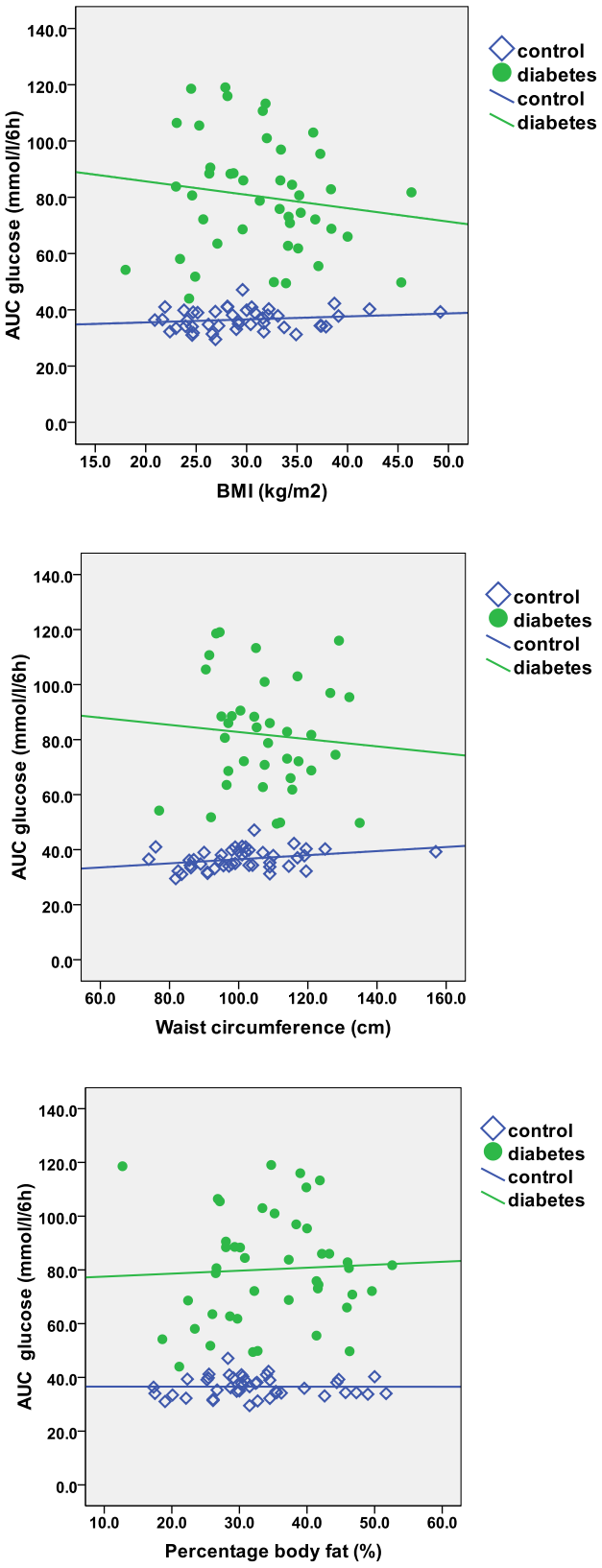


Figure 4-2: Scatterplots showing the relationship between AUC glucose and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.151, ns; DM, r=*-0.164* ns), waist circumference (CON r=0.323, p=0.032; DM r=*-0.162*, ns) and % body fat (CON r= *-0.037* ns; DM r=0.091, ns). There were no interactions on multiple linear regression analysis.

4.5 HbA_{1c}

HbA_{1c} was not measured in control participants. In participants with diabetes, HbA_{1c} correlated positively with BMI (r=0.319, p=0.035) and % body fat (r=0.316, p=0.037), but not significantly with waist circumference (r=0.252, p=0.133) (Figure 4.3).

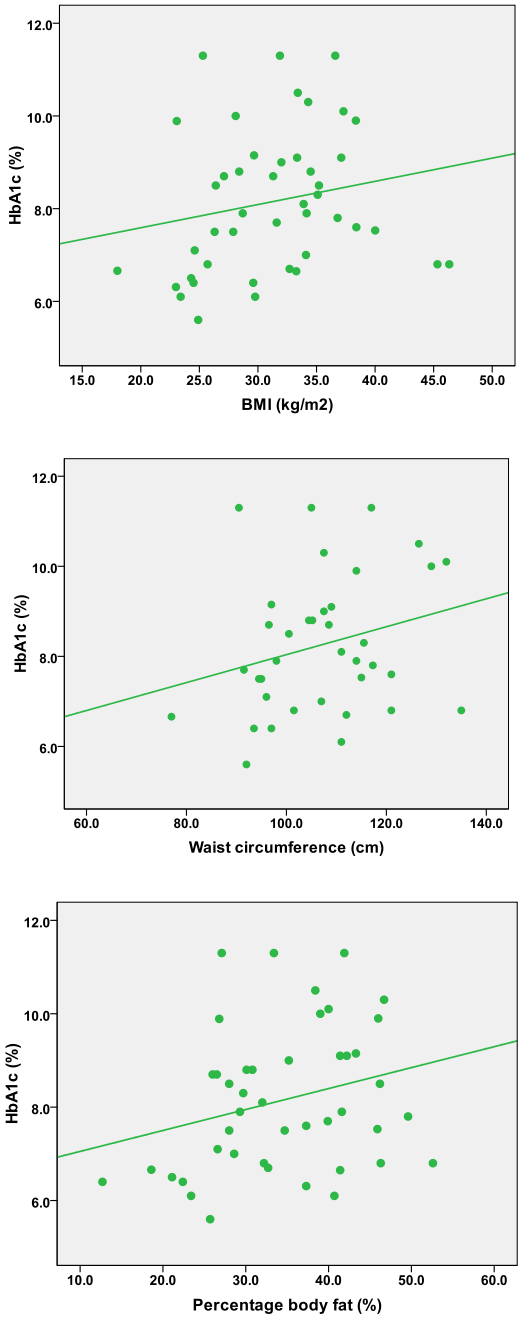


Figure 4-3: Scatterplots showing the relationship between HbA_{1c} and adiposity in participants with diabetes.

BMI (r=0.319, p=0.035), waist circumference (r=0.252, p=0.133) and % body fat (r=0.316, p=0.037).

4.6 Triglycerides

4.6.1 Fasting TAG

Controls

In control participants there was a significant positive correlation between fasting TAG and BMI (r=0.340, p=0.022) and waist circumference (r=0.461, p=0.001). Fasting TAG did not correlate with % body fat (r =0.23, p=0.881) (Table 4.1).

Participants with diabetes

In participants with diabetes there was a significant positive correlation between fasting TAG and BMI (r=0.338, p=0.028) and waist circumference (r=0.339, p=0.043). Fasting TAG did not correlate with % body fat (r=0.222 p=0.157) (Table 4.1).

Relationship between fasting TAG and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there was no difference in the relationship between fasting TAG and BMI in participants with diabetes compared to that in the control participants (p=0.864). There were also no significant differences found in the relationship between fasting TAG and waist circumference in participants with diabetes compared to control participants (p=0.354), or the relationship between fasting TAG and % body fat in participants with diabetes compared to control participants (p=0.502). (Figure 4.4).

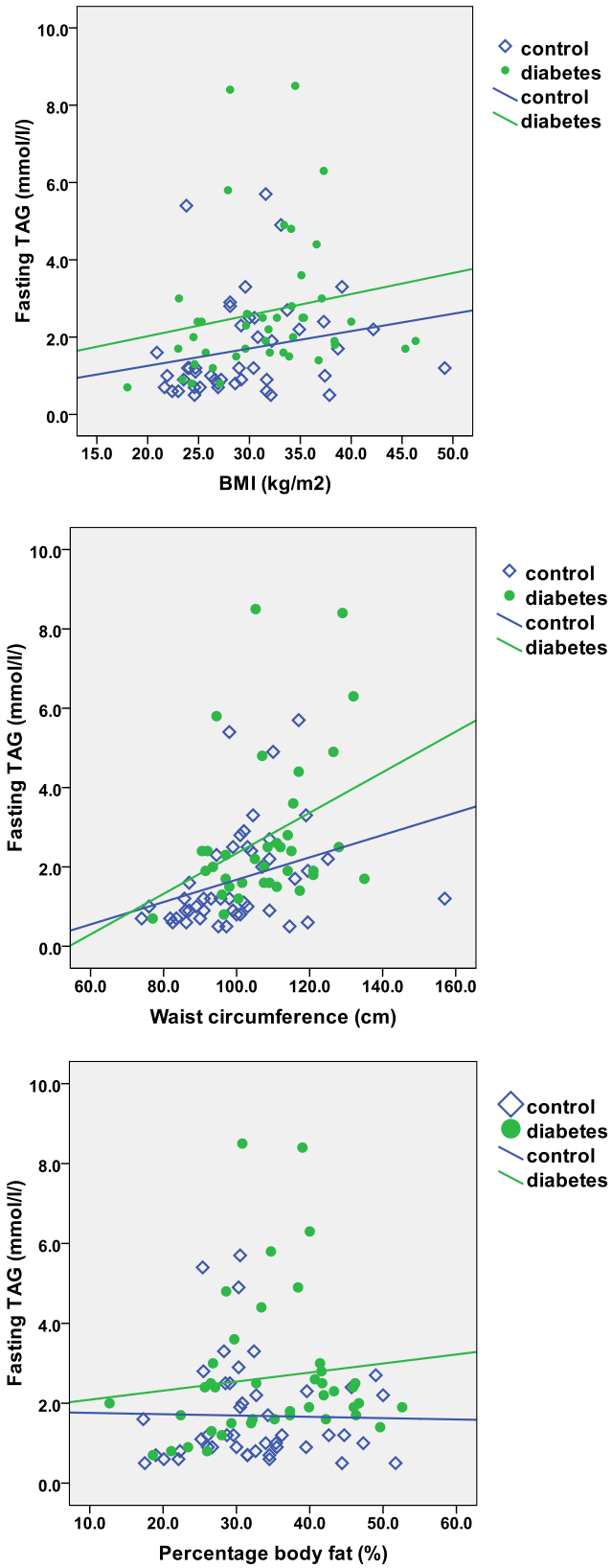


Figure 4-4: Scatterplots showing the relationship between fasting TAG and adiposity in participants with diabetes and control participants.
BMI (CON r=0.34, p=0.02; DM, r=0.328, p=0.0280), waist circumference (CON r=0.46, p=0.001; DM r=0.339, p=0.043) and % body fat (CON r= 0.23, ns; DM r=0.22, ns). There were no interactions on multiple linear regression analysis.

4.6.2 Area under the curve TAG

Controls

In control participants there was a positive correlation between area under the curve (AUC) TAG and BMI (r=0.324, p=0.03) and waist circumference (r=0.452, p=0.002). AUC TAG did not correlate with % body fat (r=-*0.018*, p=0.906) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no correlations between AUC TAG and any measure of adiposity: BMI (r=0.165, p=0.284), waist circumference (r=0.246, p=0.136) and % body fat (r=0.131, p=0.397) (Table 4.1).

Relationship between AUC TAG and adiposity in participants with diabetes compared to controls

Using multiple linear regression there were no differences in the relationships between AUC TAG and BMI (p=0.532), waist circumference (p=0.656) or % body fat (p=0.724) in participants with diabetes compared to the control participants (Figure 4.5).

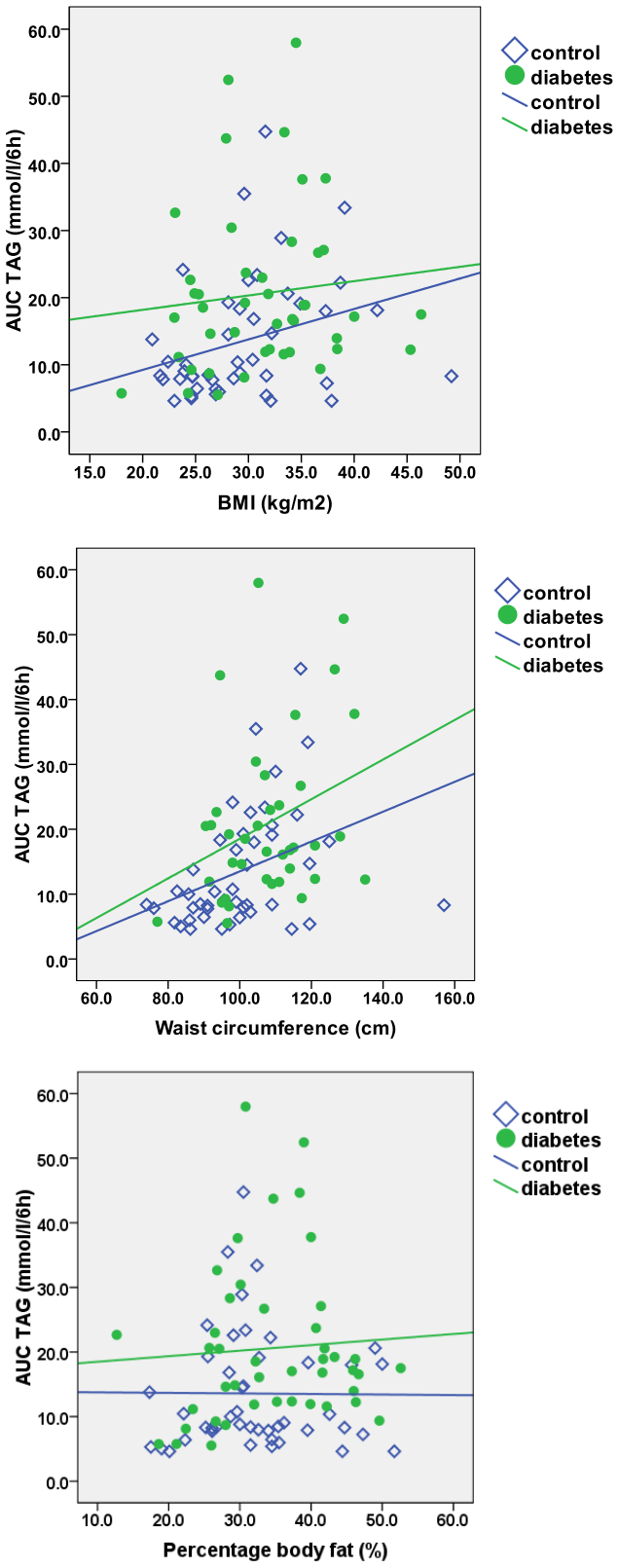


Figure 4-5: Scatterplots showing the relationship between AUC TAG and adiposity in participants with diabetes and control participants.

BMI (CON, r=0.320, p=0.03; DM, r=0.165, ns), waist circumference (CON r=0.452, p=0.002; DM r=0.246, ns) and % body fat (CON r= *-0.018*, ns; DM r=0.131, ns). There were no interactions on multiple linear regression analysis.

4.6.3 Incremental AUC TAG

Controls

In control participants there was a borderline positive correlation between incremental (INC) AUC TAG and BMI (r=0.263, p=0.080) and waist circumference (r=0.286, p=0.06), but no correlation with % body fat (r=0.080, p=0.600) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no significant correlations between INC AUC TAG and any measures of adiposity: but BMI did show a weak non-significant *negative* correlation, BMI (r=-*0.211*, p=0.180), waist circumference (r=-*0.092*, p=0.593), % body fat (r=-*0.059*, p=0.712) (Table 4.1).

Relationship between INC AUC TAG and adiposity in participants with diabetes compared to controls

Using multiple linear regression there was a significant difference in the relationship between INC AUC TAG and BMI between participants with diabetes and control participants (p=0.04). This suggests that the relationship between BMI and postprandial TAG metabolism is different in participants with diabetes compared to control participants. There were no differences in the relationships between INC AUC TAG and waist circumference (p=0.269) or % body fat (p=0.571) in participants with diabetes compared to the control participants (Figure 4.6).

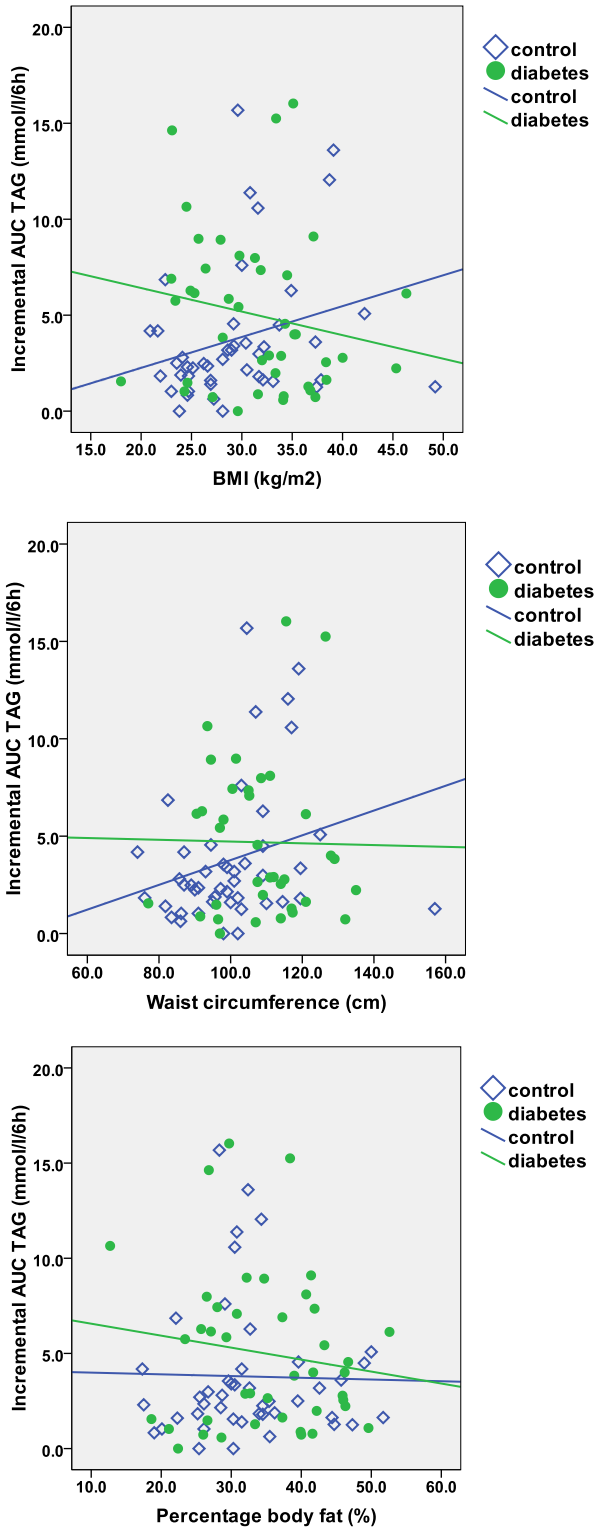


Figure 4-6: Scatterplots showing the relationship between incremental AUC TAG and adiposity in participants with diabetes and control participants. BMI (CON, r=0.260, p=0.08; DM, r=*-0.211*, ns), waist circumference (CON r=0.286, p=0.06; DM r=*-0.092*, ns) and % body fat (CON r= 0.08, p=ns; DM r=*-0.059*, ns). On multiple linear regression analysis there was a significant interaction for BMI (p=0.04).

4.6.4 ^{13}C - PA in the TAG fraction AUC

Controls

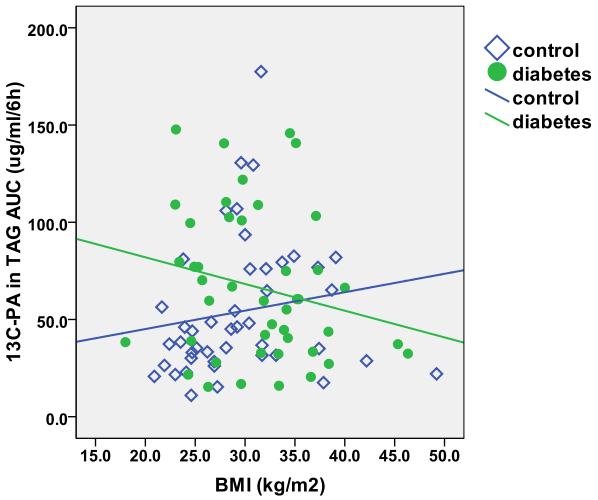
In control participants, there was a positive correlation between ^{13}C-PA AUC TAG and BMI which approached statistical significance (r=0.288, p=0.06).There was a significant positive correlation between ^{13}C-PA TAG AUC and waist circumference (r=0.296, p=0.048). There was no correlation with % body fat (r=0.080, p= 0.600) (Table 4.1).

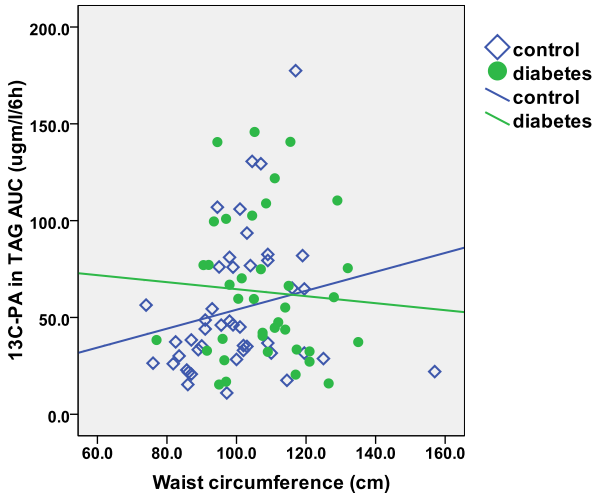
Participants with diabetes

In participants with diabetes there were no significant correlations between ^{13}C-PA AUC TAG and any measure of adiposity: BMI (r=-*0.210*, p=0.172), waist circumference (r=-*0.102*, p=0.543), % body fat (r=-*0.097*, p=0.529) (Table 4.1).

Relationship between ^{13}C-PA AUC TAG and adiposity in participants with diabetes compared to controls

Using multiple linear regression there was a trend towards a difference in the relationship between ^{13}C-PA AUC TAG and BMI and in participants with diabetes compared to that in the control participants which approached statistical significance (p=0.07) (Figure 4.7). There were no significant differences found in the relationship between ^{13}C-PA AUC TAG and waist circumference (p=0.253) or % body fat (p=0.423) in participants with diabetes compared to control participants (Figure 4.7).





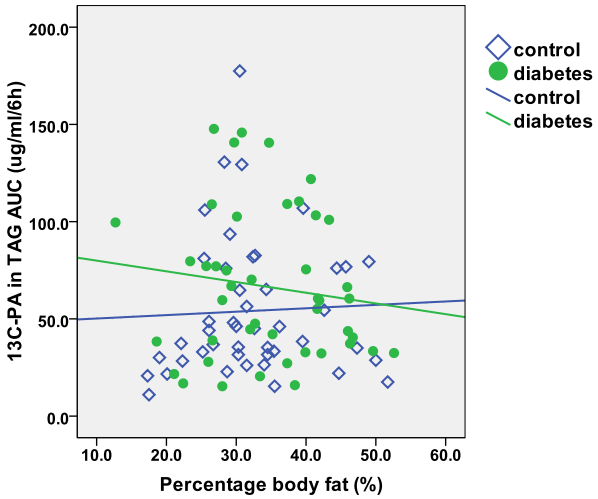


Figure 4-7: Scatterplots showing the relationship between ^{13}C-PA in TAG AUC and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.288, p=0.06; DM, r=-*0.210*, ns), waist circumference (CON r=0.296, p=0.048; DM r=-*0.102* ns) and % body fat (CON r= 0.08, p=ns; DM r=-*0.097*, ns). On multiple linear regression analysis there was a trend towards a significant interaction for BMI (p=0.07).

4.7 Non-esterified fatty acids

4.7.1 Fasting non-esterified fatty acids

Controls

In control participants there were no correlations between non-esterified fatty acids (NEFA) and measures of adiposity (BMI, r=0.166, p=0.288, waist circumference, r=0.095, p=0.544, % body fat r=0.135, p=0.390) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no correlations between fasting NEFA and BMI (r=0.252, p=0.099) or waist circumference (r=0.278, p=0.096). There was a positive correlation between fasting NEFA and % body fat (r=0.330, p=0.029) (Table 4.1). It can be seen on Figure 4.8 that there is one participant with diabetes with very elevated fasting NEFA. Removing this outlying participant from the analysis did not change the results above, so this participant was not removed from the final analysis.

Relationship between fasting NEFA and adiposity in participants with diabetes compared to controls

Using multiple linear regression there were no differences in the relationships between fasting NEFA and BMI (p=0.493), waist circumference (p=0.575) or % body fat (p=0.792) in participants with diabetes compared to the control participants (Figure 4.8).

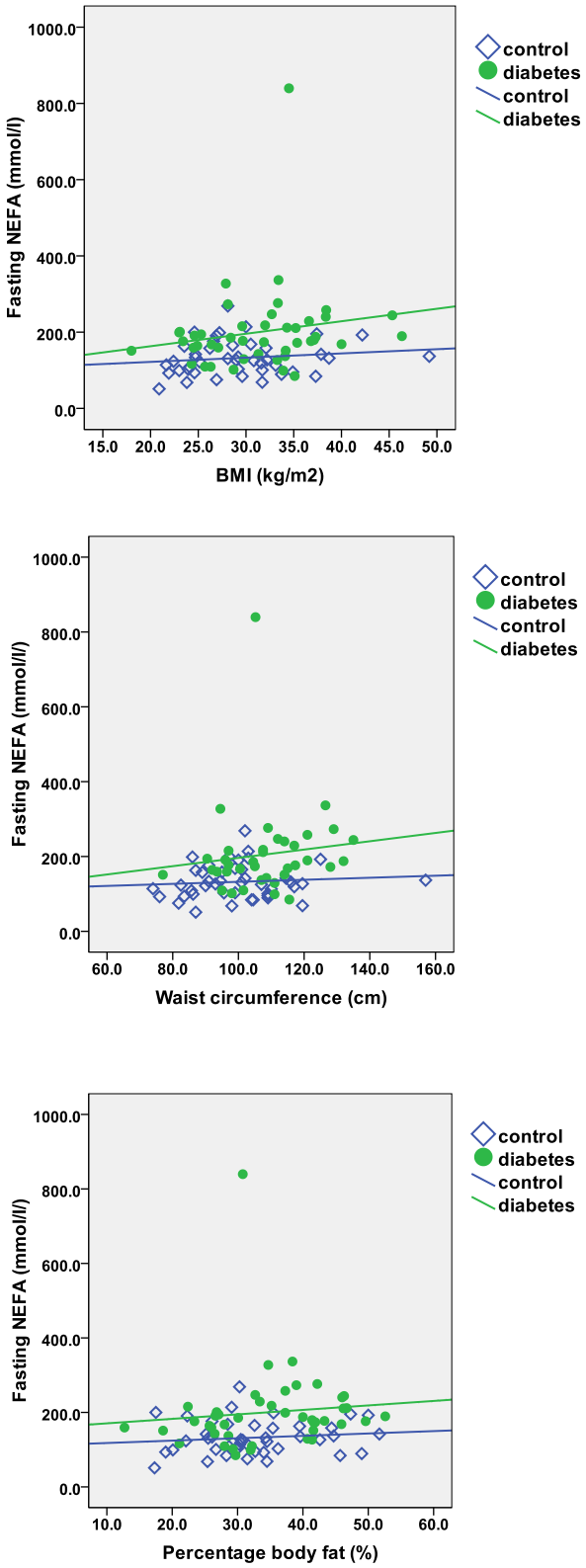


Figure 4-8: Scatterplots showing the relationship between fasting NEFA and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.166, ns; DM, r=0.252, ns), waist circumference (CON r=0.095, ns; DM r=0.278, ns) and % body fat (CON r= 0.135, ns; DM r=0.330, p=0.029). There were no interactions on multiple linear regression analysis. Removal of the outlier does not affect the results.

4.7.2 Non-esterified fatty acids AUC

Controls

In control participants there was a positive correlation between AUC NEFA and BMI which approached statistical significance (r=0.278, p=0.068). There was no correlation between AUC NEFA and waist circumference (r=0.135, p=0.381). AUC NEFA positively correlated with % body fat (r=0.326, p=0.031) (Table 4.1).

Participants with diabetes

In participants with diabetes there were no correlations between AUC NEFA and measures of adiposity: BMI (r=0.159, p=0.304), waist circumference (r=0.124, p=0.465), and a weak, non-statistically significant correlation with % body fat (r=0.240, p=0.117) (Table 4.1).

Relationship between AUC NEFA and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between AUC NEFA and BMI (p=0.905), waist circumference (p=0.748) or % body fat (p=0.885) in participants with diabetes compared to the control participants (Figure 4.9).

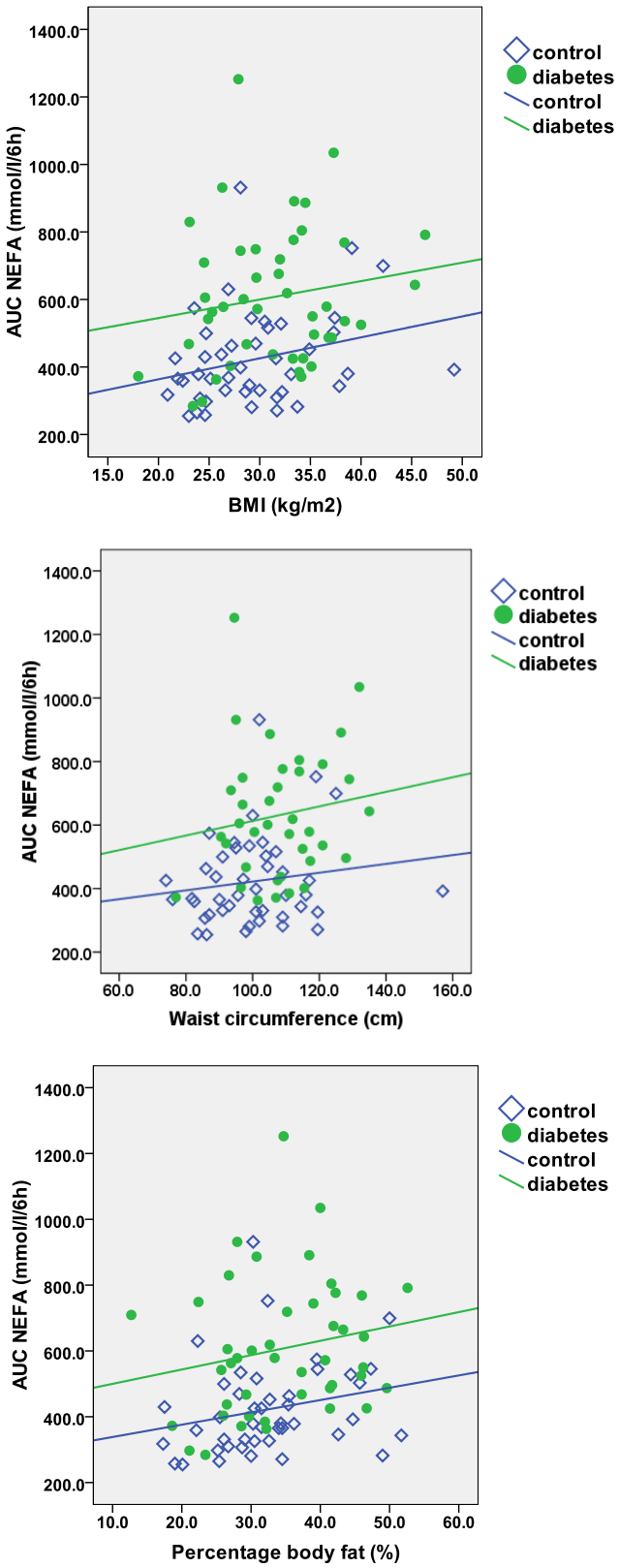


Figure 4-9: Scatterplots showing the relationship between AUC NEFA and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.278, p=0.068; DM, r=0.159, ns), waist circumference (CON r=0.135, ns; DM r=0.124, ns) and % body fat (CON r= 0.326, p=0.031; DM r=0.240, ns). There were no interactions on multiple linear regression analysis.

4.7.3 ^{13}C - palmitic acid in the non-esterified fatty acid fraction AUC

Controls

In control participants, ^{13}C - palmitic acid in the NEFA fraction AUC (^{13}C-PA NEFA AUC) did not correlate with BMI (r=*-0.112*, p=0468), correlated *negatively* with waist circumference (r=*-0.311*, p=0.04) and *positively* with % body fat (r=0.366, p=0.015) (Table 4.1).

Participants with diabetes

In participants with diabetes, ^{13}C-PA NEFA AUC *negatively* correlated with BMI (r=*-0.352*, p=0.018) and waist circumference (r=*-0.486*, p=0.002). ^{13}C-PA NEFA AUC did not correlate with % body fat (r =-0.100, p=0.513) (Table 4.1).

Relationship between ^{13}C-PA NEFA AUC and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between ^{13}C-PA NEFA AUC and BMI (p=0.265), waist circumference (p=0.178) or % body fat (p=0.158) in participants with diabetes compared to control participants (Figure 4.10).

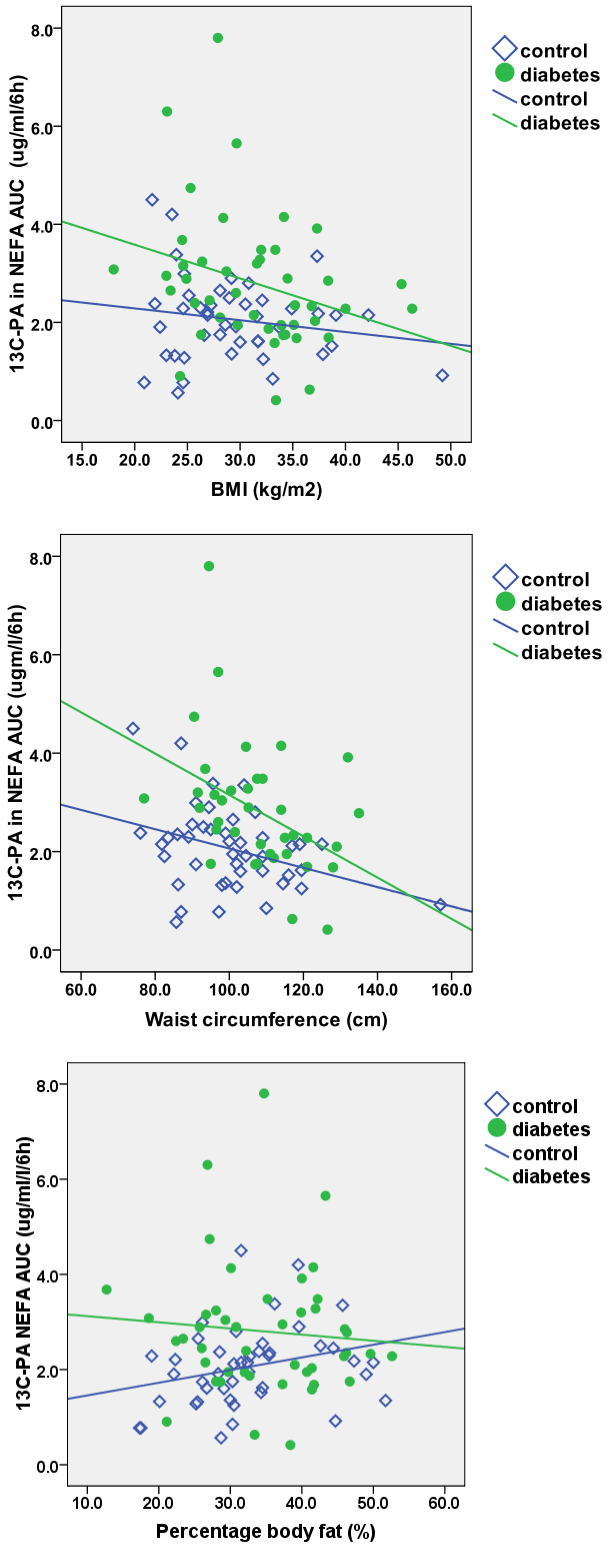


Figure 4-10: Scatterplots showing the relationship between ^{13}C-PA in the NEFA fraction AUC and adiposity in participants with diabetes and control participants.
BMI (CON, r=*-112*, ns; DM, r=*-0.352*, p=0.018), waist circumference (CON r=-*0.311*, p=0.04; DM r=*-0.486*, p=0.002) and % body fat (CON r= 0.366, p=0.015; DM r=*-0.100*, ns). There were no interactions on multiple linear regression analysis.

4.8 Insulin

4.8.1 Fasting insulin

Controls

In control participants there was a significant positive correlation between fasting insulin and all measures of adiposity: BMI ($r=0.676$, $p<0.0001$), waist circumference ($r=0.676$, $p<0.0001$), % body fat ($r=0.471$, $p=0.001$) (Table 4.1).

Participants with diabetes

In participants with diabetes, there were no statistically significant correlations between fasting insulin and adiposity, although those with BMI and waist circumference approached statistical significance ($r=0.288$, $p=0.058$ and $r=0.304$, $p=0.068$ respectively). % body fat showed no correlation with fasting insulin ($r=0.165$, $p=0.285$) (Table 4.1).

Relationship between fasting insulin and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between fasting insulin and BMI ($p=0.448$), waist circumference ($p=0.996$) or % body fat ($p=0.146$) in participants with diabetes compared to the control participants (Figure 4.11).

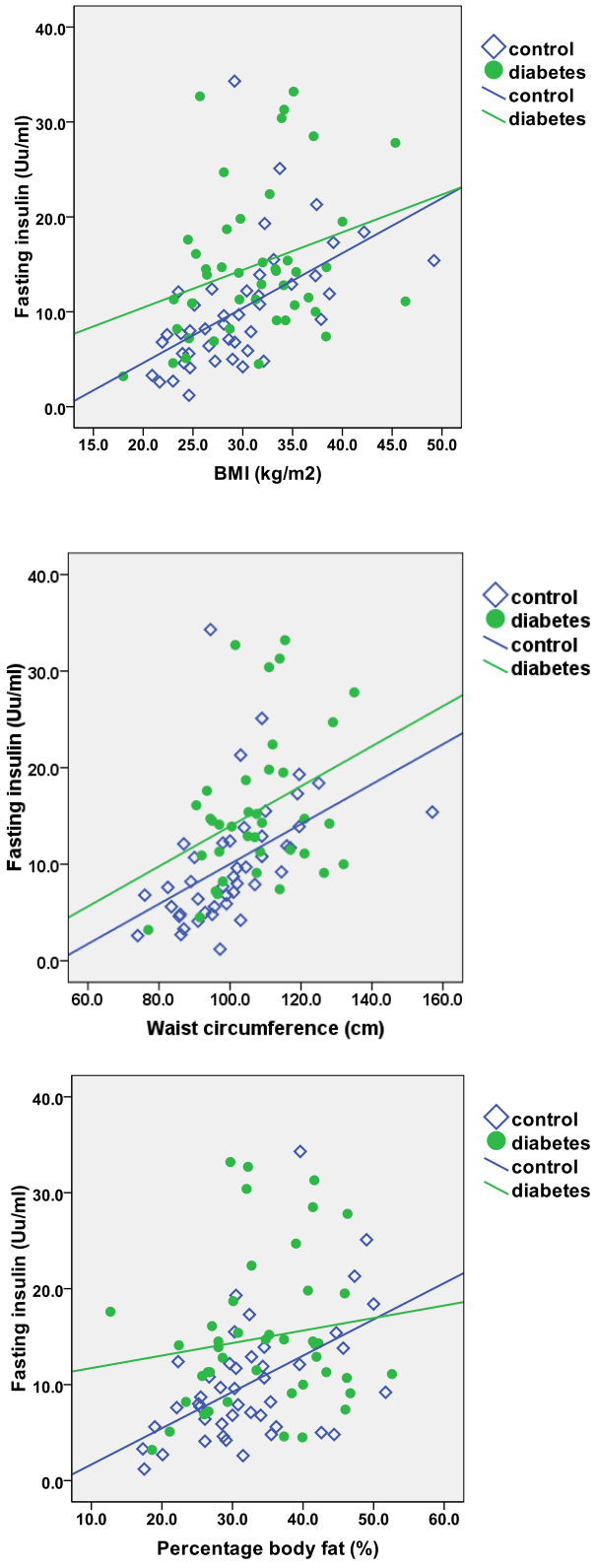


Figure 4-11: Scatterplots showing the relationship between fasting insulin and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.676, p<0.0001; DM, r=0.288, p=0.058), waist circumference (CON r=0.676, p<0.0001; DM r=0.304, p=0.068) and % body fat (CON r= 0.471, p=0.001; DM r=0.165, ns). There were no interactions on multiple linear regression analysis.

4.8.2 30 minute postprandial insulin

Controls

In control participants, there was a positive correlation between 30 minute insulin and BMI (r=0.364, p=0.014) and waist circumference (r=0.320, p=0.032), but not % body fat (r =0.137, p =0.369) (Table 4.1).

Participants with diabetes

In participants with diabetes, there was a significant positive correlation between 30 minute insulin and BMI (r=0.320, p=0.032), waist circumference (r=0.441, p=0.006) but not % body fat (r =0.111, p=0.468) (Table 4.1).

Relationship between 30 minute insulin and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between 30 minute insulin and BMI (p=0.152), waist circumference (p=0.580) or % body fat (p=0.915) in participants with diabetes compared to the control participants (Figure 4.12).

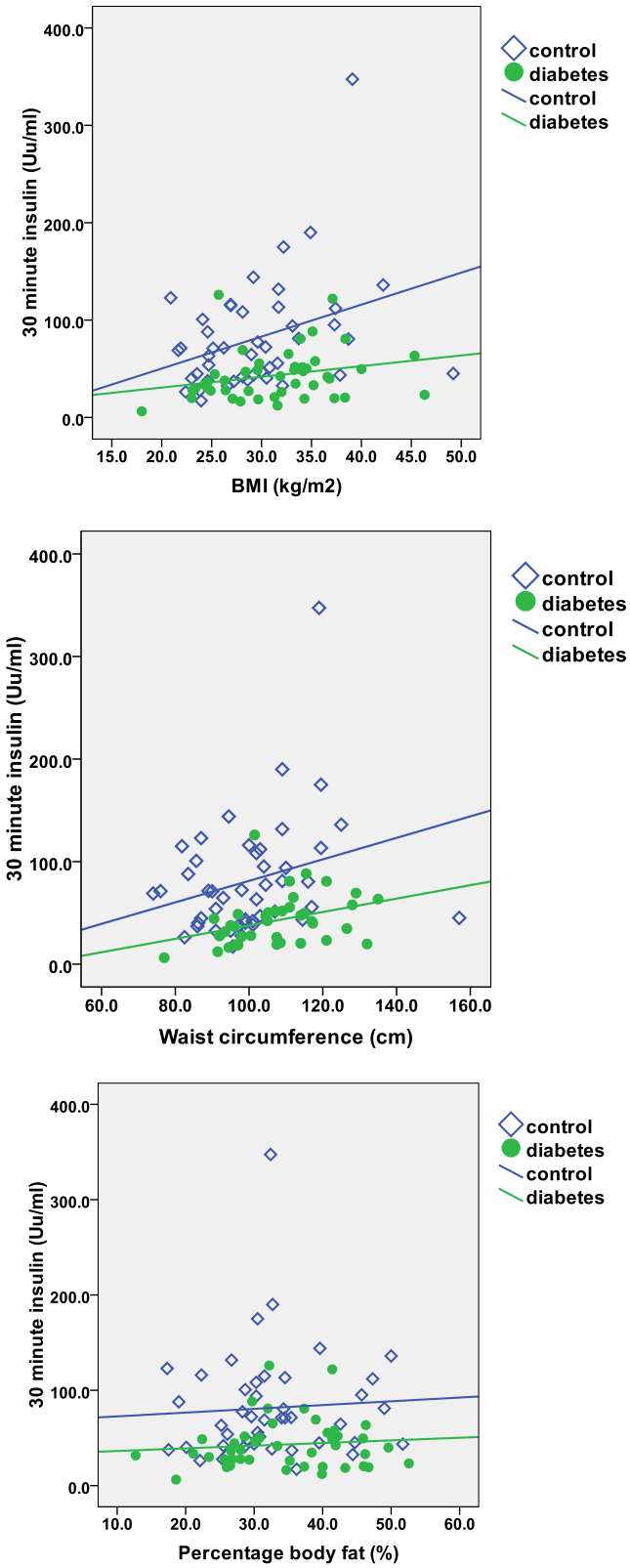


Figure 4-12: Scatterplots showing the relationship between 30 minute insulin and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.364, p=0.014; DM, r=0.320, p=0.032), waist circumference (CON r=0.320, p=0.032; DM r=0.440, p=0.006) and % body fat (CON r= 0.137, ns; DM r=0.110, ns). There were no interactions on multiple linear regression analysis.

4.8.3 AUC insulin

Controls

There was a significant positive correlation between AUC insulin and BMI ($r=0.497$, $p<0.001$), waist circumference ($r=0.409$, $p=0.005$) and % body fat ($r=0.511$, $p<0.0001$) (Table 4.1).

Participants with diabetes

There was a positive correlation between AUC insulin and BMI ($r=0.323$, $p=0.03$) in participants with diabetes, borderline significance with waist circumference ($r=0.299$, $p=0.068$) but not with % body fat ($r=0.199$, $p=0.191$) (Table 4.1).

Relationship between AUC insulin and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between AUC insulin and BMI ($p=0.606$), waist circumference ($p=0.823$) or % body fat ($p=0.338$) in participants with diabetes compared to the control participants (Figure 4.13).

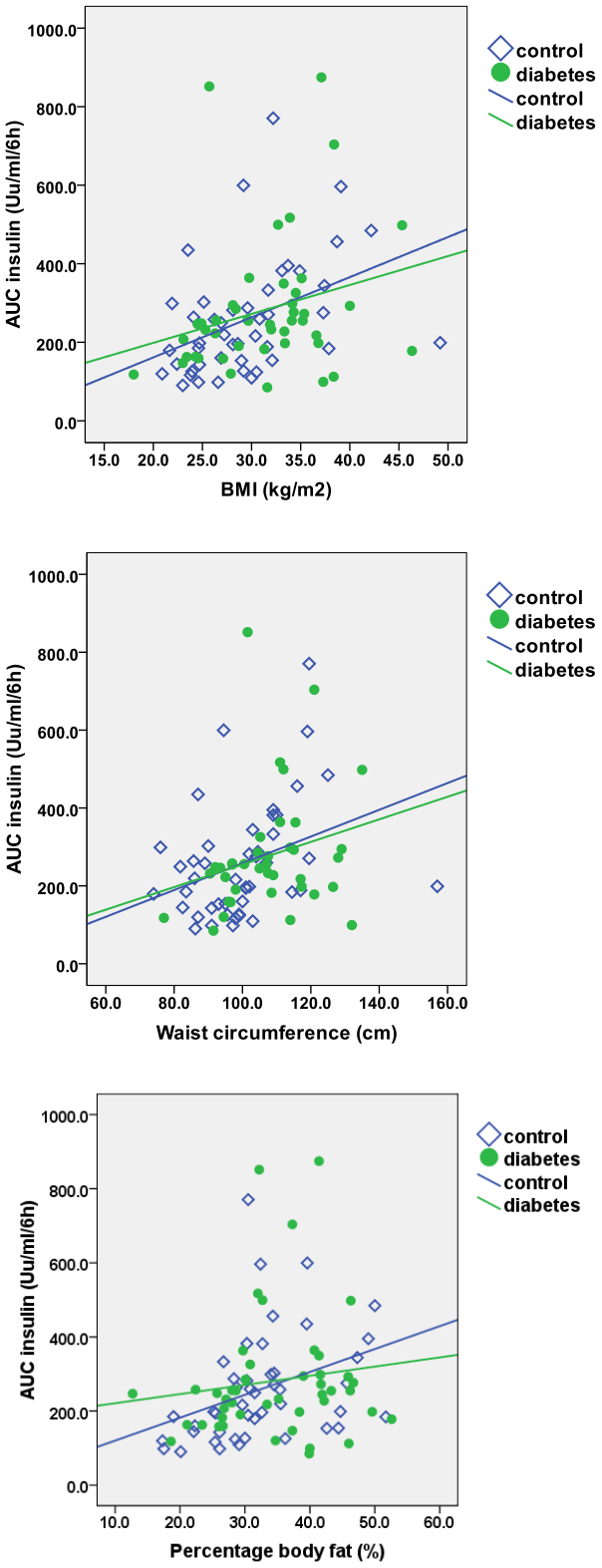


Figure 4-13: Scatterplots showing the relationship between AUC insulin and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.497, p<0.0001; DM, r=0.323, p=0.030), waist circumference (CON r=0.409, p=0.005; DM r=0.299, p=0.068) and % body fat (CON r= 0.511, p<0.0001; DM r=0.119, ns). There were no interactions on multiple linear regression analysis.

4.8.4 HOMA-IR

Control participants

HOMA-IR strongly correlated with measures of adiposity in control participants (BMI; r=0.72, p=0.0001, waist circumference; r=0.71, p=0.0001, % body fat; r=0.45, p=0.003). Mean HOMA-IR across the different quartiles of adiposity can be seen in Figure 4.14.

Participants with diabetes

HOMA-IR was not significantly associated with measures of adiposity in participants with diabetes (BMI; r=0.21, p=0.17, waist circumference; r=0.27, p=0.11, percentage (%) body fat; r=0.17, p=0.28). Mean HOMA-IR across the different quartiles of adiposity can be seen in Figure 4.14.

Relationship between HOMA- IR and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no interactions found for the differences in the relationships between HOMA- IR and BMI (p=0.451), waist circumference (p=0.465) and % body fat (p=0.425) in participants with diabetes compared to the control participants.

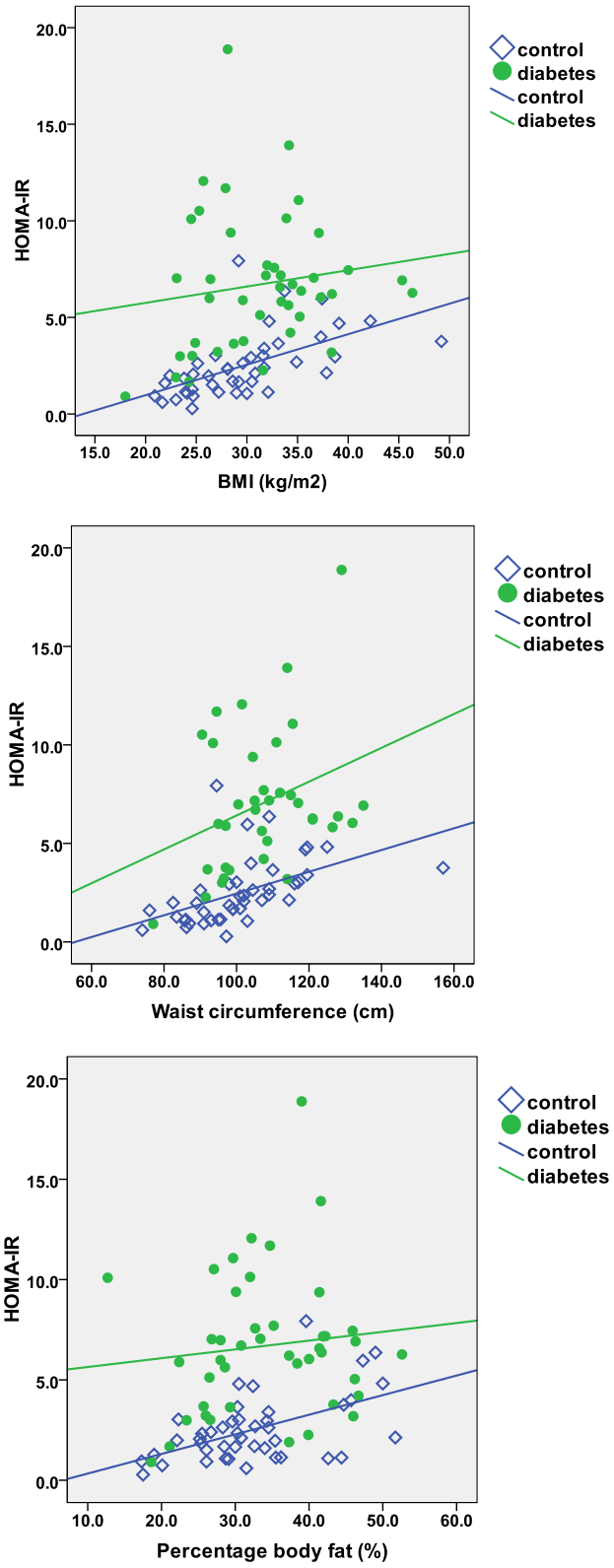


Figure 4-14: Scatterplots showing the relationship between HOMA-Insulin resistance and adiposity in participants with diabetes and control participants. BMI (CON, r=0.717, p<0.0001; DM, r=0.214, ns), waist circumference (CON r=0.712, p<0.0001; DM r=0.270, ns) and % body fat (CON r= 0.448, p=0.003; DM r=0.167, ns). There were no interactions on multiple linear regression analysis.

4.8.5 HOMA- B%

Control participants

HOMA-B% strongly positively correlated with measures of adiposity in control participants (BMI; r=0.64, p<0.0001, waist circumference; r=0.63, p<0.0001, % body fat; r=0.53, p<0.0001) (Table 4.1). The HOMA- B% across the different measures of adiposity can be seen in Figure 4.15.

Participants with diabetes

HOMA-B% was not significantly associated with measures of adiposity in participants with diabetes (BMI; r=0.11, p=0.48, waist circumference; r=0.02, p=0.90, % body fat; r=-*0.09*, p=0.58) (Table 4.1). The HOMA- B% across the different measures of adiposity can be seen in Figure 4.15.

Relationship between HOMA- B% and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis interactions were found for the differences in the relationships between HOMA-B% and BMI (p=0.026), waist circumference (p=0.059) and % body fat (p=0.009) in participants with diabetes compared to the control participants, with a positive correlation with increasing adiposity in the control participants, but no correlation in participants with diabetes (Figure 4.15).

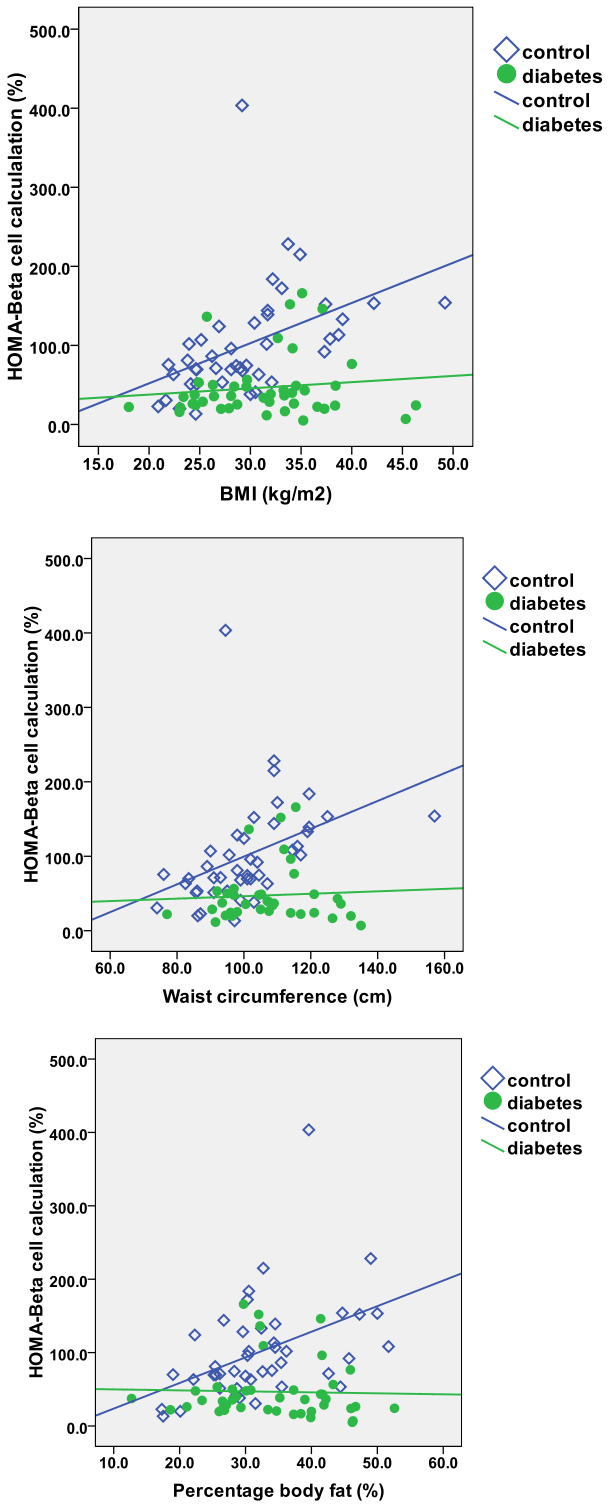


Figure 4-15: Scatterplots showing the relationship between HOMA-beta cell function and adiposity in participants with diabetes and control participants. BMI (CON, r=0.641, p<0.0001; DM, r=0.110, ns), waist circumference (CON r=0.630, p=0.0001; DM r=0.021, ns) and % body fat (CON r= 0.579, p<0.0001; DM r=0.583, ns). There were interactions on multiple linear regression analysis for BMI (p=0.026), waist circumference (p=0.059), and % body fat (p=0.009).

4.9 Lipid oxidation

4.9.1 Breath $^{13}CO_2$

Controls

In control participants, there were no correlations between breath $^{13}CO_2$ and measures of adiposity: BMI (r=0.127, p=0.405), waist circumference (r=0.099, p=0.519) and % body fat (r=-*0.025*, p=0.872) (Table 4.2).

Participants with diabetes

In participants with diabetes, there were no correlations between breath $^{13}CO_2$ and measures of adiposity: BMI (r=-*0.014*, p=0.928), a weak negative correlation with waist circumference (r=-*0.280*, p=0.098) and no correlation with % body fat (r=-*0.089*, p=0.572) (Table 4.2).

Relationship between breath $^{13}CO_2$ and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no differences in the relationships between breath $^{13}CO_2$ and BMI (p=0.300) or % body fat (p=0.527), **but for waist circumference there was a statistically significant interaction in the relationship between breath $^{13}CO_2$ and waist circumference (p=0.023)** in participants with diabetes compared to the control participants (Figure 4.16).

This data suggests that in participants with diabetes, those with a lower waist circumference oxidize more dietary triglyceride than those with a higher waist circumference but that this is not the case in control participants. This is likely to reflect the concentration of substrate available (^{13}C-PA labelled NEFA) which was higher in the diabetic participants with the lowest waist circumference (see later for further discussion).

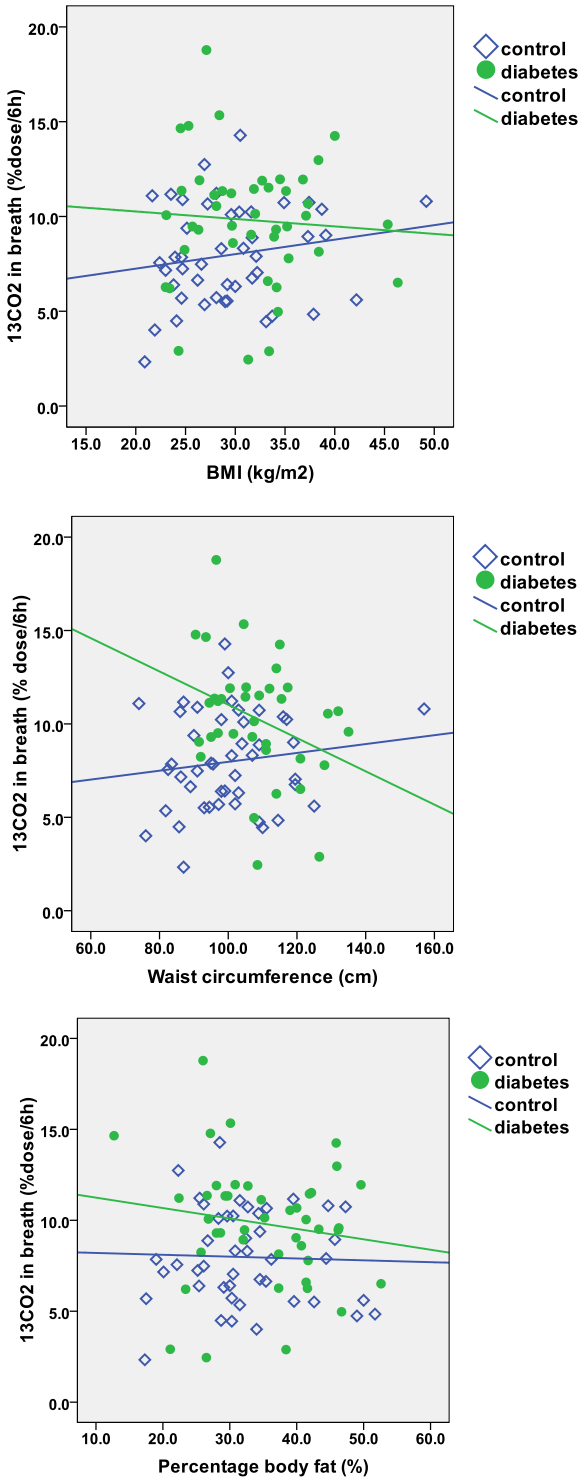


Figure 4-16: Scatterplots showing the relationship between $^{13}CO_2$ in the breath over 6 hours and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.127, ns; DM, r=*-0.014*, ns), waist circumference (CON r=0.099, ns; DM r=*-0.280*, p=0.098) and % body fat (CON r= *-0.025*, ns; DM r=*-0.089*, ns). On multiple linear regression analysis there was a statistically significant interaction in the relationship between $^{13}CO_2$ in the breath and waist circumference (p=0.023).

4.9.2 Fasting Fat Oxidation

Controls

In control participants there were positive correlations between fasting fat oxidation and BMI (r=0.314, p=0.040), waist circumference (r=0.302, p=0.049), but not with % body fat (r =0.057, p =0.717) (Table 4.2).

Participants with diabetes

In participants with diabetes, there was a positive correlation between fasting fat oxidation and BMI (r=0.322, p=0.043) but not waist circumference (r=0.290, p=0.102), or % body fat (r =0.180, p =0.267) (Table 4.2).

Relationship between fasting fat oxidation and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there was no significant difference in the relationship between fasting fat oxidation and BMI and (p=0.818), waist circumference (p=0.633) or % body fat (p=0.711) in participants with diabetes compared to the control participants (Figure 4.17).

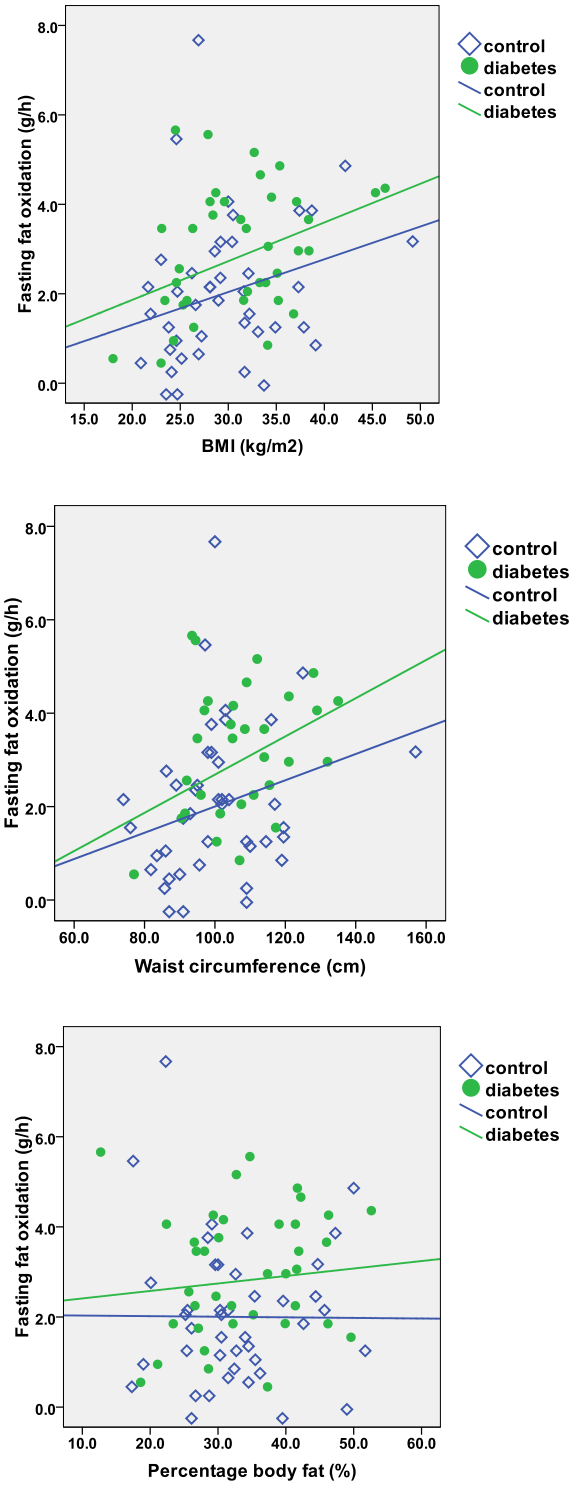


Figure 4-17: Scatterplots showing the relationship between fasting fat oxidation and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.314, p=0.040; DM, r=0.322, p=0.040), waist circumference (CON r=0.302, p=0.049; DM r=0.290, ns) and % body fat (CON r= 0.057, ns; DM r=0.180, ns). There were no interactions on multiple linear regression analysis.

4.9.3 AUC Fat Oxidation

Controls

In control participants, there were positive correlations between AUC fat oxidation and BMI (r=0.40, p=0.008) and waist circumference (r=0.305, p=0.047), but not with % body fat (r=0.197 p =0.206) (Table 4.2).

Participants with diabetes

In participants with diabetes, there was a positive correlation between AUC fat oxidation that approached statistical significance for both BMI (r=0.287, p=0.069) and waist circumference (r=0.326, p=0.060) but not with % body fat (r=0.232 p =0.144) (Table 4.2).

Relationship between AUC fat oxidation and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there was no significant difference in the relationship between AUC fat oxidation and BMI in participants with diabetes compared to the control participants (p=0.928), waist circumference (p=0.493) or % body fat (p=0.492) in participants with diabetes compared to the control participants (Figure 4.18).

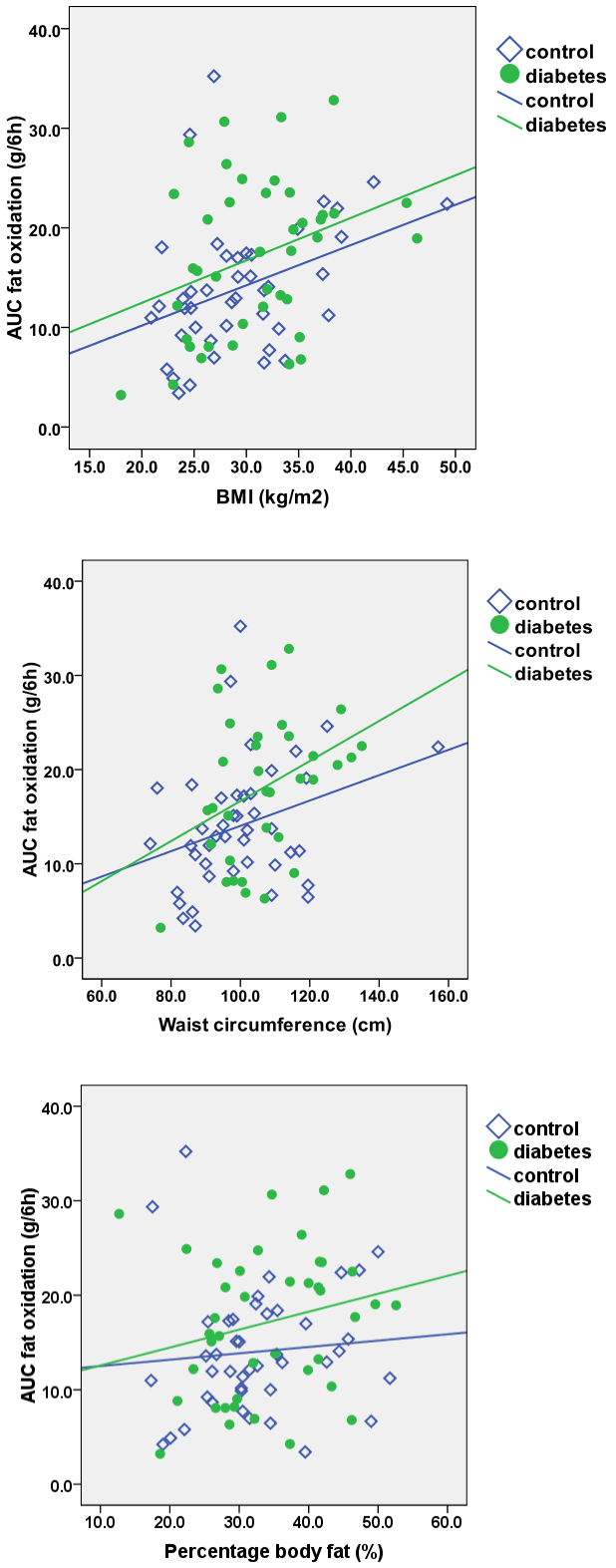


Figure 4-18: Scatterplots showing the relationship between AUC fat oxidation and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.400, p=0.008; DM, r=0.287, p=0.069), waist circumference (CON r=0.305, p=0.047; DM r=0.326, p=0.06) and % body fat (CON r= 0.197, ns; DM r=0.232, ns). There were no interactions on multiple linear regression analysis.

4.10 Carbohydrate oxidation

4.10.1 Fasting CHO oxidation

Controls

In control participants, there was no correlation between fasting CHO oxidation and BMI (r=0.239, p=0.123), a borderline correlation with waist circumference (r=0.296, p=0.054) and no correlation with % body fat (r=*-0.172*, p =0.270) (Table 4.2).

Participants with diabetes

In participants with diabetes, there were no correlations between fasting CHO oxidation and measures of adiposity: BMI (r=0.183, p=0.253), waist circumference (r=0.274, p=0.117) and % body fat (r=0.033, p=0.836) (Table 4.2).

Relationship between fasting CHO oxidation and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there was no significant difference in the relationship between fasting CHO oxidation and BMI (p=0.405), waist circumference (p=0.439) or % body fat in participants with diabetes compared to the control participants (p=0.484) (Figure 4.19).

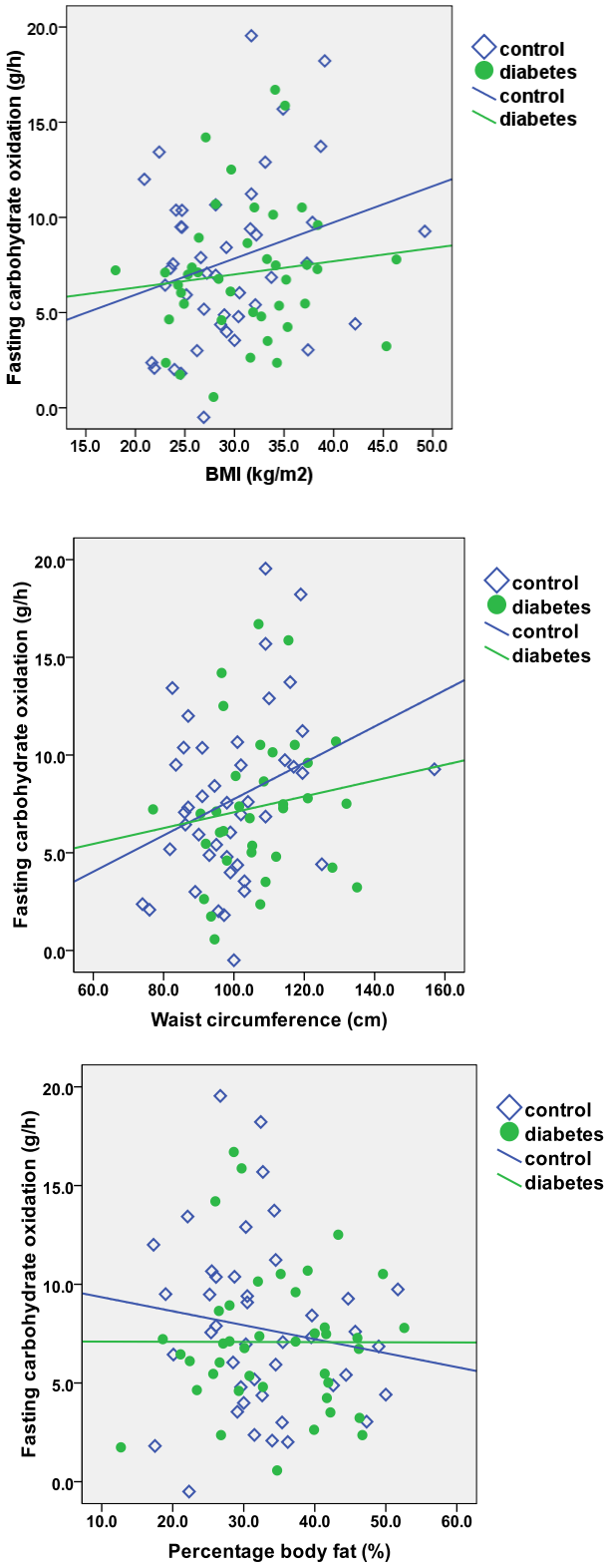


Figure 4-19: Scatterplots showing the relationship between fasting carbohydrate oxidation and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.239, ns; DM, r=0.183, ns), waist circumference (CON r=0.296, p=0.054; DM r=0.274, ns) and % body fat (CON r= *-0.172*, ns; DM r=0.033, ns). There were no interactions on multiple linear regression analysis.

4.10.2 AUC CHO Oxidation

Controls

In control participants there were significant positive correlations between AUC CHO oxidation and BMI (r=0.321, p=0.036) and waist circumference (r=0.416, p=0.006), but not with % body fat (r =*-0.219*, p=0.159) (Table 4.2).

Participants with diabetes

In participants with diabetes, there were no correlations between AUC CHO oxidation and measures of adiposity: BMI (r=0.123, p=0.445), waist circumference (r=0.121, p=0.496) and % body fat (r=*-0.161*, p=0.314) (Table 4.2).

Relationship between AUC CHO oxidation and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no significant differences in the relationships AUC CHO oxidation and BMI (p=0.377), waist circumference (p=0.185) or between % body fat (p=0.810) in participants with diabetes compared to the control participants (Figure 4.20).

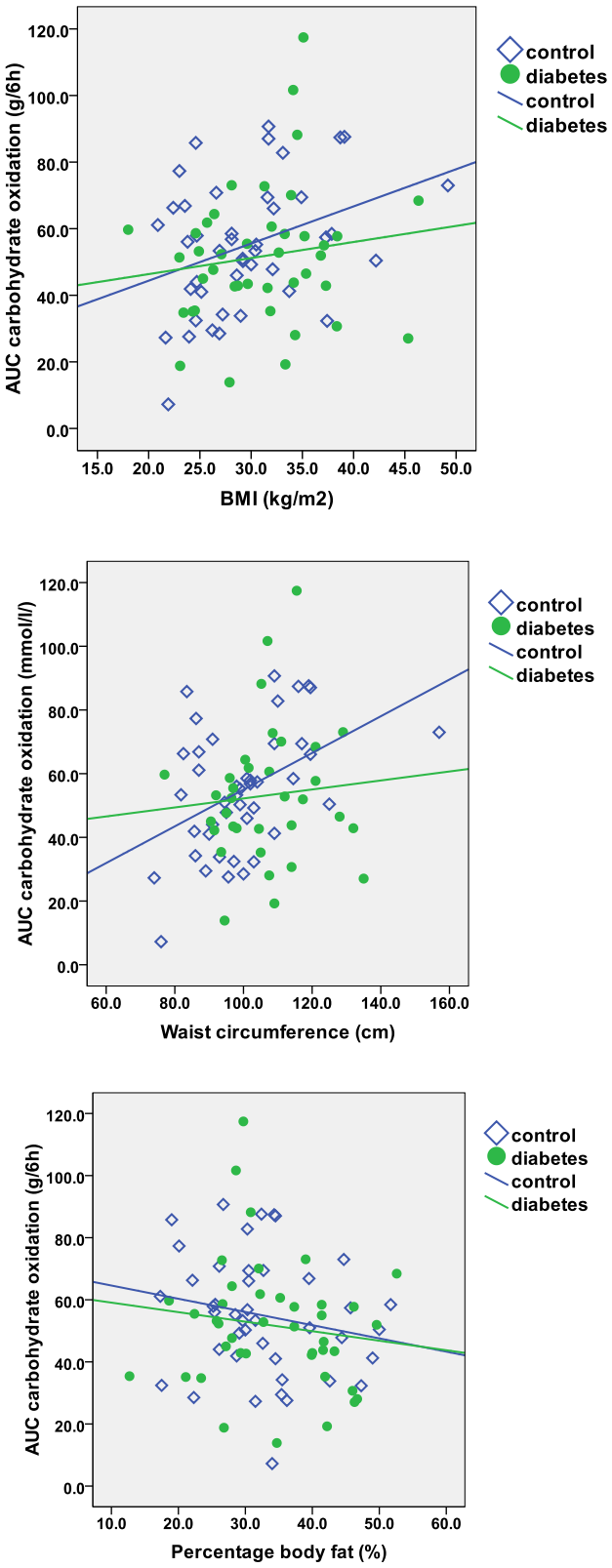


Figure 4-20: Scatterplots showing the relationship between AUC carbohydrate oxidation and adiposity in participants with diabetes and control participants. BMI (CON, r=0.321, p=0.036; DM, r=0.123, ns), waist circumference (CON r=0.416, p=0.006; DM r=0.121, ns) and % body fat (CON r= *-0.219*, ns; DM r=-0.161, ns). There were no interactions on multiple linear regression analysis.

4.11 Respiratory Quotient

Controls

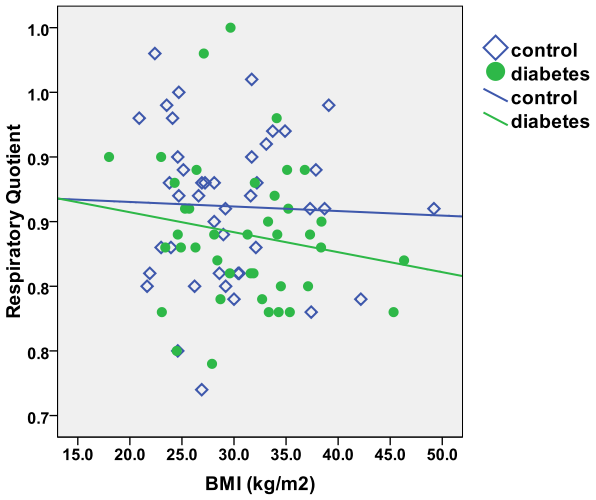
In control participants, there were no correlations between RQ and measures of adiposity. BMI (r=-*0.044*, p=0.781), waist circumference (r=-*0.010*, p=0.950) and % body fat (r=-*0.119*, p=0.446) (Table 4.2).

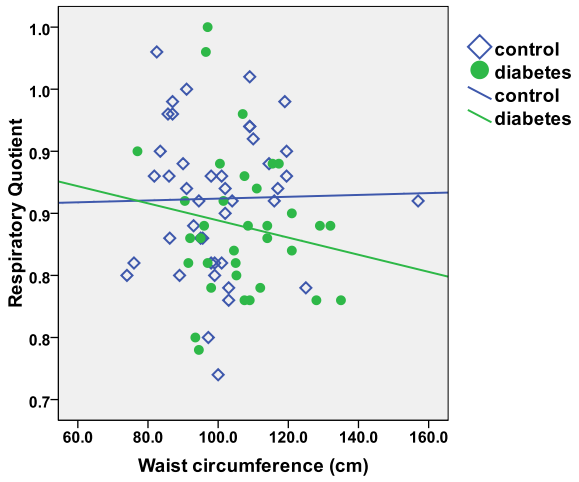
Participants with diabetes

In participants with diabetes, there were no correlations between RQ and measures of adiposity. BMI (r=-*0.145*, p=0.366), waist circumference (r=-*0.051*, p=0.774) and % body fat (r=-*0.143*, p=0.371) (Table 4.2).

Relationship between fasting RQ and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no significant differences in the relationships between fasting RQ and BMI (p=0.568), waist circumference (p=0.436), or % body fat (p=0.917) in participants with diabetes compared to the control participants (Figure 4.21).





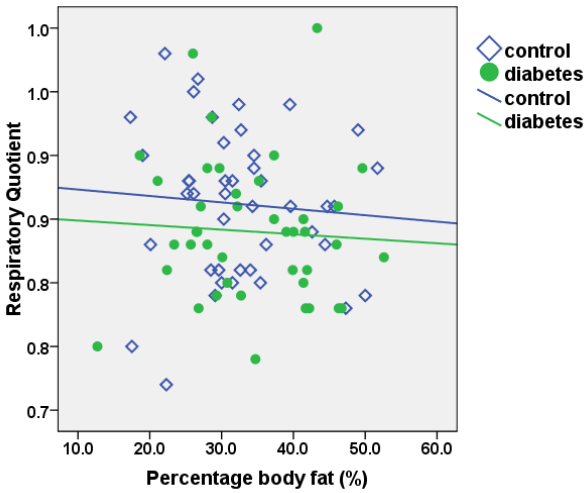


Figure 4-21: Scatterplots showing the relationship between fasting respiratory quotient and adiposity in participants with diabetes and control participants.

BMI (CON, r=-*0.044*, ns; DM, r=-*0.145*, ns), waist circumference (CON r=-*0.01*, ns; DM r=-*0.051*, ns) and % body fat (CON r= -*0.119*, ns; DM r=-*0.143*, ns). There were no interactions on multiple linear regression analysis.

4.12 Energy expenditure

4.12.1 Fasting energy expenditure

Controls

In control participants, there were strong positive correlations between fasting energy expenditure and BMI (r=0.551, p=<0.0001) and waist circumference (r=0.678, p=<0.0001), but not % body fat (r=-0.191, p=0.255) (Table 4.2).

Participants with diabetes

In participants with diabetes, there were strong positive correlations between fasting energy expenditure and BMI (r=0.493, p=0.001) and waist circumference (r=0.579, p=<0.0001), but not % body fat (r=0.079, p=0.620) (Table 4.2).

Relationship between fasting energy expenditure and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no significant differences in the relationships between fasting energy expenditure and BMI (p=0.340), waist circumference (p=0.558) or % body fat (p=0.286) in participants with diabetes compared to the control participants (Figure 4.22).

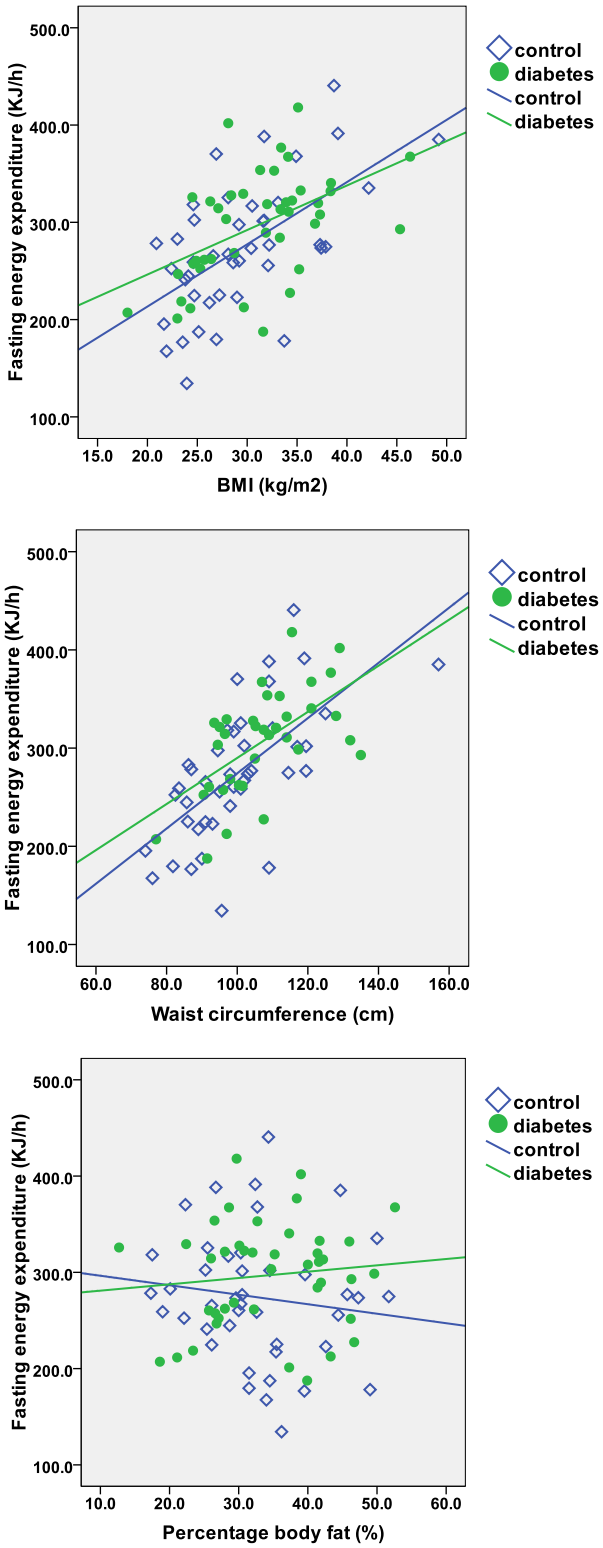


Figure 4-22: Scatterplots showing the relationship between fasting energy expenditure and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.551, p<0.0001; DM, r=0.493, p=0.001), waist circumference (CON r=0.678, p<0.0001; DM r=0.579, p<0.0001) and % body fat (CON r= -*0.191*, ns; DM r=0.079, ns). There were no interactions on multiple linear regression analysis.

4.12.2 AUC energy expenditure

Controls

In control participants, there were strong positive correlations between AUC energy expenditure and BMI (r=0.580, p=<0.0001) and waist circumference (r=0.655, p=<0.0001), but not with % body fat (r=-*0.155*, p=0.320) (Table 4.2).

Participants with diabetes

In participants with diabetes, there were strong positive correlations between AUC energy expenditure and BMI (r=0.490, p=0.001) and waist circumference (r=0.504, p=0.02), but not with % body fat (r=0.065, p=0.681) (Table 4.2).

Relationship between AUC energy expenditure and adiposity in participants with diabetes compared to controls

Using multiple linear regression analysis there were no significant differences in the relationships between AUC energy expenditure and BMI (p=0.347), waist circumference (p=0.338) or between % body fat (p=0.469) in participants with diabetes compared to the control participants (Figure 4.23).

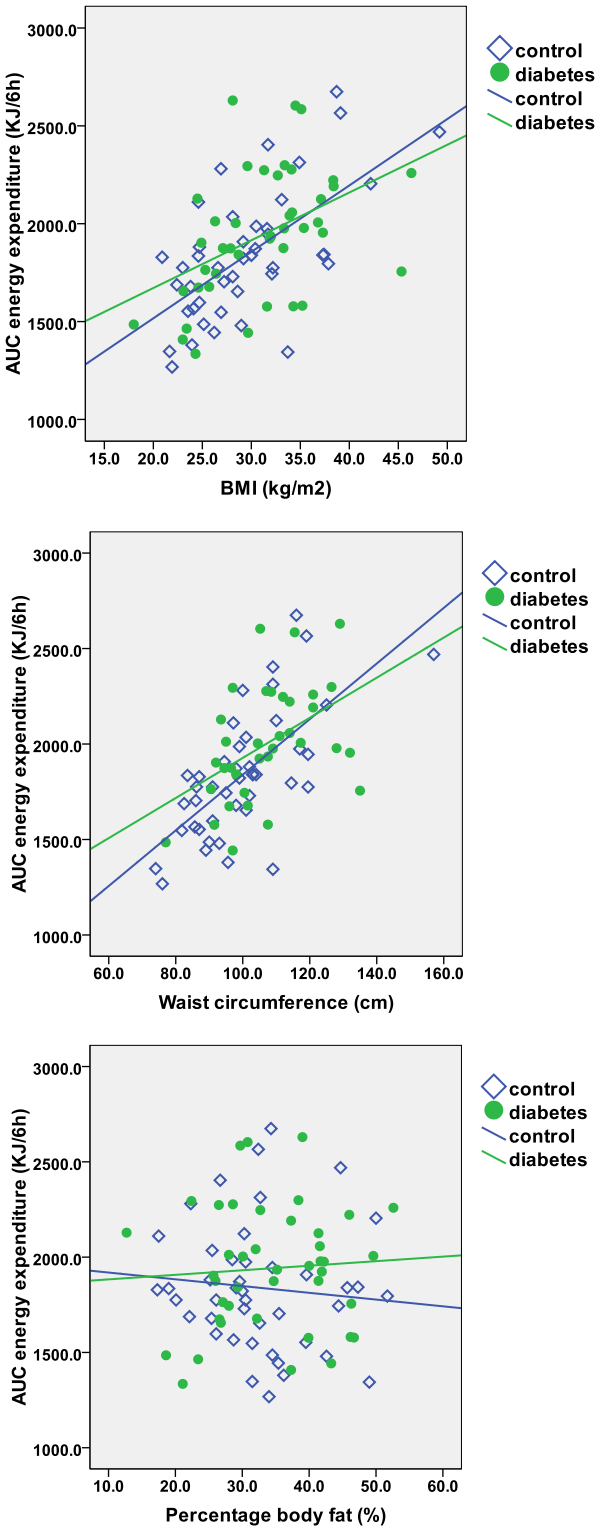


Figure 4-23: Scatterplots showing the relationship between AUC energy expenditure and adiposity in participants with diabetes and control participants.
BMI (CON, r=0.580, p<0.0001; DM, r=0.490, p=0.001), waist circumference (CON r=0.655, p<0.0001; DM r=0.504, p=0.02) and % body fat (CON r= *-0.155*, ns; DM r=0.065, ns). There were no interactions on multiple linear regression analysis.

		CONTROLS			PARTICIPANTS WITH DIABETES		
		BMI	**Waist**	**% body fat**	**BMI**	**Waist**	**% body fat**
Fasting TAG	Correlation coefficient	.340	.461	.023	.338	.339	.222
	p value	.022	.001	.881	.028	.043	.157
AUC TAG	Correlation coefficient	.324	.452	*-.018*	.165	.246	.131
	p value	.030	.002	.906	.284	.136	.397
INC AUC TAG	Correlation coefficient	.263*	.286	.081	*-.211**	*-.092*	*-.059*
	p value	.080	.057	.598	.180	.593	.712
^{13}C-PA in TAG AUC	Correlation coefficient	.288†	.296	.080	*-.210†*	*-.102*	*-.097*
	p value	.055	.048	.600	.172	.543	.529
Fasting NEFA	Correlation coefficient	.166	.095	.135	.252	.278	.330
	p value	.288	.544	.390	.099	.096	.029
NEFA AUC	Correlation coefficient	.278	.135	.326	.159	.124	.240
	p value	.068	.381	.031	.304	.465	.117
^{13}C-PA in NEFA AUC	Correlation coefficient	*-.112*	*-.311*	.366	*-.352*	*-.486*	*-.100*
	p value	.468	.040	.015	.018	.002	.513
Fasting glucose	Correlation coefficient	.214	.315	*-.119*	*-.020*	.040	.095
	p value	.164	.037	.443	.899	.813	.541
AUC glucose	Correlation coefficient	.151	.323	*-.037*	*-.164*	*-.162*	.091
	p value	.328	.032	.809	.287	.339	.558
Fasting insulin	Correlation coefficient	.676	.676	.471	.288	.304	.165
	p value	.0001	.0001	.001	.058	.068	.285
30 min insulin	Correlation coefficient	.364	.320	.137	.320	.441	.111
	p value	.014	.032	.369	.032	.006	.468
AUC insulin	Correlation coefficient	.497	.409	.511	.323	.299	.199
	p value	.001	.005	.0001	.030	.068	.191
HOMA-IR	Correlation coefficient	.717	.712	.448	.214	.270	.167
	p value	.0001	.0001	.003	.169	.112	.284
HOMA-Beta cell	Correlation coefficient	.641*	.630†	.529*	.110*	.021†	*-.086**
	p value	.0001	.0001	.0001	.481	.903	.583

Table 4-1: Spearman correlation coefficients between metabolic variables and measures of adiposity. Negative relationships are in *italics*. *Indicates a significant interaction in the relationship between metabolic variable and adiposity measure between diabetes and control participants, †indicates a borderline interaction (p>0.05 but <0.08).

		CONTROLS			PARTICIPANTS WITH DIABETES		
		BMI	**Waist**	**% body fat**	**BMI**	**Waist**	**% body fat**
$^{13}CO_2$ in breath over 6 hours	Correlation coefficient	.127	.099*	*-.025*	*-.014*	*-.280**	*-.089*
	p value	.405	.519	.872	.928	.098	.572
Fasting fat oxidation	Correlation coefficient	.314	.302	.057	.322	.290	.180
	p value	.040	.049	.717	.043	.102	.267
AUC fat oxidation	Correlation coefficient	.400	.305	.197	.287	.326	.232
	p value	.008	.047	.206	.069	.060	.144
Fasting CHO oxidation	Correlation coefficient	.239	.296	*-.172*	.183	.274	.033
	p value	.123	.054	.270	.253	.117	.836
AUC CHO oxidation	Correlation coefficient	.321	.416	*-.219*	.123	.121	-.161
	p value	.036	.006	.159	.445	.496	.314
Fasting RQ	Correlation coefficient	*-.044*	*-.010*	*-.119*	*-.145*	*-.051*	*-.143*
	p value	.781	.950	.446	.366	.774	.371
Fasting energy expenditure	Correlation coefficient	.551	.678	*-.191*	.493	.579	.079
	p value	.0001	.0001	.225	.001	.0001	.620
AUC energy expenditure	Correlation coefficient	.580	.655	*-.155*	.490	.504	.065
	p value	.0001	.0001	.320	.001	.002	.681

Table 4-2: Spearman correlation coefficients between fasting and postprandial substrate oxidation and energy expenditure and measures of adiposity in participants with diabetes and control participants.

Negative relationships are in *italics*. *Indicates a significant interaction in the relationship between metabolic variable and adiposity measure between diabetes and control participants ($p<0.05$).

	CONTROLS			PARTICIPANTS WITH DIABETES		
	BMI	**Waist**	**% body fat**	**BMI**	**Waist**	**% body fat**
Fasting TAG	+	++	↔	+	+	↔
AUC TAG	+	++	↔	↔	↔	↔
INC AUC TAG	+	+	↔	↔	↔	↔
^{13}C-PA in TAG AUC	+	+	↔	↔	↔	↔
Fasting NEFA	↔	↔	↔	+	+	+
NEFA AUC	+	↔	+	↔	↔	↔
^{13}C-PA in NEFA AUC	↔	-	+	-	--	↔
Fasting glucose	↔	+	↔	↔	↔	↔
AUC glucose	↔	+	↔	↔	↔	↔
Fasting insulin	++	++	++	+	+	↔
30 min insulin	+	+	↔	+	++	↔
AUC insulin	++	++	++	+	+	↔

Table 4-3: Summary of spearman correlations between metabolic variables and measures of adiposity; + r=0.25-0.39, ++ r=0.4-0.7 (positive correlation), --r=0.25-0.39, -- r=0.4-0.7 (negative correlation), ↔ p>0.05 (NB. borderline significant correlations are in blue)

	CONTROLS			PARTICIPANTS WITH DIABETES		
	BMI	**Waist**	**% body fat**	**BMI**	**Waist**	**% body fat**
$^{13}CO_2$ in breath over 6 hours	↔	↔	↔	↔	-	↔
Fasting fat oxidation	+	+	↔	+	↔	↔
AUC fat oxidation	++	+	↔	+	+	↔
Fasting CHO oxidation	↔	+	↔	↔	↔	↔
AUC CHO oxidation	+	++	↔	↔	↔	↔
Fasting RQ	↔	↔	↔	↔	↔	↔
Fasting energy expenditure	++	++	↔	++	++	↔
AUC energy expenditure	++	++	↔	++	++	↔

Table 4-4: Summary of spearman correlations between metabolic variables and measures of adiposity; + r=0.25-0.39, ++ r=0.4-0.7 (positive correlation), --r=0.25-0.39 (negative correlation), ↔ p>0.05 (NB. borderline significant correlations are in blue)

	BMI	Waist	% body fat
Fasting TAG			
p value for interaction	ns	ns	ns
AUC TAG			
p value for interaction	ns	ns	ns
INC AUC TAG			
p value for interaction	0.040	ns	ns
^{13}C-PA in TAG AUC			
p value for interaction	0.078	ns	ns
Fasting NEFA			
p value for interaction	ns	ns	ns
NEFA AUC			
p value for interaction	ns	ns	ns
^{13}C-PA in NEFA AUC			
p value for interaction	ns	ns	ns
Fasting glucose			
p value for interaction	ns	ns	ns
AUC glucose			
p value for interaction	ns	ns	ns
Fasting insulin			
p value for interaction	ns	ns	ns
30 min insulin			
p value for interaction	ns	ns	ns
AUC insulin			
p value for interaction	ns	ns	ns
HOMA-IR			
p value for interaction	ns	ns	ns
HOMA-%B			
p value for interaction	0.026	0.059	0.007

Table 4-5: Summary of differences in the relationship between the metabolic variables and measures of adiposity in participants with diabetes compared to control participants (interaction effects).

	BMI	Waist	% body fat
$^{13}CO_2$ in breath over 6 hours			
p value for interaction	ns	0.023	ns
Fasting fat oxidation			
p value for interaction	ns	ns	ns
AUC fat oxidation			
p value for interaction	ns	ns	ns
Fasting CHO oxidation			
p value for interaction	ns	ns	ns
AUC CHO oxidation			
p value for interaction	ns	ns	ns
Fasting RQ			
p value for interaction	ns	ns	ns
Fasting energy expenditure			
p value for interaction	ns	ns	ns
AUC energy expenditure			
p value for interaction	ns	ns	ns

Table 4-6: Summary of differences in the relationship between the lipid and carbohydrate oxidation variables and measures of adiposity in participants with diabetes compared to control participants (interaction effects).

4.13 Summary and discussion: comparison of the relationship between metabolic variables and adiposity in participants with diabetes and control participants

4.13.1 TAG

Fasting TAG correlated positively with both BMI and waist circumference in both participants with diabetes and control participants. There was no correlation with % body fat in either group. A previous study has shown weak positive correlations between fasting TAG and BMI (r=0.118) and waist circumference (r=0.138) in 539 participants with type 2 diabetes, and stronger correlations between fasting TAG and BMI (r=0.275) and waist circumference (r=0.303) in 100 participants with impaired fasting glucose, this study did not have a healthy control group and did not measure % body fat (91).

The measures of postprandial triglyceride correlated positively with BMI and waist circumference in controls but this relationship was not seen in the participants with diabetes. There were no relationships detected with % body fat in either group. There was a significant *interaction* on multiple linear regression analysis in the relationship between BMI and INC AUC TAG (p=0.040) and a trend to an interaction for ^{13}C-PA TAG AUC (p=0.078), both independent measures of meal derived TAG, in controls compared with participants with diabetes. This suggests that the relationship between BMI and dietary TAG metabolism may be different in participants with diabetes and control participants. There were similar relationships between postprandial TAG and waist circumference, ie there was a positive correlation between postprandial TAG and waist circumference in control participants which was absent in participants with diabetes but there were no significant interactions.

A potential confounding factor in these conclusions may be the influence of glycaemic control on TAG metabolism and the relationship between glycaemic control and adiposity. Poor glucose control in patients with diabetes is independently associated with impaired TAG metabolism. This is thought to be secondary to increased adipose tissue lipolysis and reduced lipoprotein lipase (LPL) action due to

insulin deficiency or impaired insulin action which causes elevated circulating NEFA and TAG concentrations (173). In clinical practice 'glycaemic control' is estimated by fasting glucose concentrations in the short term and by glycated haemoglobin (HbA1c) in the medium/long term (3-4 months).

In this study fasting glucose was measured in both participants with diabetes and control participants but HbA1c was only measured in participants with diabetes. In this study there was a positive correlation between BMI and glycated haemoglobin (HbA1c) (r=0.319, p=0.035). This suggests that the leanest participants had better glycaemic control compared with the more obese participants. There were positive realtaionships between fasting TAG and fasting glucose/HbA1c in participants with diabetes, but no association was found between ^{13}C-PA TAG AUC or INC AUC TAG and HbA1c or fasting glucose in participants with diabetes (Table 6.X). It is therefore unlikely that hyperglycaemia per se was the primary defect affecting postprandial TAG metabolism and this is unlikely to be an important confounding variable. This is discussed further in Chapter 6.

In this study the correlations between postprandial TAG and adiposity in control participants were strongest with waist circumference. This is in agreement with previous studies showing that central adiposity has an important effect on postprandial TAG metabolism in participants without diabetes (91;187). In previous studies, obese participants without diabetes had up to three times higher postprandial TAG concentrations than non-obese controls (104-111). An abnormality in chylomicron metabolism in obese participants has been described in some of these studies (104;106;108). There are few previous studies examining the effect of adiposity on postprandial TAG metabolism in participants with diabetes. One study in 539 participants with type 2 diabetes found weak positive correlations between postprandial TAG at 90 minutes post a standard meal and BMI (r=0.108) and waist circumference (r=0.123), but stronger correlations between postprandial TAG and BMI (r=0.246) and waist circumference (r=0.266) in participants with impaired fasting glucose, but not diabetes (91). Other studies of the effect of adiposity on postprandial TAG metabolism in participants with diabetes have reported conflicting results. Postprandial triglyceride responses to a standard oral fat challenge was studied in forty-four participants who were divided after an OGTT into normal

glucose tolerance, impaired fasting glucose, impaired glucose tolerance and type 2 diabetes. In this study postprandial TAG responses did not correlate with body mass index, or waist circumference. In another study by the same author, TAG AUC correlated significantly with BMI (r=0.7), but not with waist-hip ratio. These relationships were described for the participants with diabetes and control groups combined and not separately (93;94).

4.13.2 NEFA

Fasting and AUC NEFA

NEFA circulating after an overnight fast is derived from lipolysis of stored TAG in adipose tissue. In this study there was no relationship between fasting NEFA and any measure of adiposity in control participants. In participants with diabetes there was a significant correlation between fasting NEFA and % body fat and there were weak non-significant relationships with BMI and waist circumference. The lack of a relationship between fasting NEFA and adiposity in control participants may be surprising when compared to much of the published literature (188), but is in agreement with recent findings in insulin resistant men in a study by Bickerton et al, where fasting NEFA was not higher in insulin resistant men who had a higher BMI and higher fasting insulin concentration compared to the healthy control group (118). Increasing adiposity may to contribute to higher fasting NEFA in participants with diabetes, possibly due to greater insulin resistance to hormone sensitive lipase (HSL) in obese participants with diabetes, or possibly due to relative insulin deficiency in participants with diabetes. The suggestion that lipolysis rates are only elevated in obese patients with diabetes, but not those without diabetes has been mooted previously (189). Although this may be thought to be unlikely due to insulin resistance in obese patients, it could be speculated that the degree of elevation of lipolysis is not as high in obese patients with diabetes compared to patients without diabetes, this is supported by recent studies by McQuaid et al(190).

^{13}C-PA NEFA AUC

^{13}C-PA NEFA AUC is derived from lipoprotein lipase (LPL) mediated lipolysis of circulating dietary derived TAG. ^{13}C-PA NEFA AUC correlated *negatively* with BMI and waist circumference in participants with diabetes. In control participants there was a negative relationship with waist circumference but a positive relationship with % body fat. The observation that dietary derived ^{13}C-PA NEFA AUC concentrations were lower with increasing BMI and waist circumference in participants with diabetes suggests that, in participants with diabetes, there is greater entrapment of dietary fat in individuals with more adipose tissue. A central distribution of adipose tissue may also be important. This may be directly secondary to the larger adipose tissue mass, and/or greater adipose tissue blood flow or it may be that individuals with more adipose tissue have more efficient postprandial NEFA entrapment due to an associated factor, for example higher insulin concentrations, as NEFA entrapment is an insulin sensitive process (89). A recent publication suggests that re-esterification of NEFA into TAG after adipose tissue uptake may also be an important mechanism in the control of NEFA storage in adipose tissue (191). This process may be up-regulated in participants with diabetes individuals with more adipose tissue. The converse argument may of course be true, that is lean participants with diabetes, may be *less* good at entrapping dietary derived NEFA due to relative insulin deficiency or a defect with adipose tissue storage capacity function (eg late onset autoimmune diabetes with insulin deficiency or unrecognised lipodystrophy). The latter hypothesis is supported by Tan et al who showed reduced entrapment of dietary derived ^{13}C-PA NEFA in a patient with partial lipodystrophy and type 2 diabetes (88). An alternative explanation for the negative correlation between adiposity and ^{13}C-PA NEFA AUC could be that participants with diabetes with higher BMI or waist circumference oxidise more dietary NEFA. In this study there was a borderline positive correlation in participants with diabetes between AUC fat oxidation and BMI/waist circumference, but there was a borderline *negative* correlation between $^{13}CO_2$ in the breath over 6 hours and waist circumference (r=-0.28, p=0.09). These results do not suggest that dietary fat oxidation rates are increased significantly in participants with diabetes with a higher waist circumference.

4.13.3 Fat oxidation

There was a positive correlation between fasting fat oxidation measured by indirect calorimetry and BMI in participants with diabetes and control participants. There was a positive correlation between AUC fat oxidation measured by indirect calorimetry and BMI in control participants. This relationship was of borderline significance in the participants with diabetes. Similar relationships were found between AUC fat oxidation and waist circumference. There were no relationships between fat oxidation and % body fat. The relationship between measures of adiposity and fat oxidation measured by indirect calorimetry did not differ between the participants with diabetes and control participants, ie there were no interactions. The direct measure of oxidation of dietary derived triglyceride ($^{13}CO_2$ in breath over 6 hours) only showed a weak *negative* correlation with waist in participants with diabetes, but there were no significant correlations in the control subjects or for BMI or % body fat. There was a significant *interaction* for $^{13}CO_2$ in breath and waist circumference (p=0.023). This suggests that the relationship between dietary fat oxidation and waist circumference was different in participants with diabetes and control participants.ie the participants with diabetes with the lowest waist circumference had the tendency to oxidise the highest amount of 13-labelled triglyceride, but this was not shown in the control participants. This may have been due to the higher concentration of substrate for oxidation (^{13}C-PA NEFA) in the participants with diabetes with the lowest waist circumference. There is a possibility of a degree of 'fat failure' in participants with diabetes who have a low waist circumference, who are unable to efficiently store postprandial NEFA, and therefore oxidize more to compensate for this. In this study this phenomenon is not seen in the control participants.

4.14 Conclusions and hypothesis for differences in the relationship between adiposity and postprandial triglyceride metabolism in participants with diabetes and control participants

In control participants increasing BMI/waist circumference was associated with increasing insulin resistance as estimated by fasting insulin concentrations or HOMA calculation (Table 4.1). Insulin resistance contributes to higher TAG production by

the liver in the fasting state and impaired clearance of dietary TAG hence elevated fasting and postprandial TAG with increasing obesity in control participants. In control participants however the higher insulin concentrations associated with increasing BMI/waist circumference facilitate adequate regulation of fasting NEFA and together with the increased adipose tissue depot, facilitates efficient disposal of postprandial NEFA so an adverse effect of increasing adiposity on NEFA metabolism is not seen in control participants. In participants with diabetes however, fasting and postprandial TAG metabolism is impaired at *all levels* of BMI/waist circumference due to a combination of more severe insulin resistance and relative insulin deficiency. This is especially evident in the postprandial state due to impairment of the first phase insulin response (Table 3.8, Figure 3.6, Figure 4.12). There are therefore higher fasting and postprandial TAG in participants with diabetes compared to control participants all levels of adiposity. In fact, participants with diabetes with a higher BMI/waist circumference may be protected in the postprandial state by their greater adipose tissue stores as these patients possess more efficient entrapment of dietary fatty acids (Table 4.1, Figure 4.10). Alternatively or in addition, lean participants with diabetes may represent a subset of the heterogeneous population of patients with type 2 diabetes, who may have underlying lipodystrophy, and/or more advanced beta cell failure. They may for example have undiagnosed late onset Type 1 diabetes- sometimes known as late onset autoimmune diabetes (LADA), or maturity onset diabetes of the young/monogenic diabetes (MODY), although these diagnoses are relatively rare. These mechanisms are discussed further in Chapter 6.

4.15 Differences in results found with different measures of adiposity

In both participants with diabetes and control participants, the relationships between the metabolic variables and both BMI and waist circumference were generally concordant and of similar order of magnitude. In control participants the relationships between TAG and glucose and waist circumference were stronger than those found with BMI, supporting the concept of an important effect of central adiposity on impaired lipid and glucose metabolism due to increased metabolic activity of central (‘visceral’) fat (67). In participants with diabetes and control

participants, the negative relationships with ^{13}C-PA NEFA AUC were also strongest with waist circumference.

% body fat had few correlations with plasma lipids or with lipid or carbohydrate oxidation in participants with diabetes or control participants (except positive relationships with fasting NEFA in participants with diabetes and a positive relationship with ^{13}C-PA NEFA AUC and NEFA AUC in controls). There is much less information in the published literature regarding the relationship between metabolic variables and % body fat as this measurement is not frequently measured in routine clinical practice. % body fat measurement using bioelectrical impedance does not include any measure of lean mass which may have affected the results found for BMI and waist circumference. In this study percentage (%) body fat was measured using bioelectrical impedance (Bodystat 1500, Isle of Man, UK). The Bodystat 1500 works by passing a battery generated signal through the whole body and measures the bioelectrical impedance at a fixed frequency of 50 kHz. The model used was a lightweight, hand-held, battery operated device which had two main cable leads and each lead has two crocodile/alligator clips. These clips were attached to tabs on the sticky electrodes which were attached on the skin of the participant's right hand and foot. The subject's gender, age, height and weight were then entered into the device. A complete body composition analysis was displayed on the screen within three seconds with readings for percentage body fat, lean body mass and total body water. Bioelectrical impedance techniques are not the most accurate measure of % body fat and the impedance technique used does not specify where the fat is located in the body. It would be interesting to analyse the relationships of the metabolic measurements measured using DXA measures of % body fat which were not available in our centre at the time of the study. Other possible explanations for the lack of associations between the metabolic variables with % body fat are reduced power because the normal healthy distribution of % body fat is different in men and women. Further discussion for the non-concordant results between BMI/waist circumference and % body fat can be found in Chapter 7.

Chapter 5 Comparison of triglyceride metabolism between the participants in highest and lowest quartiles of body mass index

5.1 Introduction

Chapter 4 described the finding that the participants with diabetes with a low BMI or waist circumference may have equal or worse impairment of postprandial triglyceride metabolism than the participants with diabetes with greater BMI or wasit circumference. This may explain why in Chapter 3, no significant difference was found between participants with diabetes and control participants in area under the curve (AUC) ^{13}C-palmitic acid (^{13}C-PA) labelled dietary triglyceride (TAG) (Table 3.5, Figure 3.2). In the current chapter a post hoc analysis has been performed, where triglyceride metabolism in the participants with diabetes and control participants in the lowest and highest quartiles of body mass index (BMI) have been compared. Quartiles were calculated so that the 'cut off' value for participants and controls were the same and therefore directly comparable in terms of BMI. This approach has the disadvantage of different numbers of participant being included in the quartiles. The lowest quartile of BMI included participants with BMI 18.0-25.3 kg/m^2 and highest quartile of BMI included participants with BMI 34.2-49.2 kg/m^2. The mean BMI in participants with diabetes in the lowest quartile was 23.5kg/m^2 and in controls was also 23.5kg/m^2 (p=0.95). The mean BMI in highest quartile in participants with diabetes was 37.9 kg/m^2 and in controls was 39.6 kg/m^2 (p=0.35) (Table 5.1).

5.2 Differences in metabolism between the lowest and highest BMI quartiles in participants with diabetes, compared to differences in metabolism between the lowest vs highest BMI quartiles in control participants

5.2.1 Fasting lipids

Participants with diabetes

Fasting TAG was significantly lower in participants with diabetes in the lowest BMI quartile compared with participants with diabetes in the highest BMI quartile 1.7 (0.9-2.4) vs 2.5 (1.9-3.8) mmol/l, p=0.03. (Table 5.1, Figure 5.1). Fasting non-esterified fatty acids (NEFA) were numerically lower in participants with diabetes in

the lowest BMI quartile compared with participants with diabetes in the highest BMI quartile but this did not reach statistical significance 176.1(155.3-196.7) vs 200.2 (175.3-241.2) umol/l, p=0.08. (Table 5.1, Figure 5.3).

Controls

Fasting TAG (1.0 (0.7-1.2) vs 2.0 (1.1-2.4) mmol/l, p=0.06) and fasting NEFA (111.3 (93.3-135.8) vs 136.7 (95.8-192.5) umol/l, p=0.23) were both numerically but not significantly lower in the controls in the lowest BMI quartile compared to the controls in the highest BMI quartile (Table 5.1, Figures 5.1 and 5.3).

5.2.2 Postprandial lipids

Participants with diabetes

^{13}C - palmitic acid in the triglyceride fraction AUC (^{13}C-PA TAG AUC), was numerically higher in the participants with diabetes in the lowest compared with the highest BMI quartile, but this did not reach statistical significance 77.1(38.6-104.3) vs 52.1(33.2-82.4) ug/ml/6h, p=0.25. Incremental (INC) TAG AUC (6.2 (1.5-8.8) vs 3.4(1.5-6.4) mmol/l/6h, p=0.41) showed a similar trend. These are both measures of study meal derived TAG.(Table 5.1, Figure 5.1 and Figure 5.2).

There was no significant difference in AUC NEFA in the participants with diabetes in the lowest BMI quartile compared to the participants with diabetes in the highest BMI quartile (542.2 (333.4-657.4) vs 542.9 (487.0-774.3) umol/l/6h, p=0.34) (Table 5.1, Figure 5.3). ^{13}C - palmitic acid in the non-esterified fatty acid fraction AUC (^{13}C-PA NEFA AUC) (study meal derived NEFA) was significantly higher in the participants with diabetes in the lowest BMI quartile compared to the participants with diabetes in the highest BMI quartile (3.1(2.8-4.2) vs 2.3(1.7-2.8) μg/ml/6h, p=0.01) (Table 5.1, Figure 5.4).

Controls

^{13}C-PA TAG AUC was numerically lower in the controls in the lowest BMI quartile compared to the control participants in the highest BMI quartile but this did not

reach statistical significance (34.2 (22.6-44.5) vs 50.1 (23.7-80.6) ug/ml/6h, p=0.34), (Table 5.1, Figure 5.4). INC AUC TAG was numerically, but not significantly lower in controls in the lowest BMI quartile compared to those in the highest BMI quartile (2.1(1.0-3.1) vs 4.3 (1.4-10.6) mmol/l/6h, p=0.15), (Table 5.1, Figure 5.1). This was an opposite trend to that seen in the participants with diabetes.

AUC NEFA was significantly lower in the controls in the lowest BMI quartile than the controls in the highest BMI quartile (362.3(289.5-426.6) vs 477.6(383.0-660.9) umol/l/6h), (p=0.01) (Table 5.1, Figure 5.3).

^{13}C-PA NEFA AUC was not significantly different in the controls in the lowest BMI quartile compared to those in the highest BMI quartile (2.1(1.2-3.1) vs 2.2 (1.4-2.3), p=0.89) (Table 5.1, Figure 5.4). This was different to the participants with diabetes, where the lowest BMI quartile had the highest ^{13}C-PA NEFA AUC.

5.2.3 Fat oxidation

Participants with diabetes

Fasting (2.16±1.63 vs 3.37±1.09 g/h, p=0.06) and AUC fat oxidation (13.35±8.52 vs 19.22±6.54 g/6h, p=0.09) were numerically lower (both borderline significance) in the participants with diabetes in the lowest BMI quartile than the participants with diabetes in the highest BMI quartile (Table 5.3). There was no significant difference in oxidation of dietary ^{13}C-PA TAG in the breath in the participants with diabetes in the lowest BMI quartile compared to the participants with diabetes in the highest BMI quartile (9.3±4.2 vs 10.0 ± 2.6 % dose/6h, p=0.66) (Table 5.3, Figure 5.5).

Controls

Fasting (1.2±1.6 vs 2.7±1.5 g/h, p=0.05) and AUC fat oxidation (11.3±6.6 vs 19.7±4.4 g/6h, p=0.005) measured by indirect calorimetry were significantly lower in the controls in the lowest BMI quartile compared to those in the highest BMI quartile. There was no difference in ^{13}C-PA oxidation between controls in the lowest BMI quartile and controls in the highest BMI quartile (7.4±2.7 vs 8.9±2.4 %dose/6h, p=0.20) (Table 5.3, Figure 5.5).

5.2.4 Insulin

Participants with diabetes

Fasting insulin (8.2 (4.9-13.7) vs14.2 (193.0-396.8) μU/ml, p=0.04), 30 minute insulin (30.0 (23.7-35.1) vs 45.7 (22.6-67.8) μU/ml, p=0.08) and AUC insulin (163.0 (153.3-239-1) vs 274.5 (193.0-396.8) μU/ml, p=0.04) were lower in the participants with diabetes in the lowest BMI quartile compared with the participants with diabetes in the highest BMI quartile (Table 5.2, Figure 5.6).

Controls

Fasting (5.6 (3.2-7.8) vs 14.6 (12.2-18.1) μU/ml, p<0.0001), 30 minute (58.6 (35.1-75.4) vs 103.6 (54.0-176.5) μU/ml, p=0.02) and AUC insulin (161.9 (118.8-272.5) vs 362.9 (217.9-477.4) μU/ml, p=0.005) were all significantly lower in the controls in the lowest BMI quartile compared to the controls in the highest BMI quartile (Table 5.2, Figure 5.6).

5.2.5 HOMA

Participants with diabetes

There was no difference in HOMA-IR or HOMA-%B in the participants with diabetes in the lowest BMI quartile compared with the participants with diabetes in the highest BMI quartile (3.0 (1.8-8.6) vs 6.4 (5.5-7.2) , p=0.10) and (26.2% (21.8-36.2) vs 26.4% (21.1-62.7), p=0.62) respectively. (Table 5.2)

Controls

HOMA-IR and HOMA-%B were both significantly lower in the controls in the lowest BMI quartile compared to the controls in the highest BMI quartile (1.1 (0.8-1.9) vs 3.9 (2.8-4.8), p<0.0001) and 63.3% (26.7-78.3) vs 142.6% (109.5-153.8), p<0.0001) respectively. (Table 5.2)

5.3 Comparison of metabolism in participants with diabetes vs control participants in the lowest BMI quartile

5.3.1 Fasting lipids

Fasting TAG was significantly higher in participants with diabetes in the lowest BMI quartile compared to control participants in the lowest BMI quartile (1.7 (0.9-2.4) vs 1.0 (0.7-1.2) mmol/l, p=0.05) (Table 5.1, Table 5.4, Figure 5.1). Fasting NEFA was also higher in the participants with diabetes in the lowest BMI quartile compared with the control participants in the lowest BMI quartile (176.1(155.3-196.7) vs 111.3 (93.3-135.8) umol/l, p=0.002) (Table 5.1, Table 5.4, Figure 5.3).

5.3.2 Postprandial lipids

^{13}C-PA TAG AUC (a measure of dietary TAG) was significantly higher in the participants with diabetes in the lowest BMI quartile compared with the control participants in the lowest BMI quartile (77.1 (38.6-104.3) vs 34.2 (22.6-44.5) μg/ml/6h, p=0.01). INC AUC TAG was numerically higher in the participants with diabetes compared with the control participants in the lowest BMI quartile and this approached statistical significance (6.2 (1.5-8.8) vs 2.1(1.0-3.1) mmol/l/6h, p=0.07). (Table 5.1, Table 5.4, Figure 5.1 and Figure 5.2).

^{13}C-PA NEFA AUC (dietary derived NEFA) was significantly higher in participants with diabetes in the lowest BMI quartile compared with controls in the lowest BMI quartile (3.1(2.8-4.2) vs 2.1(1.2-3.1) μg/ml/6h, p=0.04) (Table 5.1, Table 5.4, Figure 5.4).

5.3.3 Insulin

There was no significant difference in fasting insulin was higher between the participants with diabetes vs controls in the lowest BMI quartile (8.2 (4.9-13.7) vs 5.6 (3.2-7.8) μU/ml, p=0.10). However, 30 minute insulin was significantly *lower* in the participants with diabetes in the lowest BMI quartile vs the controls in the lowest BMI quartile (30.0 (23.7-35.1) vs 58.6 (35.1-75.4) μU/ml, p=0.01). This confirms, as expected, that the participants with diabetes in the lowest BMI quartile are insulin

deficient in the early postprandial state compared to the controls in the lowest BMI quartile (Table 5.2, Table 5.6, Figure 5.6).

5.3.4 HOMA

HOMA-IR was significantly higher in the participants with diabetes in the lowest BMI quartile compared with the control participants in the lowest BMI quartile (3.0 (1.8-8.6) vs 1.1 (0.8-1.9), p=0.006).

HOMA-%B was was significantly lower in the participants with diabetes in the lowest BMI quartile compared with the control participants in the lowest BMI quartile (26.2(21.8-36.2) vs 63.3(26.7-78.3), p=0.04). This confirms that, as expected, the participants with diabetes in the lowest BMI quartile were more insulin resistant and insulin deficient in the fasting state compared with the control participants in the lowest BMI quartile (Table 5.2, Table 5.6).

5.4 Comparison of metabolism in participants with diabetes vs control participants in the highest BMI quartile

5.4.1 Fasting lipids

Fasting TAG was numerically higher in the participants with diabetes in the highest quartile of BMI compared to the control participants in the highest quartile of BMI and this approached statistical significance (2.5 (1.9-3.8) vs 2.0 (1.1-2.4) mmol/l, p=0.08). Fasting NEFA was significantly higher in the participants with diabetes in the highest quartile of BMI compared to the control participants in the highest quartile of BMI (200.2 (175.3-241.2) vs 136.7 (95.8-192.5) umol/l , p=0.02). (Table 5.1, Table 5.5 Figure 5.1- Figure 5.4).

5.4.2 Postprandial lipids

There were no detectable differences in any measure of postprandial TAG or NEFA between the participants with diabetes and control participants in the highest quartile of BMI (Table 5.1, Table 9.7 Figure 5.1- Figure 5.3). ^{13}C-PA TAG AUC 52.1(33.2-

82.4) vs 50.1(23.7-80.6) μg/ml/6h, p=0.63), INC AUC TAG 3.4 (1.5-6.4) vs 4.3 (1.4-10.6), p=0.71 and ^{13}C-PA NEFA AUC (2.3 (1.7-2.8) vs 2.2 (1.4-2.3), p=0.35), in diabetes vs control participants respectively (Table 5.1, Table 5.5 Figure 5.1-Figure 5.4).

5.4.3 Insulin

There were no detectable differences in fasting insulin between the participants with diabetes and control participants in the highest quartile of BMI (14.2 (193.0-396.8) vs 14.6 (12.2-18.1) μU/ml, p=0.74). 30 minute insulin was significantly *lower* in the participants with diabetes vs the controls in the highest BMI quartile (45.7 (22.6-67.8) vs 103.6 (54.0-176.5) μU/ml, p=0.01) (Table 5.2, Table 5.7, Figure 5.6). This suggests that, as expected, the participants with diabetes in the highest BMI quartile are more insulin deficient in the postprandial state than the controls in the highest BMI quartile.

5.4.4 HOMA

HOMA-IR was significantly higher in the participants with diabetes in the highest BMI quartile compared with the control participants in the highest BMI quartile (6.4 (5.5-7.2) vs 3.9 (2.8-4.8), p=0.002). (Table 5.2, Table 5.7).

HOMA-%B was was significantly lower in the participants with diabetes in the highest BMI quartile compared with the control participants in the highest BMI quartile (26.4 (21.1-62.7) vs 142.6 (109.5-153.8) p=0.003). This confirms that, as expected, the participants with diabetes in the highest BMI quartile were more insulin resistant and insulin deficient in the fasting state compared with the control participants in the highest BMI quartile. (Table 5.2, Table 5.7).

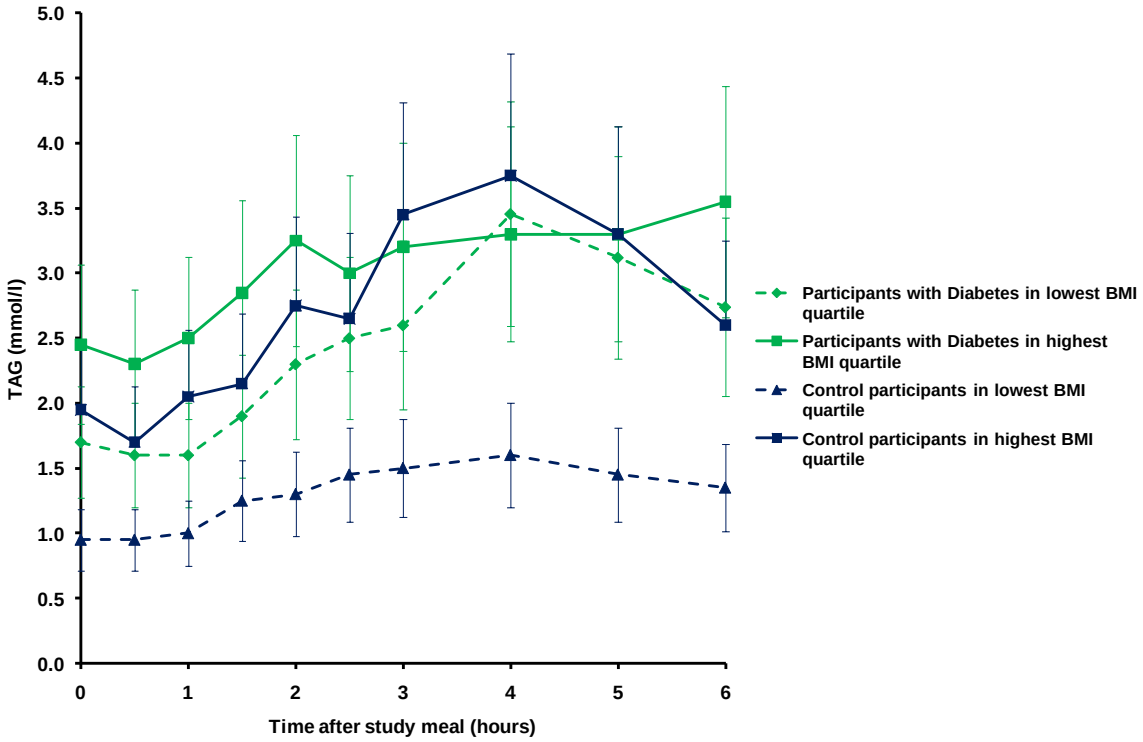


Figure 5-1: Plasma triglyceride before and after the standard meal in participants with diabetes (DM) (green) in lowest and highest quartiles of BMI and control (CON) (blue) participants in lowest and highest quartiles of BMI (median (interquartile range)).

Fasting TAG was significantly lower in DM in the lowest BMI quartile compared with DM in the highest BMI quartile, (p=0.03). Fasting TAG, was numerically lower and showed borderline significance in the CON in the lowest BMI quartile compared to the CON in the highest BMI quartile (p=0.06).

Incremental (INC) TAG AUC was numerically, but not significantly, *higher* in DM in the lowest compared with DM in the highest BMI quartile, (p=0.41). INC AUC TAG was numerically, but not significantly *lower* in CON in the lowest BMI quartile compared to CON in the highest BMI quartile (p=0.15).

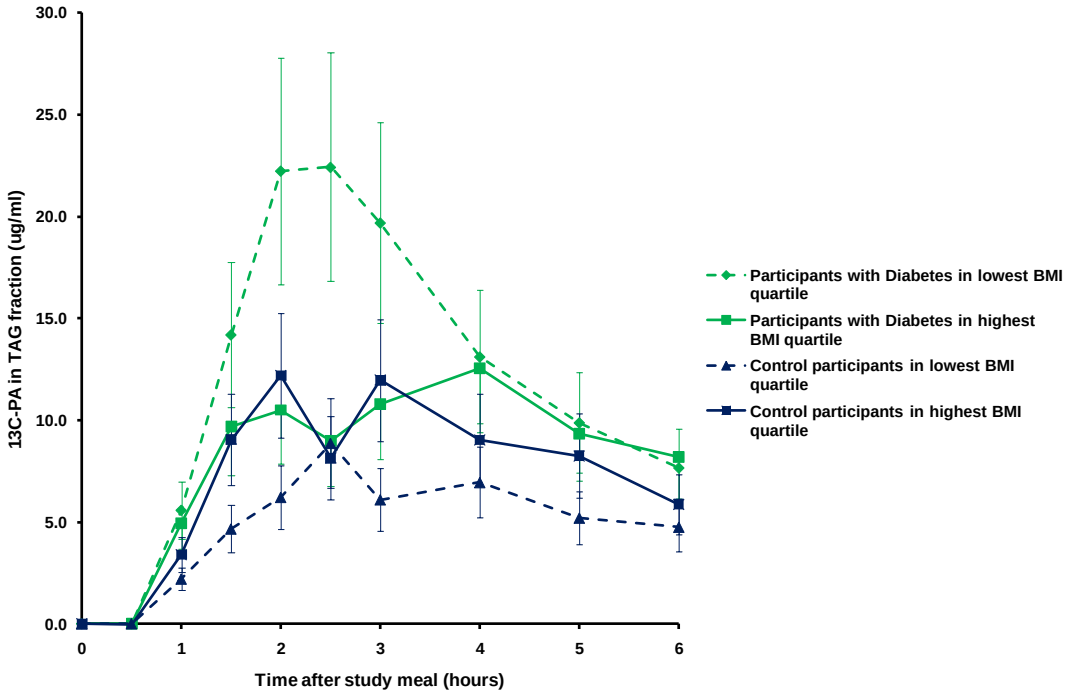


Figure 5-2: ^{13}C-palmitic acid in triglyceride fraction (^{13}C-PA in TAG) following standard meal in participants with diabetes (DM) (green) in lowest and highest quartiles of BMI, and control (CON) (blue) participants in the lowest and highest quartiles of BMI (median (interquartile range)).

^{13}C-PA TAG AUC was numerically, but not statistically, *higher* in DM in the lowest compared with the highest BMI quartile (p=0.25). ^{13}C-PA TAG AUC was numerically, but not statistically, *lower* in CON in the lowest BMI quartile compared to CON in the highest BMI quartile (p=0.34). ^{13}C-PA TAG AUC was significantly higher in DM in the lowest BMI quartile compared with CON in the lowest BMI quartile (p=0.01).

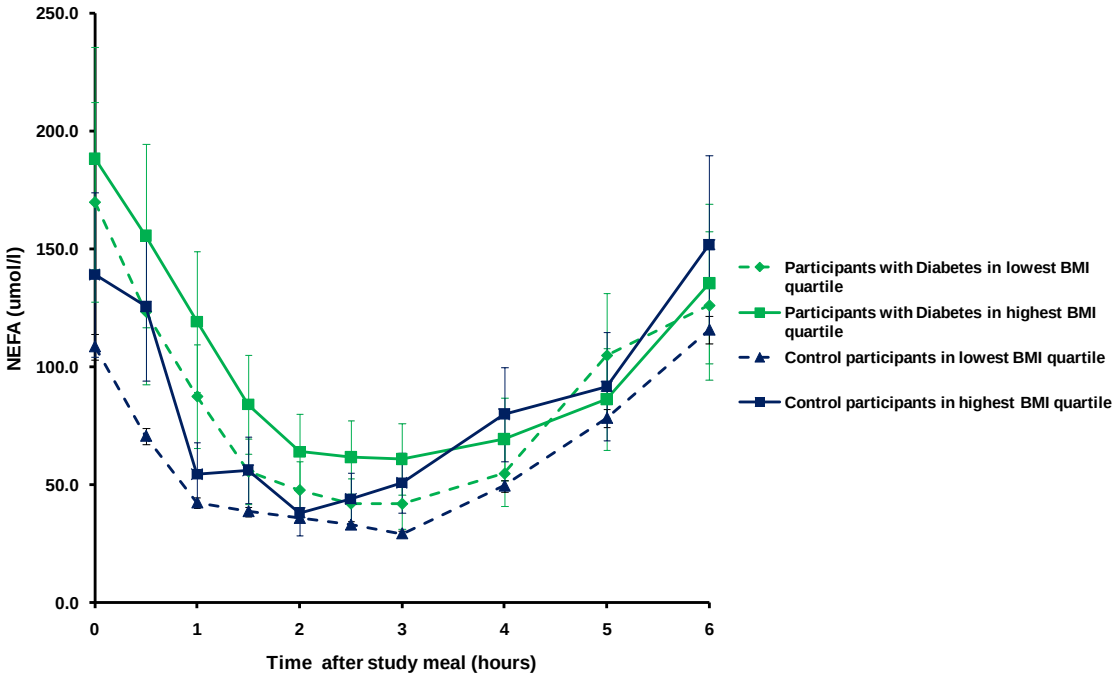


Figure 5-3: Plasma non-esterified fatty acid before and after the standard meal in participants with diabetes (green) in lowest and highest quartiles of BMI and control (blue) participants in lowest and highest quartiles of BMI (mean ± SE)

Fasting NEFA was numerically lower, but with only borderline statistical significance, in DM in the lowest BMI quartile compared with DM in the highest BMI quartile (p=0.08). Fasting NEFA was numerically, but not significantly, lower in the CON in the lowest BMI quartile compared to CON in the highest BMI quartile (p=0.23)

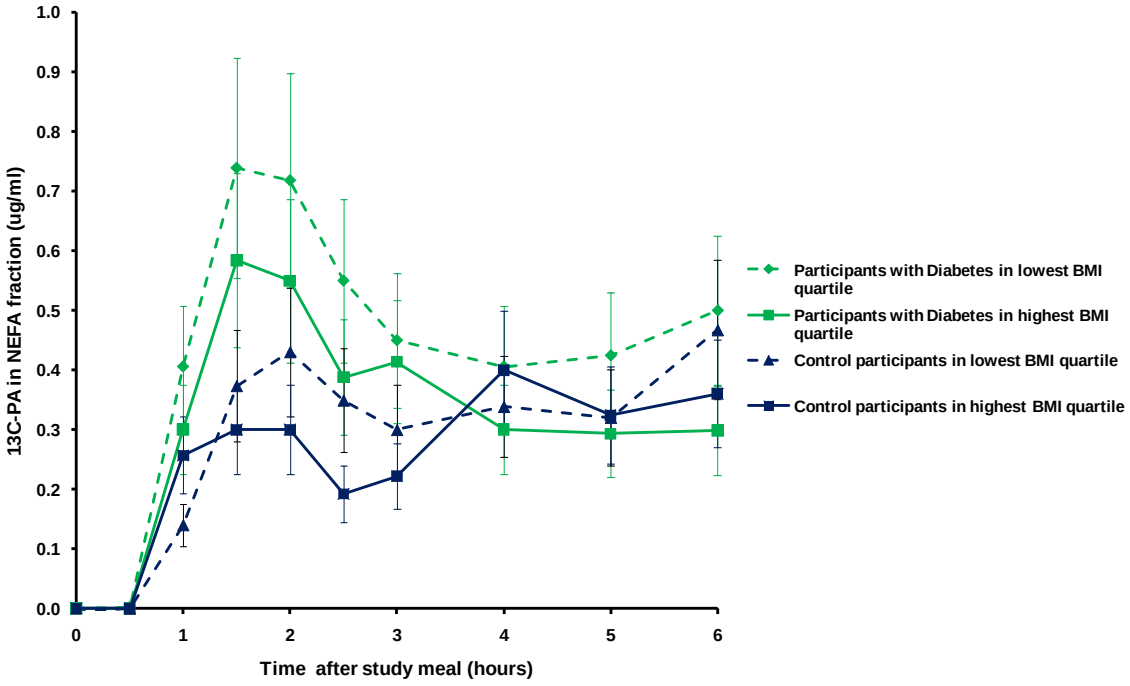


Figure 5-4: ^{13}C-palmitic acid in NEFA fraction following standard meal in participants with diabetes (DM) (green) in lowest and highest quartiles of BMI and control (blue) participants in lowest and highest quartiles of BMI (median (interquartile range)).

^{13}C-PA NEFA AUC was significantly *higher* in DM in the lowest BMI quartile compared to DM in the highest BMI quartile (p=0.01). ^{13}C-PA NEFA AUC was not significantly different in CON in the lowest BMI quartile compared to CON in the highest BMI quartile (p=0.89). ^{13}C-PA NEFA AUC was significantly higher in DM in the lowest BMI quartile compared with CON in the lowest BMI quartile (p=0.04).

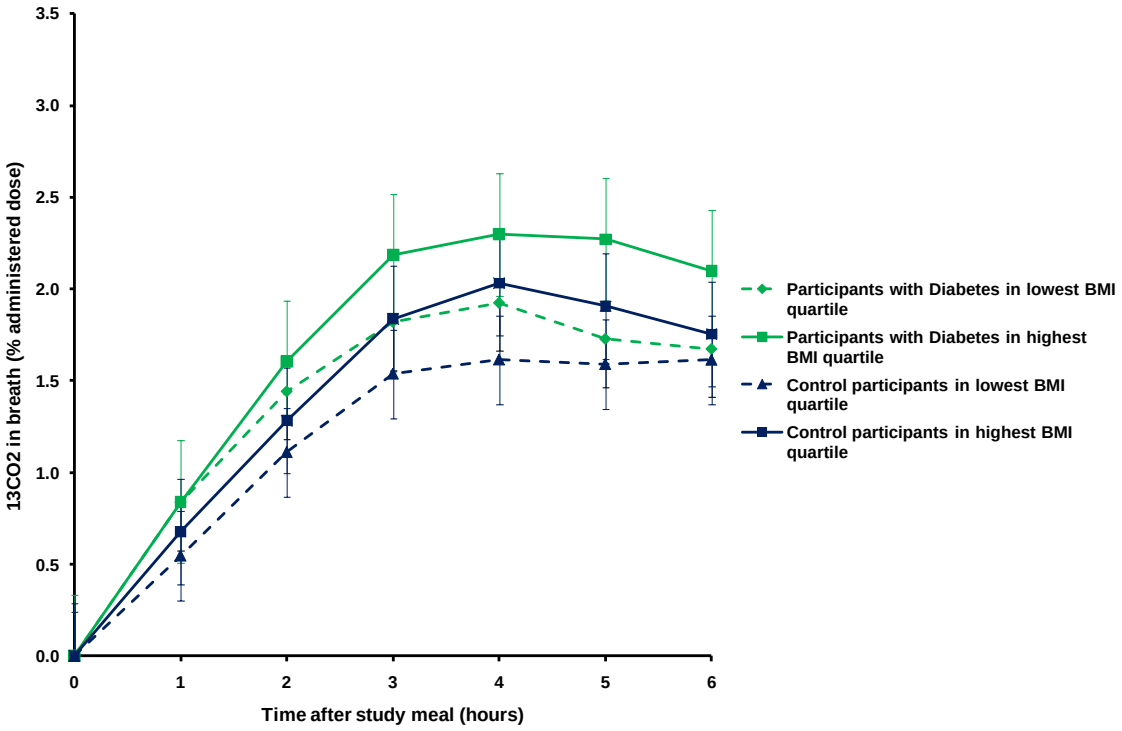


Figure 5-5:Oxidation of ingested dietary fat estimated by appearance of ingested ^{13}C-labelled lipid measured in expired breath in participants with diabetes (DM) (green) in lowest and highest quartiles of BMI and control (CON) (blue) participants in lowest and highest quartiles of BMI (mean ± SE)

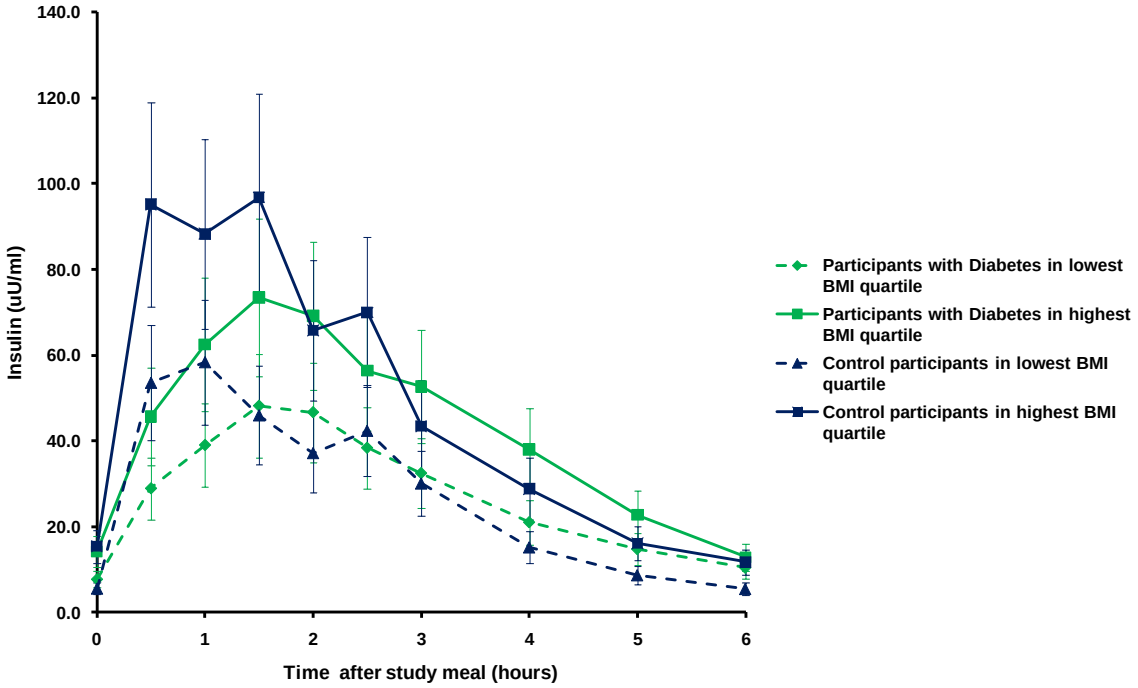


Figure 5-6:Plasma insulin before and after the standard meal in participants with diabetes (green) in lowest and highest quartiles of BMI and control (blue) participants in lowest and highest quartiles of BMI (median (interquartile range).

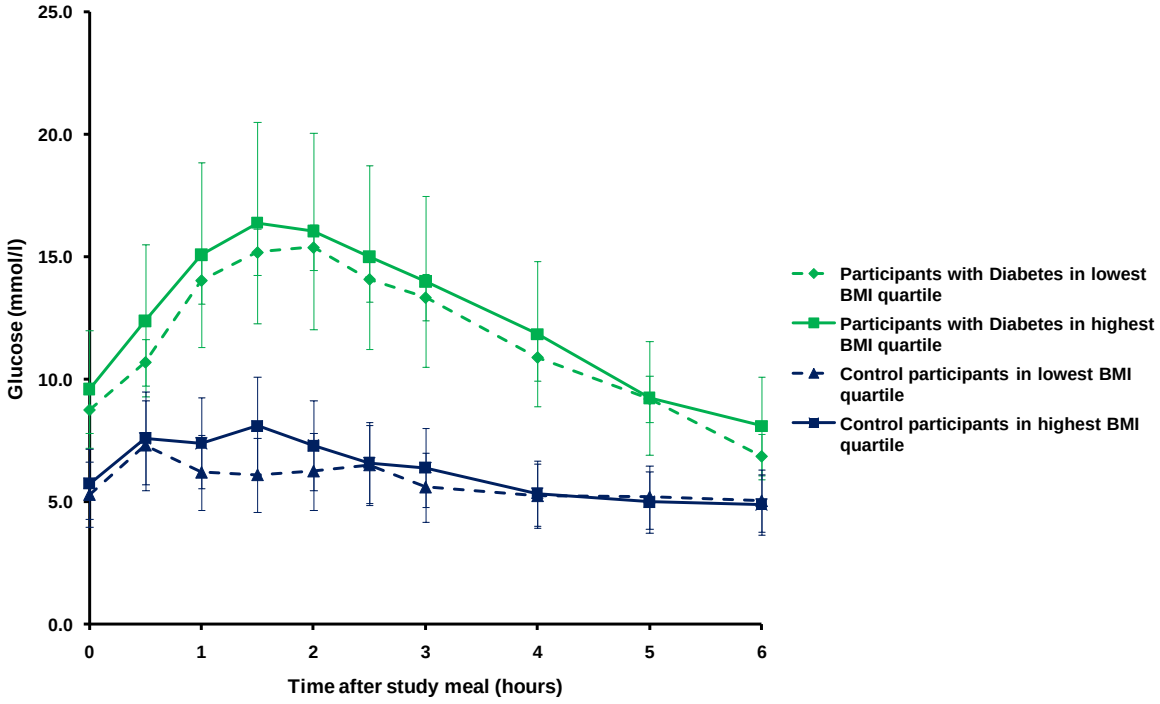


Figure 5-7: Plasma glucose before and after the standard meal in participants with diabetes (DM) (green) in lowest and highest quartiles of BMI and control (CON) (blue) participants in lowest and highest quartiles of BMI (median (interquartile range)).

	Controls			Participants with diabetes		
	Lowest BMI quartile	**Highest BMI quartile**	**p**	**Lowest BMI quartile**	**Highest BMI quartile**	**p**
BMI (kg/m^2)	23.5±1.3	39.6±4.4	<0.0001	23.5±2.2	37.9±3.7	<0.0001
Fasting TAG (mmol/l)	1.0* (0.7-1.2)	2.0 (1.1-2.4)	0.06	1.7* (0.9-2.4)	2.5 (1.9-3.8)	0.03
AUC TAG (mmol/l/6h)	8.3* (6.2-10.1)	18.1 (7.5-21.5)	0.13	17.0* (7.5-21.6)	18.2 (13.6-29.7)	0.23
INC AUC TAG (mmol/l/6h)	2.1 (1.0-3.1)	4.3 (1.4-10.6)	0.15	6.2 (1.5-8.8)	3.4 (1.5-6.4)	0.41
^{13}C-PA in TAG AUC (µg/ml/6h)	34.2* (22.6-44.5)	50.1 (23.7-80.6)	0.34	77.1* (38.6-104.3)	52.1 (33.2-82.4)	0.25
Fasting NEFA (umol/l)	111.3* (93.3-135.8)	136.7† (95.8-192.5)	0.23	176.1* (155.3-196.7)	200.2† (175.3-241.2)	0.08
AUC NEFA (umol/l/6h)	362.3* (289.5-426.6)	477.6 (383.0-660.9)	0.01	542.2* (333.4-657.4)	542.9 (487.0-774.3)	0.34
^{13}C-PA in NEFA AUC (µg/ml/6h)	2.1* (1.2-3.1)	2.2 (1.4-2.3)	0.89	3.1* (2.8-4.2)	2.3 (1.7-2.8)	0.01

Table 5-1: Fasting and postprandial lipids in control participants and participants with diabetes in lowest BMI quartile vs highest BMI quartile.

All data are expressed as median (interquartile values)), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test). * indicates a significant difference between participants with diabetes and control participants in the lowest BMI quartile, † indicates a significant difference between participants with diabetes and control participants in the highest BMI quartile.

	Controls			Participants with diabetes		
	Lowest BMI quartile	**Highest BMI quartile**	**p**	**Lowest BMI quartile**	**Highest BMI quartile**	**p**
BMI (kg/m^2)	23.5±1.3	39.6±4.4	<0.0001	23.5±2.2	37.9±3.7	<0.0001
Fasting glucose (mmol/l)	5.3* (5.2-5.9)	5.8† (5.3-6.3)	0.23	9.3* (7.5-13.5)	9.7† (8.3-11.1)	0.77
AUC glucose (mmol/l/6h)	36.1* (32.9-39.1)	36.1† (34.1-40.0)	0.47	80.7* (53.0-106.0)	73.3† (65.0-83.3)	0.90
Fasting insulin (µU/ml)	5.6 (3.2-7.8)	14.6 (12.2-18.1)	<0.0001	8.2 (4.9-13.7)	14.2 (193.0-396.8)	0.04
30 min insulin (µU/ml)	58.6* (35.1-75.4)	103.6† (54.0-176.5)	0.02	30.0* (23.7-35.1)	45.7† (22.6-67.8)	0.08
AUC insulin (µU/ml/6h)	161.9 (118.8-272.5)	362.9 (217.9-477.4)	0.005	163.0 (153.3-239-1)	274.5 (193.0-396.8)	0.04
HOMA-IR	1.1* (0.8-1.9)	3.9† (2.8-4.8)	<0.0001	3.0* (1.8-8.6)	6.4† (5.5-7.2)	0.10
HOMA-%B	63.3* (26.7-78.3)	142.6† (109.5-153.8)	<0.0001	26.2* (21.8-36.2)	26.4† (21.1-62.7)	0.62

Table 5-2: Fasting and postprandial glucose and insulin in control participants and participants with diabetes in lowest BMI quartile vs highest BMI quartile.

All data are expressed as median (interquartile values), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test). * indicates a significant difference between participants with diabetes and control participants in the lowest BMI quartile, † indicates a significant difference between participants with diabetes and control participants in the highest BMI quartile

	Controls			Participants with diabetes		
	Lowest BMI quartile	**Highest BMI quartile**	**p**	**Lowest BMI quartile**	**Highest BMI quartile**	**p**
BMI (kg/m^2)	23.5±1.3	39.6±4.4	<0.0001	23.5±2.2	37.9±3.7	<0.0001
$^{13}CO_2$ in breath (% dose/6h)	7.4±2.7	8.9±2.4	0.20	9.3±4.2	10.0±2.6	0.66
Fasting fat oxidation (g/h)	1.2±1.6	2.7±1.5	0.05	2.2±1.6	3.8±1.1	0.06
AUC fat oxidation (g/6h)	11.3±6.6	19.7±4.4	0.005	13.4±8.5	19.2±6.5	0.09
Fasting carbohydrate oxidation (g/h)	7.2±3.9	10.2±5.3	0.14	5.3±2.0	7.2±3.7	0.19
AUC carbohydrate oxidation (g/6h)	49.5±21.7	64.5±18.8	0.12	43.6±13.6	56.0±26.1	0.21

Table 5-3: Substrate oxidation in control participants and participants with diabetes in lowest BMI quartile vs highest BMI quartile.

All data are expressed as mean±sd. Differences between groups were tested for using the independent samples t-test. NB. There were no significant differences in substrate oxidation between participants with diabetes and control participants in the lowest BMI quartile, or between participants with diabetes and control participants in the highest BMI quartile

	Controls in lowest BMI quartile	Participants with diabetes in lowest BMI quartile	P value
BMI (kg/m^2)	23.50±1.32	23.45±2.20	0.95
Fasting triglyceride (mmol/l)	1.0(0.7-1.2)	1.7(0.9-2.4)	0.05
Postprandial (AUC) triglyceride (mmol/l/6h)	8.3(6.2-10.1)	17.0(7.5-21.6)	0.05
Postprandial incremental (AUC) TAG (mmol/l/6h)	2.1(1.0-3.1)	6.2(1.5-8.8)	0.07
^{13}C-PA in TAG AUC (µg/ml/6h)	34.2(22.6-44.5)	77.1(38.6-104.3)	0.01
Fasting NEFA (umol/l)	111.3(93.3-135.8)	176.1(155.3-196.7)	0.002
Postprandial (AUC) NEFA (umol/l/6h)	362.3(289.5-426.6)	542.2(333.4-657.4)	0.04
^{13}C-PA in NEFA AUC (µg/ml/6h)	2.1(1.2-3.1)	3.1(2.8-4.2)	0.04

Table 5-4: Control participants in lowest BMI quartile vs participants with diabetes in lowest BMI quartile: fasting and postprandial lipids.

All data are expressed as median (interquartile values), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test).

	Controls in highest BMI quartile	Participants with diabetes in highest BMI quartile	p value
BMI (kg/m^2)	39.58±4.39	37.90±3.73	0.35
Fasting triglyceride (mmol/l)	2.0(1.1-2.4)	2.5(1.9-3.8)	0.08
Postprandial (AUC) triglyceride (mmol/l/6h)	18.1(7.5-21.5)	18.2(13.6-29.7)	0.38
Postprandial incremental (AUC) TAG (mmol/l/6h)	4.3(1.4-10.6)	3.4(1.5-6.4)	0.71
^{13}C-PA in triglyceride fraction (µg/ml/6h)	50.1(23.7-80.6)	52.1(33.2-82.4)	0.63
Fasting NEFA (umol/l)	136.7(95.8-192.5)	200.2(175.3-241.2)	0.02
Postprandial (AUC) NEFA (umol/l/6h)	477.6(383.0-660.9)	542.9(487.0-774.3)	0.13
^{13}C-PA in NEFA fraction (µg/ml/6h)	2.2(1.4-2.3)	2.3(1.7-2.8)	0.35

Table 5-5: Control participants in highest BMI quartile vs participants with diabetes in highest BMI quartile: fasting and postprandial lipids.

All data are expressed as median (interquartile values), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test).

	Controls in lowest BMI quartile	Participants with diabetes in lowest BMI quartile	p value
BMI (kg/m^2)	23.50±1.32	23.45±2.20	0.95
Fasting glucose (mmol/l)	5.3(5.2-5.9)	9.3(7.5-13.5)	<0.0001
Postprandial (AUC) glucose (mmol/l/6h)	36.1(32.9-39.1)	80.7(53.0-106.0)	<0.0001
Fasting insulin (µU/ml)	5.6(3.2-7.8)	8.2(4.9-13.7)	0.10
30 minute insulin (µU/ml)	58.6(35.1-75.4)	30.0(23.7-35.1)	0.01
Postprandial (AUC) insulin (µU/ml/6h)	161.9(118.8-272.5)	163.0(153.3-239-1)	0.61
HOMA-IR	1.1(0.8-1.9)	3.0(1.8-8.6)	0.006
HOMA-%B	63.3(26.7-78.3)	26.2(21.8-36.2)	0.04

Table 5-6: Controls in lowest BMI quartile vs participants with diabetes in lowest BMI quartile: fasting and postprandial glucose and insulin.

All data are expressed as median (interquartile values), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test).

	Controls in highest BMI quartile	Participants with diabetes in highest BMI quartile	p value
BMI (kg/m^2)	39.58±4.39	37.90±3.73	0.35
Fasting glucose (mmol/l)	5.8(5.3-6.3)	9.7(8.3-11.1)	<0.0001
Postprandial (AUC) glucose (mmol/l/6h)	36.1(34.1-40.0)	73.3(65.0-83.3)	<0.0001
Fasting insulin (µU/ml)	14.6(12.2-18.1)	14.2(193.0-396.8)	0.74
30 minute insulin (µU/ml)	103.6(54.0-176.5)	45.7(22.6-67.8)	0.01
Postprandial (AUC) Insulin (µU/ml/6h)	362.9(217.9-477.4)	274.5(193.0-396.8)	0.37
HOMA-IR	3.9(2.8-4.8)	6.4(5.5-7.2)	0.002
HOMA-%B	142.6(109.5-153.8)	26.4(21.1-62.7)	0.003

Table 5-7: Control participants in highest BMI quartile vs participants with diabetes in highest BMI quartile: fasting and postprandial glucose and insulin.

All data are expressed as median (interquartile values), except BMI (mean (sd)). Differences between groups were tested for using the Mann Whitney U test, except BMI (independent samples t-test).

	Controls in lowest BMI quartile	Participants with diabetes in lowest BMI quartile	p value
BMI (kg/m^2)	23.50±1.32	23.45±2.20	0.95
Breath $^{13}CO_2$ (% dose/6h)	7.36±2.70	9.31±4.21	0.20
Fasting net fat oxidation (g/h)	1.21±1.58	2.16±1.63	0.18
Postprandial (AUC) net fat oxidation (g/6h)	11.31±6.62	13.35±8.52	0.53
Fasting carbohydrate oxidation (g/h)	7.19±3.92	5.33±2.04	0.21
Postprandial (AUC) carbohydrate oxidation (g/6h)	49.48±21.71	43.56±13.56	0.47
Fasting RQ	0.88±0.07	0.84±0.05	0.17

Table 5-8: Controls in lowest BMI quartile vs participants with diabetes in lowest BMI quartile: substrate oxidation.

All data are expressed as mean±sd. Differences between groups were tested for using the independent samples t-test.

	Controls in highest BMI quartile	Participants with diabetes in highest BMI quartile	p value
BMI (kg/m^2)	39.58±4.39	37.90±3.73	0.35
Breath $^{13}CO_2$ (% dose/6h)	8.88±2.38	9.97±2.63	0.35
Fasting net fat oxidation (g/h)	2.65±1.49	3.37±1.09	0.24
Postprandial (AUC) net fat oxidation (g/6h)	19.65±4.41	19.22±6.54	0.88
Fasting carbohydrate oxidation (g/h)	10.21±5.33	7.16±3.65	0.15
Postprandial (AUC) carbohydrate oxidation (g/6h)	64.50±18.80	55.97±26.13	0.44
Fasting RQ	0.86±0.06	0.830.04	0.13

Table 5-9: Control participants in highest BMI quartile vs participants with diabetes in lowest BMI quartile: substrate oxidation

All data are expressed as mean±sd. Differences between groups were tested for using the independent samples t-test.

5.5 Summary and discussion

5.5.1 Fasting state

In the fasting state, participants with diabetes in the lowest BMI quartile had significantly lower fasting TAG than the participants with diabetes in the highest BMI quartile. There was a similar trend, but this not statistically significantly in control participants. The data suggests that it is likely that obesity contributes to a metabolic defect causing increased fasting production of TAG by the liver in both participants with diabetes and control participants, but more so in participants with diabetes. This defect might be due to obesity-induced resistance to the insulin mediated inhibition of fasting TAG production by the liver, which is not adequately compensated for by increased insulin production in participants with diabetes due to beta cell dysfunction.

5.5.2 Postprandial state

Differences in postprandial triglyceride metabolism (^{13}C-PA TAG AUC and ^{13}C-PA NEFA AUC) were more pronounced between participants with diabetes and controls in the lowest quartile of BMI than between participants with diabetes and controls in the highest quartile of BMI. This may explain why no significant difference was found between participants with diabetes and controls for postprandial ^{13}C-PA TAG metabolism in the whole cohort combined (see Chapter 3).

Although not statistically significant, the data suggests that in control subjects postprandial TAG in the lowest quartile of BMI was lower or equal to that found in control subjects in the highest quartile of BMI, however the reverse pattern was seen in participants with diabetes in the lowest quartile of BMI compared to the participants with diabetes in the highest quartile of BMI. This is best illustrated in Figure 5.2. In addition as previously described in Chapter 4, the leaner participants with diabetes had a significantly higher concentration of dietary NEFA (^{13}C-PA NEFA AUC) than the obese participants with diabetes, suggesting reduced adipose tissue uptake of dietary NEFA or increased ‘spillover’.

These results again suggest that lean participants with type 2 diabetes have a defect in the metabolism of dietary fat at least equal to or perhaps worse than that found in obese participants with type 2 diabetes. This finding is biologically plausible as uptake of dietary NEFA into adipose tissue and clearance of dietary TAG are insulin dependent processes as the participants with diabetes patients in the lowest quartile of BMI also had the lowest 30 minute insulin. The participants with diabetes in the lowest quartile of BMI may have latent autoimmune (type 1) diabetes or maturity onset diabetes of the young (MODY) and thus have more severe beta cell dysfunction than the participants with diabetes in the highest quartile of BMI. An alternative explanation is that lean participants with diabetes may have inadequate adipose tissue stores for storage of dietary NEFA. Finally there did not appear to be a defect in oxidation of meal derived triglyceride in the lean participants with diabetes ^{13}C breath oxidation was not significantly affected by BMI in this study.

5.6 Possible confounding variables for the metabolic differences between highest and lowest BMI quartiles of participants with diabetes

The findings of differences between BMI quartiles may be secondary to differences in gender distribution or medication taken in the lowest and highest quartiles of BMI and not due to true metabolic differences (or similarities) between the quartiles. This possibility was explored.

5.6.1 Gender

There were 4 female and 5 male participants in the lowest BMI quartile and 9 female and 5 male participants in highest BMI quartile (Table 5.10). The distribution of gender between the highest and lowest quartiles of BMI was not significantly different ($p=0.613$, using chi-squared test). In addition when the difference in ^{13}C-PA NEFA AUC between the lowest and highest quartiles of BMI in participants with diabetes was corrected for gender (using chi-squared test), the difference between the lowest and highest quartile remained statistically significant ($p= 0.023$).

BMI quartile (kg/m^2)	Gender
18.0-25.3	4F, 5M
25.3-29.7	2F, 8M
29.7-34.2	6F, 6M
34.2-49.2	9F, 5M

Table 5-10: Gender distribution in the different BMI quartiles of participants with diabetes (M=male, F=female)

BMI quartile (kg/m^2)	Sulphonylurea	Metformin	TZD	Number of hypoglycaemic drugs		
				0	1	2+
18.0-25.3	4 (44%)	2 (22%)	0 (0%)	5 (56%)	2 (22%)	2 (22%)
25.3-29.7	7 (70%)	4 (40%)	1 (10%)	1 (10%)	4 (40%)	3 (30%)
29.7-34.2	3 (25%)	3 (25%)	0 (0%)	8 (67%)	1 (8%)	3 (25%)
34.2-49.2	5 (36%)	8 (57%)	2 (14%)	3 (21%)	3 (21%)	5 (36%)

Table 5-11: Diabetes medication in the different BMI quartiles of participants with diabetes

5.6.2 Diabetes medication

The lowest BMI quartile had 5/9 participants (55.5%) on no diabetes medication, 4/9 (44%) on a sulphonylurea , 2/9 (22%) on metformin and none on a thiazolidendione (TZD). The highest BMI quartile had 3/14 (21%) on no medication, 5/14 (35.7%) on a sulphonylurea, 8/14 (57%) on metformin and 2/14 (14%) on a TZD (Table 5.11). The effects on increased entrapment of ^{13}C-PA NEFA in the more obese patients may be due to the TZD. When the effects of medication were examined in the whole cohort of participants with diabetes patients, there was no significant effect of medication on ^{13}C-PA NEFA AUC (metformin ;p=0.877, sulphonylurea;p=0.770 and glitazone; p=0.756) or on ^{13}C-PA TAG AUC (metformin ;p=0.316, sulphonylurea;p=0.478 and glitazone; p=0.406). There were proportionally more patients on no oral hypoglycaemic medication in the lowest BMI group which may have had an effect on the postprandial triglyceride handling in these participants, although when the effects of 'no medication' were examined in the total cohort of participants with diabetes patients by chi squared test, there was no significant effect of 'absence of oral hypoglycaemic medication' on ^{13}C-PA NEFA AUC (p=0.969) or on ^{13}C-PA TAG AUC (p=0.351). In addition when the difference in ^{13}C-PA NEFA AUC was corrected for metformin, glitazone, sulphonylurea and no medication, the difference between the lowest and highest BMI quartiles remained statistically significant (p= 0.042, p=0.015, p=0.036, p=0.040 respectively).

5.6.3 Glycaemic control

There was no difference in fasting glucose between the participants with diabetes in the lowest and highest quartiles of BMI (Table 5.2). The mean HbA1c in the participants with diabetes in the lowest BMI quartile was 7.3% (56mmol/mol) and in the participants with diabetes in the highest BMI quartile was 8.7% (8.7mmol/mol) (p=0.07). It was also shown in Chapter 4 that there was a positive relationship between adiposity and HbA_{1c} in participants with diabetes. The impairment of lipid metabolism in the lean participants with diabetes was therefore not likely to be due to worse glycaemic control in these participants

5.7 Conclusions

Differences in postprandial triglyceride metabolism (^{13}C-PA TAG AUC and ^{13}C-PA NEFA AUC) were much more pronounced between participants with diabetes and controls in the lowest quartile of BMI than between participants with diabetes and obese controls in the highest quartile of BMI (Figures 5.3 and 5.4, Table 5.1). This is likely to be partly because the more obese controls are more insulin resistant than leaner controls and therefore have impaired metabolism which masks differences between participants with diabetes and control participants in the highest quartile of BMI. However participants with diabetes in the lowest quartile of BMI had impaired postprandial triglyceride metabolism even when compared to the participants with diabetes in the highest quartile of BMI. This may be because the leanest participants with diabetes are the most insulin deficient and also because the lean participants with diabetes may have undiagnosed lipodystrophy, maturity onset diabetes of the young/monogenic diabetes (MODY) or latent autoimmune (type 1) diabetes.

The relationships found in this study between lipid metabolism and insulin resistance, glycaemic control and beta cell function are explored further in Chapter 6.

Chapter 6 Relationships between triglyceride metabolism and insulin resistance, beta cell function and glycaemic control

6.1 Introduction

The mechanism for abnormal triglyceride and glucose metabolism in type 2 diabetes is likely to be due to a combination of insulin resistance in the peripheral tissues and relative insulin deficiency. The mechanism for abnormal triglyceride and glucose metabolism in obese patients in the absence of type 2 diabetes is likely to be due to insulin resistance in the peripheral tissues without insulin deficiency. This chapter compares the relationships between insulin resistance and beta cell function and the metabolic variables found in the participants with diabetes and control participants in this study. The chapter also describes the relationships between insulin resistance and beta cell function and measures of adiposity in the participants with diabetes compared to the control participants. This may help elucidate the pathophysiological mechanisms underlying the differences and similarities in the relationships found between adiposity and metabolism in participants with diabetes compared to control participants which have previously been described in Chapters 4 and 5.

6.2 Estimates of insulin resistance and beta cell function

6.2.1 Homeostatic model assessment

This study has utilised the homeostatic model assessment (HOMA) for estimating insulin resistance (HOMA-IR) and beta cell function (HOMA-%B) in the fasting state (see Chapter 2 for explanation of HOMA). This method uses fasting insulin and glucose concentrations. Fasting insulin concentrations can also be used as a marker of fasting insulin sensitivity in non-participants with diabetes.

6.2.2 30 minute postprandial insulin concentration

Thirty minute postprandial insulin can be used as an estimate of the ‘first phase’ insulin response, which is a proxy measure for beta cell reserve (192) (see Chapter 2 for more detail on the first phase insulin response).

6.3 HOMA-IR and metabolism

HOMA-IR was significantly higher in participants with diabetes compared with control participants (6.75±3.53 vs 2.51±1.65, p<0.0001) (Table 3.2).

6.3.1 Participants with diabetes

In participants with diabetes fasting TAG (r=0.43, p=0.005), area under the curve (AUC) TAG (r=0.46, p=0.002), incremental (INC) AUC TAG (r=0.31, p=0.05) and ^{13}C - palmitic acid in the TAG fraction AUC (^{13}C-PA TAG AUC) (r=0.31, p=0.05) all correlated significantly with HOMA-IR (Table 6.1).

Fasting NEFA did not correlate with HOMA-IR. AUC NEFA approached a significant correlation with HOMA-IR (r=0.28, p=0.08). ^{13}C - palmitic acid in the non-esterified fatty acid fraction AUC (^{13}C-PA NEFA AUC) did not correlate with HOMA-IR (r=-*0.007*, p=0.967) (Table 6.1).

$^{13}CO_2$ in breath AUC over 6 and 24 hours (r=0.33, p=0.03, and r=0.34, p=0.04) correlated significantly with HOMA-IR, as did fasting and AUC fat oxidation (r=0.42, p=0.007 and r=0.46, p=0.003). Measures of carbohydrate (CHO) oxidation did not correlate with HOMA-IR (Table 6.2).

6.3.2 Control participants

In control participants fasting TAG (r=0.42, p=0.006), AUC TAG (r=0.40, p=0.008) and fasting glucose (r=0.31, p=0.04) all correlated significantly with HOMA-IR. ^{13}C-PA AUC TAG (r=0.27, p=0.08) approached a significant correlation with HOMA-IR (Table 6.1).

Fasting NEFA and ^{13}C-PA NEFA AUC did not show a relationship with HOMA-IR. AUC NEFA (r=0.30, p=0.06) approached a significant correlation with HOMA-IR (Table 6.1).

AUC CHO oxidation correlated significantly with HOMA-IR (r=0.31, p=0.05), but measures of fat oxidation did not show a relationship with HOMA-IR in control participants (Table 6.2).

		CONTROLS		PARTICIPANTS WITH DIABETES	
		HOMA-IR	**Fasting insulin**	**HOMA-IR**	**Fasting insulin**
Fasting TAG	Correlation coefficient	.416	.380	.431	.361
	p value	.006	.011	.005	.021
AUC TAG	Correlation coefficient	.399	.353	.456	.329
	p value	.008	.019	.002	.031
INC AUC TAG	Correlation coefficient	.243	.212	.314	.273
	p value	.117	.167	.048	.084
^{13}C-PA in TAG AUC	Correlation coefficient	.267	.227	.309	.299
	p value	.083	.138	.046	.052
Fasting NEFA	Correlation coefficient	*-.042*	*-.036*	.085	*-.055*
	p value	.792	.821	.593	.727
NEFA AUC	Correlation coefficient	.296	.283	.277	.118
	p value	.057	.066	.076	.450
^{13}C-PA in NEFA AUC	Correlation coefficient	*-.007*	*-.003*	.141	*-.048*
	p value	.967	.987	.368	.756
Fasting glucose	Correlation coefficient	.314	.238	.288	*-.066*
	p value	.040	.124	.061	.672
AUC glucose	Correlation coefficient	.219	.180	.235	*-.063*
	p value	.158	.247	.129	.687

Table 6-1: Spearman correlation coefficients between metabolic variables and measures of insulin resistance in control participants and participants with diabetes

		CONTROLS		PARTICIPANTS WITH DIABETES	
		HOMA-IR	**Fasting insulin**	**HOMA-IR**	**Fasting insulin**
^{13}CO2 in breath over 6 hours	Correlation coefficient	.117	.101	.332	.222
	p value	.455	.516	.034	.158
Fasting fat oxidation	Correlation coefficient	.090	.048	.424	.402
	p value	.575	.763	.007	.011
AUC fat oxidation	Correlation coefficient	.164	.128	.461	.383
	p value	.305	.418	.003	.015
Fasting CHO oxidation	Correlation coefficient	.245	.244	.060	.123
	p value	.122	.119	.712	.451
AUC CHO oxidation	Correlation coefficient	.305	.306	.130	.240
	p value	.052	.048	.425	.136
Fasting RQ	Correlation coefficient	.115	.138	*-.233*	*-.166*
	p value	.475	.384	.166	.307

Table 6-2: Spearman correlation coefficients of substrate oxidation variables with measures of insulin resistance in control participants and participants with diabetes

6.4 Summary and discussion – HOMA-IR and metabolism

The participants with diabetes were significantly more insulin resistant in the fasting state (estimated using HOMA-IR) than the control participants. As expected, fasting TAG concentrations were positively associated with insulin resistance in both participants with diabetes and control participants, however for meal-derived TAG measures the positive association with insulin resistance appeared stronger in the participants with diabetes than the control participants. Perhaps insulin resistance affects postprandial triglyceride handling to a more severe degree in patients with diabetes.

An unexpected finding was that fasting NEFA and dietary ^{13}C-PA NEFA AUC concentrations did not correlate with insulin resistance in participants with diabetes or control participants. This does not support the widely-held hypothesis that elevated circulating NEFA is an important cause of insulin resistance. Indeed, fasting NEFA concentrations are incorporated into the modified QUICKI, a method for estimating insulin resistance in individuals (modified QUICKI=1/[log(fasting insulin)+log(fasting blood glucose)+log(fasting NEFA)]). The modified QUICKI has been found to increase the power of the QUICKI in the detection of mild insulin-resistant states (193).The contribution of NEFA to insulin resistance causation remains an area of controversy and the need for further research in this field has recently been highlighted (194).

Another unexpected finding was that fasting and postprandial fat oxidation rates correlated positively with insulin resistance in participants with diabetes (but not control participants). There is evidence in the literature to suggest that fat oxidation is decreased in patients with insulin resistance (184). It has been proposed that the reduction in fat oxidation in patients with insulin resistance is due to impairment of mitochondrial oxidative function, but the direction of causation in the relationship between insulin resistance and mitochondrial dysfunction remains controversial (195;196).

Insulin resistance correlated positively with increasing adiposity in control participants but not in participants with diabetes. The reasons for this lack of

association in participants with diabetes is unclear, but may be because the underlying pathophysiology in patients with type 2 diabetes is heterogeneous and that adiposity may not be the most important causative factor of insulin resistance in patients who already have established diabetes. This may be one of the explanations why there was not a relationship between postprandial TAG metabolism in participants with diabetes, but there was a relationship in control participants (Chapter 4).

6.5 HOMA: Beta cell function and metabolism

Beta cell function as estimated by HOMA-B% was significantly lower in participants with diabetes compared with control participants (46.60 ± 39.45% vs 101.47±69.10% respectively, $p<0.0001$) (Table 3.2).

6.5.1 Participants with diabetes

In the participants with diabetes, there were no correlations between HOMA-B% and fasting or postprandial TAG or with ^{13}C-PA labelled TAG or NEFA (Table 6.3).

In the participants with diabetes, there were significant negative correlations of HOMA-B% with fasting glucose (r=-*0.43*, p=0.004), AUC glucose (r=-*0.35*, p=0.02) and fasting NEFA (r=-*0.35*, p=0.02).

There was a significant positive correlation of HOMA-B% with AUC CHO oxidation (r=0.40, p=0.01) and fasting CHO oxidation approached significance (r=0.29, p=0.07). There were no correlations with fat oxidation (Table 6.4).

6.5.2 Control participants

In the control participants HOMA-B% had a significant positive correlation with fasting TAG (r=0.30, p=0.05) and approached significance with AUC TAG (r=0.26, p=0.09) (Table 6.3).

There were no associations between HOMA-B% and measures of fat or carbohydrate oxidation in control participants (Table 6.4).

		CONTROLS		PARTICIPANTS WITH DIABETES	
		HOMA-Beta cell	**30-minute insulin**	**HOMA-Beta cell**	**30-minute insulin**
Fasting TAG	Correlation coefficient	.299	.278	.096	.208
	p value	.051	.065	.554	.185
AUC TAG	Correlation coefficient	.259	.242	.074	.158
	p value	.093	.109	.640	.305
INC AUC TAG	Correlation coefficient	.100	.244	.137	.089
	p value	.521	.106	.399	.576
^{13}C-PA in TAG AUC	Correlation coefficient	.144	.000	.206	.060
	p value	.357	.999	.190	.700
Fasting NEFA	Correlation coefficient	-.094	-.235	-.350	-.061
	p value	.560	.130	.023	.693
NEFA AUC	Correlation coefficient	.157	.180	-.176	-.139
	p value	.320	.241	.265	.367
^{13}C-PA in NEFA AUC	Correlation coefficient	-.010	-.064	-.174	-.424
	p value	.952	.679	.265	.004
Fasting glucose	Correlation coefficient	-.094	.073	-.427	-.260
	p value	.547	.638	.004	.088
AUC glucose	Correlation coefficient	.046	.047	-.354	-.322
	p value	.771	.762	.020	.033

Table 6-3: Spearman correlation coefficients between metabolic variables and measures of beta cell function in control participants and participants with diabetes

		CONTROLS		PARTICIPANTS WITH DIABETES	
		HOMA-Beta cell	**30-minute insulin**	**HOMA-Beta cell**	**30-minute insulin**
$^{13}CO_2$ in breath over 6 hours	Correlation coefficient	*-.005*	*-.145*	.064	.074
	p value	.973	.341	.690	.636
Fasting fat oxidation	Correlation coefficient	*-.041*	*-.054*	.116	.273
	p value	.800	.732	.482	.088
AUC fat oxidation	Correlation coefficient	.114	.140	.102	.184
	p value	.478	.371	.532	.250
Fasting CHO oxidation	Correlation coefficient	.213	.262	.291	.120
	p value	.181	.089	.069	.453
AUC CHO oxidation	Correlation coefficient	.243	.195	.401	.336
	p value	.125	.210	.010	.032
Fasting RQ	Correlation coefficient	.158	.170	.085	*-.078*
	p value	.325	.275	.603	.628

Table 6-4: Spearman correlation coefficients between metabolic variables and measures of fasting and postprandial beta cell function in control participants and participants with diabetes

6.6 Fasting and 30 minute insulin and metabolism

Fasting insulin concentrations can also be used a proxy measure for fasting insulin resistance in non-participants with diabetes patients, but the concentrations fall as beta cells begin to fail. The 30-minute insulin can be used as a proxy for postprandial beta cell function. Fasting insulin concentrations were higher in the participants with diabetes than the control participants (14.90±7.85 vs 10.16±6.47μU/ml, p=0.003) (Table 3.8). There was a lower 30 minute insulin in the participants with diabetes than the control participants (43.18±25.86 vs 81.51±57.19 μU/ml, p <0.0001) (Table 3.8).

6.6.1 Participants with diabetes

Fasting insulin

In participants with diabetes fasting TAG (r=0.36, p=0.21), AUC TAG (r=0.33, p=0.03), ^{13}C-PA TAG AUC (r=0.30, p=0.05) correlated positively with fasting insulin (Table 6.1).

Fasting fat oxidation (r=0.40, p=0.01) and AUC fat oxidation (r=0.38, p=0.02) also correlated positively with fasting insulin (Table 6.2).

30 minute postprandial insulin

In participants with diabetes ^{13}C-PA NEFA AUC (r=-*0.42*, p=0.004), fasting glucose (r=-*0.26*, p=0.08) and AUC glucose (r=-*0.32*, p=0.03) correlated *negatively* with 30 minute insulin (Table 6.3, Figure 6.1).

Carbohydrate oxidation correlated positively with 30 minute insulin (r=0.34, p=0.03) (Table 6.4).

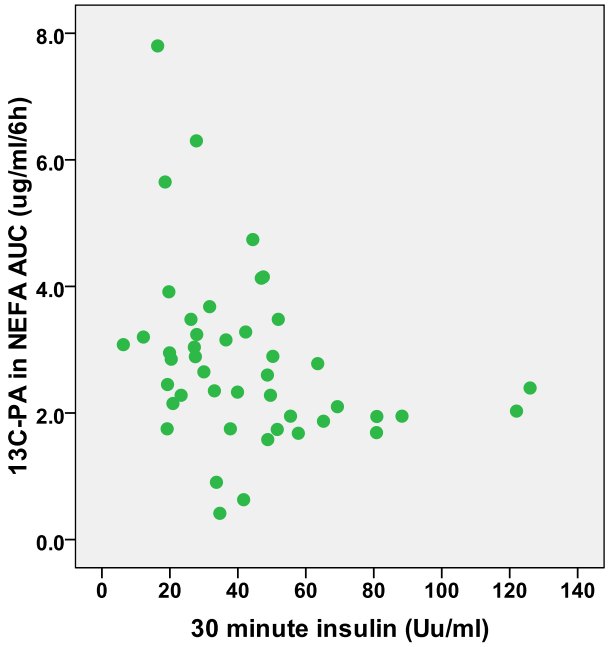


Figure 6-1: Scatter plot showing negative relationship between ^{13}C-PA AUC NEFA and 30 minute insulin in participants with diabetes (r=*-0.42*, p=0.004)

Control participants

Fasting insulin

In control participants fasting and AUC TAG correlated positively with fasting insulin (r=0.38, p=0.01 and r=0.35, p=0.02). NEFA AUC approached a significant correlation with fasting insulin (r=0.28, p=0.07) (Table 6.1).

AUC carbohydrate oxidation correlated positively with fasting insulin (r=0.31, p=0.05) (Table 6.2).

30 minute postprandial insulin

In control participants, 30 min insulin approached a significant positive correlation with fasting TAG (r=0.28, p=0.07) (Table 6.3) and correlated *negatively* with $^{13}CO_2$ in the breath over 24 hours (r=-*0.31*, p=0.04) (Table 6.4).

6.7 Fasting and 30 minute insulin and metabolism relationships with adiposity in participants with diabetes compared to control participants

This has been previously discussed in Chapter 4. Fasting insulin and 30 minute insulin correlated with increasing adiposity in control participants. In participants with diabetes the correlations for fasting insulin were weaker, but for 30 minute insulin were similar to those seen for the control participants (Table 4.1, Figures 4.11-4.13).

6.8 Summary and discussion: relationship between insulin concentrations and triglyceride metabolism

Fasting and AUC TAG correlated positively with fasting insulin with in both participants with diabetes and control participants, this is likely to reflect the

impairment of TAG metabolism associated with insulin resistance, as increasing fasting insulin concentrations are associated with increasing insulin resistance. In participants with diabetes, there was a negative relationship between dietary derived ^{13}C-PA NEFA AUC and 30 minute insulin. This suggests that postprandial uptake of ^{13}C-PA labelled dietary NEFA is influenced by postprandial insulin concentrations, and that the uptake of dietary NEFA into adipose tissue is reduced when 30 minute insulin concentrations are lower as found in participants with diabetes. This may explain why the participants with diabetes patients in the lowest quartiles of BMI and waist circumference showed higher postprandial ^{13}C-PA NEFA AUC concentrations compared to the participants with diabetes in the highest quartile of BMI.

6.9 Glycaemic control

Poor glucose control in patients with diabetes is independently associated with impaired lipid metabolism in patients. This is thought to be secondary to increased adipose tissue lipolysis and reduced lipoprotein lipase (LPL) action due to insulin deficiency or impaired insulin action which causes elevated circulating NEFA and TAG concentrations (173). In clinical practice 'glycaemic control' is estimated by fasting glucose concentrations in the short term and by glycated haemoglobin (HbA1c) in the medium/long term (3-4 months).

In this study fasting glucose was measured in both participants with diabetes and control participants but HbA1c was only measured in participants with diabetes.

6.9.1 Fasting glucose

Participants with diabetes

In participants with diabetes fasting and AUC TAG correlated positively with fasting glucose ($r=0.39$, $p=0.01$ and $r=0.45$, $p=0.002$ respectively). Fasting and AUC NEFA also correlated positively with fasting glucose ($r=0.38$, $p=0.011$ and $r=0.52$, $p<0.0001$ respectively) (Table 6.5).

There was a weak positive correlation between fasting fat oxidation and fasting glucose (r=0.27, p=0.09) and a stronger positive correlation between AUC fat oxidation and fasting glucose (r=0.35, p=0.03). There was a weak negative correlation with between fasting RQ and fasting glucose (r=-*0.28*, p=0.08) (Table 6.6).

There was a negative correlation of fasting glucose with AUC insulin (r=-*0.30*, p=0.05) and a weak negative correlation with 30 minute insulin (r=-*0.26*, p=0.09).

Control participants

In control participants fasting TAG, AUC TAG and INC AUC TAG correlated positively with fasting glucose (r=0.34, p=0.02, r=0.35, p=0.019 and r=0.30, p=0.05 respectively) (Table 6.5).

There was a weak positive correlation between fasting fat oxidation and fasting glucose (r=0.30, p=0.06) (Table 6.6).

6.9.2 $\underline{HbA_{1c}}$

Participants with diabetes

Fasting and AUC TAG correlated positively with HbA_{1c} (r=0.37, p=0.016 and r=0.37, p=0.014 respectively). Fasting and AUC NEFA also correlated positively with HbA_{1c} (r=0.37, p=0.015 and r=0.33, p=0.03 respectively) (Table 6.5).

There was a weak positive correlation between dietary ^{13}C-PA oxidation (breath $^{13}CO_2$) and HbA_{1c} (r=0.27, p=0.08) and AUC fat oxidation and HbA_{1c} (r=0.27, p=0.09) (Table 6.6).

Control participants

No HbA_{1c} data was available.

		CONTROLS	PARTICIPANTS WITH DIABETES	
		Fasting glucose	**Fasting glucose**	**HbA_{1c}**
Fasting TAG	Correlation coefficient	.339*	.398**	.372*
	p value	.024	.010	.016
AUC TAG	Correlation coefficient	.354*	.452**	.374*
	p value	.019	.002	.014
INC AUC TAG	Correlation coefficient	.298*	.145	.072
	p value	.050	.365	.653
^{13}C-PA TAG AUC	Correlation coefficient	.191	.073	.021
	p value	.215	.643	.891
Fasting NEFA	Correlation coefficient	.022	.384*	.368*
	p value	.888	.011	.015
NEFA AUC	Correlation coefficient	.147	.517**	.334*
	p value	.348	.000	.029
^{13}C-PA NEFA AUC	Correlation coefficient	-.164	.274	.153
	p value	.293	.072	.320
Fasting insulin	Correlation coefficient	.238	-.066	.025
	p value	.124	.672	.873
30 minute insulin	Correlation coefficient	.073	-.260	-.061
	p value	.638	.088	.693
AUC insulin	Correlation coefficient	.070	-.296	-.055
	p value	.650	.051	.725

Table 6-5: Spearman correlation coefficients between fasting glucose and HbA_{1c} with metabolic variables in control participants (no HbA_{1c} data available) and participants with diabetes

		CONTROLS	PARTICIPANTS WITH DIABETES	
		Fasting glucose	**Fasting glucose**	**HbA_{1c}**
$^{13}CO_2$ in breath over 6 hours	Correlation coefficient	.161	.171	.272
	p value	.295	.278	.081
Fasting fat oxidation	Correlation coefficient	.298	.269	.066
	p value	.056	.093	.688
AUC fat oxidation	Correlation coefficient	.089	.349	.272
	p value	.576	.026	.089
Fasting CHO oxidation	Correlation coefficient	.062	-.133	.132
	p value	.697	.408	.416
AUC CHO oxidation	Correlation coefficient	.221	-.176	-.108
	p value	.161	.270	.506
Fasting RQ	Correlation coefficient	-.118	-.280	-.049
	p value	.457	.076	.762

Table 6-6: Spearman correlation coefficients between fasting glucose and HbA_{1c} with substrate oxidation variables in control participants (no HbA_{1c} data available) and participants with diabetes

6.10 Summary and discussion

Participants with diabetes had worse fasting insulin resistance as estimated by HOMA-IR and impairment of fasting and postprandial beta cell function as estimated by HOMA-B% and 30-minute insulin compared with control participants.

The positive relationship between BMI and waist circumference and fasting insulin resistance and beta cell function as estimated by HOMA found in control participants were absent in participants with diabetes. However the positive relationship between BMI and waist circumference and 30-minute insulin, an estimate of postprandial beta cell function was similar in participants with diabetes and control participants. A possible explanation for the absence of a relationship between ^{13}C-PA TAG metabolism and adiposity found in participants with diabetes (see Chapter 4) may be that there is already substantial insulin resistance and beta cell dysfunction across all participants with diabetes patients regardless of their degree of adiposity, whereas in control participants, increasing obesity related insulin resistance causes increased impairment of the metabolism of ingested lipid. Lean participants with diabetes also have evidence of postprandial beta cell dysfunction as they have the lowest 30-minute insulin. This is likely to have an adverse effect on^{13}C-PA NEFA uptake by adipose tissue, as evidenced by the negative relationship between 30 minute insulin and ^{13}C-PA NEFA AUC. It is unlikely that the principal defect is one of impairment of fat oxidation as a positive relationship was shown between fat oxidation rates and insulin resistance.

Finally, the impairment of lipid metabolism in the lean participants with diabetes was not likely to be due to worse glycaemic control in these participants as there was a positive relationship between adiposity and glycaemic control in participants with diabetes. There was no difference in fasting glucose between the participants with diabetes in the lowest and highest quartiles of BMI (Table 5.2). The mean HbA1c in the participants with diabetes in the lowest BMI quartile was 7.3% (56mmol/mol) and in the participants with diabetes in the highest BMI quartile was 8.7% (8.7mmol/mol) (p=0.07). It was also shown in Chapter 4 that there was a positive relationship between adiposity and HbA_{1c} in participants with diabetes. The impairment of lipid metabolism in the lean participants with diabetes was therefore

not likely to be due to worse glycaemic control in these participants. However the impairment of fasting TAG in the obese participants with diabetes may be have been related to the worse glycaemic control and worse insulin resistance in this participant group but it is unclear if this is a causative effect or simply an association.

Chapter 7 Comparison of results found with different measures of adiposity

7.1 Introduction

One of the secondary research aims of this study was to investigate whether different measures of adiposity have different relationships with triglyceride and glucose metabolism, and if possible to identify which measure of adiposity is the best predictor of metabolic phenotype.

7.2 Why the measures of adiposity used were selected

In this study the measures used to estimate degree of adiposity were body mass index (BMI), waist circumference and percentage (%) body fat measured using bioelectrical impedance.

These measures were selected for the following reasons:

1. BMI is the most commonly used measure of adiposity in clinical practice after simple body weight and takes account of the participants' height.
2. Waist circumference is easy to measure repeatedly in clinical practice and is a good estimate for 'central' adiposity. However waist circumference can be difficult to measure accurately and reproducibly, especially on obese subjects as the anatomical landmarks are difficult to detect.
3. % body fat gives an estimation of pure fat mass as compared with BMI and weight which also measure lean mass. Bioelectrical impedance was the method available to us at the time of the study, although some units prefer to use other measures such as dual-emission X-ray absorptiometry (DXA) scanning, 'Bod Pod', or magnetic resonance imaging (MRI) scanning. From the % body fat result the fat mass in kg can be calculated.

7.3 Associations between the different measures of adiposity

7.3.1 Control participants

BMI correlated strongly with waist circumference ($r=0.85$, $p=<0.0001$), % body fat ($r=0.54$, $p=<0.0001$) and fat mass ($r=0.93$, $p=<0.0001$) (Table 7.1, Figure 7.1-7.2).

Waist circumference correlated strongly with BMI (r=0.85, p=<0.0001), less strongly with % body fat (r=0.29, p=0.05) and strongly with fat mass (r=0.79, p=<0.0001). % body fat correlated strongly with BMI (r=0.54, p=<0.0001), less strongly with waist circumference (r=0.29, p=0.05), and strongly with fat mass (r=0.71, p<0.0001).

7.3.2 Participants with diabetes

BMI correlated strongly with waist circumference (r=0.82, p=<0.0001), % body fat (r=0.73, p=<0.0001) and fat mass (r=0.89, p=<0.0001) (Table 7.2, Figure 7.1-7.2).

Waist circumference correlated strongly with BMI (r=0.82, p=<0.0001), strongly with % body fat (r=0.64, p<0.0001) and strongly with fat mass (r=0.87, p=<0.0001).

% body fat correlated strongly with BMI (r=0.73, p=<0.0001), strongly with waist circumference (r=0.264, p<0.0001), and strongly with fat mass (r=0.89, p<0.0001).

		CONTROLS				
		BMI	**Waist**	**% body fat**	**Fat mass**	**Fat free mass**
BMI	Correlation coefficient	N/A	.850**	.542**	.932**	.422**
	p value	N/A	<.0001	<.0001	<.0001	.004
Waist	Correlation coefficient	.850**	N/A	.292	.789**	.623**
	p value	<.0001	N/A	.051	<.0001	<.0001
% body fat	Correlation coefficient	.542**	.292	N/A	.712**	-.418**
	p value	<.0001	.051	N/A	<.0001	.004

Table 7-1: Spearman correlation coefficients between different measures of adiposity in control participants

		PARTICIPANTS WITH DIABETES				
		BMI	**Waist**	**% body fat**	**Fat mass**	**Fat free mass**
BMI	Correlation coefficient	N/A	.815**	.734**	.889**	.327*
	p value	N/A	<.0001	<.0001	<.0001	.028
Waist	Correlation coefficient	.815**	N/A	.637**	.868**	.391*
	p value	<.0001	N/A	<.0001	<.0001	.015
% body fat	Correlation coefficient	.734**	.637**	N/A	.890**	-.248
	p value	<.0001	<.0001	N/A	<.0001	.100

Table 7-2: Spearman correlation coefficients between different measures of adiposity in participants with diabetes

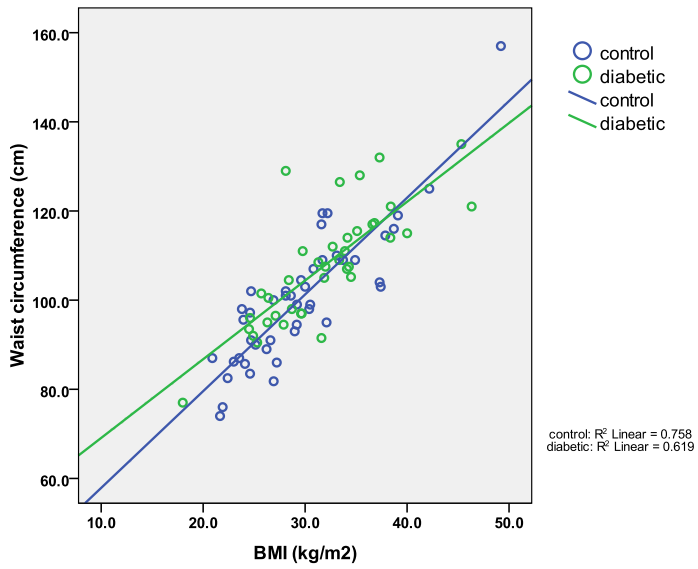


Figure 7-1: Scatterplot showing relationship between BMI and waist circumference in participants with diabetes and control participants

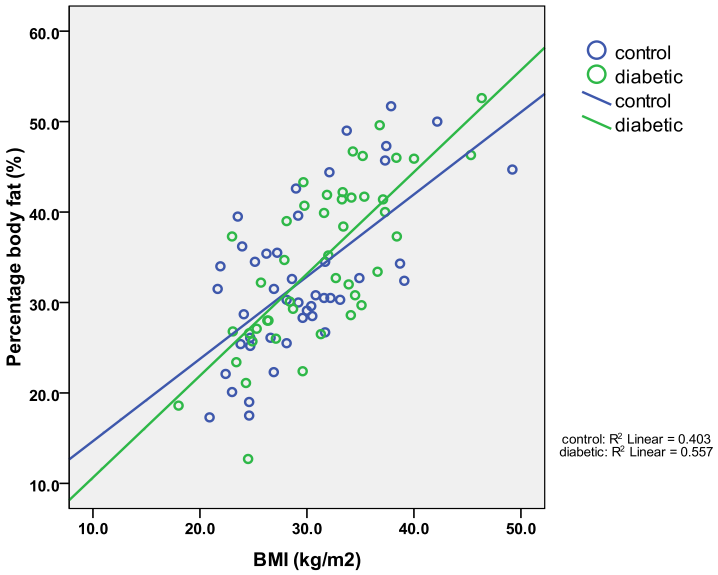


Figure 7-2: Scatterplot showing relationship between BMI and % body fat in participants with diabetes and control participants

7.4 Comparison of relationships between the different measures of adiposity and metabolic variables

In both participants with diabetes and control participants, the relationships between the metabolic variables and both BMI and waist circumference were generally concordant and of similar order of magnitude, with relationships between TAG and glucose and waist circumference being stronger than with BMI in control participants, supporting the concept of the adverse effect of central adiposity on lipid metabolism (67). In participants with diabetes and control participants, the negative relationship with ^{13}C - palmitic acid in the non-esterified fatty acid fraction AUC (^{13}C-PA NEFA AUC) and adiposity was also strongest with waist circumference.

% body fat had fewer correlations with plasma lipids or with lipid or carbohydrate oxidation in participants with diabetes or control participants. The positive relationships found were with fasting NEFA in participants with diabetes, and with ^{13}C-PA NEFA AUC and NEFA AUC in controls. The positive ^{13}C-PA NEFA AUC relationship with % body fat in control participants was *opposite* to the negative relationship found for waist circumference.

Reasons for why BMI and waist circumference on one hand and % body fat on the other often show different correlations with the metabolic outcomes measured here may be:

7.4.1 <u>% body fat is a better measure of adipose tissue mass</u>

% body fat is a better measure of true 'adipose tissue mass' than BMI or waist circumference. BMI and waist circumference correlate strongly with lean (muscle, liver) mass, whereas % body fat does not. Therefore if some of the metabolic effects and differences in rates of oxidation were affected by differences in muscle metabolism between participants with diabetes and control participants then this is more likely to co-vary with BMI and waist circumference than with % body fat.

7.4.2 Waist circumference is a better estimate of central fat distribution in the body

Waist circumference is a better estimate of central adiposity than % body fat, as peripheral adiposity is thought to be 'healthier' fat metabolically.

7.4.3 Percentage fat vs total fat mass

Patients with the same *percentage* body fat can have very different total *fat mass* as the percentage body fat also depends on their lean mass. As an extreme example, a 100 kg person with 50% fat mass will have 50 kg of fat whereas a 50 kg person with 50% fat mass will only have 25 kg of fat. It is unknown if a persons' actual total fat mass in kg has more influence on their metabolism than their % body fat.

7.4.4 Gender effect

'Normal range' % body fat is higher in women than in men, but to increase the power of the study, the male and female data have been pooled. This may dilute any gender specific influences of differences in % body fat on metabolism.

7.5 What measure of adiposity should be used in clinical practice to predict 'metabolic health'?

This question has been asked many times before. The current consensus is that a measure of waist circumference or waist to hip ratio is the best predictor of metabolic health and cardiovascular risk in an individual (1). The results of this study would be in concordance with this view as the strongest association with impaired lipid metabolism was seen with waist circumference in both participants with diabetes and control participants, although the relationship was weaker in the participants with diabetes. It would be interesting to repeat this study with a more accurate quantification of regional fat distribution for example using DXA or MRI scanning, to elucidate if differences in regional adiposity in participants with type 2 diabetes are associated with differences in postprandial triglyceride metabolism.

Chapter 8 Final discussion and conclusions

8.1 Introduction

This chapter summarises the original aims of the study and the key results obtained. It puts the results in the context of the existing published literature and discusses the possible pathophysiological explanations behind the results. The limitations of the study are also discussed. Finally there is a discussion of how the study findings may change clinical practice and what further research is needed to answer questions raised by this study.

8.2 Study background and aims

There is a well described positive association between obesity and type 2 diabetes (5;6). The prevalence of both is rising and they are projected to have far reaching effects on public health and to have negative effects on the world economy mainly due to increased cardiovascular disease (197). Less is known regarding the effect of obesity on mortality and morbidity outcomes once an individual has established diabetes, although recent data suggests that there may be an 'obesity paradox' in individuals with diabetes (8-10). A better understanding of the effects of obesity on outcomes in patients with established diabetes is important as some therapeutic interventions increase body mass index (BMI) and some interventions are only made available to patients with a BMI above a specific value ($35kg/m^2$) (170).

This study was designed to determine the effect of easily accessible, clinical measures of adiposity on fasting and postprandial triglyceride and glucose metabolism in men and women with and without type 2 diabetes. The primary outcome measure was a comparison of the relationship between postprandial dietary-derived triglyceride concentrations (^{13}C - palmitic acid in the triglyceride fraction AUC (^{13}C-PA TAG AUC)) and BMI in participants with diabetes compared with control participants.

Secondary outcome measures included:

i) a comparison of the relationships between other measures of fasting and postprandial triglyceride and glucose metabolism and BMI in participants with diabetes compared with control participants.

ii) a comparison of the relationships between fasting and postprandial triglyceride and glucose metabolism and waist circumference in participants with diabetes compared with control participants.

iii) a comparison of the relationships between fasting and postprandial triglyceride and glucose metabolism and percentage body fat in participants with diabetes compared with control participants.

iv) A comparison of fasting and postprandial triglyceride and glucose metabolism between participants with diabetes and control participants after controlling for adiposity.

8.3 Choice of primary outcome measure

^{13}C-PA TAG AUC was used as the primary measure of lipid metabolism as this reflects metabolism of dietary-derived triglyceride as distinguished from endogenous circulating triglyceride and therefore potentially adds more mechanistic information about the abnormalities in lipid metabolism known to be associated with obesity and diabetes than a measurement of 'total' circulating TAG or NEFA would provide. Another measure of dietary TAG metabolism used in the study was the incremental area under the postprandial triglyceride curve (INC AUC TAG), which is the total postprandial area under the TAG curve minus the fasting area under the curve TAG. There is a known association between abnormalities in postprandial triglyceride metabolism and cardiovascular risk (12). BMI was used as the primary measure of adiposity as this is the measure used most frequently in clinical practice and the measure which is used in clinical guidelines to stratify therapeutic interventions (170), other measures of adiposity used were the waist circumference, a measure of abdominal adiposity and percentage body fat.

8.4 Discussion of primary outcome results

A weak non-statistically significant *negative* correlation was found between ^{13}C-PA TAG AUC and BMI in the participants with diabetes (r=-*0.210*, p=0.172), whereas in control participants there was a positive correlation between ^{13}C-PA TAG AUC and BMI which was not statistically significant but which approached statistical significance (r=0.288, p=0.06) (Table 4.1, Figure 4.7). Using multiple linear regression there was a trend towards a difference in the relationship between ^{13}C-PA AUC TAG and BMI and in participants with diabetes compared to that in the control participants which approached statistical significance (p=0.07) (Figure 4.7). Although these results did not reach predefined criteria for statistical significance, they are still of interest as the results for another measure of postprandial TAG metabolism, INC AUC TAG were similar. In participants with diabetes there was a weak non-statistically significant *negative* correlation between INC AUC TAG and BMI (r=-*0.211*, p=0.180), whereas in control participants there was a borderline positive correlation between INC AUC TAG and BMI (r=0.263, p=0.080). (Figure 4.6). Using multiple linear regression there was a significant difference in the relationship between INC AUC TAG and BMI between participants with diabetes and control participants (p=0.04). (Figure 4.6).

In a post hoc analysis measures of postprandial TAG metabolism (^{13}C-PA TAG AUC and INC AUC TAG) were compared between in the participants in the lowest and highest quartiles of BMI. ^{13}C-PA TAG AUC, was numerically higher in the participants with diabetes in the lowest BMI quartile compared with the highest BMI quartile, but this did not reach statistical significance (77.1(38.6-104.3) vs 52.1(33.2-82.4) ug/ml/6h, p=0.25). Incremental (INC) TAG AUC (6.2(1.5-8.8) vs 3.4(1.5-6.4) mmol/l/6h, p=0.41) showed a similar trend. In control subjects an opposite trend was found.^{13}C-PA TAG AUC was numerically lower in the controls in the lowest BMI quartile compared to the control participants in the highest BMI quartile but this did not reach statistical significance (34.2 (22.6-44.5) vs 50.1 (23.7-80.6) ug/ml/6h, p=0.34). INC AUC TAG was also numerically, but not significantly lower in controls in the lowest BMI quartile compared to those in the highest BMI quartile (2.1(1.0-3.1) vs 4.3 (1.4-10.6) mmol/l/6h, p=0.15) (Table 5.1, Figures 5.1 and 5.2).

These data suggest that the relationship between BMI and postprandial TAG metabolism is different in participants with diabetes compared to control participants. It appears that in control participants that postprandial TAG metabolism is impaired with increasing BMI, but in participants with diabetes that postprandial TAG metabolism is impaired regardless of the BMI of the participant or possibly postprandial TAG metabolism is impaired further in non-obese participants with diabetes. This is the first study to show this finding. As the results for the primary endpoint meet borderline statistical significance, this study would need to be repeated in a larger participant population for confirmation. However this may go some way to explain the obesity paradox that is becoming increasingly apparent in patients with diabetes (8-10).This is an important finding as impairment of postprandial triglyceride metabolism is an independent cardiovascular risk factor and currently useful therapies such as GLP-1 analogues are not routinely offered to diabetes patients with a BMI less than 35kg/m^2 (170).

8.4.1 Previous literature

There are no previous data in the literature describing the relationship between dietary TAG metabolism and BMI in patients with type 2 diabetes, or how this compares to the relationship found in individuals without diabetes. There is a known association between postprandial dyslipidaemia and obesity in individuals without diabetes with impaired chylomicron clearance with increasing obesity observed in some studies (106;108).

8.4.2 Pathophysiological explanation for results

In this study, non-obese participants with diabetes were found to have impairment of metabolism of dietary TAG which was as severe as the impairment found in obese participants with diabetes. In control participants however, postprandial dietary TAG concentrations increased as BMI increased. The latter is not a unique finding (106;108) and is likely to be due to increasing insulin resistance with increasing BMI. This study confirmed a strong positive relationship between insulin resistance estimated by the homeostatic model assessment-insulin resistance (HOMA-IR) and

BMI in control participants (r=0.72, p=0.0001), but not in participants with diabetes (r=0.21, p=0.17) (Table 4.1, Figure 4.14).

Normal processing of dietary TAG involves many metabolic pathways including gut absorption, LPL-mediated hydrolysis of chylomicron-TAG (and VLDL-TAG) into NEFA and glycerol, storage of dietary NEFA in adipose tissue and other peripheral tissues, and lipid oxidation (see Chapter 1). Impairment of metabolism may occur during one or more of these processes. ^{13}C-PA TAG measured in this study was likely to be contained mainly in the chylomicron-TAG fraction, but may also have been incorporated early in the postprandial period into VLDL-TAG (198). Likewise, INC AUC TAG is a measure of TAG both in chylomicrons and VLDL particles.

It is likely that in obese control participants, resistance to insulin-stimulated LPL-mediated clearance of dietary TAG in chylomicrons contributed to elevated postprandial TAG with increasing obesity. In participants with diabetes, the absence of a relationship between postprandial dietary TAG concentrations and BMI may be explained by the hypothesis that in participants with diabetes, metabolism of postprandial TAG is impaired at *all levels of BMI.* In an attempt to explore the pathophysiology of this further, the results from the study will be used to address the possible underlying mechanism(s) sequentially below. It must be born in mind that adiposity may change in a patient with diabetes with time and that duration of diabetes may have an effect on metabolism, but the latter was not recorded in this cross sectional study:

8.4.3 Gut absorption rates of dietary TAG

There are no previous studies which directly examine the effect of BMI on gut absorption of TAG in participants with diabetes, but previous studies in participants with type 2 diabetes and other insulin resistant states suggest increased production rate of apolipoprotein B-48 containing particles. This is speculated as secondary to overproduction of lipoprotein particles by enterocytes in response to elevated plasma NEFA (199). In this study ^{13}C-PA TAG entry into the circulation over the first hour appeared higher in participants with diabetes compared to non-obese control

participants (Figure 5.4) which may suggest increased gut TAG absorption in participants with diabetes. ^{13}C-PA TAG entry into the circulation over the first hour was similar in the lowest and highest quartiles of BMI in participants with diabetes. Peak ^{13}C-PA TAG was reached at 2 hours in both quartiles. The ^{13}C-PA TAG peak at 2 hours was greatest in the participants with diabetes in in the lowest quartile of BMI. Further analysis of the higher peak in the non-obese participants with diabetes would require mathematical modelling of gut absorption rates vs clearance rates of ^{13}C-PA TAG. However the timecourse data suggests that ^{13}C-PA TAG gut absorption rates in the first hour were similar in the highest and lowest BMI quartiles in participants with diabetes, but that ^{13}C-PA TAG clearance appeared reduced in lowest quartile of BMI in participants with diabetes.

8.4.4 Clearance of ^{13}C-PA TAG and ^{13}C-PA NEFA from the circulation

^{13}C-PA dietary TAG undergoes lipolysis by endothelial LPL to ^{13}C-PA NEFA and monoacylglycerol. ^{13}C-PA NEFA is then taken up for storage in adipose tissue and to a lesser extent liver and skeletal muscle. ^{13}C-PA NEFA which is not stored may be re-esterified into ^{13}C-PA VLDL-TAG by the liver. ^{13}C-PA labelled VLDL will be detected in the ^{13}C-PA TAG fraction. In this study, in participants with diabetes there was a significant negative correlation between ^{13}C - palmitic acid in the non-esterified fatty acid fraction AUC (^{13}C-PA in NEFA AUC) and BMI ($r=-0.352$, $p=0.018$) (Table 4.1, Figure 4.10). In addition ^{13}C-PA NEFA AUC was significantly higher in the lowest quartile of BMI compared with the highest quartile of BMI (3.1(2.8-4.2) vs 2.3(1.7-2.8) μg/ml/6h, $p=0.01$). (Table 5.1, Figure 5.4). This suggests that storage of dietary NEFA was impaired in non-obese participants with diabetes, with reduced entrapment and increased 'overspill' of ^{13}C-PA NEFA into the circulation. The observation that there was a negative correlation between ^{13}C-PA NEFA and BMI in participants with diabetes supports this (Table 4.1). The ^{13}C-PA NEFA 'overspill' may be taken up by liver and incorporated into VLDL and re-circulated. The ^{13}C-PA VLDL may contribute to the elevated ^{13}C-PA TAG seen in the non-obese participants with diabetes. The elevated ^{13}C-PA TAG may also be secondary to reduced LPL mediated lipolysis of chlyomicron ^{13}C-PA TAG which

may be lower in the non-obese participants with diabetes. Further analysis would require mathematical modelling of clearance of ^{13}C-PA TAG in chylomicrons and appearance of ^{13}C-PA TAG in VLDL together with measurement of ^{13}C-PA TAG in the different lipoproteins by ultracentrifugation. However it might be expected that appearance of ^{13}C-PA NEFA would be reduced if LPL lipolysis of ^{13}C-PA chylomicron TAG was impaired.

There is no previous data examining the relationship between uptake of circulating ^{13}C-PA dietary NEFA and BMI in participants with diabetes, although there are previous studies describing dietary TAG metabolism in participants with rare PPAR gamma mutations and partial lipodystropy. Tan et al. studied postprandial triglyceride metabolism in a participants with diabetes patient with a mutation in peroxisome proliferator-activated receptor gamma (PPARG) P467L and found that meal-derived ^{13}C-PA accumulated rapidly in the NEFA fraction (88). The authors suggested that this was due to impaired fatty acid trapping in adipose tissue. They also found that ^{13}C-PA TAG was higher in the participants with the PPARG mutation compared to healthy and participants with diabetes controls. Interestingly, this patient had a BMI of 24.1 and the investigators struggled to find participants with diabetes of a similar BMI to use as a comparator group. It is possible therefore that our non-obese participants with diabetes cohort contained participants with undiagnosed partial lipodystrophy which may account for our results. Evidence against this however is that fasting and postprandial insulin concentrations are often elevated in patients with lipodystrophy and this was not the case in our cohort (45).

8.4.5 Beta-oxidation of ^{13}C-PA TAG

There were no significant correlations detected between ^{13}C-PA oxidation and BMI in participants with diabetes when assessed by % excretion of $^{13}CO_2$ in the breath (r=*-0.014*, p=0.928), (Table 4.2). There was no difference in oxidation rates of dietary ^{13}C-PA TAG (measured as $^{13}CO_2$ in the breath) in the participants with diabetes in the lowest BMI quartile compared to the participants with diabetes in the highest BMI quartile (9.3±4.2 vs 10.0 ± 2.6 % dose/6h, p=0.66) (Table 5.3, Figure 5.5). This was unexpected as the substrate concentration (^{13}C-PA NEFA) was

significantly higher in the lowest BMI group. There was a positive correlation between both fasting and AUC fat oxidation and BMI measured by indirect calorimetry (Table 4.2). Calorimetry derived fasting and AUC fat oxidation rates were lower in the participants with diabetes in the lowest BMI quartile than the participants with diabetes in the highest BMI quartile and this was of borderline statistical significance (p=0.06 and p=0.09 respectively). These data suggest that there may be a defect in oxidation of dietary lipid in non-obese participants with diabetes relative to the total circulating substrate. This would be supported by experiments by Kelley, which showed reduced muscle oxidation in patients with diabetes (184); however, when all participants were analysed together, participants with diabetes showed higher oxidation rates than controls and therefore it is unlikely that defects in fat oxidation are a major contributor to impairment of postprandial triglyceride metabolism in the participants with diabetes (Table 3.10). In the study of a non-obese participants with diabetes patient with a PPAR gamma mutation described above, there was no defect detected in dietary fat oxidation (88).

8.4.6 Fasting TAG

Fasting TAG has been shown to make an important contribution to postprandial dyslipidaemia (95) due probably to competition between circulating VLDL from the liver with recently ingested chlomicron-TAG. In this study there was a positive relationship between fasting TAG and BMI in both participants with diabetes and control participants (Table 4.1, Figure 4.1). Fasting TAG was significantly *lower* in participants with diabetes in the lowest BMI quartile compared with participants with diabetes in the highest BMI quartile (1.7 (0.9-2.4) vs 2.5 (1.9-3.8) mmol/l, p=0.03) (Figure 5.1). Fasting NEFA was also lower in participants with diabetes in the lowest BMI quartile but this did not reach statistical significance (176.1(155.3-196.7) vs 200.2 (175.3-241.2) umol/l, p=0.08), (Figure 5.3). Therefore the effect of fasting TAG is unlikely to be the primary factor adversely affecting postprandial TAG metabolism in the non-obese participants with diabetes over and above the effect seen in obese participants with diabetes.

8.4.7 Glycaemic control

Poor glycaemic control is independently associated with impaired TAG metabolism (173). In this study there was a positive correlation between BMI and glycated haemoglobin (HbA1c) in participants with diabetes (r=0.319, p=0.035). This suggests that the leanest participants with diabetes had better glycaemic control compared with the more obese participants. No association was found between ^{13}C-PA TAG AUC or INC AUC TAG and HbA1c or fasting glucose in participants with diabetes. It is therefore unlikely that hyperglycaemia per se was the primary defect affecting postprandial TAG metabolism.

8.4.8 Beta cell function

Insulin action is central to many of the physiological processes important in metabolism of dietary lipid. Estimates of beta cell function used in this study were in the fasting state, homeostatic model assessment-% beta cell function (HOMA-B%) and in the postprandial state, the 30 minute insulin concentration.

In control participants HOMA-B% strongly positively correlated with measures of adiposity (BMI; r=0.64, p<0.0001, waist circumference; r=0.63, p<0.0001, % body fat; r=0.53, p<0.0001). In participants with diabetes HOMA-B% was *not* significantly associated with measures of adiposity (BMI; r=0.11, p=0.48, waist circumference; r=0.02, p=0.90, % body fat; r=-*0.09*, p=0.58) (Table 4.1). Using multiple linear regression analysis interactions were found for the differences in the relationships between HOMA-B% and BMI (p=0.026), waist circumference (p=0.059) and % body fat (p=0.009) in participants with diabetes compared to the control participants, with a positive correlation with increasing adiposity in the control participants, but no correlation in participants with diabetes (Figure 4.15). These data suggest that in the fasting state in obese control subjects, beta cells produce more insulin to overcome the insulin resistance associated with obesity, but that this does not occur to the same extent in participants with diabetes. In the participants with diabetes, it is likely that beta cell function is impaired across all BMI measurements.

There was, however, a significant positive correlation between 30-minute insulin and BMI in participants with diabetes (r=0.320, p=0.032) and fasting insulin (8.2 (4.9-13.7) vs14.2 (193.0-396.8) μU/ml, p=0.04), 30 minute insulin (30.0 (23.7-35.1) vs 45.7 (22.6-67.8) μU/ml, p=0.08) and AUC insulin (163.0 (153.3-239-1) vs 274.5 (193.0-396.8) μU/ml, p=0.04) were all lower in the participants with diabetes in the lowest BMI quartile compared with the participants with diabetes in the highest BMI quartile (Table 5.2, Figure 5.6). There was also a significant negative correlation between ^{13}C-PA in NEFA AUC and 30-minute insulin (r=-*0.424*, p=0.004) (Table 6.3, Figure 6.1). It is therefore likely that relative insulin deficiency contributed to impaired postprandial TAG metabolism in the non-obese participants with diabetes. This may be due to impaired insulin-induced activity of LPL and chylomicron TAG lipolysis and/or reduced insulin mediated uptake of dietary NEFA for storage in adipose and other tissues. In the study by Tan et al. described above, the subject with the PPARG mutation had plasma insulin concentrations which were lower than that of participants with diabetes control participants in the fasting state, but were similar in the postprandial state, and were similar to those of the non-participants with diabetes controls (88).

8.4.9 Insulin resistance

Insulin resistance was estimated by homeostatic model assessment-insulin resistance (HOMA-IR). When all participants were considered together, HOMA-IR was significantly greater in participants with diabetes compared to control participants ($p<0.0001$). HOMA-IR was not significantly associated with BMI in participants with diabetes (r=0.21, p=0.17) but strongly correlated in control participants (BMI; r=0.72, p=0.0001) (Figure 4.14). HOMA-IR was numerically lower in the lowest quartile of BMI compared to the highest quartile of BMI in the participants with diabetes but this was not statistically significant (3.0 (1.8-8.6) vs 6.4 (5.5-7.2) , p=0.10) In contrast, in control participants HOMA-IR was lower in the lowest quartile of BMI compared to the highest quartile of BMI, and this was highly statistically significant (1.1 (0.8-1.9) vs 3.9 (2.8-4.8), $p<0.0001$) (Table 5.2). The data therefore suggest that in participants with established diabetes, there is not an important effect of BMI on insulin resistance. It is therefore unlikely that the non-

obese participants with diabetes were significantly more insulin resistant than the obese participants with diabetes, but it is important to remember that HOMA-IR only reflects insulin resistance in the fasting state and not in the postprandial state.

8.4.10 Medication differences

The oral hypoglycaemic medications used by the participants are summarized in Table 5.11. The participants with diabetes in the lowest quartile of BMI consisted of 55.5% on no medication, 44% on a sulphonylurea, 22% on metformin and none on a thiazolidenedione (TZD). The participants with diabetes in the highest quartile of BMI consisted of 21% on no medication, 35.7% on a sulphonylurea, 57% on metformin and 14% on a TZD. The effects on higher entrapment of ^{13}C-PA NEFA in the obese patients may therefore be due to the TZD, but this was not statistically significant. There were proportionally more patients on no oral hypoglycaemic medication in the lowest BMI group which may have had an effect on the postprandial triglyceride handling in these participants but this was not statistically significant. For this observation to be validated, the study would need to be repeated with patients preferably taking no oral hypoglycaemic medication, although this may be difficult to achieve in practice due to the ethical considerations of withholding medication.

8.4.11 Gender distribution

There were 4 female and 5 male participants in the lowest quartile of BMI and 9 female and 5 male participants in the highest BMI quartile. These distributions were not significantly different (Table 5.10). It is possible however, that females with diabetes have better postprandial triglyceride handling than males and that this has influenced the results. However we have previously shown that the protective effect of female gender on lipid handling is lost in patients with type 2 diabetes (90).

8.4.12 Were the non-obese participants with diabetes a subset of participants with diabetes with lipodystrophy, late onset autoimmune diabetes or maturity onset diabetes of the young/monogenic diabetes?

A more detailed review of the medical history of the participants with diabetes to establish age of onset of diabetes and family history would help establish whether there were any features to suggest lipodystrophy, late onset autoimmune diabetes (LADA) or maturity onset diabetes of the young/monogenic diabetes (MODY). Also an analysis of auto-antibodies including anti-glutamic acid decarboxylase (GAD) and anti-islet cell antibodies would be useful as would any subsequent genetic testing results. Unfortunately access to the medical notes is not currently possible and these details were not taken as part of the study dataset. It is entirely plausible that the participants have one of these conditions as the cause of their diabetes as these diagnoses are often missed when a patient first presents with diabetes. Most, but not all types of MODY are associated with favourable lipid profiles (43) and therefore the lean participants with diabetes were more likely to have undiagnosed lipodystrophy or LADA than MODY.

8.4.13 Erroneous result

It is possible that the results are erroneous especially as the results for the difference in the relationship between ^{13}C-PA TAG AUC and BMI in participants with diabetes and control participants only reached borderline statistical significance (p=0.07). However a similar pattern of results was found for the for the difference in the relationship between INC AUC TAG and BMI in participants with diabetes and control participants which was statistically significant (p=0.04) (Table 4.1, Figure 4.6). These are both measures of postprandial TAG metabolism, and were measured independently of each other. Bonferroni corrections for multiple comparisons were not made and therefore this exploratory study would need to be repeated to confirm the findings.

8.5 Summary: primary outcome

In this study, non-obese participants with diabetes were found to have impairment of metabolism of dietary TAG which was as severe as the impairment found in obese participants with diabetes. In control participants however, postprandial dietary TAG concentrations increased as BMI increased. Non-obese participants with diabetes showed worse impairment of postprandial ^{13}C-PA NEFA uptake than obese participants with diabetes which may contribute to elevated ^{13}C-PA TAG concentrations due to subsequent uptake of ^{13}C-PA NEFA in the liver and formation of ^{13}C-PA-labelled VLDL. Oxidation of dietary TAG was possibly also impaired in non-obese participants with diabetes relative to the concentration of available substrate, although fat oxidation in participants with diabetes was increased compared with control participants overall, so this is unlikely to be the primary mechanism causing impairment of postprandial triglyceride metabolism in participants with diabetes. The data suggest that it is likely that both postprandial insulin deficiency and insulin resistance play a key role in the impairment of postprandial TAG metabolism in participants with diabetes patients regardless of BMI, but in the non-obese participants insulin deficiency is likely to be more important.

A higher BMI (fat mass and/or lean mass), may be *protective* in type 2 diabetes, as there was greater entrapment of dietary fatty acids in these individuals (Figure 4.10). This would be in keeping with the 'adipose expandability' hypothesis of diabetes and the metabolic syndrome (54;200). Alternatively, non-obese participants with diabetes may have an *additional* impairment of dietary TAG metabolism compared with participants with diabetes with a higher BMI, due to insulin deficiency, reduced oxidation of dietary fat, or relative adipose tissue failure in efficient postprandial storage of dietary TAG.

8.6 Secondary outcomes

8.6.1 Relationship between ^{13}C-PA TAG AUC and other measures of adiposity in participants with diabetes vs control participants

There was a significant positive correlation between ^{13}C-PA TAG AUC and waist circumference in control participants (r=0.296, p=0.048) but there was no correlation in participants with diabetes (r=-0.102, p=0.543). There was no statistically significant difference (interaction) in the relationship between ^{13}C-PA TAG AUC and waist circumference in participants with diabetes and control participants, but it can be seen in Figure 4.7 that the participants with diabetes in the lowest quartile of waist circumference have a numerically higher ^{13}C-PA TAG AUC than that found in the highest waist circumference quartile. There was also a significant negative correlation between ^{13}C-NEFA AUC and waist circumference in participants with diabetes and control participants, again suggesting that individuals with less central adiposity remove dietary NEFA less efficiently from the circulation.

There was no correlation between ^{13}C-PA TAG AUC and % body fat in the participants with diabetes or control groups. Results found throughout the study were generally less concordant with % body fat than between waist circumference and BMI. This may be because the measure of % body fat using bioelectrical impedance is not always reliable. It would be interesting to repeat the study using another method of body composition eg dual-emission X-ray absorptiometry (DXA) scanning.

8.6.2 Relationship between other measures of fasting and postprandial triglyceride metabolism and measures of adiposity in participants with diabetes compared to control participants

TAG

Fasting TAG had a positive relationship with BMI in both participants with diabetes and control participants (Table 4.1, Figure 4.4), suggesting that production of VLDL-TAG by the liver overnight was increased with increasing adiposity in both groups. This relationship was also similar for waist circumference, but for % body fat no relationship was found in participants with diabetes or controls. In the postprandial state INC AUC TAG is a further estimate of dietary TAG metabolism.

The results for INC AUC TAG were consistent with those found for ^{13}C-PA TAG AUC and this adds validity to the results of the study (Table 4.1).

NEFA

Fasting NEFA is generated from lipolysis from adipose tissue TAG stores overnight. Adipose tissue TAG lipolysis is inhibited by insulin. In this study fasting NEFA had a stronger positive correlation with all measures of adiposity in participants with diabetes than control participants, in whom no correlations were found (Table 4.1). The difference in the relationship between NEFA and adiposity in participants with diabetes and control participants was not statistically significant. It has previously been presumed that increasing adiposity is positively associated with fasting NEFA in non-participants with diabetes, but this is not the first study to refute this (118;194). It may be that the increased insulin concentrations in obese participants can overcome the increase in lipolysis rates in obese non-participants with diabetes, but that the insulin concentrations are not high enough in the obese participants with diabetes patients. In the postprandial state however in participants with diabetes there is clearly a *negative* relationship between adiposity (BMI and waist circumference) and ^{13}C-PA NEFA AUC and it could be suggested that the obese participants with diabetes have adipose tissue which is functioning more efficiently postprandially than the non-obese participants with diabetes. However in the control participants the correlation between ^{13}C-PA NEFA AUC with BMI was not statistically significant and the relationships with waist circumference and % body fat show opposite results, so it is difficult to draw conclusions. The circulation of 'spillover' dietary NEFA is important as it is likely that this NEFA will be stored ectopically in other tissues such as the liver and skeletal muscle, and contribute to worsening insulin resistance and fatty liver disease (201).

Lipid oxidation

Previous studies have shown both increased and decreased fat oxidation in obese individuals without diabetes (202;203). A reduction in mitochondrial oxidative function of skeletal muscle has been found in in-vitro studies in participants with diabetes. The effect of adiposity was not examined in the latter in vitro studies

(183;204). In an in vivo study where patients with lipodystrophy, some of whom had type 2 diabetes, were given a high fat meal, fat oxidation rates were increased but the direct effect of BMI was not determined (205).

In the current study, whole body fat oxidation was measured by determining the oxidation of dietary derived ^{13}C-PA as $^{13}CO_2$ in the breath, and also by indirect calorimetry. In participants with diabetes, there were no correlations between breath $^{13}CO_2$ and BMI (r=*-0.014*, p=0.928), a weak *negative* correlation with waist circumference (r=*-0.280*, p=0.098) and no correlation with % body fat (r=*-0.089*, p=0.572) (Table 4.2). In control participants, there were no correlations between breath $^{13}CO_2$ and BMI (r=0.127, p=0.405), waist circumference (r=0.099, p=0.519) or % body fat (r=*-0.025*, p=0.872) (Table 4.2).Using multiple linear regression analysis there were no differences in the relationships between breath $^{13}CO_2$ and BMI (p=0.300) or % body fat (p=0.527) in participants with diabetes compared to the control participants, but for waist circumference there was a statistically significant interaction in the relationship between breath $^{13}CO_2$ excretion and waist circumference (p=0.023) in participants with diabetes compared to the control participants (Figures 4.16). These data suggest that in participants with diabetes, those with a lower waist circumference oxidized more dietary triglyceride than those with a higher waist circumference but that this was not the case in control participants. This is likely to reflect the concentration of substrate available (^{13}C-PA labelled NEFA) which was higher in the diabetic participants with the lowest waist circumference.

When measured by indirect calorimetry, in participants with diabetes, there was a positive correlation between AUC fat oxidation that approached statistical significance for both BMI (r=0.287, p=0.069) and waist circumference (r=0.326, p=0.060) but not with % body fat (r=0.232 p =0.144) (Table 4.2). In control participants, there were positive correlations between AUC fat oxidation and BMI (r=0.40, p=0.008) and waist circumference (r=0.305, p=0.047), but not with % body fat (r=0.197 p =0.206) (Table 4.2). Using multiple linear regression analysis there was no significant difference in the relationship between AUC fat oxidation and BMI in participants with diabetes compared to the control participants (p=0.928),

waist circumference (p=0.493) or % body fat (p=0.492) in participants with diabetes compared to the control participants.

Overall, in this study there did not appear to be significant differences in the relationships between postprandial fat oxidation and adiposity between participants with diabetes and control participants, except in the relationship between dietary fat oxidation and waist circumference.

8.7 Differences in triglyceride metabolism between participants with diabetes and control participants after controlling for measures of adiposity

Postprandial TAG (^{13}C-PA TAG AUC and INC TAG AUC)

^{13}C-PA TAG AUC and INC TAG AUC were not statistically different between participants with diabetes and control participants when the whole BMI-matched cohort was analysed and no difference emerged when the results were adjusted for the measures of adiposity. However there was a statistically significant difference in ^{13}C-PA TAG AUC when the participants with diabetes vs controls in the lowest BMI quartile were compared (p=0.01), but not participants with diabetes vs controls in the highest BMI quartile were compared, p=0.63 (Figure 5.2). This finding is important in two respects, firstly it suggests that the non-obese participants with diabetes are not more metabolically healthy than the obese participants with diabetes, but also that obese controls are as metabolically unhealthy, at least when considering postprandial triglyceride metabolism, as the obese participants with diabetes. In fact when comparing postprandial lipid data in the patients in the highest quartile of BMI, there were no significant differences between participants with diabetes and controls (Table 5.5). This of course may be because the study was not powered to look at these small groups of patients.

Other metabolic variables

When comparing the whole BMI-matched cohort of participants with diabetes vs control participants, all other aspects of plasma fasting and postprandial metabolism were worse in the participants with diabetes (Tables 3.5 and 3.8). This was confirmed when the data were corrected for other measures of adiposity (Tables 3.13-3.18). It is therefore clear that participants with diabetes patients have impairments in lipid and glucose metabolism beyond those caused by excess adipose tissue. Insulin deficiency is likely to make a substantial contribution to this, especially in the postprandial period, as the 30-minute post study meal insulin was significantly lower in the participants with diabetes patients (Figure 3.6, Table 3.8).

NEFA suppression

It is interesting that the participants with diabetes and control groups showed similar rates of NEFA suppression over the first 30 minutes postprandially despite loss of the 'first phase' insulin response (30-minute insulin) in the participants with diabetes group (Figure 3.3, Table 3.6). The 30-minute NEFA is likely to be the best proxy for inhibition of lipolysis immediately after eating. The NEFA suppression data suggest that inhibition of adipose tissue lipolysis is very sensitive in the early postprandial period in both participants with diabetes and control participants, and that reduction of the first phase insulin response in participants with diabetes does not affect inhibition of adipose tissue lipolysis. It is likely that increased rates of adipose tissue lipolysis overnight, plus poor adipose tissue entrapment of dietary NEFA in participants with diabetes contribute more to elevated NEFA in participants with diabetes than does failure of suppression of adipose tissue lipolysis postprandially. This study confirms that in participants with diabetes there was a reduction of adipose tissue capacity to store dietary NEFA postprandially (ie increased 'spillover').

Fat oxidation

An unexpected and important finding in this study was that fasting fat oxidation and oxidation of ^{13}C-PA was significantly higher in participants with diabetes vs control

participants, even after adjustment for adiposity (Figures 3.7 and 3.10, Table 3.10). This is the opposite result than would have been expected following the studies of Kelley et al., who showed reduced lipid oxidation in muscle in participants with diabetes (184). These findings supports Randle's hypothesis that there is uptake and metabolism of fat possibly preferentially to glucose metabolism in patients with diabetes (162) and refute the theory that elevated lipid concentrations and excess adiposity in patients with type 2 diabetes are due to reduced fat oxidation. The oxidation in this study reflects total body lipid oxidation and correlates with the amount of substrate (NEFA) in both participants with diabetes and control participants (Figure 3.11, Tables 3.11 and 3.12). It would be interesting to calculate whether the increase in oxidation is in proportion to the excess substrate in participants with diabetes, or if the participants with diabetes are not able to up-regulate oxidation rates enough to compensate for the increased substrate concentration. However, when the energy expenditure was calculated per unit of fat free mass this remains higher in participants with diabetes, which suggests that mitochondrial activity is increased, not decreased in these patients (Table 3.9). It is unlikely therefore that reduced fat oxidation was the primary reason for increased fasting or postprandial NEFA and TAG concentrations in the participants with diabetes patients.

8.8 Limitations of the study

8.8.1 Subject selection

The study participants were a heterogenous group with different ages and both male and female participants. Recruitment of both males and females was required to facilitate the relatively large numbers of patients required in this study, but is likely to have contributed to an increased variance of the results. Lipid metabolism varies between males and females and also in pre and postmenopausal women. Several studies with a more homogenous study population would most likely give more precise information about lipid metabolism in these subgroups.

8.8.2 Gender and measures of adiposity

Men and women have different adiposity and fat distribution. The 'normal' values for percentage body fat measured by bioelectrical impedance and waist circumference are different in men and women (206;207). This study pooled male and female data to increase the power of the study, and although the groups were well matched for numbers of male and female participants some inaccuracies may occur due to pooling the data.

8.8.3 Genetic polymorphisms

Genetic polymorphisms have been described which contribute to abnormalities in lipid metabolism (80). We have not screened for polymorphisms in this study.

8.8.4 Matching of diabetes and control groups

The two groups were well matched for gender, age, weight and BMI, but the participants with diabetes had a significantly higher waist circumference (107.74cm vs 100.27cm respectively, p=0.02). The higher waist circumference in the diabetes participants is likely to affect overall lipid metabolism, but not the analysis of effects of waist circumference in the individual groups.

8.8.5 Numbers of participants

Whereas this study has one of the largest study populations of a study of its kind, the numbers of participants in some of the subgroups of BMI, waist circumference and other measures of adiposity are relatively small. This reduces the power of the study to find differences between these groups, but may also lead to misleading results in the extreme (ie highest and lowest) groups.

8.8.6 Medication

The majority of the participants with diabetes were taking oral hypoglycaemic medication (Table 3.3). These medications have effects insulin concentrations and

degree of insulin resistance and are therefore likely to affect lipid metabolism. The study medication was not taken on the morning of the study but this was unlikely to have given enough time for complete elimination of the drug.

8.8.7 Meal content

Metabolism patterns vary according to the composition of the meal. The study meal given to our study participants was relatively fat rich. Therefore our findings may not reflect metabolism of meals of different composition eg carbohydrate rich, or protein rich meals.

8.8.8 Measures of adiposity

The measures of adiposity used in this study have limitations. BMI is not the best measure of fatness, especially when used to assess relatively small numbers of participants. There is a range of body fat content within different participants at the same BMI, even within participants with a 'normal' BMI.

Bioelectrical impedance is an imprecise measure of body fat and the calculations used by the machine depend on anthropmetric measures such as height and weight of the participants (206;207). More accurate measures currently being used are DXA scanning, magnetic resonance imaging (MRI) and air-displacement plethysmography ('BOD POD'). These methods have recently been reviewed. (208;209).

The location of body fat may be more important than the total amount of body fat. We have not assessed how much fat is contained in the tissues, for example skeletal muscle and liver. Instead, we have used waist circumference as a proxy measure of central or visceral adiposity.

8.8.9 Measures of Insulin Resistance and β Cell Function

The HOMA calculation was used to give an estimate of insulin resistance in the study population. This model has been validated against many other methods for

measuring insulin resistance and beta cell function including the euglycaemic and hyperglycaemic clamp techniques although the model is sometimes criticised as it is not the 'gold standard measure' especially when used in relatively small study populations. It is also only a reflection of insulin sensitivity in the fasting state. A hyperinsulinaemic-euglycaemic clamp may have given a more accurate estimation of muscle / whole body insulin resistance, although clamp techniques use insulin and glucose concentrations outside the normal physiological range (179). The use of oral hypoglycaemic drugs in some of the participants with diabetes will have influenced the HOMA and beta cell function results in these participants.

8.8.10 Duration of diabetes

It must be born in mind that adiposity may change over time in a patient with diabetes due to changes in lifestyle and medication changes and rarely with bariatric surgery (although none of the participants in this study had had bariatric surgery. The duration of diabetes may have an effect on glucose and triglyceride metabolism via changes with time in insulin resistance and beta cell function. Duration of diabetes was not recorded in this cross sectional study.

8.9 Clinical implications of the study

The recognition that a lean phenotype is not benign in patients with type 2 diabetes is important as this study has shown that lean patients with type 2 diabetes may have worse metabolic control of lipids in the postprandial period than patients with co-existing obesity and type 2 diabetes. This may put non-obese participants with diabetes patients at higher risk of cardiovascular disease. This view has recently been reinforced by findings from larger studies suggesting an inverse relationship between BMI and cardiovascular mortality in patients with type 2 diabetes (8-10). Current National Institute for Clinical Excellence (NICE) guidance advises 'rationing' the newer interventions for the treatment of type 2 diabetes for example, exenatide and the gliptins to patients above a specified BMI of 35kg/m^2 (170) (Figure 1.5). This would exclude all but the highest BMI quartile of participants in this study from access to a class of drug shown to benefit postprandial triglyceride metabolism

(137). Also there is historically more focus on ensuring that obese participants with diabetes are treated with metformin, whereas non-obese patients also benefit from this treatment. Prandial insulin therapy may be beneficial early in the disease course in non-obese patients with type 2 diabetes, even with good glucose control, as this may help overcome effects of loss of the first phase insulin response and reduce cardiovascular risk from impaired postprandial triglyceride metabolism.Weight loss and healthy eating remain important for all patients with type 2 diabetes. Even non-obese patients with diabetes would be likely to benefit from a low fat intake as this study has shown that their adipose tissue appears less able to store prandial fat. This fat is likely to be stored in depots outside the adipose tissue (eg liver and muscle) and this will exacerbate insulin resistance and metabolic derangements. This is known to be the case in patients with lipodystrophy where a strict low fat diet remains a central component of treatment (45;205).

In the future clinical assessment of patients with type 2 diabetes should include better phenotypic characterisation of patients based on BMI and fat distribution and careful examination for presence/absence of features of severe insulin resistance such as acanthosis nigricans, a typical pigmented appearance in the axillae. Biochemical investigations such as anti-GAD and anti-islet cell autoantibodies are also useful. This should help with identification of monogenic syndromes of diabetes including MODY and lipodystrophy and also identify patients with LADA (44;210). This will help with the individualisation of management of each patient and is also important as many of the monogenic syndromes are familial and this management approach would encourage genetic counselling and family screening.

It is important also to emphasise that obesity can be associated with an unhealthy metabolic phenotype in patients who do *not* have type 2 diabetes, and that whilst some individuals may be obese and have a healthy metabolic phenotype (211), others, whilst not having overt diabetes, can have impairment of fasting and postprandial lipids similar to those found in a patient with a similar BMI who has type 2 diabetes.

8.10 Future studies

This is a relatively small study which proposes that postprandial triglyceride metabolism is impaired across participants with diabetes patients of any BMI. This finding needs to be replicated. Future studies should also be performed to investigate the pathophysiological mechanisms involved more fully and with clearer phenotyping of patients. The existence of whole exome sequencing also makes it possible to perform phenotype/genotype studies in families with a high prevalence of type 2 diabetes.

An important potential study would be to examine the deposition of dietary fat into tissues outside adipose tissue. A similar study performed by Ravikumar et al used ^{13}C magnetic resonance spectroscopy following ingestion of ^{13}C-labelled fat and showed that postprandial uptake of fatty acids by liver and skeletal muscle are increased in 12 participants with type 2 diabetes (201). It would be interesting to repeat this study with a larger cohort of participants with diabetes patients and examine the relative uptake in patients across a range of BMI. It would be important that the patients were carefully phenotyped and ideally their fat distribution more accurately determined using MRI or DXA scanning. A useful follow on study would be to give these participants meals with different fat content to see what quantity of fat they can ingest with before 'overspill' of NEFA is found. This data may help guide dietary advice given to patients with diabetes and hypertriglyceridaemia.

Intervention studies using newer agents for example the GLP-1 agonist exenatide and the dipeptidyl peptidase IV (DPPIV) inhibitors for example sitagliptin in patients with type 2 diabetes who are carefully phenotypically characterised may guide future use of these therapies and may reduce the inequality of access to these therapies. Recent studies which have looked at adipose tissue functionality in obese and lean participants without diabetes have suggested that obese non-participants with diabetes have adipose tissue which is less metabolically active (increased NEFA spillover) (190). This is opposite to the finding in this study which shows that dietary NEFA concentrations are lower in obese participants with diabetes than non-obese participants with diabetes. It would be interesting to perform similar studies in

participants with type 2 diabetes to see whether the adipose tissue is functionally different in these participants to control participants.

Finally more longitudinal studies of long term outcomes in well phenotyped patients with type 2 diabetes are needed so that risk stratification and prognosis in these patients can be clarified and so that energies and resources are directed into the most useful interventions for improving outcomes in patients with type 2 diabetes.

8.11 Conclusion

Despite the widely accepted strong association between obesity and type 2 diabetes, it remains unknown whether increasing adiposity adversely affects cardiovascular and other outcomes in patients with type 2 diabetes as it does in obese individuals without type 2 diabetes. Recent studies have suggested that there may be an 'obesity paradox' in patients with type 2 diabetes.

The aims of this study were to investigate the effects of adiposity as measured by easily available clinical measures on fasting and postprandial glucose and lipid metabolism in 90 participants with and without diabetes over a range of BMI. The study design included administration of a standard meal which contained a stable isotope ^{13}C-palmitic acid (PA), which gave the advantage of being a marker of dietary triglyceride. ^{13}C-PA was then measured in the TAG and NEFA plasma fractions and excretion in the breath as $^{13}CO_2$, the latter being a measure of beta oxidation of ingested fat by the peripheral tissues. Indirect calorimetry was also used to enable calculation of whole body fat and carbohydrate oxidation rates.

The results of the study showed that there was a positive relationship between fasting TAG and BMI/waist circumference in both participants with diabetes and control participants, but that postprandial TAG was positively related to BMI/waist circumference only in control participants, but not in participants with diabetes. The results also showed that postprandial NEFA concentrations had a *negative* relationship with BMI/waist circumference in participants with diabetes, with significantly higher ^{13}C-PA NEFA in participants with diabetes in the lowest quartile

of BMI compared to those participants in the highest quartile of BMI. 30-minute postprandial insulin was significantly lower in the in participants with diabetes in the lowest quartile of BMI compared to those participants in the highest quartile of BMI and there was a *negative* correlation between ^{13}C-PA NEFA and concentrations. This data suggests that insulin deficiency is likely to make a contribution to the pathophysiological mechanism of impairment of dietary NEFA uptake by adipose tissue in the non-obese patients with diabetes. Another important and novel finding was that there were higher rates of fasting and postprandial fat oxidation and energy expenditure in participants with diabetes compared to control participants. This finding is opposite to findings in isolated skeletal muscle, but is in agreement with whole body studies in participants with diabetes patients with lipodystrophy. There was a positive relationship between fat oxidation measured by calorimetry and BMI in both control and participants with diabetes, but no relationship was found for $^{13}CO_2$ in the breath, a measure of oxidation of recently ingested fat.

These findings have important implications for clinical management of patients with diabetes. Whilst fasting TAG concentrations were higher in participants with diabetes patients with a higher BMI/waist circumference, metabolism of dietary fat was impaired in participants with diabetes regardless of their degree of adiposity, and was significantly more impaired in the non-obese participants with diabetes. It is likely that excess circulating dietary TAG and NEFA will be taken up into tissues outside the adipose tissue for example the liver and skeletal muscle, and will cause further metabolic problems such as worsening insulin resistance and fatty liver disease. It is therefore important to consider that postprandial fat metabolism may be impaired in non-obese patients with diabetes even if the fasting TAG is relatively normal. It is also important that health policy makers such as NICE are made aware that limiting accessibility to therapies for diabetes such as metformin, GLP-1 agonists and even in extreme cases bariatric surgery, only to those patients with a BMI>35kg/m^{2,} may be denying patients access to important therapies. Finally it is important that when clinicians assess patients with type 2 diabetes, they think carefully about the phenotype and pathophysiology of diabetes in that individual, and do not concentrate their efforts to encourage low fat diets only in the obese patients.

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