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An Investigation of the Oxidation of Carbohydrate versus Fat for Energy in Individuals with Type 2 Diabetes

by

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Abstract

Impaired fat oxidation has been associated with increased plasma free fatty acid concentrations, ectopic fat deposition and insulin resistance, which may contribute to the development of Type 2 Diabetes (T2D). The objectives of this research were to determine whether individuals with T2D oxidized a greater proportion of carbohydrate (CHO oxidizers) versus fat (fat oxidizers) over a 24 hour period, measured by the cumulative respiratory quotient (RQ_{24}), and to identify differences in metabolic variables between CHO and fat oxidizers. Ten participants spent two non-consecutive days in the Whole Body Calorimetry Unit to determine their RQ_{24}. More (n=7) were classified as CHO oxidizers than fat oxidizers (n=3); and CHO oxidizers had a greater central fat distribution (*P*=0.041) and a higher resting systolic blood pressure (*P*=0.031) than fat oxidizers. Results of this pilot study suggest that differences in substrate oxidation exist in people with T2D and these differences may be clinically important.

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List of Abbreviations

AHS	Alberta Health Services
ATP	Adenosine Triphosphate
BEE	Basal Energy Expenditure
BMI	Body Mass Index
BP	Blood Pressure
CDA	Canadian Diabetes Association
CHO	Carbohydrate
CO_2	Carbon Dioxide
CSEP:CEP	Canadian Society for Exercise Physiology: Certified Exercise Physiologist
CV	Coefficient of Variation
DXA	Dual-Energy X-Ray Absorptiometry
FAO	Food and Agriculture Organization of the United Nations
FFA	Free Fatty Acid
FFM	Fat Free Mass
FM	Fat Mass
HOMA-IR	Homeostasis Model Assessment-Estimated Insulin Resistance
HR	Heart Rate
IGT	Impaired Glucose Tolerance
MPH	Mile Per Hour
N	Nitrogen
NEFA	Non-Esterified Fatty Acid
NGT	Normal Glucose Tolerance
NPRQ	Non-Protein Respiratory Quotient
O_2	Oxygen
PADL	Physical Activity and Diabetes Laboratory
PAR-Q+	Physical Activity Readiness Questionnaire for Everyone
PHAC	Public Health Agency of Canada

REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
RQ	Respiratory Quotient
RQ_{24}	24 Hour Respiratory Quotient
$RQ_{Fasting}$	Fasting Respiratory Quotient
$RQ_{Postprandial}$	Postprandial Respiratory Quotient
RQ_{Sleep}	Sleep Respiratory Quotient
SEE	Sleep Energy Expenditure
SNS	Sympathetic Nervous System
SPA	Spontaneous Physical Activity
T2D	Type 2 Diabetes
TEE	Total Energy Expenditure
TEF	Thermal Effect of Food
TG	Triglyceride
UNU	United Nations University
VCO_2	Carbon Dioxide Production
VO_2	Oxygen Production
VO_2max	Maximal Oxygen Consumption
VT	Ventilatory Threshold
WHO	World Health Organization
Wmax	Maximal Aerobic Power
WTR	Waist to Thigh Ratio
$\Delta RQ_{Exercise}$	Metabolic Flexibility from Pre-Exercise to Exercise Conditions
ΔRQ_{Meal}	Metabolic Flexibility from Fasting to Postprandial Conditions
ΔRQ_{Sleep}	Metabolic Flexibility during an Overnight Fast

Chapter 1: Introduction

1.1 Overview

The Canadian Diabetes Association identifies three main types of diabetes. Type 1 diabetes occurs when the pancreas is unable to produce insulin. Type 2 diabetes (T2D) is characterized by a deficiency in insulin production by pancreatic β-cells (Porte, 1991) and/or by the body's inability to use the insulin it produces (insulin resistance) (Reaven, 1988). While T2D can be diagnosed in people of all ages, it is usually diagnosed in individuals 40 years of age or older (Canadian Diabetes Association, 2009). The third type of diabetes is gestational diabetes and it occurs when there is hyperglycemia during pregnancy (Canadian Diabetes Association, 2012). When left untreated or if not properly managed, diabetes can lead to various complications including cardiovascular disease, neuropathy, retinopathy, chronic kidney disease and amputations (CDA, 2009; Mortaz, Wessman, Duncan, Gray, & Badawi, 2012). The prevalence of diabetes is increasing worldwide. The number of people with diabetes was estimated to be 153 million in 1980 (Goodarz, et al., 2011), however, in 2012 it was estimated that number had increased to 371 million cases, and the most recent reports estimate that 522 million people worldwide (or 1 in 10 adults) will have diabetes by 2030(International Diabetes Federation, 2012). In Canada alone, the number of adults diagnosed with diabetes has risen from 1.1 million in 1998 to 3.7 million in 2009, and it is projected to increase to 3.7 million by 2019 (Public Health Agency of Canada, 2011) While these statistics included all types of diabetes, the World Health Organization (WHO; 2012) estimates that 90% of diabetes cases

worldwide are T2D. This increase in the prevalence of T2D translates to a greater number of people living with the daily burden of the disease, as well as its complications and an increasing financial pressure on the healthcare system (CDA, 2009). While the development of T2D is believed to be multifactorial (Rosenthal, 2009), there is growing evidence that suggests that substrate oxidation may play an important role in the development of certain physiological changes associated with the disease. A better understanding of the etiology of T2D in relation to substrate utilization is therefore important for treatment and prevention initiatives.

It is well recognized that individuals with T2D often have elevated plasma free fatty acid (FFA) concentrations (Kelley & Simoneau, 1994; Galgani, Heilbronn, et al., 2008) and ectopic lipid accumulation (Jacob, et al., 1999; Krssak, Petersen, Dresner, et al., 1999). An increased circulating FFA concentration can cause alterations in the functions of the liver and pancreas that have been associated with the development of insulin resistance and T2D. Studies have shown that increased delivery of FFA to the liver can impair the insulin-mediated suppression of hepatic glucose output (Lewis, Vranic, Harley, & Giacca, 1997), cause an increase in the synthesis of VLDL (Frayn, Williams, & Arner, 1996) and reduce hepatic insulin clearance (Hennes, Dua, & Kissbah, 1997). An increase in plasma FFA may also reduce insulin secretion byβ-cells in the pancreas (Prentki & Corkey, 1996). Furthermore, an increase in ectopic lipid accumulation, specifically the accumulation of lipid in the form of triglyceride (TG) in skeletal muscle, has been positively associated with insulin resistance.

The question as to how these intramyocellular lipids (IMCL) contribute to the development of insulin resistance and T2D still remains unanswered. One well recognized theory is the suggestion that excess lipid can induce lipotoxicity that ultimately impairs insulin signaling in tissues and organs, however recently, other plausible theories have implicated inflammation, endoplasmic reticulum stress, mitochondrial stress and redox imbalance (Muoio, 2012). While it has been suggested that increased fasting rates of lipolysis caused by enlarged adipose tissue and an impaired insulin-mediated suppression of lipolysis may be responsible for elevated circulating plasma FFA (Solaranta & Groop, 1996), there is also research that suggests that elevated plasma FFA may be caused by impairments in substrate uptake and oxidation (Kelley & Simoneau, 1994). Imbalances between FFA uptake and oxidation may also play an important role in the increased lipid accumulation in skeletal muscle and potentially other organs in individuals with T2D (Jacob, et al., 1999; Krssak, et al., 1999).

The ratio of carbohydrate to fat oxidation can be determined by measuring the ratio of carbon dioxide produced to oxygen consumed. This ratio is known as the respiratory quotient (RQ) and is measured using indirect calorimetry. In general, during fasting conditions, energy needs are predominantly derived from fat oxidation, while carbohydrate oxidation is reduced in order to preserve glycogen stores (Flatt, 1995). When there is a transition to a fed state, the increased availability of carbohydrate results in an increased rate of carbohydrate oxidation (Flatt, 1995; Jebbs, Prentice, Goldberg, Mutrgatroyd, & Black, 1996). Increased fat availability, however, does not increase fat oxidation in the same

manner. Instead, fat uptake and oxidation is initially suppressed allowing for carbohydrate to be the main fuel source during insulin-stimulated conditions (Flatt, 1995; Jebbs, et al., 1996). In addition to elevated rates of oxidation, carbohydrate uptake and storage is also increased (Flatt, 1995). Eventually, the availability of carbohydrate will slowly diminish resulting in a slow increase in the rate of fat oxidation (Flatt, Ravussin, Acheson, & Jequier, 1985). As a result, during long-term energy balance substrate utilization tends to match substrate availability (Flatt, 1995). Kelley and Mandarino (2000) defined a systems "capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation and storage under insulin-stimulated conditions" as metabolic flexibility. Metabolic flexibility can be determined by measuring the change in RQ from a fasting to a fed state (insulin-stimulated conditions)(Galgani, Moro, & Ravussin, 2008). Both RQ and changes in RQ are influenced by the amount and type of nutrients consumed (Galgani, Moro, et al., 2008).

Impairments in the ability of a system to readily switch substrate uptake and oxidation during fasting and insulin-stimulated conditions have been reported in studies of individuals with various health conditions including obesity and T2D. Kelley and Simoneau (1994) compared substrate utilization in leg skeletal muscle of individuals with T2D and age and weight matched controls during fasting and postprandial conditions. During the fasting state, individuals with T2D had a lower rate of FFA uptake and oxidation compared to controls, however FFA

release was the same. In postprandial conditions, controls experienced a greater suppression of FFA release, uptake and oxidation compared to the participants with T2D. Similarly, Chomentowski, et al. (2011) and Stull, et al. (2010) reported higher whole-body $RQ_{Fasting}$ values and smaller changes in RQ from fasting to postprandial conditions in people with T2D compared to controls. This indicates that individuals with T2D had lower levels of fat oxidation during fasting conditions and a decreased suppression of fat oxidation in the postprandial state. Similar findings have been reported in individuals with a familial history of T2D (Pergola, et al., 2003; Ukropcova, et al., 2007).

Despite implications that impairments in fat oxidation may ultimately lead to T2D, studies to date that have examined substrate oxidation in this population have been performed in the short-term(over the course of a few hours) only. These studies have included work that examined nutrient utilization during fasting and insulin-stimulated conditions, which have included the previously mentioned work that examined substrate oxidation during the postprandial state, as well as studies that measured substrate oxidation using hyperinsulinemic euglycemic clamp methodology. There have also been a number of studies that have examined substrate oxidation in people with T2D during various intensities of exercise. A lack of research exists however that has examined fat and CHO oxidation over the 24 hour period. Measuring substrate oxidation in people with T2D over the course of 24 hours using a whole body calorimetry unit is important, as it provides a better reflection of nutrient utilization in this population during normal living conditions. In addition, understanding whether

individuals with T2D predominantly use fat versus CHO for energy over the course of a day may lead to a better understanding of how differences in substrate oxidation may play a role in the physiological changes associated with T2D such as increased circulating plasma FFA concentrations and ectopic fat accumulation.

1.2 Purpose

The overall purpose of this pilot study was to describe the metabolic profile of people with T2D and to determine whether people with T2D used predominantly fat (fat oxidizers) or carbohydrate (CHO) (CHO oxidizers) for energy. A second goal was to determine whether physical characteristics, metabolic variables, and plasma/serum substrate concentrations differed between fat oxidizers and CHO oxidizers.

1.3 Research Questions

Primary research questions:

a) What is the metabolic profile of people with T2D?

b) Can people with T2D be defined as either fat oxidizers or CHO oxidizers, and if so, will people with T2D who are fat oxidizers differ from those who are CHO oxidizers in:

   i. participant characteristics (age, BMI, body composition, blood pressure), metabolic variables (BEE/ kg, measures of RQ, metabolic flexibility) and/or fasting biochemical variables (glucose, insulin, FFA, TG)?

   ii. changes to RQ (metabolic flexibility) during an overnight fast, from fasting to 2-hours postprandial or during a bout of moderate-intensity exercise?

   iii. changes in blood glucose, insulin, FFA, TG after consuming a standard meal?

c) Is 24 hour RQ (RQ_{24}) in people with T2D associated with participant characteristics (age, BMI, body composition, blood pressure), metabolic variables (BEE/kg, other measures of RQ, metabolic flexibility) and/or biochemical variables (fasting glucose, insulin, FFA, TG)?

d) Is $RQ_{Fasting}$ in people with T2D associated with participant characteristics (age, BMI, body composition, blood pressure), metabolic variables (BEE/kg, other measures of RQ, metabolic flexibility) and/or biochemical variables (fasting glucose, insulin, FFA, TG)?

1.4 Objectives

The objectives of this research were:

a) To describe the metabolic profile of people with T2D using whole body indirect calorimetry, whereby participants stayed in the calorimetry unit for 24 hours on two separate occasions.

b) To determine the RQ from respiratory gas exchange measurements and the associations between RQ and metabolic and biochemical characteristics of people with T2D.

c) To establish appropriate RQ_{24} and $RQ_{Fasting}$ values in which to classify people with T2D as either fat oxidizers or CHO oxidizers.

d) To assess changes between fasting and postprandial plasma glucose, insulin, FFA and TG in people with T2D when fed a standard meal of 50% carbohydrate, 20% protein and 30% fat.

Chapter 2: Literature Review

2.1 Introduction

The accumulation of fat in non-adipose tissue, known as ectopic fat, has been strongly associated with insulin resistance and T2D (Snel, et al., 2012). It is often suggested that a reduced capacity of tissues to adjust fat oxidation to fat availability can lead to ectopic fat stores. To efficiently endure fluctuations in energy supplies, excess energy from increased energy intake and/or reduced energy expenditure is stored as TG in subcutaneous adipose tissue. However, a the pathophysiologic state, it is thought that adipose tissue dysfunction occurs, resulting in an impaired up take and release of FFA from adipocytes (Muoio, 2012). The resulting elevated levels of circulating FFA are redirected to tissues that normally contain only small amounts of fat such as the liver, heart, skeletal muscle and pancreas (Snel, et al., 2012). If the flux of FFA is greater than the oxidative capacity of the tissue, accumulation of fat occurs which interferes with cellular functions resulting in impaired organ function. Defects in cellular function appear to be dependent on cell type and are therefore different for each tissue. The accumulation of the ectopic fat metabolites diacylglycerol, ceramide and long chain fatty acyl-CoA in the liver and skeletal muscle impair insulin signaling leading to insulin resistance and T2D (Snel, et al., 2012).

Despite the implications that impaired fat oxidation may be involved in the development of T2D, there are many gaps in the literature that examines substrate utilization in this population. The purpose of this review is therefore to discuss the research to date that examined nutrient oxidation in individuals with

T2D over a 24 hour period, as well as during various metabolic conditions. In addition, metabolic variables associated with substrate oxidation in this population will be identified. Since the ability to achieve energy balance has been associated with both changes in substrate oxidation and ectopic fat accumulation, energy expenditure of individuals with T2D will be initially described.

2.2 Energy Expenditure of Individuals with Type 2 Diabetes

2.2.1 Energy Expenditure during Resting Conditions

Basal energy expenditure (BEE)is the minimal amount of energy expended to support life, and it has been estimated to comprise between 60 – 75% of an individual's total energy expenditure (TEE) (Ravussin, Burnand, Schultz, & Jequier, 1982; Huang, Kormas, Steinbeck, Loughnan, & Caterson, 2004).According to a joint report by the Food and Agriculture Organization of the United Nations (FAO), WHO, and United Nations University (UNU) (1981), BEE should be measured under strict experimental conditions. Participants must be measured in the post-absorptive state (minimum of a 12 hour fast) and in a thermal neutral environment. They are required to lie awake in a supine position with complete muscular relaxation and minimal emotional disturbances. It is also recommended that females be measured during the follicular phase of their menstrual cycle in order to achieve the lowest possible measurement. The rigorous conditions described by the FAO, WHO, and UNU can often be difficult to achieve, and as a consequence, many researchers choose to report resting energy expenditure (REE) as an alternative. Conditions in which REE is estimated

resemble those of BEE however they are usually not as strict (Shils, Shike, Ross, & Cabattero, 2006). For example, Careau et al. (2013) reported that REE does not have to be measured in the post-absorptive state. The extent to which REE differs from BEE is therefore dependent on the experimental conditions in which REE is measured; differences have been reported to be as high as 10% (Matarese, 1997). Although BEE and REE may be estimated under different conditions, the terms are often used interchangeably (Westerterp & Schols, 2008) which introduces a challenge when interpreting and comparing results. Since details required to distinguish BEE from REE are not always fully reported, the term REE will be used in this review for all measurements of energy expenditure taken during resting conditions after a 4 - 12 hour fast.

Metabolic rate during sleep is another measure of resting energy expenditure often studied. The FAO, WHO and UNU (1985) reported sleeping energy expenditure (SEE) to be 1.0 times that of BEE (i.e. the same values for both). While some study results have supported the definition of SEE set by the FAO, WHO and UNU (Seale & Conway, 1999), others have reported that SEE is 5 – 12% lower than BEE (Garby, Kurzer, & Nielsen, 1987; Goldberg, Prentice, Davies, & Murgatroyd, 1988; Fredrix, et al., 1990). Some researchers have therefore studied SEE independently of REE while others have not. Sleeping energy expenditure will be studied independently from REE in this review.

The majority of studies that have examined metabolic rate in people with T2D have reported REE to be elevated in this population compared to healthy individuals (Fontvielle, et al., 1992; Gougeon, Lamarche, Yale, & Venuta, 2002;

Bitz, et al., 2004; Huang, et al., 2004;Bosy-Westphal, et al., 2008). In addition, results from multiple-regression analysis found T2D status to be an independent determinant of REE (Weyer, Bogardus, & Pratley, 1999; Martin, Wallace, Rust, & Garvey, 2004). Hyperglycemia is one suggested mechanism responsible for the elevated metabolic rate during resting conditions in people with T2D. Gougeon et al. (2002) examined the relationship between glycemia and REE in a cross-sectional study of 65 obese individuals with T2D. Resting energy expenditure was measured for 20 minutes using a hooded metabolic cart. Results from this study indicated that fasting plasma glucose concentrations were positively correlated with REE ($P = 0.013$). Researchers from this study also reported that those with poor glycemic control (fasting plasma glucose > 10 mmol/L) had an increase in REE of up to 8%. Results from studies performed by Nair et al. (1986) and Buscemi et al. (2007)that compared REE in obese people with T2D and healthy controls provide support for these results. Resting energy expenditure has also been found to be positively associated with hepatic glucose production (Franssila-Kallunki & Groop, 1992). Ryan et al., 2006 reported no significant difference in REE between individuals with T2D and healthy controls, however in this study, the individuals with T2D were reported to have well-controlled glycemia. Studies that have reported an elevated REE in individuals with T2D have examined individuals who had either not taken anti-diabetic medications (Fontvielle, et al., 1992; Weyer, Snitker, Rising, Bogardus, & Ravussin, 1999), those who had not taken medications for at least one week prior to the study (Bitz, et al., 2004; Martin, et al., 2004), or those who had a wide range of fasting plasma glucose

concentrations (Gougeon, et al., 2002). It has been reported that when individuals with T2D were treated with anti-diabetic therapies, improvements in plasma glucose concentrations were accompanied by reductions in REE. Bogardus, et al. (1986) demonstrated that after 6 weeks of sulphonylureas therapy, obese individuals (n = 9) experienced both an improvement in fasting glucose (13.1 ± 0.9 to 8 ± 0.8 mmol/L, $P < 0.01$), as well as a significant decrease in REE (31.9 ± 0.8 to 30.2 ± 0.6 kcal/day per kg FFM, $P < 0.04$) measured by indirect calorimetry with the use of a metabolic cart. Additionally, Franssila-Kallunki & Groop(1992)reported that 12 months of insulin therapy resulted in both improvements in glycemic control (15.6 ± 0.8 to 9.3 ± 1.0 mmol/L, $P < 0.05$) and a decrease in REE (115.5 ± 5.6 to 103.1 ± 5.7 J•kg/FFM/min, $P < 0.01$). Insulin therapy was also reported to decrease REE in a group of obese individuals with T2D with poor glycemic control (Gougeon, 2001). In this study, participants (n = 9) experienced a decrease in REE after 8 days of insulin therapy (-8 ± 2%, $P<0.05$). A subsequent 20 days on a very low energy diet resulted in an additional decrease in REE (-14 ±3%, $P<0.05$). The magnitude in which REE decreased during the anti-diabetic therapies was related to the magnitude of change in hyperglycemia. Results from these studies suggest that reductions in plasma glucose concentration may result in an improved efficiency in fuel utilization and therefore a reduction in energy expenditure in the resting state to a rate similar to those without T2D.

The association between glycemic control and REE may also explain why an increased REE has been found in people diagnosed with impaired glucose

tolerance (IGT). Weyer, Bogardus, et al. (1999) sought to identify at what point in the development of T2D that changes in REE occurred. A group of Pima Indians (n = 404) between 20 and 50 years of age with normal glucose tolerance (NGT) were recruited into this longitudinal study. At baseline and once a year for 5 years, REE was measured by indirect calorimetry using a hooded metabolic cart. Seventeen of the enrolled 404 participants developed T2D and had REE measurements at every stage of the progression of the disease (NGT, IGT, T2D). After adjustments for fat free mass (FFM) and fat mass (FM), results indicated that participants experienced a significant increase in REE from NGT to T2D (1,755 ± 277 kcal/day and 1,821 ± 302 kcal/day respectively, $P < 0.05$). When changes in REE were examined throughout the progression to T2D, REE increased by 4.2% during the transition from NGT to IGT ($P = 0.06$), and increased an additional 2.6% through the transition from IGT to T2D ($P = 0.06$). Weyer, Bogardus, et al. also performed a cross-sectional study designed to examine the difference in SEE in people with different levels of glucose tolerance. Researchers recruited 560 Pima Indians with NGT (n = 365), IGT (n = 127) and T2D (n = 68) and determined SEE (measured between 11:30 p.m. – 5 a.m.) using a whole body calorimetry unit. Results from this study supported those of their longitudinal study. After adjustments for age, sex, FFM and FM, SEE was 2.7% higher in those with IGT ($P < 0.01$) and 4.6% higher in those with T2D ($P< 0.001$) compared to those that had NGT. These results are consistent with those of Weyer, Snitker et al. (1999) who also compared SEE (measured between 1:30 a.m. and 5 a.m.) in people with NGT (n = 720) and IGT (n = 196).

They reported that SEE was significantly higher in those with IGT when compared to people with NGT (+38 ±12 kcal/day, $P < 0.01$). When the relationship between length of time from diagnosis of T2D and REE was considered, no significant association was found (Bitz, et al., 2004). These results suggest that changes in REE may occur early in the development of T2D with the onset of hyperglycemia, and that the relationship is independent of changes in body composition. It is important to note that although REE has been associated with current glycemia, hemoglobin A1c (HbA1c), a measure of glycemic control over the previous three months, has shown no correlation with metabolic rate(Gougeon, et al., 2002; Ryan, et al., 2006). This suggests that current metabolic rate may be impacted by current conditions of glycemia only.

Other Determinants of Metabolic Rate during Resting Conditions

In addition to diabetes status, other determinants of inter-individual differences amongst measures of REE and SEE have been examined (Ravussin, Lillioja, Anderson, Christin, & Bogardus, 1986). Fat free mass, the most metabolically active tissue in the body, was reported to be the single most important determinant of both REE and SEE (Ravussin,et al., 1986; Cunningham, 1991; Klausen, Toubro, & Astrup, 1997; Nielsen, Hensrud, Romanski, Levine, Burguera, & Jensen, 2000; Bitz, et al., 2004;Ryan, et al., 2006), explaining 60 – 85% of the variance amongst individuals (Klausen, et al., 1997; Nielsen, et al., 2000; Bitz, et al., 2004). The differences in the reported effect of FFM on REE and SEE may be attributable to differences in the methods used to assess body composition. Fat free mass has been estimated using various techniques including

bioelectrical impedance (Klausen, et al., 1997; Gougeon, et al., 2002), hydrodensitometry (Ravussin, et al., 1986; Fontvielle, et al, 1992), and dual-energy X-ray absorptiometry (DXA) (Nielsen, et al., 2000; Bitz, et al., 2004). The ability to accurately assess body composition has been shown to differ amongst the various assessment tools (Pritchard, et al., 1993; Bolanowski & Nilsson, 2001). Differences have also been reported in SEE depending on the time in which metabolic rate was measured throughout the overnight period (Seale & Conway, 1999). Despite these reported differences in the effect of FFM on REE and SEE, it is generally accepted that FFM is the most important determinant of metabolic rate at rest.

Similar to FFM, FM is an energy utilizing tissue. Adipose tissue consumes oxygen (O_2) at a rate of 0.4ml/kg/min (Simonsen, Bulow, & Madsen, 1994). This is considerably less than the rate of O_2 consumption in lean tissue (Jensen, Johnson, Cryer, & Murray, 1995), however, since adipose tissue it approximately 85% fat(Jequier & Tappy, 1999), it is logical to assume that FM would explain a proportion of the difference in the variance in REE and SEE amongst individuals, but to a lesser extent than FFM. Although some researchers have been able to confirm that FM is an independent predictor of REE and SEE (Nelson, Weinsier, Long, & Schutz, 1992; Klausen, et al., 1997; Dionne, Despres, Bouchard, & Tremblay, 1999; Nielsen, et al., 2000; Bitz, et al., 2004) others have not (Ravussin, et al., 1986; Huang, et al., 2004; Ryan, et al., 2006). It is possible that the size of sample used or the use of less reliable measures of body composition may have affected the ability to detect independent effects of FM on REE

(Nielsen, et al., 2000). It has also been suggested that the effects of FM on metabolic rate during rest may only be evident in a sample where absolute FM is above normal (Bernstein, Thornton, & Yang, 1983; Nelson, et al., 1992). However, in studies performed in both severely obese (body mass index (BMI) ≥ 35 kg/m^2) and obese (BMI ≥ 30kg/m^2) participants, no independent effects of FM on REE were reported (Ryan, et al., 2006). Since the effect of FM on REE and SEE is small, it may therefore be more reasonable to assume that its effect can only be detected when a sample includes individuals with a wide range of body fat as seen in the study done by Neilsen et al. where normal weight, overweight and obese participants with varying amounts of FM (ranged from 11.2 – 37.4 kg) were examined. In this study, authors concluded that FM was positively correlated to REE, and when multiple linear regression analysis was performed, FM was found to be an independent predictor. Although it appears that FM may be an important determinant of REE, more research with participants of varying levels of adiposity is required to confirm these results.

Gender and age are two other variables that have been suggested to be independent determinants of metabolic rate during resting conditions, but study results have been equivocal (Arciero, Goran, & Poehlman, 1993; Klausen, et al., 1997; Weyer, Snitker, et al., 1999; Nielsen, et al., 2000; Bitz, et al., 2004). Both gender and age are known to be associated with body composition, and therefore it is possible that associations found between gender and age with REE may be in large part due to effects of body composition, particularly FFM. There are other factors however, such as changes in thermogenic hormones and reduced

metabolism of the mitochondria that may play a role in an age related decline in energy expenditure (Klausen, et al, 1997). Sample size and the range of age of participants being studied may also affect whether gender and age effects on metabolic rate at rest can be detected (Gougeon, et al., 2002). Similar to FM, more research is still required to determine whether gender and age play a significant role in energy expenditure during resting conditions.

2.2.2 Energy Expenditure during Physical Activity

Other components of TEE are more variable than REE; however, they each encompass a significant proportion of an individual's daily energy expenditure. Physical activity has been reported to comprise between 10 – 40% of TEE (Ravussin & Bogardus, 1989). It includes both planned exercise, as well as spontaneous physical activity (SPA) performed throughout the day. The amount of energy expended during physical activity is dependent on both the length and intensity of the activity performed (Li, et al., 2012). Those that participate in regular exercise or perform a greater amount of SPA may therefore have a greater proportion of TEE from physical activity than sedentary individuals. Since the cost of SPA is proportional to body weight, absolute energy expenditure from physical activity may not however, be greater in more physically active individuals compared to sedentary individuals (Ravussin, et al., 1986). In a study performed by Fagour et al. (2013), individuals with T2D were found to do less physical activity than age, gender and employment matched controls. An inverse relationship was also reported between BMI and physical activity levels. Since those with T2D were reported to have a significantly higher mean BMI, it is not

clear in this study whether BMI is a cofounding factor in the relationship between T2D and physical activity levels or whether these two variables are in fact independent. Morrato et al. (2007) also found that T2D status and BMI were associated with participation in physical activity in a large sample (n = 23,226) of individuals of varying ages and body weight. They reported that although it was found that physical activity decreased with increasing BMI in adults without T2D, normal-weight adults with T2D were no more likely to participate in physical activity than overweight or obese adults. Their results therefore suggest that a relationship between diabetes status and physical activity levels may be independent of BMI; however, more research is needed to examine this relationship and to determine whether their results can be generalized to the larger population. If individuals with T2D are found to perform less physical activity than healthy individuals, a lower proportion of their daily energy expenditure may be attributed to physical activity.

2.2.3 Thermic of Effect Food

The increase in energy expenditure after the ingestion of a meal or the infusion of nutrients is referred to as the thermic effect of food (TEF). There are two main components to this thermogenic response. The obligatory component consists of the energy required to digest, absorb, transport and store nutrients. It accounts for approximately two-thirds of the TEF. The facultative component accounts for the remainder of the energy expended after ingestion of nutrients (Watanabe, et al., 2006). It involves the processes not accounted for by the obligatory component, and therefore, includes such mechanisms as stimulation of

the sympathetic nervous system (SNS), protein turnover, substrate cycling, and sodium pumping (Segal, Albu, Edano, Legaspi, & Pi-Sunyer, 1992). When consuming a mixed diet, TEF accounts for approximately 10% of TEE. The TEF depends on both the amount of food (Kinabo & Durnin, 1990) and the type of macronutrient consumed (Ravussin, et al 1989). Protein produces the greatest TEF response (Segal, et al., 1992). The TEF of carbohydrate (CHO) is less than protein, but greater than fat (D'Alessio, et al., 1988). Thermic effect of food is determined by comparing the energy expended at rest in the postabsorptive state to energy expended at rest after the digestion of a meal. The TEF can occur for up to 8 hours after the digestion of a meal (de Jonge & Bray, 1997), however, it has been reported that 70% of the TEF occurs within the first 3 hours after food intake (Segal,et al., 1992).

Age and fitness level have been suggested to affect TEF, however results have been inconsistent. Some studies have shown an age related decline in TEF (Schwartz, Jaeger, & Veith, 1990), however other studies have reported no age related effect (Melanson, Slatzman, & Vinken, 1998). When fitness levels were examined, Davis et al. (1983) reported that individuals with a higher maximal oxygen uptake (VO_2max) had a greater TEF, especially to large meals. Other researchers however, have reported a blunted TEF in trained compared to untrained individuals (LeBlanc, Diamond, Cote, & Labrie, 1984; Poehlman, Melby, & Badylak, 1988). In a review by Granata and Brandon in 2002, it was reported that even though inconsistencies in findings of age related effects on TEF existed, 37 of the 50 studies they reviewed controlled for aging. Only 10 of the 50

studies controlled for fitness level. Since more research is still needed to better understand the effects age and fitness level have on TEF, controlling for both age and fitness level should be considered in all analysis.

The presence of obesity has also been suggested to have an effect on TEF, although results from studies examining its effect have been somewhat equivocal. Research performed using glucose infusions have consistently reported that TEF is blunted in obese compared to lean individuals (Bogardus, et al., 1985; Golay, Schutz, Felber, DeFronzo, & Jequier, 1986). Conversely, results from oral feeding studies have been inconsistent (de Jonge & Bray, 1997). In a review by de Jonge and Bray, the authors attempted to determine if consistencies could be found if studies were separated based on whether participants were fed liquid versus solid meals or if they were fed single nutrient versus mixed meals. They concluded that even when studies were separated based on meal type, results remained inconsistent. Authors of this study went on to examine the methods used to classify participants as obese versus lean. They suggested that the use of BMI in the majority of the studies examined might have led to misclassification of individuals resulting in the inability to detect the effect of obesity on TEF. Few studies actually used percent FM to classify participants and only three studies considered fat distribution with the use of waist-to-hip ratios. When only the studies that had a significant difference in BMI between obese and lean groups were considered, 22 of the 29 studies concluded that TEF was significantly lower in participants with obesity. Studies that have examined the effect of percent FM on TEF have also reported a lower TEF in those with a greater percent FM;

however, inter-individual differences in TEF existed. When fat distribution was examined, results suggest that abdominal FM is negatively associated with TEF. Differences in percent FM and FM distribution could therefore account for some of the inter-individual differences in TEF in obese participants.

It has been suggested that the observed blunting of the thermogenic response to a meal or infusion of glucose in people with obesity is related to insulin resistance (Ravussin, Acheson, Vernet, Danforth, & Jequier, 1985). Results have consistently shown that the TEF was reduced in people with varying levels of insulin resistance in both glucose infusion, as well as oral administration studies. Segal, et al. (1992) examined whether insulin resistance was responsible for the impaired TEF response often reported in people with obesity by observing the thermogenic response of four groups of individuals – lean controls, lean with insulin resistance, obese controls and obese with insulin resistance. All four groups were matched for age, FFM, and VO_2max. The two lean groups were also matched for body weight and percent FM, as were the two groups with obesity. Investigators found that regardless of body composition, those with insulin resistance had a lower TEF than those with a normal insulin response. Insulin resistance and obesity were both found to be independent predictors of TEF. These results supported early studies that reported impaired TEF in lean individuals with T2D (Gumbiner, Thorburn, & Henry, 1991), as well as those studies that showed that when obese individuals with T2D achieved weight loss, TEF improved but was still blunted compared to lean controls (Ravussin, et al.,

1983). Research to-date therefore suggests that obesity and insulin resistance are independent determinants of TEF.

Impaired glucose disposal related to insulin resistance appears to be an important mechanism involved in the depression of the thermogenic response to a meal. When the hyperinsulinemic euglycemic clamp technique was employed with controls and individuals with T2D, energy expenditure above resting conditions was lower in people with T2D compared to controls (Ravussin, et al., 1983; Gumbiner, et al., 1991; Segal, et al., 1992). However, when glucose uptake was controlled by varying rates of glucose infusion, similar rates of glucose disposal amongst controls and individuals with T2D was achieved, and no difference in rates of energy expenditure above resting conditions existed (Gumbiner, et al., 1991; Segal, et al., 1992). When glucose disposal was partitioned into glucose oxidation and storage, it was determined that glucose oxidation was not significantly different between those that were insulin sensitive compared to those that were insulin resistant, however, rates of glucose storage was significantly different between these two groups (Segal, et al., 1992). The energy cost of glucose storage is greater than that of oxidation (Segal, et al., 1992), therefore, impaired TEF may be related to the impaired ability to store glucose in those with T2D.

2.2.4 Total Energy Expenditure

Since REE is responsible for the majority of the energy that is expended throughout a 24 hour period, it is not unexpected that similar variables that have been found to be possible predictors of REE have also been found to be predictors

of TEE. Fat free mass is the greatest determinant of TEE (Ravussin, et al., 1986; Klausen, et al., 1997; Bitz, et al., 2004). Fat mass (Ravussin, et al., 1986; Klausen, et al., 1997; Bitz, et al., 2004) and ethnicity (i.e. after adjusting for body composition, TEE was 9% lower in black women than white women; Weinsier, et al., 2007) are also significant determinants of TEE. Similar to findings of those for REE, reports of whether sex and age are significant determinants of TEE are inconsistent and appear to be dependent on the heterogeneity of the cohort studied (Ravussin & Bogardu, 1989;Klausen, et al., 1997; Bitz, et al., 2004). In addition to these variables, 24 hour SPA, as well as the duration of exercise have also been reported to be significant predictors of TEE (Bitz, et al., 2004).

The effect diabetes status has on TEE is still somewhat unclear. When controlling for the previously mentioned metabolic variables, some studies have reported elevated rates of TEE in people with T2D compared to healthy individuals (Fontvielle, et al., 1992; Weyer, Bogardus, et al., 1999; Bitz, et al., 2004). It has been suggested that an elevated TEE in those with T2D may be due to increased REE, increased plasma glucose, an increased circulating FFA (Weyer, Bogardus, et al., 1999; Bitz, et al., 2004), and a disturbance in metabolic rate due to the discontinuation of anti-diabetic medication prior to the study (Bitz, et al., 2004). A greater amount of sleep disturbances and elevated SPA during sleep have been reported in individuals with T2D possibly due to increased thirst and bathroom breaks (Bitz, et al., 2004). Despite the number of studies reporting elevated TEE in T2D, other studies have reported no significance differences when compared to controls (Ravussin, et al., 1986). While some researchers have

suggested that impaired TEF in T2D cancels out the effect of the elevated REE (Nair, et al., 1986),this seems rather unlikely since REE accounts for up to 75% of TEE and TEF only accounts for approximately 10% of TEE. Inconsistent results in this area may instead be due to differences in study protocol including differences in diet, the ability to achieve energy balance throughout the measurement, as well as the ability to control for activities performed by participants throughout the 24 hour period.

Although uncertainty remains surrounding the effects that some metabolic variables have on energy expenditure, evidence suggests that T2D status appears to be an important determinant of both REE and TEF, and therefore may affect energy expenditure over the 24 hour period.

2.3 Overview of Fuel Utilization and how it is Quantified

The availability of exogenous CHO, fat and protein varies throughout a 24 hour period. Therefore, in order to maintain homeostasis, the body must have a highly regulated process to store substrates in the postprandial state when they are in abundance so they can later be mobilized and used for energy in fasting conditions (Flatt, 1995). The ability of the body to store each nutrient varies considerably. It has been proposed that the auto-regulation of macronutrient oxidation is related to their capacity for being stored within the body (Jebbs, Prentice, Goldberg, Mutrgatroyd, & Black, 1996).

Glucose, the primary form of CHO in the body, is stored as glycogen in the liver and muscle. Studies that have examined the storage capacity of glycogen

have reported it to be as little as 800-900g in healthy individuals (Acheson, Schultz, Bessard, Flatt, & Jequier, 1988). It is well accepted, however, that glycogen is not stored at capacity. Instead, total muscle and hepatic glycogen storage is approximately 200-500g depending on the size of the individual and CHO intake (Acheson, et al., 1988; Flatt, 1995). Furthermore, glycogen storage fluctuates considerably throughout a 24 hour period in response to CHO intake and physical activity (Galgani & Ravussin, 2008). In contrast to CHO, both protein and fat stores are large. Total protein stores in a lean adult male account for approximately one third of total energy stored. These stores are highly controlled however, and a growth in protein stores only occurs in response to growth stimuli such as growth hormone, androgens, physical training and weight gain (Galgani & Ravussin, 2008). The excess consumption of protein in the absence of these stimuli therefore does not result in an increase in skeletal muscle mass. In contrast to CHO and protein, the storage of fat is theoretically limitless and it is very susceptible to periods of over- and underfeeding. In lean adults, FM is approximately 8-20 kg. In individuals who are obese, FM is typically much larger and can reach amounts greater than 100 kg (Jequier & Tappy, 1999).

In a healthy individual, the release of insulin in response to the ingestion of a standard meal promotes glucose uptake, hepatic and skeletal muscle glycogen synthesis, and inhibits the release of hepatic glucose (Flatt, 1995). This highly regulated process prevents postprandial hyperglycemia. Since glycogen stores are small, the non-oxidative disposal of blood glucose is limited and as a result, the ingestion of CHO must also elicit an increased rate of glucose oxidation. In the

postprandial period, CHO is therefore the primary fuel source (Jebbs, et al., 1996). Since protein stores are also highly controlled, protein intake stimulates an increase in protein oxidation. Given that the protein content of a standard meal is small (approximately 15%) (Galgani & Ravussin, 2008), its contribution to the fuel mixture is considerably less than that of CHO. Fat intake does not elicit the same response. The ingestion of fat stimulates no or little fat oxidation (Abbott, et al., 1988; Schutz, Flatt, & Jequier, 1989). Therefore, when a mixed diet is consumed there is an increase in CHO and protein oxidation but not in fat oxidation. Instead, enhanced CHO and protein oxidation causes the suppression of fat oxidation, and the release of fatty acids from adipose tissue is blunted. Fatty acids are therefore pushed toward storage and out of the blood stream (Flatt, et al., 1985; Flatt, 1995; Jebbs, et al., 1996). Enhanced CHO oxidation and the reduction of fat oxidation are reflected by an increase in RQ to a value close to 1.0.As the postprandial period progresses, there will be a gradual drop in plasma glucose concentrations. In order to preserve glycogen stores, CHO oxidation is reduced (Galgani & Ravussin, 2008). An increase in the mobilization of fatty acids from adipose tissue will occur and fat oxidation gradually contributes more to the fuel mixture; shifts in the primary fuel source are reflected by a drop in the RQ (Flatt, et al., 1985). It is suggested that the regulation off at oxidation is not determined by the appearance of exogenous fat, but rather by the intake of the other macronutrients. This idea has been supported by studies that have demonstrated that unlike the rapid increase in CHO oxidation in response to increased CHO availability, greater dietary fat causes a much slower progressive rise in fat

oxidation (Hill, et al., 1991; Schrauwen, van Marken Lichtenbelt, Saris, & Westererp, 1997; Smith, et al., 2000).

Fat stores acts as an energy buffer to the small day-to-day negative and positive energy imbalances. Excesses in energy intake will be stored as fat in adipose tissue, whereas a deficit in caloric intake will cause a decrease in fat stores. A strong positive relationship therefore exists between energy balance and fat balance over the long-term (Abbott, et al., 1988).

The chemical composition of fats, carbohydrates and proteins differ, and the amount of VCO_2 and VO_2 during the oxidation of these macronutrients vary. The ratio of VCO2 to VO_2 measured by open circuit indirect calorimetry systems can therefore be used to provide a ratio of the fuel mixture being oxidized. The RQ refers of the ratio of VCO_2 to VO_2 at the tissue level. Indirect calorimetry systems provide measurements of gas exchange at the lungs and are therefore a measure of whole body O_2 consumption and CO_2 production.

A RQ of 1.0 reflects the exclusive oxidation of CHO. The ratio of VCO2/VO2 varies with the length of the fatty acid chain and the type of protein oxidized, therefore, the RQ value for these macronutrients will differ depending on the fat and protein being metabolized (McArdle, Katch, & Katn, 2001). It is generally accepted, however, that a value of 0.7 indicates the exclusive metabolism of fat, and a RQ of 0.8 reflects the complete consumption of protein (Ferrannini, 1988; Brandi, Bertonili, & Calafa, 1997). Mixed fuel metabolism is reflected by a RQ of approximately 0.84 (Brandi, et a., 1997). When urinary nitrogen losses are measured concurrently with indirect calorimetry, a non-protein

RQ (NPRQ) can be calculated (Ferrannini, 1988). The NPRQ reflects the proportion of CHO to fat oxidized for fuel only, and does not include oxidized protein. Urinary nitrogen excretion is also used to determine net protein oxidation. The net rates of CHO and fat oxidized in the body is quantified with the use of the NPRQ and tables created by Lusk (1924), or by one of the various equations created based on the stoichiometry of fat and CHO oxidation (Brouwer, 1957; Ferrannini, 1988; Frayn, 1983; Peronnet & Massicotte, 1991). Each of the existing equations differs based on slight differences in the stoichiometry of the substrate selected for its determination. In a review performed by Jeukendrup and Wallis (2005), a comparison in the CHO oxidation rate calculated with Lusk's table and the various published equations resulted in a difference as little as 6%.

Indirect calorimetry is based on the assumption that all O_2 is used in the oxidation of fuel and all CO_2 is a product of those reactions (Ferrannini, 1988; Schultz, 1997). It is also assumed that all resulting CO_2 is recovered (Ferrannini, 1988). While the assumption for O_2 is fundamentally correct (Tappy & Schneiter, 1997), situations can arise however, that may invalidate the assumption for CO_2. During situations where there is a shift in the acid-base balance, such as post-exercise oxygen consumption, acidosis or alkalosis, or hyper- and hypoventilation, changes to the CO_2/bicarbonate pool can occur that affect the measurement of VCO_2 as it causes the production of nonoxidative CO_2. (Ferrannini, 1988; Tappy & Schneiter, 1997).

Other metabolic processes including lipogenesis, gluconeogenesis, ketogenesis can also influence the measurement of the RQ. Lipogenesis from

CHO results in more than twice as many moles of CO_2 produced compared to O_2 consumed. The RQ of lipogenesis is therefore very high. The concurrent oxidation of CHO and lipogenesis results in a RQ greater than 1 (Ferrannini, 1988; Schultz, 1997). It is therefore difficult to distinguish the rates of fat oxidation from fat synthesis, the true rate of CHO oxidation, as well as the corresponding rates of energy production during lipogenesis. The influence gluconeogenesis has on RQ is dependent on the source of the amino acid utilized in the process and the fate of the glucose produced. Typically, the RQ during gluconeogenesis is approximately 0.4 (Schultz & Ravussin, 1980). Gluconeogensis therefore results in an underestimation of CHO oxidation and an overestimation of protein oxidation (Tappy, Paquot, Tounian, Schneiter, & Jequier, 1995). Although ketogenesis involves the consumption of O_2, CO_2 is not produced during this process. The RQ of ketogenesis is therefore zero. The ultimate fate of the ketones produced has a corresponding influence on the measured RQ value (Schultz & Ravussin, 1980).

2.4 Twenty-Four Hour Substrate Oxidation

The measurement of RQ_{24} with the use of a whole body indirect calorimetry unit allows for the assessment of substrate utilization during daily activities such as sleep, rest, physical activity, as well as during postprandial conditions. Despite the numerous studies examining RQ_{24} in healthy lean and obese individuals, there is a lack of research that has examined 24 hour substrate oxidation in individuals with T2D. Weyer, Snitker et al. (1999) studied the effects

of IGT on 24 hour substrate utilization using data obtained between 1985 and 1998. Data from 916 Pima Indian and Caucasian males and females who were either diagnosed as NGT or IGT were included in the analysis. Whole group RQ_{24} ranged from 0.771 to 0.931 and mean RQ_{24} was 0.854 ± 0.026. When adjusted for FFM, FM, sex, age, and waist to thigh ratio RQ_{24} did not differ between individuals with NGT and IGT. Inter-individual differences were instead attributed to percent FM, age, and energy balance throughout the measurement. Although results from this study suggest that glucose tolerance is not a determinant of substrate utilization over a 24 hour period, several limitations of this study should be acknowledged. Since data were included from various studies over the span of four years, differences in study protocols such as differences in the length of the run in diet, as well as physical activity while in the unit may have influenced substrate utilization. While a large sample size was used for analysis, considerably fewer people with IGT (n = 196) were included in the study than people with NGT (n=720), which may have impacted the ability to detect differences between the two groups. Genetic differences in Pima Indians also make it difficult to generalize these findings to the general population (Ellis, Hyatt, Gower, & Hunter, Respiratory Quotient Predicts Fat Mass Gain in Premenopausal Women, 2010). While the work performed by Weyer, Snitker et al. (1999) provides initial insights into 24 hour substrate oxidation in individuals with impaired insulin sensitivity, more research is needed in this population, as well as in individuals with T2D.

Although research in the area of substrate utilization over the 24 hour period in individuals with T2D is lacking, research performed with healthy lean and obese individuals has provided information about other factors that may be associated with RQ_{24}. Similar to the inter-individual differences in substrate oxidation reported by Weyer, Snitker et al. (1999), variances in RQ_{24} have also been reported in healthy lean and obese individuals (Ravussin & Bogardus, 1989; Ferros, et al., 1993; Sorensen, Hindsberger, Christensen, & Atrup, 1998; Stefan, et al., 2002; Toubro, Ukropcova, et al., 2007). These differences were found despite the fact that individuals were fed similar standard diets (approximately 50% CHO, 30% fat, 20% protein) estimated to maintain individual energy balance. Even though a sizable range in RQ_{24} has been described by many researchers, the mean RQ_{24} has often been reported to be approximately 0.85 (Zurlo, et al., 1990; Ferros, et al., 1993; Snitker, Tatarani, & Ravussin, 1998; Toubro, et al., 1998; Ravussin & Bogardu, 1989; Stefan, et al., 2002; Brons, Lilleore, et al., 2013). Acute energy balance has consistently been found to be a major determinant of RQ_{24} (Zurlo, et al., 1990; Astrup, et al., 1992; Snitker, et al., 1998; Toubro, et al., 1998;). Results from these studies have shown that acute positive energy balance is positively associated with RQ_{24}.Since the nutrient composition of the diet also affects substrate utilization, these results indicate that RQ_{24} is dependent on both diet quality and quantity (Ravussin & Bogardu, 1989). Evidence also suggests that diet composition and the number of calories consumed in the days prior to the measurement of RQ_{24}may also impact substrate utilization (Zurlo, et al., 1990; Toubro, et al., 1998), and it has been suggested that

strictly controlled run-in diets should be administered over the two days prior to the measurement (Toubro, et al., 1998).

Attempts to explain the remaining observed differences in RQ_{24} amongst individuals have provided conflicting results. Age (Snitker, et al., 1998; Toubro, et al., 1998; Weyer, Snitker, et al., 1999) and gender (Zurlo, et al., 1990; Toubro, et al., 1998) have both been reported to be independent predictors of RQ_{24}however, others have reported no significant correlation amongst these variables(Zurlo, et al., 1990; Weyer, Snitker, et al., 1999). Whether substrate oxidation over the 24 hour period differs between males and females and whether changes occur with age are therefore still unclear. Differences in methods used to assess body composition and FM distribution have created challenges when comparing results from different studies. Results that described FM distribution by measures of waist circumference reported it to be an independent determinant of RQ_{24} (Astrup, et al., 1992; Weyer, Snitker, et al., 1999). Other measures of FM distribution such as waist-to-hip (Snitker, et al., 1998), WTR (Ferros, et al., 1993), and upper and lower body obesity (Buemann, Astrup, Quaade, & Madsen, 1994) were not found to be associated with 24 hour substrate utilization. The relationship between body composition and RQ_{24} remains unclear. Since being overweight or obese, as well as FM content and distribution are often associated with T2D, understanding the relationship between body composition and RQ_{24}could be important in the prevention and management of T2D.

When biochemical variables were investigated, an inverse relationship was found between fasting plasma FFA and RQ_{24} (Zurlo, et al., 1990; Toubro, et

al., 1998). Since circulating FFA concentrations are often elevated in individuals with T2D, these findings may have an important implication in this population. A positive relationship was found between plasma insulin concentrations and RQ_{24} (Astrup, et al., 1992; Toubro, et al., 1998). Toubro et al., also reported that the insulin mediated glucose disposal rate was not associated with substrate oxidation over the 24 hour period, which suggests that the relationship between insulin concentrations and RQ_{24} is due to the action of insulin on suppressing fat oxidation and facilitating fat storage (Ellis, et al., 2010) rather than the role of insulin on glucose utilization. Reports from Astrup et al., however, contradict these conclusions. They examined RQ_{24} in a group of premenopausal women of varying body weight (n = 50), and reported that plasma insulin concentrations were an independent determinant of CHO oxidation and not a determinant of fat oxidation. While a positive relationship between plasma insulin concentrations and RQ_{24} has been reported in some studies, it has not been described in others (Ravussin, et al., 1983; Zurlo, et al., 1990; Snitker, et al., 1998). Zurlo et al. reported a weak but significant inverse relationship between fasting plasma insulin concentrations and RQ_{24} ($r = -0.17$, $P = 0.04$) in a group of obese men and women. When men and women were considered separately however, the relationship only remained significant in the men. While results from the studies performed by Zurlo et al. and Astrup et al. suggest that gender may be a confounding variable, Toubro et al., who studied the relationship between insulin concentrations and RQ_{24} in both men and women, reported both insulin concentrations and gender to be independent determinants of RQ_{24}. Other possible

reasons for contradicting results amongst studies are sample sizes and range of insulin concentrations within each study. Other biochemical variables have been examined in relation to RQ_{24} including fasting plasma glucose concentrations (Zurlo, et al., 1990), fasting plasma triglycerides concentrations, norepinephrine, plasma free triiodinethyronine (Toubro, et al., 1998), and plasma adiponectin levels (Stefan, et al., 2002), however no significant relationships were found.

Other factors that have been reported to be independent determinants of RQ_{24} are family membership (Ravussin, et al., 1983; Zurlo, et al., 1990) and SNS activity (Snitker, et al., 1998). Family membership has been reported to account for up to 28% of the inter-individual variance in RQ_{24}. This suggests that genetics may play an important role in nutrient partitioning and oxidation. Low SNS activity has been found to be a predictor of weight gain, and is therefore thought to be involved in the regulation of body weight. Therefore, an association between SNS activity and RQ_{24} suggests that impaired SNS activity may contribute to impaired fat oxidation and increased fat storage (Snitker, et al., 1998). A summary of the results from studies that have examined the relationship between RQ24 and metabolic and biochemical variables is described in **Table 2.1**.

Table 2.1: Summary of Reported Relationships between24 Hour Respiratory Quotient and Metabolic and Biochemical Variables

Source	Variable Examined	Relationship with Respiratory Quotient
Zurlo, et al., 1990; Astrup, et al., 1992; Snitker, et al., 1998; Toubro, et al., 1998; Weyer, Snitker, et al., 1999	Energy Balance	Positive acute energy balance = ↑ RQ_{24}
Zurlo, et al., 1990; Toubro, et al., 1998	Run in diet	Positive energy balance of run in diet = ↑ RQ_{24} ↑ FQ^{1} two days prior to testing = ↑ RQ_{24}
Buemann, et al., 1994; Weyer, Snitker, et al., 1999	Age	↑ Age = ↑ RQ_{24}
Zurlo, et al., 1990	Age	NS
Weyer, Snitker, et al., 1999	Gender	NS
Weyer, Snitker, et al., 1999	Percent FM	↑ Percent FM = ↓ RQ_{24}
Astrup, et a., 1992; Weyer, Snitker, et al., 1999	Distribution of FM - waist circumference	↑ Waist circumference = ↓ RQ_{24}

Source	Variable Examined	Relationship with Respiratory Quotient
Ferros, et al., 1993; Buemann, et al., 1994; Snitker, et al., 1998	Distribution of FM waist-to-hip, waist-to-thigh, upper	NS
Weyer, Snitker, et al., 1999	Glucose tolerance	NS
Zurlo, et al., 1990	Fasting glucose concentrations	NS
Astrup, et a., 1992; Toubro, et al., 1998	Fasting insulin concentrations	↑ Insulin Concentrations = ↑ RQ_{24}
Zurlo, et al., 1990	Fasting insulin concentrations	↑ Insulin Concentrations = ↓ RQ_{24}
Toubro, et al., 1998; Astrup, et al., 1992	Fasting TG concentrations	NS
Astrup, et al., 1992; Toubro, et al., 1998	Fasting FAA concentrations	↑ FFA Concentrations = ↓ RQ_{24}
Zurlo, et al. 1990	Fasting FFA concentrations	NS
Toubro, et al., 1998	Glucose disposal rate	NS
Toubro, et al., 1998	Norepinephrine	NS

Source	Variable Examined	Relationship with Respiratory Quotient
Toubro, et al., 1998	Free triiodinethryronine	NS
Toubro, et al., 1998	Adiponectin concentrations	NS
Toubro, et al., 1998	Family membership	↑ FQ[1] = ↑ RQ_{24}

[1]FQ = $\frac{0.207 \times \text{carbohydrate}(\%) + 0.159 \times \text{fat}(\%) + 0.193 \times \text{protein}(\%) + 0.137 \times \text{alcohol}(\%)}{0.207 \times \text{carbohydrate}(\%) + 0.226 \times \text{fat}(\%) + 0.243\ 3\ \text{protein}(\%) + 0.206 \times \text{alcohol}(\%)}$

Abbreviations: RQ24 (24 hour respiratory quotient), FQ (food quotient), NS (not significant), FM (fat mass), TG (triglycerides), FFA (fat free mass)

2.5 Classification of Fat and Carbohydrate Oxidizers

The utilization of nutrients varies greatly amongst individuals even when intake is well controlled. The ability to appropriately identify individuals as those that predominantly use CHO for energy (CHO oxidizers) or those that predominantly use fat for energy (fat oxidizers), and to recognize what metabolic factors are associated with each group, may assist in the prevention and treatment of certain metabolically related conditions such as T2D. Only two studies however, have attempted to classify individuals as CHO oxidizers or fat oxidizers. The methods in which these studies used to classify individuals were quite different and neither study included individuals with T2D. Zurlo et al. (1990) used data from Pima Indians (n = 152) who spent 24 hours in a whole body calorimetry unit. All participants consumed similar diets for at least two days before the calorimetry tests, as well as during the measurements. Weight maintenance diets were composed of 50% CHO, 30% fat and 20% protein. After adjusting for weight change during the run-in diet, acute energy balance, percent FM and sex, the mean RQ_{24} was 0.847 ± 0.020. Participants who had an adjusted RQ_{24} at or above the 90^{th} percentile (RQ = 0.877) were considered CHO oxidizers and those with a RQ_{24} at or below the 10^{th} percentile (RQ = 0.822) were classified as fat oxidizers. One hundred and eleven participants were followed up to 3 years. Individuals who were classified as predominantly CHO oxidizers were found to be at 2.5 greater risk of gaining more than 5 kg body weight than those classified as fat oxidizers. No other differences between fat and CHO oxidizers were examined.

Claessens et al. (2007) were the second group to classify individuals based on measured substrate utilization. In this study, classification was made based on the rate of fat oxidation during fasting conditions, as well as during postprandial conditions in response to a liquid saturated fat load consisting of 3% CHO, 2% protein, and 95% fat of which 60% was saturated. Those with fasting and postprandial fat oxidation rates greater than the 50^{th} percentile (expressed relative to energy expenditure) were considered high fat oxidizers (RQ < 0.825) and those with fat oxidation rates lower than the 50% percentile were considered low fat oxidizers (RQ > 0.825). Of the 99 participants included in the study, six high fat oxidizers and six low fat oxidizers were chosen and matched for body weight, age, and FFM which were included in their analysis. Researchers found that $RQ_{Fasting}$ was significantly different between groups; no significant differences in age, BMI, FFM, and HOMA-IR were found. Body weight, waist circumference, fasting FFA concentrations, mean REE and postprandial energy expenditure tended to be lower in the low fat oxidizers however, these differences did not reach significance. Together, these two studies indicate that the classification of individuals as CHO or fat oxidizers may assist in the identification of variables related to differences in substrate utilization, as well as potential health consequences associated with these differences

2.6 Metabolic Flexibility in Individuals with Type 2 Diabetes

Metabolic flexibility is the capacity of a system to switch from high rates of fat oxidation and low rates of CHO oxidation in fasting conditions to the

suppression of fat oxidation and the increase in glucose uptake, oxidation and storage in the fed state (Kelley & Mandarino, 2000). It can also be considered as the body or cells' ability to match fuel oxidation to fuel availability (Galgani & Ravussin, 2008). Metabolic flexibility is often assessed by the change in RQ from the postabsorptive state to insulin-stimulated or postprandial conditions. Changes in RQ throughout an overnight fast, in response to exercise, or in response to an isoenergenic change in the macronutrient composition of the diet can also be considered as measurements of metabolic flexibility(Galgani, Heilbronn, et al., 2008).

2.6.1 Metabolic Flexibility during Fasting Conditions

The progressive fall in RQ throughout an overnight fast reflects an increase in the proportion of fat being oxidized for energy. An impaired ability to switch from CHO to fat oxidation during this time has been defined by Galgani, Moro, et al. (2008) as metabolic inflexibility to fat. Studies that have examined the change in RQ throughout an overnight fast are lacking. It is thought however, that impaired substrate switching during the overnight period is reflected by a high $RQ_{Fasting}$ (Galgani, Moro, et al., 2008). Since fat oxidation is thought to be impaired in individuals with T2D,it has been frequently hypothesized that fat oxidation during fasting conditions is diminished in this population. Results from studies that have tested this hypothesis however, have been equivocal. In a study performed by van de Weije, et al. (2013), it was reported that whole body $RQ_{Fasting}$ was significantly higher in a group of men with T2D compared to age and weight matched controls, which indicated that the men with T2D oxidized a

greater proportion of CHO than fat for energy. When rates of substrate oxidation were examined, differences in RQ between the two groups were a result of higher rates of CHO oxidation in the men with T2D; no differences in rates of fat oxidation were found. In contrast, Golay, et al. (1984) and Paolisso, et al. (1994) reported that $RQ_{Fasting}$ was lower in both men and women with T2D. Those with T2D were reported to have lower rates of CHO oxidation and higher rates of fat oxidation. While these studies suggest that fasting substrate oxidation is altered in individuals with T2D, other studies have reported that no significant differences exist in $RQ_{Fasting}$ or rates of fasting CHO and fat oxidation in individuals with T2D compared to healthy controls (Borghouts, Wagenmakers, Goyens, & Keizer, 2002; Galgani, Heilbronn, et al., 2008; Meex, et al., 2010; Stull, et al., 2010; Russell, Kraemer, & Nelson, 2013). In consistent results in this area may be due to methodological differences amongst studies. Pretest maintenance of energy balance and nutrient composition of meals have been shown to have marked effects on resting substrate utilization (McNeil, Bruce, Ralph, & James, 1988; Schutz, 1997). Therefore, differences in the energy and nutrient composition of reported run-in diets may account for differences in results amongst studies. Use of glucose lowering medications by participants may also affect $RQ_{Fasting}$ values. Often studies require participants to discontinue their medications prior to testing; however the discontinuation of medications has been reported to vary from two days (Golay, et al., 1984) to one week (Meex, et al., 2010; van de Weijer, et al., 2013). Differences in medication protocols may cause differences in plasma substrate concentrations, which have been shown to affect substrate utilization

(Blaak, et al., 2006). It therefore remains unclear as to whether T2D status is associated with whole body $RQ_{Fasting}$, however $RQ_{Fasting}$ has been reported to be associated with other variables such as age, BMI, insulin sensitivity, plasma FFA concentrations and mitochondrial function (Blaak, et al., 2006; van de Weijer, et al., 2013).

Since ectopic fat accumulation in skeletal muscle is thought to impair the capability of the muscle to oxidize fat for energy, Kelley and Simoneau (1994) examined nutrient utilization in the skeletal leg muscle of people with T2D during fasting conditions using regional indirect calorimetry. They found that muscle $RQ_{Fasting}$ was significantly higher in individuals with T2D compared to age and weight matched controls. The rates of both fasting CHO and fat oxidation appeared to be altered; individuals with T2D were reported to have higher rates of glucose oxidation and lower rates of fat oxidation compared to controls. These reported differences in $RQ_{Fasting}$ in the skeletal leg muscle of individuals with T2D were supported by Kelley et al. (1999) who reported a strong negative association between leg $RQ_{Fasting}$ and insulin sensitivity. Although Kelley and Simoneau (1994) reported that skeletal leg muscle $RQ_{Fasting}$ was elevated in those with T2D, these differences were not found at the whole body level. Whole body $RQ_{Fasting}$ was measured simultaneously with leg RQ, and it was not significantly different between individuals with T2D and healthy controls, nor were rates of CHO and fat oxidation. Therefore, skeletal leg muscle substrate oxidation does not appear to reflect gas exchange at the whole body level during resting conditions. Although fat is the primary oxidative fuel in skeletal muscle during resting

conditions, muscle O_2 consumption is fractionally small compared to whole body consumption (~30%) during this time. Furthermore, muscle glucose oxidation has been reported to be one-tenth of whole body utilization during fasting conditions. Estimating whole body RQ based on muscle RQ therefore appears to be problematic during fasting conditions (Kelley & Simoneau, 1994). Lower rates of fat oxidation in skeletal muscle may be compensated by higher rates of fat oxidation in other tissues such as the liver (Despres, 1991). Impaired FFA uptake of the skeletal muscle observed in T2D may provide additional fuel for hepatic oxidation (Kelley & Simoneau, 1994). Gluconeogenesis may also contribute to differences in $RQ_{Fasting}$ between whole body and skeletal muscle. Gluconeogenesis, which has been reported to be elevated in individuals with T2D (Consoli, 1992), does not influence muscle RQ but can affect whole body RQ. The cause of differences in skeletal muscle RQ between individuals with and without T2D is still being explored. Proposed mechanisms include differences in glycemia (Kelley & Simoneau, 1994) and mitochondrial function (Meex, et al., 2010; van de Weijer, et al., 2013).

2.6.2 Metabolic Flexibility to Exogenous Nutrients

Metabolic Flexibility during a Hyperinsulinemic Clamp

The hyperinsulinemic euglycemic clamp has become a frequent method used for determining metabolic flexibility from fasting to insulin-stimulated conditions. This method allows for the careful control of plasma glucose and insulin levels in people with different metabolic conditions (Galgani, Moro, et al., 2008). Typically, the RQ will increase in response to a hyperinsulinemic clamp

reflecting the suppression of fat oxidation and an increase in CHO oxidation. This response, however, seems to be impaired in people with insulin resistance and T2D in both skeletal muscle and at the whole body level (Kelley, et al., 1999; Galgani, Moro, et al., 2008; Meex, et al., 2010; Stull, et al., 2010;Russell, et al., 2013; van de Weijer, et al., 2013), reflecting a lower capacity to increase CHO oxidation and a blunted suppression of fat oxidation in insulin-stimulated conditions (Paolisso, et al., 1994; Meex, et al., 2010; van de Weijer, et al., 2013). Russell, et al. (2013) demonstrated that healthy individuals with a family history of T2D also exhibited impaired changes in RQ during insulin-stimulated conditions. Their results indicate that impairments in substrate switching may precede the onset of insulin resistance and T2D, and that an important genetic component may be present. While results of some studies have supported their results (Lattuada, et al., 2005), others have found no differences in metabolic flexibility in individuals with a family history of T2D when compared to those with no family history of the disease (Ukropcova, et al., 2007).

Other mechanisms involved in the impaired substrate switching in response to insulin-stimulated conditions have been examined. In a study done by Stull, et al. (2010), metabolic flexibility was examined in 168 African American and Caucasian individuals with and without diabetes. The difference between $RQ_{Fasting}$ and insulin-stimulated RQ was determined to assess metabolic flexibility. It was found that metabolic flexibility was found to be inversely associated with age and $RQ_{Fasting}$, as well as fasting plasma glucose, insulin, and TG concentrations. It was positively associated with insulin sensitivity. When these

variables, with the addition of gender and diabetes status were included in stepwise multiple regression analysis, the only variables that remained significant were insulin sensitivity, $RQ_{Fasting}$, fasting TG concentrations, race and diabetes status. Seventy-one percent of the variability in metabolic flexibility was explained by these variables. Insulin sensitivity was the greatest contributor explaining 48% of the variance in metabolic flexibility.

Galgani, Heilbronn, et al. (2008) also demonstrated that fasting plasma glucose and insulin concentrations were inversely associated with substrate switching during a clamp in a group of individuals with and without T2D (n = 101) matched for BMI, gender and race. In addition, they reported that metabolic flexibility had an inverse relationship with insulin stimulated FFA concentrations and glycerol and a positive association with adiponectin. Glucose disposal rate, a measure of insulin sensitivity, was positively associated with metabolic flexibility. Glucose disposal rate encompasses both oxidative and non-oxidative glucose disposal rates. Therefore, differences in glucose disposal will reflect differences in glucose oxidation and/or glucose storage. Since glucose transport is impaired in people with T2D, these impairments may ultimately affect glucose oxidation. When Galgani, Heilbronn, et al. performed multiple stepwise regression analysis, glucose disposal rate was the most important predictor of metabolic flexibility, explaining 46% of the variance. Insulin-stimulated FFA concentrations explained an additional 3% of the variance. Stull et al. (2010) reported similar results in a comparable study examining substrate oxidation in individuals with T2D during a hyperinsulinemic euglycemic clamp. Results

published by van de Wijer, et al. (2013) also support the implication that glucose disposal rate and insulin-stimulated FFA concentrations are important determinants of metabolic flexibility. These results therefore suggest that a reduced metabolic flexibility during insulin-stimulated conditions may not be due to impaired CHO oxidation but rather blunted glucose uptake, and that elevated insulin-stimulated FFA concentrations in T2D may impair substrate switching. Higher plasma FFA may result in elevated fat oxidation, reducing the need for glucose oxidation.

Although results from these studies suggest that glucose disposal rate are important determinants of metabolic flexibility, studies that have compared substrate oxidation controlling for glucose disposal rates in individuals with and without T2D are limited. The few that have been published have reported mixed results. Some researchers have found that CHO oxidation was similar amongst individuals with T2D and controls when the rate of glucose disposal was matched (Kelley & Mandarino, 1990; Thorburn, Gumbiner, Bulacan, Wallace, & Henry, 1990). Others however, reported that CHO oxidation rates remained lower in individuals with T2D even when glucose disposal rates were the same as healthy controls (Koska, Ortega, Bogardus, Krakoff, & Blunt, 2007). In a study that examined the effects of exercise on glucose disposal rates and metabolic flexibility, it was found that a prescribed 12 week exercise program that included both aerobic exercise and weight training significantly increased the glucose disposal rate of people with T2D, which was accompanied by an improvement in metabolic flexibility to levels similar to those of healthy controls who also

participated in the exercise program. These results support the hypothesis that glucose disposal rate is an important determinant of impaired substrate switching of individuals with T2D during insulin-stimulated conditions. More research however, is still need to confirm these findings, as well as to determine what other metabolic parameters and mechanisms may be involved.

Metabolic Flexibility to Fat

Metabolic flexibility in response to glucose in individuals with T2D has been well considered with the use of hyperinsulinemic euglycemic clamps. Despite evidence suggesting that the impaired ability to adjust fat oxidation to fat availability may lead the accumulation of ectopic fat, few studies have examined substrate switching in individuals with T2D in response to fat. Rigalleau, et al. (1997) studied the effects of a flux in lipid by comparing lipid infusion and substrate oxidation compared to a saline infusion in two groups of individuals with T2D. They demonstrated that the group that was exposed to a flux in exogenous lipid experienced a rapid drop in CHO oxidation and an increase in fat oxidation to levels that were significantly higher than those measured in the control group receiving a saline solution. The suppressed rate of CHO oxidation and the increased rate of fat oxidation then remained constant for the remainder of the infusion. Although this study effectively demonstrated that significant changes in CHO and fat oxidation occurred in people with T2D when given a lipid infusion, since healthy matched controls were not used, it did not provide information about whether these changes in substrate oxidation differed from a healthy population.

A limited number of studies have examined metabolic flexibility in response to an orally administered high fat load. Kelley and Simoneau (1994) compared the postprandial RQ ($RQ_{postprandial}$) in the skeletal muscle of the leg in people with T2D and weight matched controls in response to a fat load. Participants were given a high fat meal (62% of energy from fat) and gas exchange was measured throughout the subsequent six hours. Results indicated that individuals with T2D had a significantly higher $RQ_{postprandial}$ than controls, which was found to reflect a significantly lower rate of fat oxidation and a higher rate of CHO oxidation. In addition, individuals with T2D were reported to have elevated postprandial glycemia and a reduced skeletal muscle FFA uptake, both of which may have influenced fat and CHO oxidation. Markers of mitochondrial function were found to be preserved in leg muscle of individuals with T2D, and therefore, did not appear be to a factor in the differences in RQ.

Substrate oxidation in response to a high fat meal (50% energy from fat) was further examined at the whole body level in lean and obese individuals without T2D but with varying levels of insulin resistance. Insulin resistance, as determined by HOMA-IR, was positively associated with postprandial fat oxidation after controlling for cofounding variables. In addition to HOMA-IR, fasting fat oxidation, gender, BMI and postprandial plasma FFA concentrations were found to be significant determinants of postprandial fat oxidation (Blaak, et al., 2006). These results do not support those of Kelley and Simoneau (1994) as they suggest that T2D status may be a factor in the ability to increase fat oxidation in response to a high fat load.

The postprandial response to a high fat meal (76% of energy from fat) in individuals with a family history of T2D has also been examined. In this study by Heilbronn, et al. (2007), RQ was compared in individuals with and without a family history of T2D. All participants had similar insulin sensitivity. Although changes in plasma glucose, FFA and insulin concentrations were similar, individuals with a family history of T2D experienced an impaired drop in RQ after the ingestion of the high fat meal, and those without a family history of T2D had an enhanced rate of fat oxidation. These results indicate that offspring of individuals with T2D may be predisposed to lower rates of postprandial fat oxidation and that this impairment may precede the development of insulin resistance.

2.6.3 Metabolic Flexibility during Exercise

It is often recommended that individuals with T2D participate in moderate to high levels of physical activity as part of their treatment plan (CDA, 2013). Regular exercise by those with T2D has been shown to improve glycemic control, improve plasma lipid concentrations, reduce insulin resistance, and help maintain weight loss (Wing, et al., 2001; Sigal, Kenny, Wasserman, Castaneda-Sceppa, & White, 2006). Altered substrate utilization at rest in the skeletal muscle of individuals with T2D however, has given rise to the question as to whether similar impairments in fat and CHO oxidation occur during exercise. During exercise, the majority of plasma FFA oxidation occurs in skeletal muscle. Impairments in fat oxidation during exercise therefore, maybe linked to the accumulation of ectopic fat in this tissue (Borghouts, et al., 2002).

The hypothesis that impaired fat oxidation occurs in individuals with T2D during exercise was tested by Ghassania, et al. in 2006. Fat oxidation was measured by indirect calorimetry using a metabolic cart at varying intensities on a cycle ergometer in sedentary men and women with T2D (n = 38) and a group of controls (n = 30) matched for age, weight and training status. The exercise test performed consisted of five six minute intervals at incremental workloads of 20, 30, 40, 50 and 60% of calculated theoretical maximal aerobic power (Wmax). Researchers found that the rate of fat oxidation was significantly lower in men and women with T2D than controls at all intensity levels, and that the shift to the predominant use of CHO during exercise occurred at a lower intensity in the group with T2D. Although results from this study support the idea that altered fat oxidation occurs in people with T2D during exercise, many others have reported contradicting results. Boon et al. (2007) reported that the rate of whole body fat oxidation measured using a metabolic cart was significantly higher in men with T2D (n = 10) when compared with healthy men (n = 10) of similar age, body weight and when performing 60 minutes of moderate-intensity exercise (50% of Wmax) on a cycle ergometer. The rate of CHO oxidation was not significantly different. With the continuous infusion of[6,6-$^{2}H_{2}$] glucose (0.3 μmol/kg/min) and [U-^{13}C] palmitate (0.1 μmol/kg/min) tracers throughout the exercise period, Boon et al. were also able to determine plasma glucose and FFA oxidation rates and estimate the use of muscle glycogen and/or lipoprotein derived triacylglycerol. Despite a significant drop in plasma glucose concentrations in men with T2D only, rates of plasma glucose oxidation and muscle glycogen oxidation in men

with T2D were reported to be similar to the healthy controls. Higher rates of total fat oxidation in men with T2D were attributed to elevated rates of FFA oxidation. No significant difference in muscle and/or lipoprotein derived triglyceride oxidation was found. Although Braun, et al., (2004) reported that overweight women with insulin resistance (n = 6) had a lower RQ during exercise ($RQ_{Exercise}$) while walking on a treadmill (45% of VO_2max) for 50 minutes, than overweight women with normal insulin sensitivity (n = 6) matched for percent FM, VO_2max and habitual physical activity. Unlike Boon, et al. (2007), however, the lower RQ was attributed to a reduced rate of CHO oxidation measured throughout the exercise period by an infusion of [6,6-^{2}H] glucose (6.0mmg/min).

Although some researchers have reported differences in $RQ_{Exercise}$, many others have reported no significant differences in RQ and similar rates of fat and CHO oxidation in individuals with T2D compared to controls during exercise of varying intensities (Colberg, et al., 1996; Kang, et al., 1999;Blaak, van Aggel-Leijssen, Wagenmakers, Saris, & van Baak, 2000; Borghouts, et al., 2002;Larsen, et al., 2009). As well, in contrast to the findings of Boon, et al. (2007), many have reported that although total CHO oxidation was similar in individuals with T2D and controls, the source of CHO appears to be different. Borghouts, et al. (2002) investigated substrate oxidation in individuals with T2D (n = 8) and a group of healthy controls (n = 8) during a 60 minute period of exercise (40%VO_2max) on a cycle ergometer. Throughout the exercise period stable isotope tracers [6,6-$^{2}H_2$] glucose (0.3 µmol/kg/min) and [U-^{13}C] palmitate (0.1 µmol/kg/min) were also simultaneously infused, and the rate of appearance and disappearance of these

metabolites was measured. Individuals with T2D utilized a significantly greater amount of plasma glucose and less glycogen for CHO oxidation than the healthy controls. Results from this study supported the previous work of Colberg, et al. (1996) and Kang, et al.(1999). The preferential use of plasma glucose over glycogen for energy during exercise in people with T2D may explain why individuals with T2D have been reported to have a significant reduction in plasma glucose concentrations during a bout of exercise (Kang, et al., 1999; Borghouts, et al., 2002). Whether the source of fatty acids being oxidized during exercise is the same in T2D as healthy controls seems to be less clear. While some researchers have reported reduced plasma fatty acid oxidation and increased muscle-derived triglyceride oxidation in individuals with T2D (Blaak, et al., 2000), others have reported no differences in the source of fatty acid oxidation (Borghouts, et al., 2002; Larsen, et al., 2009).

Contrasting reports of substrate utilization in individuals with T2D may be due to differences in study design. Some studies reported that controls were well matched for age, weight, and training status (Kang, et al., 1999; Blaak, et al., 2000; Ghanassia, et al., 2006; Boon, et al., 2007) however, others used controls that were not as coordinated (Borghouts, et al., 2002; Larsen, et al., 2009). Differences in substrate oxidation may have also occurred between those that are newly diagnosed with T2D and those that had long-term diagnosis. Newly diagnosed individuals may still have experienced compensatory hyperinsulinemia. Those that had a long-term T2D diagnosis may no longer experience this compensatory effect, which may result in elevated plasma FFA concentrations

due to failed inhibition of adipose tissue lipolysis. Higher concentrations of plasma FFA may lead to alterations in FFA oxidation (Boon, et al., 2007). While Boon, et al. reported excluding newly diagnosed individuals, many other studies did not report the length of time since diagnosis (Blaak, et al., 2000; Boon, et al., 2007; Larsen, et al., 2009). Differences in the type and intensity of exercise performed may also impact measurements of substrate utilization, however, all the studies used in this review included exercise performed on a cycle ergometer at mild or moderate intensity.

In addition to the need for more research examining newly versus long-term diagnosed individuals with T2D, other research gaps exist in this area. There is a lack of studies that examine substrate utilization in trained individuals with T2D, as studies often only include those that are sedentary. Since physical activity is often part of an individual's treatment plan, it is important to examine if differences in substrate utilization occur in individuals with T2D who become more physical active. The majority of studies have also only examined substrate oxidation in obese individuals with T2D. Having a better understanding of whether substrate oxidation differs in obese and non-obese individuals with T2D may provide important insight into the development of the disease in these heterogeneous groups (Borghouts, et al., 2002).

2.7 Conclusions

Most of the work on energy expenditure and substrate oxidation in individuals with T2D has been completed over the course of a few hours. Less is

known about energy and substrate utilization over a 24-hour period. Type 2 diabetes appears to influence both REE and TEF however, how they are affected indifferent ways. Since REE contributes to a greater proportion of TEE, higher rates of REE may be responsible for elevated TEE seen in some but not all studies reviewed. Evidence suggests that elevated rates of energy expenditure during rest may be associated with increased plasma glucose concentration, and therefore, REE is often normalized once anti-diabetic treatments are started.

The examination of substrate oxidation over a 24 hour period in individuals with T2D is lacking. Studies performed in healthy, normal weight and overweight individuals have indicated that RQ_{24} may be associated with fasting plasma FFA and insulin concentrations. Since it is often reported that those with T2D have elevated fasting FFA concentrations, a relationship between these variables may have important implication for 24 hour substrate oxidation in individuals with T2D. A compensatory hyperinsulinemic effect is often noted in individuals newly diagnosed with T2D, therefore, results obtained from studies of healthy individuals suggest that newly diagnosed individuals may have elevated RQ_{24} in comparison to those without T2D or those who have had T2D for a considerable period of time. More research is needed however before any such conclusions can be made.

In resting conditions, whole body substrate utilization appears to be similar in healthy individuals and those with T2D. When measured in skeletal leg muscle however, $RQ_{Fasting}$ is greater in those with T2D, reflecting a higher rate of CHO oxidation and impaired fat oxidation. Since total substrate oxidation is

relatively low in skeletal muscle during rest, altered rates of substrate oxidation in this tissue are not reflected in $RQ_{Fasting}$ measured at the whole body level. However, when whole body substrate oxidation is measured during a clamp, metabolic flexibility appears to be impaired in those with T2D. Many researchers have attributed impaired metabolic flexibility in those with T2D to a reduced glucose disposal rate. However, more research is needed that controls for glucose disposal rate while comparing individuals with T2D and healthy controls during insulin-stimulated conditions to confirm these implications. While there are a number a studies examining substrate oxidation of individuals with T2D in response to CHO, there is a limited number of studies that examine substrate utilization in response to a high fat load; studies examining metabolic flexibility to a standard meal are absent all together.

In summary, research completed to date during resting and insulin-stimulated conditions suggests that substrate oxidation is impaired in individuals with T2D. However, research that examines substrate utilization in individuals with T2Dover the 24-hour period and in response to various stimuli is either lacking or limited. The research performed in this thesis provides a preliminary examination of some of these research gaps.

Chapter 3: Materials and Method

3.1 Ethics

Approval for this observational pilot study was provided by the Physical Education and Recreation, Agricultural, Life & Environmental Sciences and Native Studies Research Ethics Board at the University of Alberta (Appendix A). This research project was designed to examine energy expenditure and substrate oxidation of people with T2D over a 24 hour period with the use of a whole body calorimetry unit.

3.2 Recruitment Strategies

The goal for this project was to recruit 10 participants (five men and five women) with T2D from the Edmonton area. Recruitment strategies took three main forms. The first method included the distribution of study information within the community. This included the placement of posters around the University of Alberta, information distributed to pertinent online groups and networks, as well as a paid advertisement in the Metro newspaper; a newspaper free of charge and distributed daily throughout the city of Edmonton. If people were interested in participating in the study they were directed to call the research office, at which time they were provided detailed information about the study and its inclusion and exclusion criteria. The second method used to recruit participants was through diabetes education classes offered by Alberta Health Services (AHS). Permission to recruit at the "Taking Charge" classes was pre-arranged with the Manager of Program Delivery. A research assistant delivered a 5 minute

presentation at the start of each class and handed out the study information sheet (Appendix B). Those who wanted to learn more about the study provided their names and contact information to the research assistant. They were then contacted by the same research assistant within three days to further discuss the project. Lastly, contact was made with participants from two previous T2D studies conducted at the University of Alberta.

3.3 Inclusion Criteria

Individuals were eligible to participate if they were between 40 –75 years of age and had been previously diagnosed with T2Dby a physician. Their diagnosis had to be at least 6 months prior to the start of the study.

3.4 Exclusion Criteria

The following individuals were ineligible to participate in the study:

- People taking insulin
- People who smoke
- People with claustrophobia
- People with dietary restrictions (e.g. severe food allergy, Celiac disease)
- People on medications with metabolic implications (e.g. beta-blockers, stimulants, thiazolidinedione, growth hormone)
- People with a change in medication within the last 6 weeks

- People with self-reported untreated high blood pressure defined as >140/90mmHg
- People with problems with diabetes management including a history of severe hypoglycemic episodes (requiring third party assistance) and hypoglycemic unawareness
- People who had lost or gained more than 2.3 kg in the previous 6 months
- People who had increased or decreased their level of physical activity by more than 1 hour per week in the previous 6 months
- People with active cardiovascular disease (e.g. myocardial infarct or revascularization in the last 6 months or angina pectoris)
- People with other significant health issues and with a high risk of a health episode occurring (e.g. anxiety, breathing difficulties, sleep difficulties)
- People with other medical or psychiatric factors that in the judgment of the principle investigators may interfere with study participation or ability to follow study protocol

All medications taken by potential participants, as well as other existing medical conditions in addition to diabetes were assessed on a case-by-case basis to ensure they would not impact the ability to examine the effect of T2D on metabolism. An endocrinologist was consulted as required.

3.5 Orientation Session

All those who expressed interest in participating in the study were asked to attend an orientation session (~1.0 – 1.5 hours) prior to their enrollment. During the session, participants received an explanation of the study's rationale and its protocol, and were given a tour of the calorimetry unit. Participants were asked to spend some time in the unit to screen for claustrophobia, and were given the opportunity to practice walking on the treadmill. If participants were still unsure of whether they would be comfortable within the calorimetry unit for 24 hours, they were given the opportunity to return on another occasion to spend additional time (~1 hour) inside the unit.

Informed consent (Appendix C) and personal information (Appendix D) was obtained from those who remained interested in participating in the study. Participants had been asked to bring all current medications and nutritional supplements with them to the orientation session. Names of the medication, frequency and length of use were recorded. Height was measured to the nearest 0.1 cm using the Quick Medical Heightronic digital stadiometer (Northbend, WA) and date of birth was collected.

3.6 Fitness Testing

In order to standardize the intensity of the exercise session performed by each participant while in the calorimetry unit, all participants completed a modification of the Bruce treadmill test(McInnis and Baladay, 1994) a minimum of two days prior to their test days in the unit. Fitness testing was performed in the

Physical Activity and Diabetes Laboratory (PADL) at the University of Alberta by a Canadian Society for Exercise Physiology: Certified Exercise Physiologist (CSEP: CEP) trained in exercise testing of clinical populations. Prior to testing, participants completed the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) (CSEP, 2011) and were given instructions on how to prepare for the test (Appendix E).

Upon arrival to the PADL, participants were weighed in light clothing without shoes to the nearest 0.1 kg using the 402KL Health o Meter scale (Sunbeam, Boca Raton, FL, USA). Height had been previously measured during the orientation session. Participants were then asked to sit quietly for 5 minutes after which time, pre-test blood pressure (BP) and heart rate (HR) measurements were performed. Blood pressure was measured using the precise N sphygmomanometer (Riester, Jungingen, Germany) and stethoscope and HR was determined by a Polar Electro HR monitor (Oy, Kempele, Finland). As per protocol, if a participant's resting BP was greater than 144/94 mmHg or HR was greater than 99 beats/minute, the participant was required to remain seated quietly for an additional 5 minutes. Blood pressure and HR measurements were then repeated. If the measurements remained higher than the aforementioned criteria, the exercise test was not performed and the participant was referred to their doctor for further evaluation (Canadian Society for Exercise Physiology, 2003).

Participants who met the pre-test BP and HR criteria performed a 5 minute warm up on the Freemotion Incline Trainer treadmill (Logan, Utah, USA) at a speed of 1.7 miles per hour (mph) and 0% grade for the first 2.5 minutes and 2.5

mph and 0% grade for the following 2.5 minutes. Blood pressure and HR were measured using the same method as above between minute four and five of the warm up. Participants were then given a 5 minute break. During this time, the headgear and mouth piece (Hans Rudolf, Shawnee, KS, USA) were properly positioned to the participant's head and mouth, and attached to the TrueOne 2400 metabolic measurement system (ParvoMedics, Sandy, UT, USA) that was calibrated for air flow and O_2 and CO_2 gas concentration according to the manufacturer. After one minute of standing with the apparatus attached, BP and HR were measured and metabolic measurements were checked. The fitness test was then started with participants walking at the speed and grade as outlined in **Table 3.1** for 3 minutes. After each 3 minute interval, participants would progress to the next stage of the test until their individual ventilatory threshold (VT) was reached which was determined using the V-slope method (Beaver, Wasserman, & Whipp, 1986). This was visually observed by the trained investigator from the graphic display of VCO_2 versus VO_2 using the software program provided by the metabolic measurement system. At the end of each stage BP and HR were measured and the Borg scale (Borg, 1982) was used by participants to determine their rating of perceived exertion (RPE). Once VT was reached, treadmill speed and grade were reduced to 1.7 mph and 0% grade for 5 minutes of recovery. Blood pressure, HR and metabolic variables were after 5 minutes of recovery. After the 5 minute recovery phase on the treadmill, participants were asked to sit quietly for 5 minutes. Blood pressure and HR were measured at the end of the 5 minute period. In total, the test took approximately 45 minutes.

Results obtained were used to determine the treadmill settings for the exercise sessions performed by the study participants during their calorimetry test days. The treadmill speed and percent grade were determined as approximately 90% of their VT.

Table 3.1: Abbreviated Modified Bruce Fitness Test Treadmill Speed and Grade Performed during Each Stage

Stage 1	Speed (mph)	Grade (%)
1	1.7	0
2	1.7	5
3	1.7	10
4	2.5	12
5	3.4	14

3.7 Whole-Body Indirect Calorimetry

3.7.1 Calorimetry Unit

Twenty-four hour energy expenditure and substrate oxidation were measured in a whole-body open circuit indirect calorimetry unit located in the Alberta Diabetes Institute at that University of Alberta. The unit has a geometric volume of 28,738 L and was furnished with a bed, small table, chair, toilet, mirror, sink, television, laptop computer, telephone, intercom and specimen refrigerator. Items such as food and other items were passed in and out of the unit via two separate airlock windows. Iris ports allowed for blood draws to be performed while the participants were in the unit. The ambient temperature of unit was maintained at 22°C. Mixed, expired air was drawn out of the unit while fresh, conditioned air was passively drawn into the unit at a constant flow rate of 60 -

90L/min predetermined by research staff according to each participant's body size. Differences in carbon dioxide (CO_2) and O_2 concentrations of expired and fresh air were calculated every minute by the ABB Automated GmbH CO2 analyser (Frankfurt, Germany) and the Siemens Oxymat 6 O_2 analyser (Munich, Germany). Information was translated from the gas analysers to the computer via the National Instruments NI USB-6221 device (Vaudreuil-Dorion, Quebec, Canada).

Propane burn testing was performed 14 days prior to commencing the study and 6 days after the study completion to determine the overall accuracy of the unit. Gas analysers were calibrated the morning of each test day. Test/re-test reliability of the unit had been previously determined (unpublished results). A mean difference in TEE of the whole sample between day 1 and day 2 was 39 kcal, and TEE correlation between day 1 and day 2 was $r^2 = 0.983$ and the coefficient of variation (CV) was 2.2%.

Two research staff were always on duty while participants were in the unit. They monitored the instrumentation and kept records of flow rate (L/min), CO_2 concentrations and temperature every 30 minutes. They would also do a visual check on participants through a window in the unit approximately every hour. Participants could contact the monitor at any time using the intercom system or landline telephone.

3.7.2 Test Days

Participants spent two 24 hour periods in the calorimetry unit, one day apart. Participants were instructed to arrive at the research unit by motor vehicle

and to take the elevator up to the second floor. They had been previously instructed to refrain from exercise for the 24 hours prior to the test and to fast for at least 8 hours (water only).

They were asked to arrive at the research unit between 7:15 - 7:30 a.m. Upon arrival to the unit, participants were weighed while wearing a cloth hospital gown. Weight was measured to the nearest 0.1 kg using Health o Meter Professional Series digital scale (Sunbeam, Boca Raton, FL). Participants were weighed twice. If identical measurements were obtained, that value was recorded. If measurements were not the same, a third measurement was taken and the majority value was recorded. Upon completion of the weight measurement, a trained research staff member inserted a Medtronics continuous glucose monitor (Brampton, ON, Canada) into the subcutaneous tissue right above the participants' waist band. The continuous glucose monitor was inserted on test day 1, and was worn by the study participants until the completion of the second calorimetry unit test day. After the insertion of the continuous glucose monitor on test day 1, a venous fasting blood draw was also performed by a trained phlebotomist. Participants were then given 10 - 15 minutes to sit quietly in a room and relax. Research staff answered any remaining questions the participants had and provided them with some reminders about study protocol. At ~7:50 a.m. participants were walked to the calorimetry unit. The test began at ~8:00 a.m. The schedule of rest, meals, exercise, and sleep was the same for test day 1 and test day 2 (Appendix F). The following morning upon completion of the test, participants were weighed once more in the same cloth hospital gown.

3.7.3 Resting Energy Expenditure

Resting energy expenditure testing was done during the first hour of testing (~8:00 – 9:00 a.m.) on day 1 and day 2. Each participant was asked to lie in a supine position on the bed within the metabolic unit for the entire first hour. They were given the option of watching television during this time, otherwise no other activities could be performed. The first 30 minutes of the test was considered an acclimatization period, and the last 30 minutes were used for analysis.

3.7.4 Diet

Menus were designed by a Registered Dietitian to keep the macronutrient distribution similar for all participants (~50% carbohydrate, 30% fat, 20% protein). Caloric content and macronutrient distribution of the menus were determined using ESHA's The Food Processor Nutrition and Fitness Software version 10.6.0. Eight menus were created that ranged from 1600 – 3000 kcal (Appendix G). Participants were assigned to a menu based on their individual caloric requirements to achieve energy balance. The metabolizable energy content of each meal was estimated using a digestibility factor of 0.93 based on the work done by Smith, et al. (2000). Caloric requirements were estimated based on energy expenditure data obtained from the calorimetry unit. Three predictions of energy needs were made throughout both day 1 and day 2 as data became available. The first prediction was made prior to the first meal being served (~8:45 a.m.) and was based on the first 30 minutes of the resting energy expenditure data. Participants were assigned to one of the eight menus based on

this first prediction of TEE. A second prediction was made prior to the second meal of the day (~11:45 a.m.) and was based on the first 3.5 hours of data collected. It included data that represented both resting and sedentary activities such as sitting in a chair. A final prediction of caloric requirements was completed in the afternoon after the exercise session (~3:45 p.m.) based on energy expenditure data from the first 7.5 hours of time spent in the unit. After the second and third predictions were complete, foods with the appropriate macronutrient balance were added to or removed from the menu (in 100 kcal units) as needed to ensure caloric needed were being achieved.

All meals and snacks were prepared on site in the research kitchen by trained staff. Food served was accurately measured and weighed. Three meals and two snacks were provided to all participants at approximately 9:00 a.m., 12:00 p.m., 3:00 p.m. (snack), 6:00 p.m., 9:00 p.m. (snack). Participants were instructed to eat all the provided food within a 30 minute timeframe. The amount of food served as well as the amount of food eaten was carefully documented. Bottled water was provided at each meal/snack and as requested by participants.

3.7.5 Exercise

During the afternoon (~2:30 p.m.) of day 1 and day 2, participants performed a moderate-intensity walk on the BH T8 Sport treadmill (BH North America, Foothill Ranch, CA, USA) inside the unit. The intensity of the workload was predetermined by the results obtained from the previously described fitness test. The intensity level of the bout of exercise was standardized to 90% of VT to ensure participants could comfortably and safely complete the exercise session.

This level of exercise intensity also ensured that lactic acidosis was avoided as it causes the non-oxidative production of CO_2resulting in the overestimation of CHO oxidation. The treadmill was pre-set to the speed and grade that reflected this intensity by research staff in the morning prior to commencing the calorimetry measurement. Participants walked on the treadmill at the individually prescribed intensity for 30 minutes. Upon completion of the 30 minutes, the speed stayed constant but the percent grade was reduced to zero for a 5 minute cool down. A HR monitor was worn by the participants throughout each exercise session. They reported their HR to the research staff monitoring the session before starting and every 5 minutes while exercising. They also reported their RPE using the Borg scale at each 5 minute interval. The exercise session was timed by the research staff. Constant visual and verbal contact was made with the participants via the window and intercom system throughout the session.

3.7.6 Sleep

Eight hours of sleep time was scheduled between 10:30 p.m. - 6:30 a.m. Participants were asked not to sleep at any other time during the day. If participants were unable to fall asleep at the scheduled time or if they woke up early, they were requested to remain lying in bed for the entirety of the scheduled sleep time. A wakeup call was made to participants by the research staff at 6:30 a.m.

3.7.7 Blood Collection

Blood samples were drawn by a trained phlebotomist on day 1 of testing. A fasting blood sample (11ml) was taken from the antecubital vein prior to

participants entering the unit (~7:30 a.m.). A two hour postprandial sample (~11:30 a.m., 11ml) was achieved without disruption to the calorimetry unit measurement using an iris port. Participants slid their arm through the rubber sleeve of the iris port and rested it on a padded table on the other side in the monitoring area. Eye contact through the window in which the iris port was located and verbal contact by intercom was maintained with the participants throughout the blood draw. Both fasting and postprandial samples were collected into a grey top tube containing fluoride and oxalate for plasma glucose analysis and a gold top tube containing a clot activator and gel for serum insulin, FFA and TG analysis. Samples were immediately centrifuged at 1200 xg for 10 minutes. Plasma and serum were then stored at -80°C until analysis.

3.7.8 Urinary Nitrogen Excretion

Participants were instructed to collect all urine during their two test days in the calorimetry unit. They were asked to void prior to entering the unit. Samples were collected in 3L plastic urine jugs and stored in the specimen refrigerator inside the unit. Urine 'hats' were provided to female participants to aid with collection. Upon completion of the test, total urine volume was measured and three aliquots of 15 ml were stored at -20°C until analysis. A Shimadzu TOC-V (Shimadzu Scientific Instruments, Columbia, MD, USA) with a nitrogen analyzer attachment was used to determine total urinary nitrogen. Samples were combusted to NO and NO_x and reacted with an ozone generator to form NO_2 in an excited state. The resulting photon emission was measured by chemiluminescense

detector and then quantified using a total nitrogen standard. The analysis of total urinary nitrogen was done in duplication with a CV of less than 1%.

3.8 Blood Analysis

3.8.1 Analysis of Glucose

The assay used in the assessment of glucose in plasma (Genzyme Diagnotics P.E.I. Inc, Charlottetown, PE) was a modification of the glucose oxidase/peroxidase method described by Trinder (Trinder, 1969). This is a single reagent assay in which oxidation of glucose by glucose oxidase produced D-gluconic acid and hydrogen peroxide. The hydrogen peroxide in the presence of peroxidase caused the oxidative coupling of hydroxybenzoare and 4-aminoantipyrine yielding a quinoneimine dye. Absorbency of this coloured dye complex was measured at 505 nm to determine the concentration of glucose in the sample. The analysis of plasma glucose was done in duplicate and CV was 5.1%.

3.8.2 Analysis of Insulin

Insulin concentrations were determined using the ALPCO Ultrasensitive Insulin Enzyme-Linked Immunosorbent Assay (Salem, NH, USA). Samples, standards and controls were pipetted into a microplate coated with monoclonal antibody. The detection antibody was then added. The plate was covered and incubated at room temperature for 1 hour on a microplate shaker. Once the incubation period was complete, the wells were rinsed with a wash buffer and blotted dry. Tetramethylbenzidine substrate was then added and the microplate was incubated for a second time at room temperature on the microplate shaker. A stop solution was then added. The optical density of the colour complex generated

was measured at 450 nm. The intensity of the colour generated was directly proportional to the insulin concentration in the samples. The analysis of serum insulin was done in triplicate and the CV was 2.2%.

3.8.3 Analysis of Triglycerides

The Triglyceride-SL Assay (Genzyme Diagnotics P.E.I. Inc, Charlottetown, PE) was used to provide a quantitative measurement of TG in serum. In this single reagent assay, TGs were hydrolysed by lipoprotein lipase to form glycerol and FFA. Glycerol was phosphorylated to glycerol-1-phosphate in the presence of adenosine triphosphate (ATP) and glycerol kinase. Oxidation of glycerol kinase produced hydrogen peroxide, which caused the oxidative coupling of p-chlorophenol and 4-aminoantipyrine yielding a coloured dye complex in the presence of peroxidase. The absorbency of this coloured complex was measured at 520 nm to determine the TG concentration in the sample. The analysis of serum TG was done in triplicate and the CV was 3.2%.

3.8.4 Analysis of Free Fatty Acids

The quantification of serum fatty acids was through the coupled reaction to measure non-esterified fatty acids (NEFA). A commercial kit from Zen-Bio, Inc. (Research Triangle Park, NC, USA) was used for this assay. In the initial step, acyl-CoA synthetase acted on NEFA, ATP, magnesium and CoA to produce fatty acyl-CoA thiol esters. Acyl-CoA derivatives and oxygen reacted in the presence if acyl-CoA oxidase to produce hydrogen peroxide. The combination of hydrogen peroxide and peroxidase allowed for oxidative condensation of methyl-N-ethyl-N-(β-hydroxythyl)-aniline with 4-aminoantipyrine which formed a

coloured complex. The concentration of NEFA was then determined by measurement of optical density of this coloured complex at 550 nm. The analysis of serum insulin was done in triplicate and the CV was 3.7%.

3.9 Body Composition

After the completion of the two calorimetry test days, participants returned to the research unit a final time for a body composition measurement. Lean mass and FM were determined by DXA performed by an experienced certified Medical X-Ray Technologist. Participants were given the DXA information sheet (Appendix H) and informed consent was obtained by each participant prior to performing the DXA (Appendix I). Participants were excluded from the DXA measurement if they were pregnant, had recently undergone barium tests/exams (within 2 weeks), or if they had a nuclear medicine scan or been injected with an X-ray dye (within 1 week). Female participants performed a pregnancy test prior to the measurement unless they were post-menopausal (no menstrual cycle for ≥ 6 months), were taking oral/injection contraceptives or had a hysterectomy. Participants were given a cloth hospital gown to wear and were instructed to remove all clothing with the exception of socks and underwear. Jewelry, hair accessories and any other objects that may have contained metal were also removed. Participants were then positioned on the DXA table and told to remain still while the technician performed the measurement.

3.10 Calculations and Statistical Analysis

Equations used in the calculations of energy expenditure, NPRQ and rates of CHO and fat oxidation are listed in **Table 3.2**. Twenty-four hour energy expenditure adjusting for total urinary nitrogen losses was calculated using the complete Weir equation (1949). Resting energy expenditure and SEE were also calculated using the complete Weir equation, however, nitrogen losses were estimated using the mean rate of nitrogen loss calculated over the 24 hour period. Non-protein RQ was calculated based on the method of Ferrannini (1988). Similar to calculations made for energy expenditure, fasting, postprandial, exercise and sleep NPRQ were calculated using estimated nitrogen losses based on the rate over the entire test period. Rates of CHO and fat oxidation were estimated using the equations of Frayn (1983). For each participant, a mean value from data collected during day 1 and day 2 of their stay in the calorimetry unit was used for the analysis of metabolic data. All calculations were performed using Microsoft Excel for Windows 2010.

Metabolic flexibility to a standard mixed meal (ΔRQ_{Meal}) was calculated as the difference between the mean RQ during fasting conditions measured from ~8:30 –9:00 a.m. and the mean RQ during the 2 hour postprandial state measured from ~11:00 - 11:30 a.m. Metabolic flexibility in response to exercise ($\Delta RQ_{Exercise}$) was determined as the difference between the mean RQ measured during the 30 minutes prior to the bout of exercise on the treadmill (~1:30 –2:00 p.m.) and the mean RQ measured throughout the exercise period (~2:00 - 2:30 p.m.). Lastly, metabolic flexibility to an overnight fast ($\Delta RQ_{Overnight}$) was

calculated as the difference between the mean RQ measured during the first hour of sleep at night (~10:30 - 11:30 p.m.) and the mean RQ measured during the last hour of sleep in the morning (~5:30 - 6:30 a.m.).

The change in all blood values were all determined by calculating the difference between values obtained during fasting and postprandial conditions. The homeostasis assessment model (HOMA-IR) was calculated using the method described by Matthews et al.(1985: HOMA-IR = fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5), and was used as a proxy measure of whole-body insulin resistance.

Statistical analyses were performed using STATA for Windows version 12. A test for normality was performed for all continuous variables. The logarithm of glucose, insulin, FFA and TG concentrations, as well as HOMA-IR and years with T2D were used in the statistical analysis to normalize distributions. The equality of variances of participant characteristics, metabolic and biochemical variables, measures of metabolic flexibility and changes in blood concentrations between CHO and fat oxidizers were determined using the F-test (Ruxtin, 2006). Comparisons of variables of equal variance between CHO and fat oxidizers, as well as differences in RQ_{24} and $RQ_{Fasting}$ between men and women were performed by independent sample student's t-test. Comparisons between variables with unequal variance were performed using t-test for unequal variance. Correlations are Pearson product-moment correlations. All statistical tests were two-sided and performed at the $P<0.05$ level of significance. A trend was defined as a *P value* greater than 0.06 and less than 1.0.

Table 3.2: Equations used in the Calculation of Energy Expenditure, NPRQ and Rates of CHO and Fat Oxidation

Equation	Source
Energy Expenditure (kcal/day) = $[3.9 \cdot VO_2 + 1.1 \cdot VCO_2]$ 1.44 - $2.17 \cdot N$	Weir, 1949
NPRQ = $(VCO_2 - 4.89 \cdot N) / (VO_2 - 6.04 \cdot N)$	Ferrannini, 1988
CHO Oxidation (g/day) = $4.55 \cdot VCO_2 - 3.21 \cdot VO_2 - 2.87 \cdot N$ Fat Oxidation (g/day) = $1.67 \cdot VO_2 - 1.67 \cdot VCO_2 - 1.92 \cdot N$	Frayn, 1983

Where, VO2 = oxygen consumption (L/day)
VCO2 = carbon dioxide production (L/day)
N = urinary nitrogen (g/d)

Chapter 4: Results

4.1 Recruitment

A total of 10 individuals with T2D participated in the study. Half of the individuals recruited called into the research office in response to a newspaper advertisement. An additional four participants were recruited through AHS diabetes classes as described in the methods. One participant had participated in a previous study performed at the University of Alberta. Recruitment strategies, the number of people who initially expressed interest in participating, and the number that completed the study are listed in **Table 4.1**. The flow of participants through the recruitment process is described in **Figure 4.1**. In total, 58 individuals initially responded to the recruitment efforts; 10 people completed the study, as per the target number for this pilot study.

Table 4.1: Recruitment Strategies

Recruitment Strategies	Expressed Interest (Contact Information Provided for Follow Up)	Individuals that Completed Study
Metro Newspaper Advertisement	19	5
E-mail Listservs	2	0
Posters on University of Alberta Campus	0	0
Participated in Previous T2D Studies	7	1
AHS Taking Charge Diabetes Classes	28	4
Word of Mouth	2	0
Total	58	10

Abbreviations: T2D (type 2 diabetes), AHS (Alberta Health Services)

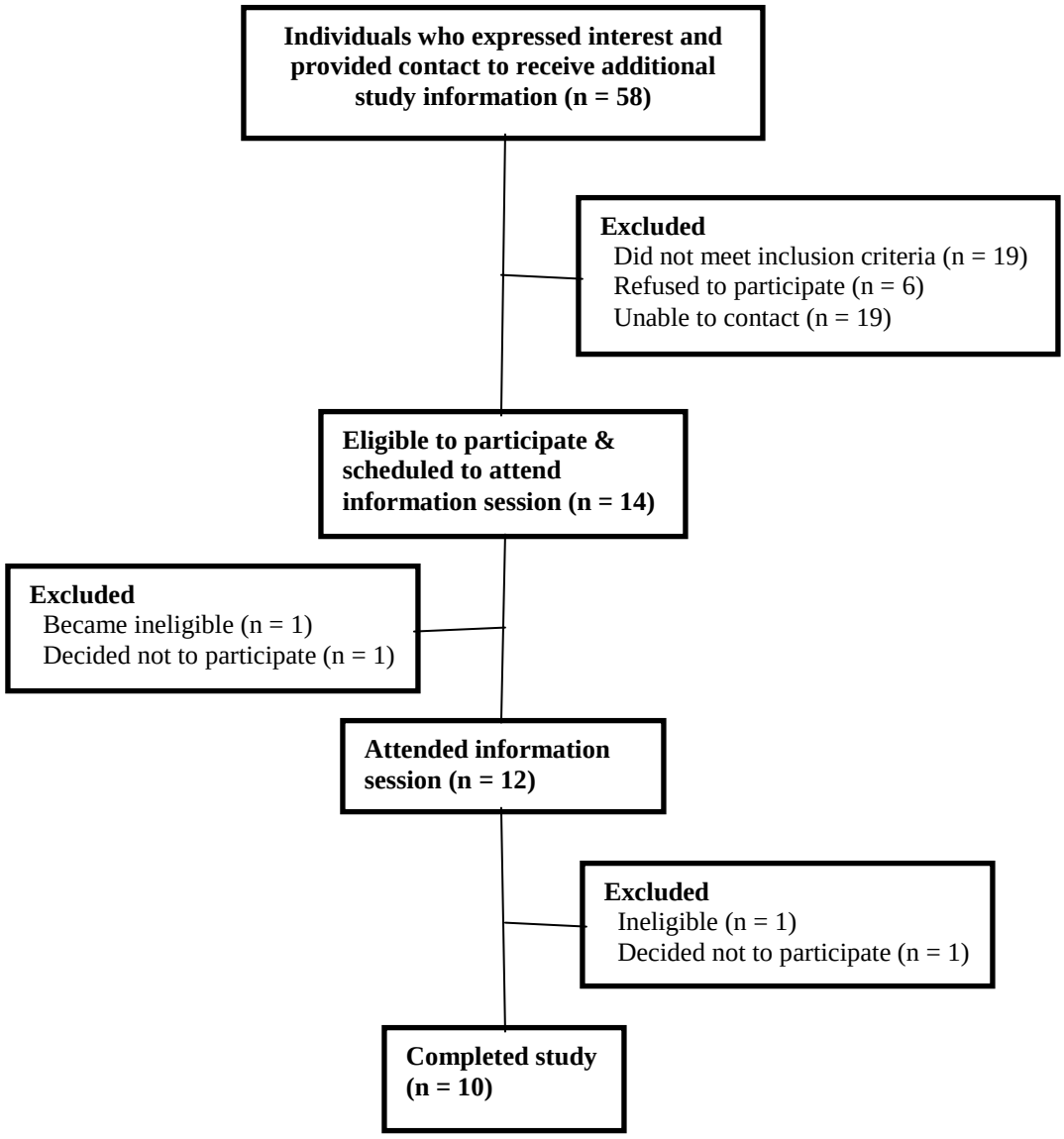

Figure 4.1: Flow of Participants through the Recruitment Process

4.2 Study Participation

Six men and four women completed the study. Of the 10 participants enrolled, data on body composition and time since diagnosis could not be obtained for one participant due an illness that began after the completion of the two calorimetry test days. Blood samples were obtained from nine participants. One of the nine participants ate a small meal (¾ cup cereal, 8 raspberries, ½ cup almond milk) 2 hours prior to the fasting blood draw; all other participants were fasted for at least 8 hours. Nine of the ten participants spent two 24 hour periods in the calorimetry unit, and one participant spent one 24 hour period in the unit. Due to technical problems, data obtained during the second day of testing for one participant was not used in the analysis. Metabolic variables are therefore expressed as a mean of day 1 and day 2 for eight participants; metabolic data for the remaining two participants are values from day 1 only. All measures of TEE and RQ have been adjusted for urinary nitrogen excretion.

4.2.1 Participant Characteristics

Participant physical characteristics are described in **Table 4.2**. Study participants ranged in age from 45 - 73 years, however over half of the participants (n = 6) were between 62 - 68 years of age. Participants presented with a wide range of body weight (50.1 - 106.3 kg) and body composition (19.0 - 43.2% FM). One participant was underweight (BMI < 18.5 kg/m^2), three were normal weight (BMI between 18.5 and 24.9 kg/m^2), one was overweight (BMI between 25 and 29.9kg/m^2) and four were obese (BMI > 30 kg/m^2). All but one participant was taking anti-diabetic medication. Three participants were taking

metformin alone, while one was taking a sulfonylurea only. The remaining five participants took metformin in combination with either a sulfonylurea and/or with a dipeptidyl peptidase-4 (DPP-4) inhibitor or a meglitinide.

Table 4.2: Participant Characteristics

(n = 10)	**Mean ± SD**	**Range**
Sex (males/females)	6/4	
Age (years)	61.2 ± 9.3	45 - 73
Weight (kg)[1]	78.7 ± 17.5	50.1 - 106.3
Height (cm)	171.4 ± 7.3	163.5 - 186.9
BMI (kg/m^2)[1]	26.8 ± 5.8	18.2 - 37.1
Fat Mass (kg) [2]	20.8 ± 7.6	11.2 - 32.9
Fat Mass (%)[2]	28.5 ± 8.9	19.0 - 43.2
Fat Free Mass (kg) [2]	52.2 ± 13.6	37.0 - 78.3
Central Fat Distribution[2,3]	0.80 ± 0.26	0.28 - 1.15
Resting Systolic BP (mmHg)	126 ± 11	109 - 142
Resting Diastolic BP (mmHg)	75 ± 8	62 - 87
Years with T2D[2]	12 ± 12	0.75 - 40
Oral Antihyperglycemic Agent(s) (yes/no)	9/1	

[1]Body weight and BMI are from the morning measurement on test day 1
[2] n = 9
[3] Calculated as the ratio of android to gynoid FM
Abbreviations: SD (standard deviation), kg (kilogram), cm (centimeter), BMI (body mass index), m (meters), mmHg (millimeter of mercury), BP (blood pressure), T2D (type 2 diabetes)

4.2.2 Participant Metabolic Variables

Measures of energy metabolism and substrate oxidation are presented in **Table 4.3**. ESHA Food Processor was used to calculate total caloric intake and the macronutrient distribution of the diet consumed by participants while in the calorimetry unit. After accounting for the adjustments made to meet individual energy requirements and foods not completely consumed by participants, the macronutrient ratio of the diet was 46 – 56% carbohydrate, 23 – 34% fat and 19 – 22% protein. The mean energy intake was 2231 ± 278 kcal/day and mean energy expenditure was 2246 ± 330 kcal/day; acute energy balance was -14 ± 126.The proportion of CHO to fat oxidized for energy differed between participants over the 24 hour period (RQ_{24} range was 0.849 - 0.919). Inter-individual differences in RQ were also found during fasting, sleep and exercise conditions (ranges were 0.774 - 0.934, 0.834 - 0.931, 0.906 - 0.993, respectively). No significant difference in RQ_{24} was found between genders (men: 0.902 ± 0.017 and women: 0.882 ± 0.028; 95% CI: -0.01, 0.05; $P = 0.201$).Fasting RQ was significantly higher in men than women (0.870 ± 0.034 and 0.811 ± 0.030, respectively; 95%CI: 0.01, 0.11; $P = 0.024$). Metabolic flexibility was determined three times throughout the 24 hour period: from fasting to postprandial conditions (range was -0.051 - 0.097), from pre-exercise to exercise conditions (range was -0.012 - 0.127), and during an overnight fast (range was -0.081 - 0.087). A positive change in RQ indicated that there was an increase in the amount of energy being oxidized from CHO and a decrease in energy from fat, while a negative change indicated

the contrary. The degree of change, therefore, corresponded to the ability to match fuel oxidation to fuel availability.

Table 4.3: Metabolic Variables

(n = 10)	**Mean ± SD**	**Range**
TEE (kcal/day)	2246 ± 330	1788 - 2798
Total Energy Intake (absorbable) (kcal/day)	2231 ± 278	1799 - 2678
Total Energy Balance (kcal/day)	-14 ± 126	-170 - 252
REE (kcal/day)	1620 ± 298	1085 - 1980
REE/kg Body Weight	19.9 ± 4.2	9.3 - 24.9
Energy Expenditure during Exercise (kcal/min)	5.4 ±1.1	3.9 - 7.4
RQ_{24}	0.894 ± 0.023	0.849 - 0.919
24-Hour CHO Oxidation (g/day)	343.4 ± 77.2	233.7 - 496.8
24-Hour Fat Oxidation (g/day)	69.6 ± 13.8	52.0 ± 96.1
$RQ_{Fasting}$	0.847 ± 0.043	0.774 - 0.934
Fasting CHO Oxidation (g/min)	0.14 ± 0.07	0.07 - 0.31
Fasting Fat Oxidation (g/min)	0.05 ± 0.01	0.03 - 0.08
$RQ_{Postprandial}$	0.885 ± 0.025	0.859 - 0.940
Postprandial CHO Oxidation (g/min)	0.23 ± 0.04	0.18 - 0.31
Postprandial Fat Oxidation (g/min)	0.06 ± 0.02	0.02 - 0.08
$RQ_{Exercise}$	0.946 ± 0.027	0.906 - 0.993
Exercise CHO Oxidation (g/min)	1.15 ± 0.33	0.68 - 1.84
Exercise Fat Oxidation (g/min)	0.09 ± 0.04	0.01 - 0.15
RQ_{Sleep}	0.886 ± 0.028	0.834 - 0.931
Sleep CHO Oxidation (g/min)	0.16 ± 0.04	0.08 - 0.24
Sleep Fat Oxidation (g/min)	0.04 ± 0.01	0.02 - 0.05
ΔRQ_{Meal}	0.038 ± 0.049	-0.051 - 0.097
$\Delta RQ_{Exercise}$	0.054 ± 0.036	-0.012 - 0.127
$\Delta RQ_{Overnight}$	-0.014 ± 0.058	-0.081 - 0.087

Abbreviations: SD (standard deviation), TEE (total energy expenditure), kcal (kilocalories), REE (resting energy expenditure), kg (kilogram), min (minute), RQ_{24} (24 hour respiratory quotient), CHO (carbohydrate), g (gram), $RQ_{Fasting}$ (fasting respiratory quotient), $RQ_{Postprandial}$ (postprandial respiratory quotient), $RQ_{Exercise}$ (exercise respiratory quotient), RQ_{Sleep} (sleep respiratory quotient), ΔRQ_{Meal} (metabolic flexibility from fasting to postprandial conditions), $\Delta RQ_{Exercise}$ (metabolic flexibility from pre-exercise to exercise conditions),$\Delta RQ_{Overnight}$ (metabolic flexibility determined throughout an overnight fast)

4.2.3 Participant Biochemical Variables

The majority of participants in the sample (n = 7) had fasting plasma glucose concentrations above the reference range described by Alberta Health Services (3.3 - 6.0 mmol/L; AHS, 2013). Of those with high values, one person had a value above 10 mmol/L. Three participants had fasting serum insulin concentrations above the reference range (5.0 - 20.0 mU/L), while two participants had insulin concentrations below this range. One participant had fasting serum TG concentrations > 1.70 mmol/L. All other participants had TG concentrations within the desirable range. Fasting FFA concentrations were within the normal range for all participants (0.29 - 0.72 mmol/L;Savage, Petersen, & Shulman, 2007) (**Table 4.4**).

Table 4.4: Biochemical Variables

(n = 9)	**Mean ± SD**	**Range**	**Reference Range[1]**
Fasting Plasma Glucose (mmol/L)	8.5 ± 4.7	5.6 - 20.5	3.3 - 6.0
Postprandial Plasma Glucose (mmol/L)	8.3 ± 4.5	4.2 - 18.6	
Fasting Serum Insulin (µIU/ml)	14.7 ± 10.9	2.4 - 24.1	5.0 - 20.0
Postprandial Serum Insulin (µIU/ml)	53.3 ± 26.5	10.4 - 108.3	
Fasting Serum TG (mmol/L)	0.85 ± 0.62	0.42 - 2.45	> 1.70
Postprandial Serum TG (mmol/L)	1.13 ± 0.65	0.61 - 2.70	
Fasting Serum FFA (mmol/L)	0.34 ± 0.12	0.12 - 0.483	0.29 - 0.72[2]
Postprandial Serum FFA (mmol/L)	0.18 ± 0.07	0.12 - 0.33	
HOMA-IR	6.5 ± 7.4	0.8 - 21.9	

[1]Reference range described by Alberta Health Services
[2]Reference range described by Savage, Petersen, & Shulman, 2007
Abbreviations: SD (standard deviation), mmol (millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG, (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)

4.3 Classification of Fat and Carbohydrate Oxidizers

Participants were classified as fat and CHO oxidizers based on measured RQ_{24}. The RQ_{24}determined for each participant is shown in **Table 4.5**. Based on a natural break in these data, a working definition of fat and CHO oxidizers was established. Participants with a $RQ_{24} \leq 0.872$ were classified as fat oxidizers (n = 3) and participants with a $RQ_{24} \geq 0.897$ were considered CHO oxidizers (n = 7). An independent sample student's t-test was used to confirm that the mean RQ_{24} of the fat oxidizers was significantly different than the mean RQ_{24} of the CHO oxidizers ($P< 0.001$). The respiratory quotient measured over the 24 hour period between fat and CHO oxidizers is illustrated in **Figure 4.2**.

Table 4.5: Twenty Four Hour Respiratory Quotient Measured in All Study Participants

Participant	RQ_{24}	Classification
DCAL8	0.849	Fat Oxidizer
DCAL7	0.870	Fat Oxidizer
DCAL10	0.872	Fat Oxidizer
DCAL4	0.897	CHO Oxidizer
DCAL3	0.898	CHO Oxidizer
DCAL2	0.903	CHO Oxidizer
DCAL5	0.908	CHO Oxidizer
DCAL9	0.912	CHO Oxidizer
DCAL6	0.913	CHO Oxidizer
DCAL1	0.919	CHO Oxidizer

Abbreviations: RQ_{24} (24 hour respiratory quotient), CHO (Carbohydrate)

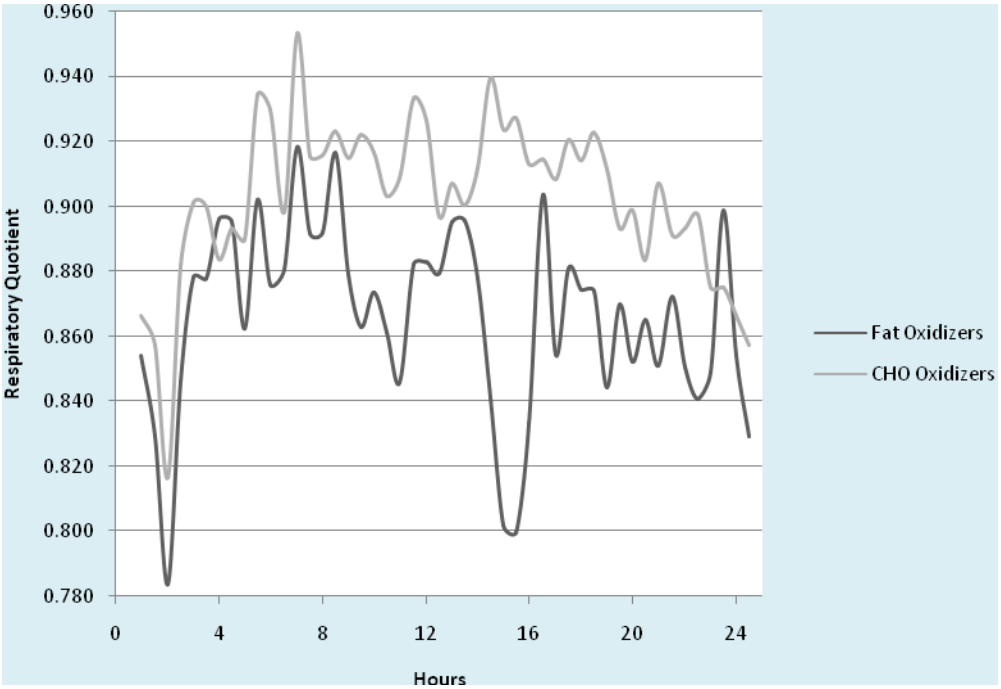

Figure 4.2: The Respiratory Quotient over the 24 Hour Period between Fat and Carbohydrate Oxidizers
The RQ was determined by calculating the mean of each 30 minute interval

4.4 Difference between Fat and Carbohydrate Oxidizers

Differences in participant characteristics, metabolic and biochemical variables between fat and CHO oxidizers were determined using a student's t-test or t-test for unequal variance.

4.4.1 Differences in Participant Characteristics between Fat and Carbohydrate Oxidizers

No significant differences in age, gender, weight, BMI or number of years with T2D were found between fat and CHO oxidizers. The central fat distribution was significantly higher in CHO oxidizers ($P = 0.04$), and was the only measure of body composition to differ between groups. Resting systolic BP was also significantly higher in CHO oxidizers compared to fat oxidizers ($P = 0.03$) (**Table 4.6**). No clear pattern in antihyperglycemic medication use was present between fat and CHO oxidizers. Amongst the fat oxidizers one participant was not taking antidiabetic medication, one participant was taking metformin, and one was taking a combination of metformin, a sulphonylureas, a meglitinide and a DPP-4 inhibitor. Of the seven CHO oxidizers, two were taking only metformin and one was taking a sulphonylureas. The remaining four CHO oxidizers were taking metformin in combination with a sulphylureas, a meglitinide, and/or a DDP-4 inhibitor.

Table 4.6: Comparison of Participant Characteristics between Fat and Carbohydrate Oxidizers

	Fat Oxidizers (n = 3) Mean ± SD	**CHO Oxidizers (n = 7) Mean ± SD**	**95% CI**	***P* value**
Gender (males/females)	1/2	5/2		0.500
Age (years)	60.3 ± 7.6	61.6 ± 10.5	-16.9, 14.4	0.860
Weight (kg)[1]	69.8 ± 17.5	82.5 ± 17.3	-40.3, 15.0	0.322
BMI (kg/m^2)[1]	24.6 ± 6.5	27.7 ± 5.8	-12.6, 6.4	0.476
Fat Mass (kg)[2]	19.7 ± 11.6	21.4 ± 6.1	-15.3, 11.8	0.769
Fat Mass (%)[2]	28.2 ± 11.1	28.7 ± 8.7	-16.3, 15.4	0.946
Fat Free Mass (kg) [2]	47.4 ± 10.3	54.6 ± 15.3	-30.8, 16.3	0.491
Central Fat Distribution[2,3]	0.56 ± 0.24	0.92 ± 0.19	-0.70, 0.02	0.041*
Resting Systolic BP (mmHg)	114.7 ± 9.0	131.1 ± 9.15	-31.0, -2.0	0.031*
Years with T2D[2]	17.9 ± 20.1	9.1 ± 5.6	-1.0, 1.1	0.928

*Independent t-test significant at $P < 0.05$

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

Abbreviations: CHO (carbohydrate), SD (standard deviation), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), BP (blood pressure), T2D (type 2 diabetes)

4.4.2 Differences in Metabolic Variables between Fat and Carbohydrate Oxidizers

The higher RQ_{24} measured in CHO oxidizers reflected a significantly higher absolute amount of CHO oxidation in this group compared to fat oxidizers over the 24 hour period (P = 0.009). Fat oxidizers showed a trend toward had a greater quantity of 24 hour fat oxidation than CHO oxidizers (P = 0.06). Mean $RQ_{Exercise}$ measured during the 30 minute bout of exercise was significantly higher in CHO oxidizers than fat oxidizers; and rates of fat oxidation during exercise were lower in CHO oxidizers than fat oxidizers (P = 0.04). Rates of CHO oxidation were similar between the two groups. Sleep RQ was also higher in CHO oxidizers and during sleep the rate of CHO oxidation was higher in the CHO oxidizers compared to fat oxidizers (P = 0.01), however, rates of fat oxidation did not differ. Fasting RQ, rates of fasting CHO and fat oxidation, $RQ_{Postprandial}$ and rates of postprandial CHO and fat oxidation were not different between fat and CHO oxidizers. When measures of metabolic flexibility (ΔRQ) were compared between fat and CHO oxidizers, no significant differences were found (**Table 4.7)**.

Table 4.7: Comparison of Metabolic Variables between Fat and Carbohydrate Oxidizers

	Fat Oxidizers (n = 3) Mean ± SD	CHO Oxidizers (n = 7) Mean ± SD	95% CI	*P* value
Total Energy Balance (kcal/day)	-34 ± 67	-6 ± 148	-239.6, 183.5	0.768
REE/kg Body Weight	20.5 ± 1.1	19.6 ± 5.1	-6.2, 8.0	0.778
RQ_{24}	0.864 ± 0.126	0.907 ± 0.008	-0.06, -0.03	<0.001*
24-Hour CHO Oxidation (g/day)	256.9 ± 27.7	380.4 ± 57.9	-206.3, -40.6	0.009*
24-Hour Fat Oxidation (g/day)	81.8 ± 14.1	64.3 ± 10.6	-0.9, 35.9	0.060
$RQ_{Fasting}$	0.821 ± 0.041	0.857 ± 0.043	-0.10, 0.03	0.253
Fasting CHO Oxidation (g/min)	0.10 ± 0.34	0.16 ± 0.08	-0.17, 0.05	0.223
Fasting Fat Oxidation (g/min)	0.06 ± 0.02	0.05 ± 0.01	-0.01, 0.03	0.502
$RQ_{Postprandial}$	0.889 ± 0.044	0.883 ± 0.017	-0.1, 0.1	0.822
Postprandial CHO Oxidation (g/min)	0.21 ± 0.03	0.24 ± 0.04	-0.10, 0.03	0.246
Postprandial Fat Oxidation (g/min)	0.05 ± 0.02	0.06 ± 0.02	-0.04, 0.01	0.207
$RQ_{Exercise}$	0.921 ± 0.013	0.957 ± 0.024	-0.07, -0.00	0.040*
Exercise CHO Oxidation (g/min)	0.99 ± 0.27	1.22 ± 0.35	-0.8, 0.3	0.325
Exercise Fat Oxidation (g/min)	0.13 ± 0.01	0.07 ± 0.04	0.00, 0.11	0.041*
RQ_{Sleep}	0.852 ± 0.016	0.901 ± 0.018	-0.08, -0.02	0.004*
Sleep CHO Oxidation (g/min)	0.11 ± 0.02	0.18 ± 0.03	-0.12, -0.02	0.014*
Sleep Fat Oxidation (g/min)	0.04 ± 0.01	0.03 ± 0.01	-0.01, 0.03	0.296
ΔRQ_{Meal}	0.068 ± 0.049	0.025 ± 0.046	-0.03, 0.12	0.221
$\Delta RQ_{Exercise}$	0.053 ± 0.070	0.055 ± 0.018	-0.2, 0.2	0.981
$\Delta RQ_{Overnight}$	0.017 ± 0.071	-0.028 ± 0.052	-0.05, 0.14	0.294

*Independent t-test significant at $P < 0.05$

Abbreviations: CHO (carbohydrate), SD (standard deviation), CI (confidence interval), TEE (total energy expenditure), kcal (kilocalories), REE (resting energy expenditure), kg (kilogram), RQ_{24} (24 hour respiratory quotient), g (gram), $RQ_{Fasting}$ (fasting respiratory quotient), min (minute), $RQ_{Postprandial}$ (postprandial respiratory quotient), $RQ_{Exercise}$ (exercise respiratory quotient), RQ_{Sleep} (sleep respiratory quotient), ΔRQ_{Meal} (metabolic flexibility from fasting to postprandial conditions), $\Delta RQ_{Exercise}$ (metabolic flexibility from pre-exercise to exercise conditions), $\Delta RQ_{Overnight}$ (metabolic flexibility determined throughout an overnight fast)

4.4.3 Differences in Biochemical Variables between Fat and Carbohydrate Oxidizers

Concentrations of fasting and postprandial glucose, insulin, TG and FFA were not found to be significantly different between fat and CHO oxidizers. Changes in plasma glucose concentrations from fasting to postprandial conditions were significantly different between the two groups ($P = 0.04$). Plasma glucose concentrations of CHO oxidizers increased from fasting to postprandial conditions (0.4 ± 1.5), however, fat oxidizers experienced a drop in plasma glucose concentrations (-2.5 ± 1.0). Fat oxidizers had a greater increase in TG concentrations over this time period compared to CHO oxidizers ($P = 0.037$). Changes in serum insulin and FFA from fasting to postprandial conditions did not differ between the two groups (**Table 4.8**).

Table 4.8: Comparison of Biochemical Variables between Fat and Carbohydrate Oxidizers

	Fat Oxidizers (n = 2) Mean ± SD	CHO Oxidizers (n = 7) Mean ± SD	95% CI	*P* value
Fasting Plasma Glucose (mmol/L)	14.0 ± 9.2	7.0 ± 1.4	-2.3, 2.8	0.448
Postprandial Plasma Glucose (mmol/L)	11.4 ± 10.2	7.4 ± 2.2	-3.6, 3.8	0.812
ΔPlasma Glucose (mmol/L)[1]	-2.5 ± 1.0	0.4 ± 1.5	-5.6, -0.2	0.038*
Fasting Serum Insulin (µIU/ml)	13.2 ± 15.4	15.1 ± 10.9	-0.9, 0.5	0.517
Postprandial Serum Insulin (µIU/ml)	35.3 ± 35.2	58.5 ± 24.3	-4.7, 4.0	0.534
ΔSerum Insulin (µIU/ml)[1]	22.0 ± 19.8	43.3 ± 26.8	-70.4, 27.8	0.339
Fasting Serum TG (mmol/L)	0.69 ± 0.38	0.90 ± 0.69	-0.5, 0.4	0.678
Postprandial Serum TG (mmol/L)	1.25 ± 0.44	1.10 ± 0.73	-0.3, 0.5	0.577
ΔSerum TG (mmol/L)[1]	0.56 ± 0.06	0.20 ± 0.19	0.03, 0.70	0.037*
Fasting Serum FFA (mmol/L)	0.41 ± 0.10	0.31 ± 0.12	-0.2, 0.5	0.366
Postprandial Serum FFA (mmol/L)	0.18 ± 0.05	0.18 ± 0.08	-0.3, 0.3	0.913
ΔSerum FFA (mmol/L)[1]	-0.23 ± 0.05	-0.13 ± 0.12	-0.3, 0.1	0.311
HOMA-IR	11.4 ± 15.0	5.1 ± 5.1	-8.1, 8.1	0.962

*Independent t-test significant at $P < 0.05$

[1]Change from fasting to postprandial conditions

Abbreviations: CHO (carbohydrate), SD (standard deviation), CI (confidence interval), mmol (millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)

4.5 Correlations between 24 Hour Respiratory Quotient and Study Variables

No significant correlation was found between RQ_{24} and age, weight, BMI, measures of body composition, years with T2D or BP. There was also no significant correlation between RQ_{24} and acute energy balance or REE/kg body weight. A positive correlation was found between RQ_{24} and both $RQ_{Exercise}$ (P = 0.019) and RQ_{Sleep} (P = 0.003) (**Figures 4.2a and 4.2b**), however it was not significantly associated to $RQ_{Fasting}$ or $RQ_{Postprandial}$. $RQ_{24}$was inversely associated with fasting plasma glucose (P = 0.018) (**Figure 4.2c**). No other significant correlations were observed between RQ_{24} and the other biochemical variables or measures of metabolic flexibility (**Table 4.9**).

Table 4.9: Pearson's Product-Moment Correlation Coefficients between 24 Hour Respiratory Quotient and Study Variables

(n = 10)	***r***	**95% CI**	***P* value**
Age (years)	0.137	-0.00, 0.00	0.707
Weight (kg)[1]	0.237	-0.00, 0.00	0.510
BMI (kg/m^2)[1]	0.156	-0.00, 0.00	0.667
Fat Mass (kg)[2]	-0.030	-0.00, 0.00	0.940
Fat Mass (%)[2]	-0.030	-0.00, 0.00	0.940
Fat Free Mass (kg) [2]	0.128	-0.00, 0.00	0.742
Central Fat Distribution[2,3]	0.559	-0.02, 0.12	0.118
Resting Systolic BP (mmHg)	0.452	-0.00, 0.00	0.190
Years with T2D[2]	-0.166	-0.04, 0.03	0.670
Acute Energy Balance (kcal/day)	0.028	-0.00, 0.00	0.937
REE/kg Body Weight	-0.148	-0.01, 0.00	0.684
$RQ_{Fasting}$	0.555	-0.07, 0.65	0.096
$RQ_{Postprandial}$	0.219	-0.7, 0.8	0.849
$RQ_{Exercise}$	0.718	0.1, 1.1	0.019*
RQ_{Sleep}	0.824	0.3, 1.0	0.003*
ΔRQ_{Meal}	-0.461	-0.6, 0.1	0.180
$\Delta RQ_{Exercise}$	-0.144	-0.6, 0.4	0.691
$\Delta RQ_{Overnight}$	-0.187	-0.4, 0.2	0.605
Fasting Plasma Glucose (mmol/L)[2]	-0.760	-0.18, -0.02	0.018*
Postprandial Plasma Glucose (mmol/L)[2]	-0.437	-0.14, 0.04	0.239
ΔPlasma Glucose (mmol/L)[2,4]	0.502	-0.00, 0.02	0.169
Fasting Serum Insulin (μIU/ml)[2]	0.235	-0.04, 0.07	0.543
Postprandial Serum Insulin (μIU/ml)[2]	0.383	-0.04, 0.10	0.309
ΔSerum Insulin (μIU/ml)[2,4]	0.277	-0.00, 0.00	0.471
Fasting Serum TG (mmol/L)[2]	0.186	-0.07, 0.11	0.632
Postprandial Serum TG (mmol/L)[2]	-0.243	-0.11, 0.09	0.884
ΔSerum TG (mmol/L)[2,4]	0.561	-0.13, 0.02	0.116
Fasting Serum FFA (mmol/L)[2]	-0.501	-0.15, 0.03	0.169
Postprandial Serum FFA (mmol/L)[2]	-0.161	-0.2, 0.1	0.679
ΔSerum FFA (mmol/L)[2,4]	0.480	-0.06, 0.25	0.191
HOMA-IR[3]	-0.092	-0.05, 0.04	0.813

*Independent t-test significant at $P< 0.05$

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: RQ_{24} (24 hour respiratory quotient), *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), BP (blood pressure), T2D (type 2 diabetes), kcal (kilocalories), REE (resting energy expenditure), $RQ_{Fasting}$ (fasting respiratory quotient), min (minute), $RQ_{Postprandial}$ (postprandial respiratory quotient), $RQ_{Exercise}$ (exercise respiratory quotient), RQ_{Sleep} (sleep respiratory quotient), mmol (millimoles), L (litre), μIU (microinternational units), ml (millilitre), TG

(triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance) , ΔRQ_{Meal} (metabolic flexibility from fasting to postprandial conditions), $\Delta RQ_{Exercise}$ (metabolic flexibility from pre-exercise to exercise conditions),$\Delta RQ_{Overnight}$ (metabolic flexibility determined throughout an overnight fast)

Figure 4.3a: The Relationship between the 24 Hour Respiratory Quotient and Exercise Respiratory Quotient
y = 0.322 + 0.604x; r = 0.718; *P* = 0.019

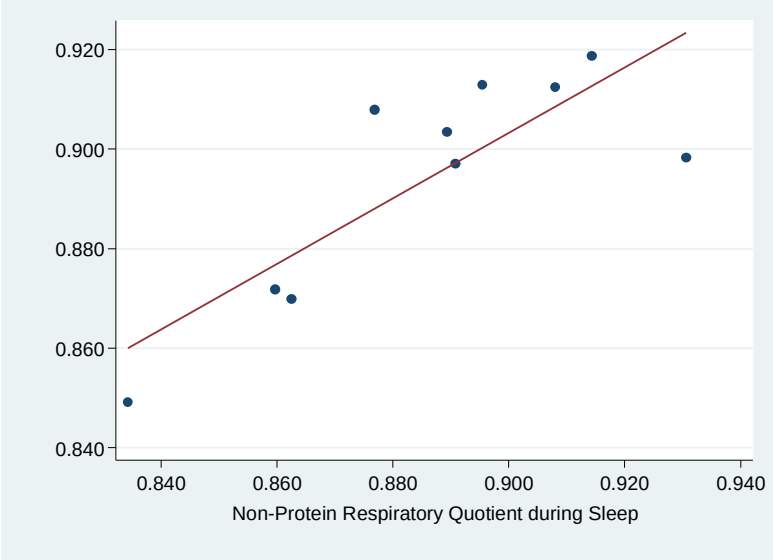

Figure 4.3b: The Relationship between the 24 Hour Respiratory Quotient and Sleep Respiratory Quotient
y = 0.311 + 0.657x; r = 0.824; *P* = 0.003

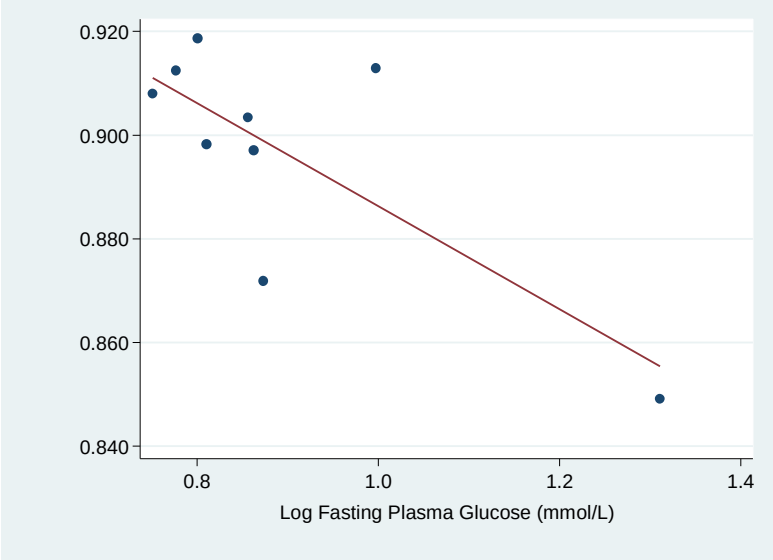

Figure 4.3c: The Relationship between 24 Hour Respiratory Quotient and Fasting Plasma Glucose Concentrations
y = 0.990 - 0.099x; r = -0.760; *P* = 0.018

4.6 Correlations between Fasting Respiratory Quotient and Study Variables

There was no significant correlation between $RQ_{Fasting}$ and age, weight, BMI, measures of body composition, years with T2D or BP. In addition, no significant correlation was found between $RQ_{Fasting}$ REE/kg body weight, $RQ_{Exercise}$ or $RQ_{Sleep.}$ An inverse association was found between $RQ_{Fasting}$ and metabolic flexibility from fasting to postprandial conditions (P = 0.002) (**Figure 4.3a**), but not with other measure of metabolic flexibility. Fasting serum FFA concentrations were inversely associated with $RQ_{Fasting}$, while the change in serum FFA concentrations from fasting to postprandial conditions was positively correlated with $RQ_{Fasting}$ (P = 0.017 and 0.039, respectively) (**Figures 4.3b and 4.3c**). There was no significant correlation with $RQ_{Fasting}$ and any other biochemical variable (**Table 4.10**).

Additional correlations between absolute substrate oxidation and study variables are listed in Appendix J.

Table 4.10: Pearson's Product-Moment Correlations between Fasting Respiratory Quotient and Study Variables

	r	***95%CI***	***P* value**
Age (years)	0.614	-0.00, 0.01	0.059
Weight (kg)[1]	0.572	-0.00, 0.00	0.084
BMI (kg/m^2)[1]	0.478	-0.00, 0.01	0.162
Fat Mass (kg)[2]	-0.351	-0.01, 0.00	0.355
Fat Mass (%)[2]	-0.570	-0.00, 0.00	0.109
Fat Free Mass (kg) [2]	0.568	-0.00, 0.00	0.110
Central Fat Distribution[2,3]	0.273	-0.01, 0.1	0.478
Resting Systolic BP (mmHg)	0.763	-0.00, 0.00	0.110
Years with T2D[2]	0.175	-0.06, 0.04	0.652
Total Energy Balance (kcal/day)	-0.431	-0.00, 0.00	0.213
REE/kg Body Weight	-0.051	-0.01, 0.01	0.890
RQ_{24}	0.551	-0.2, 2.3	0.096
$RQ_{Postprandial}$	0.079	-1.3, 1.5	0.828
$RQ_{Exercise}$	0.088	-1.2, 1.4	0.810
RQ_{Sleep}	0.241	-0.8, 1.6	0.503
ΔRQ_{Meal}	-0.854	-1.1, -0.4	0.002*
$\Delta RQ_{Exercise}$	-0.239	-1.2, 0.7	0.505
$\Delta RQ_{Overnight}$	-0.066	-0.7, 0.6	0.856
Fasting Plasma Glucose (mmol/L)[2]	-0.267	-0.3, 0.2	0.487
Postprandial Plasma Glucose (mmol/L)[2]	-0.898	-0.2, 0.2	0.807
ΔPlasma Glucose (mmol/L)[2,4]	0.373	-0.01, 0.03	0.323
Fasting Serum Insulin (μIU/ml)[2]	0.080	-0.1, 0.1	0.838
Postprandial Serum Insulin (μIU/ml)[2]	0.032	-0.1, 0.2	0.935
ΔSerum Insulin (μIU/ml)[2,4]	-0.140	-0.00, 0.00	0.719
Fasting Serum TG (mmol/L)[2]	-0.120	-0.2, 0.2	0.759
Postprandial Serum TG (mmol/L)[2]	-0.014	-0.2, 0.2	0.972
ΔSerum TG (mmol/L)[2,4]	0.005	-0.2, 0.2	0.898
Fasting Serum FFA (mmol/L)[2]	-0.762	-0.33, -0.05	0.017*
Postprandial Serum FFA (mmol/L)[2]	-0.056	-0.3, 0.3	0.888
ΔSerum FFA (mmol/L)[2,4]	0.692	0.02, 0.53	0.039*
HOMA-IR[3]	-0.035	-0.09, 0.09	0.930

*Independent t-test significant at $P< 0.05$

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: $RQ_{Fasting}$ (fasting respiratory quotient), *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), BP (blood pressure), T2D (type 2 diabetes), kcal (kilocalories), REE (resting energy expenditure), RQ_{24} (24 hour respiratory quotient), min (minute), $RQ_{Postprandial}$ (postprandial respiratory quotient), $RQ_{Exercise}$ (exercise respiratory quotient), RQ_{Sleep} (sleep respiratory quotient), mmol

(millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance) , ΔRQ_{Meal} (metabolic flexibility from fasting to postprandial conditions), $\Delta RQ_{Exercise}$ (metabolic flexibility from pre-exercise to exercise conditions),$\Delta RQ_{Overnight}$ (metabolic flexibility determined throughout an overnight fast)

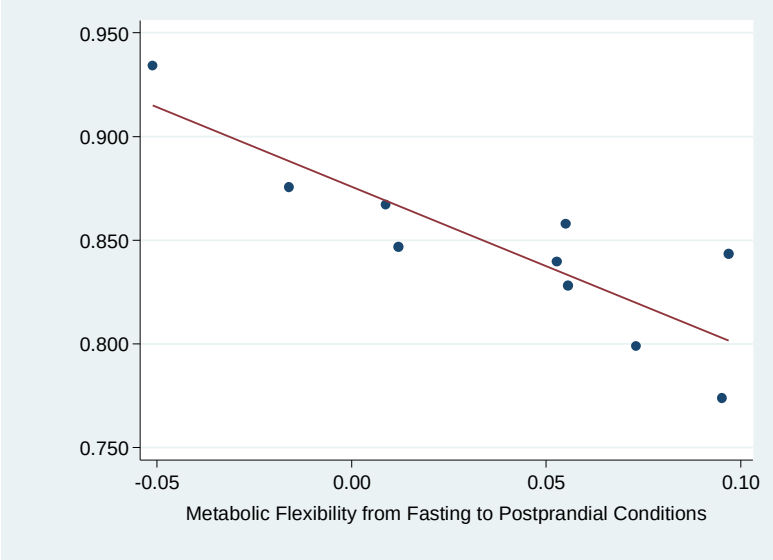

Figure 4.4a: The Relationship between Fasting Respiratory Quotient and Metabolic Flexibility Measured from Fasting to Postprandial Conditions
y = 0.876 - 0.766x; r = -0.854; *P* = 0.002

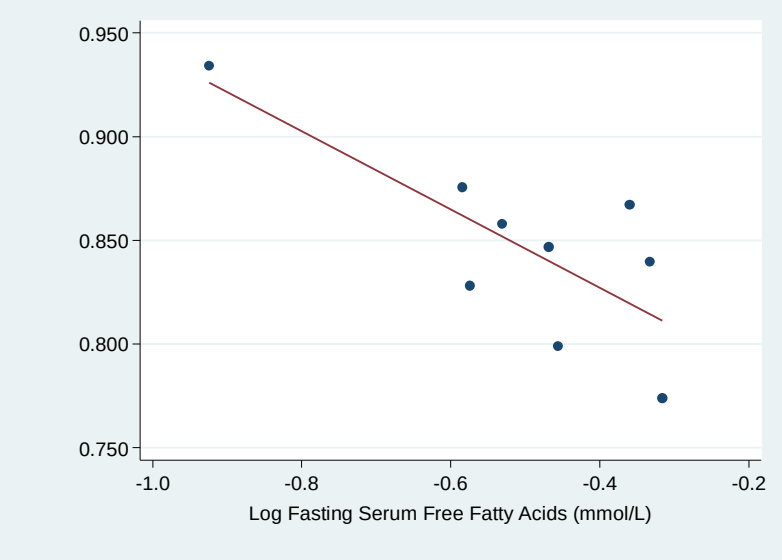

Figure 4.4b: The realtionship between Fasting Respiratory Quotient and Fasting Serum Free Fatty Acid Concentrations
y = 0.752 - 0.188x; r = 0.762; *P* = 0.017

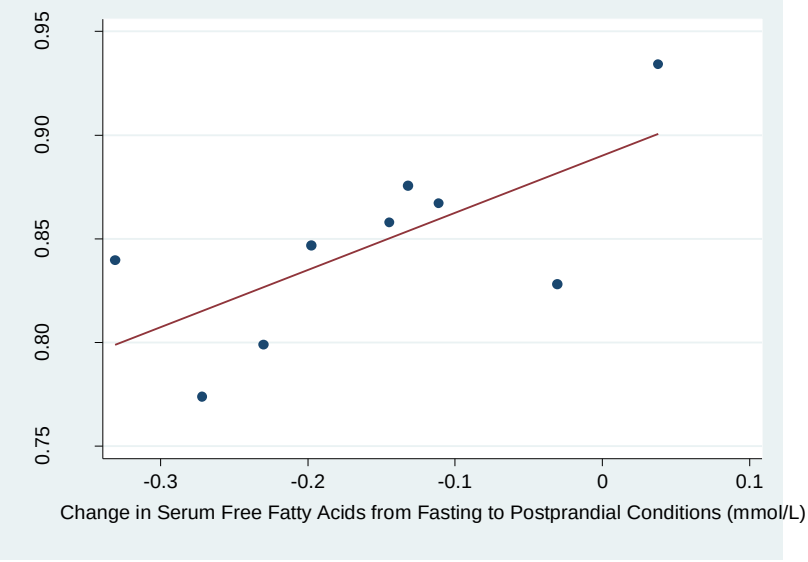

Figure 4.4c: The Relationship between Fasting Respiratory Quotient and the Change in Serum Free Fatty Acid Concentrations Measured from Fasting to Postprandial Conditions
y = 0.890 + 0.276x; r = 0.692; *P* = 0.039

Chapter 5: Discussion

An elevated concentration of circulating FFA and the accumulation of ectopic fat in skeletal muscle and the liver have been associated with insulin resistance and T2D. It has been proposed that a reduced capacity of the tissue to adjust fat oxidation to fat availability may lead to greater levels of circulating FFA and the deposition of ectopic fat. Previous reports examining substrate oxidation in individuals with T2Dhave been carried out over the course of a few hours only; therefore, the purpose of the current study was to examine 24 hour substrate utilization in individuals with T2D. The present study demonstrated that over a 24 hour period, people with T2D are more likely to oxidize a greater proportion of CHO than fat for energy, and those that were classified as CHO oxidizers had a significantly greater amount of central fat mass and a significantly higher systolic blood pressure. Additionally, important relationships between RQ_{24} and $RQ_{Fasting}$ were also found with fasting glucose concentration and fasting FFA concentration, respectively.

5.1 Differences between Fat and Carbohydrate Oxidizers

The first aim of this study was to determine whether people with T2D used predominantly fat or CHO for energy, and to determine whether participant characteristics, metabolic and biochemical variables differed between individuals classified as fat and CHO oxidizers. As previously reported in healthy normal weight and overweight individuals (Weyer, Snitker, et al., 1999; Lammert, et al., 2000), inter-individual differences in RQ_{24} were also observed in this study. Two

previous studies performed by Zurlo et al. (1990) and Claessens et al. (2007) classified individuals based on substrate oxidation. Both of these studies however, used different study methodologies and did not include individuals with T2D. Consequently, RQ values used in these previous studies to classify individuals could not be used in the current study. A working definition was therefore established to classify participants as either fat or CHO oxidizers based on RQ_{24}. Based on this definition, more people in the sample were considered CHO oxidizers than fat oxidizers (70% versus 30%, respectively). Carbohydrate oxidizers had a significantly higher RQ_{24} indicating that they used a greater proportion of CHO than fat for energy compared to fat oxidizers over a 24 hour period. In congruence with these findings, CHO oxidizers utilized a significantly greater absolute amount of CHO per day for energy, and a significantly less absolute amount of fat per day than fat oxidizers.

Although the mean $RQ_{24}$was significantly different between fat and CHO oxidizers, the RQ in response to certain metabolic stimuli was not always different between the two groups. Carbohydrate oxidizers had a higher RQ during both periods of sleep and exercise, however, their RQ was not significantly different than that of fat oxidizers during fasting and postprandial conditions. A lack of variability in $RQ_{Fasting}$ between the fat and CHO oxidizers may indicate that they experienced a similar response in substrate oxidation to fasting conditions. It may also be explained by a study performed by Kelley and Simoneau (1994) that concluded that whole body $RQ_{Fasting}$ might not be a good indicator of skeletal muscle $RQ_{Fasting}$. If differences in RQ_{24}between fat and CHO

oxidizers are primarily due to impairments in skeletal muscle substrate oxidization, these differences may not be detected during fasting conditions when participants are at rest and substrate oxidation in skeletal muscle is low.

Objective c) of this thesis was to develop both RQ_{24} and $RQ_{Fasting}$ values in which to classify fat and CHO oxidizers. In the sample studied however, a high RQ_{24} did not always correspond to a high $RQ_{Fasting}$. Due to this lack of consistency between RQ_{24} and $RQ_{Fasting}$, the establishment of fat and CHO oxidizers was based on RQ_{24}values only.

In addition to $RQ_{Fasting}$, two hour postprandial RQ was also not significantly different between fat and CHO oxidizers. Results from this study are not consistent with those of Claessens et al. (2007) who reported that both fasting and postprandial RQ was significantly lower in fat oxidizers compared to those they classified as CHO oxidizers. Differences in study methodologies and participant characteristics may explain differences in results obtained by the two studies. Although samples sizes were similar between the two studies (n = 10; five fat oxidizers and five CHO oxidizers and n = 10; three fat oxidizers and seven CHO oxidizers, Claessen et al. and the present study, respectively), other differences such as methods used to classify fat and CHO oxidizers and differences in participant characteristics including age, gender, BMI, weight, and diabetes status existed. In addition, Claessens at al. fed participants a high fat liquid meal (95% fat), while the meal consumed by participants in the current study was a standard mixed breakfast. It may therefore be possible that differences in $RQ_{Postprandial}$ may only occur between fat and CHO oxidizers when

meals provided are composed of high amounts of a single macronutrient. A final important difference between studies is differences in the use of medication. Since participants in the study by Claessens et al. did not have T2D, they were not taking any oral antihyperglycemic agents. Nine of the ten participants in the current study were prescribed at least one or more oral antihyperglycemic medication that were taken at the time of the meal challenge. Antihyperglycemic agents including metformin and sulphonylureas taken alone or in combination with other antihyperglycemic medications have been previously associated with a higher RQ and increased glucose oxidation (Nakaya, et al., 1998; Avignon, et al., 2000; Mourad, Chevalier, Morais, Lamarche, & Gougeon, 2009). There were no distinguishable differences in medication use between fat and CHO oxidizers, therefore the use of antihyperglycemic medications by the majority of study participants (n = 9) while consuming a meal of similar macronutrient composition could have resulted in similarities in postprandial nutrient oxidation.

The lack of differences in substrate oxidation between fat and CHO oxidizers during both fasting and postprandial conditions reflects a similar ability between the two groups to adjust substrate oxidation to substrate availability. In other words, metabolic flexibility in response to a meal was similar between the two groups. In addition to similarities in metabolic flexibility to a meal, metabolic flexibility to exercise and throughout an overnight fast were not different between fat and CHO oxidizers. Therefore, although CHO oxidizers had a higher RQ during exercise and sleep conditions, their ability to switch between CHO and fat as fuel sources did not differ from that of fat oxidizers. This indicates that

although CHO oxidizers used a greater proportion of CHO to fat over a 24 hour period their ability to switch fuel sources in response to a meal, exercise and an overnight fast is similar to that of fat oxidizers. Results from Claessens et al. (2007) are consistent with the results of the present study. They reported that there were no significant differences in metabolic flexibility to a meal between those classified as fat and CHO oxidizers. Researchers that have examined metabolic flexibility during a hyperinsulinemic euglycemic clamp in individuals with T2D and controls have reported that those with T2D have an impaired ability to adjust fuel oxidation to fuel availability (Kelley, et al., 1999; Galgani, et al., 2008; Meex, et al., 2010; Stull, et al., 2010; van de Weijer, et al., 2013). Impairments in metabolic flexibility were also reported by Russell et al. (2013) in people with T2D compared to controls in repsonse to a oral glucose tolerance test (50g glucose dissovled in 296ml water). In addition, it has been reported that people with T2D may have impaired fat oxidaition during exercise (Ghassania, et al., 2006). Since the present study only examined individuals with T2D and Claessens et al. only studied healthy individuals, mechanisms involved in the impairments in metabolic flexibility may be attributed to T2D status and not whether an individual is a fat or CHO oxidizer. Although the implications of these results are still unknown, they may suggest that mechanisms involved in the determining substrate oxidation over the long-term may differ from those involved in substrate switching in the short-term. Consequences of a utilizing high proportion of CHO for energy over the long-term and having an impaired metabolic flexibility may

be similar, however, as they both may lead to an increased storage of fat (Zurlo, et al., 1990; Galgani, et al., 2008)

Participant characteristics and biochemical variables were also compared between fat and CHO oxidizers. There were no significant differences between fat and CHO oxidizers in age, weight, BMI and FFM; similar to results reported by Claessen et al. (2007). Central fat distribution and resting systolic BP were greater in CHO oxidizers compared to fat oxidizers. A greater central fat distribution in CHO oxidizers may be a consequence of an increase in both subcutaneous and visceral adipose tissue. The utilization of a greater proportion of CHO to fat for energy over a 24 hour period by CHO oxidizers may result in a decreased amount of CHO stored as glycogen and a greater amount of fat pushed toward storage. This may result in an increased accumulation of subcutaneous adipose tissue (Jorgensen, Borch-Johnsen, Stolk, & Bjerregaard, 2013). If, however, adipose tissue dysfunction occurs, there may also be an increase in ectopic fat storage in visceral adipose tissue, as well as in the skeletal muscle and the liver(Muoio, 2012; Snel, et al., 2012). An increase in waist circumference as a result of an increase in visceral and subcutaneous adipose tissue has been reported to be positively associated with glucose intolerance and insulin resistance (Jørgensen, et al., 2013). Additionally, ectopic fat in skeletal muscle and liver is thought to cause lipotoxicity leading to tissue dysfunction and insulin resistance in these tissues (Snel, et al., 2012).

There is a lack of research in the role substrate oxidation may have on BP, however hypertension has been reported to be more common in those with a

greater abdominal obesity (Nagao, et al., 2013). Although results from this study cannot be used to determine the mechanisms between RQ_{24} and central fat distribution and systolic BP, findings from the present study may provide a preliminary look at the relationship between RQ_{24} and the development of metabolic syndrome, as high blood pressure and abdominal obesity are two out of the five listed criteria for the diagnosis of this condition (CDA, 2012). Metabolic syndrome is important to consider as it places individuals at greater risk for developing both T2D and cardiovascular disease (Alberti, et al., 2009).

No differences in biochemical variables measured during fasting and postprandial conditions were found between fat and CHO oxidizers. However, differences in the change in plasma glucose concentration and serum TG concentration from fasting to postprandial conditions were present. Fat oxidizers experienced a drop in glucose concentration while CHO oxidizers experienced an increase in glucose concentration. Fat oxidizers also experienced a greater increase in TG concentration two hours after breakfast compared to CHO oxidizers. Since there was no significant difference in $RQ_{Postprandial}$ or metabolic flexibility, differences in changes in glucose concentration and TG concentration may be due to disparities between the two groups in their ability to store substrates. As previously suggested, it is possible that CHO oxidizers have a decreased ability to store CHO as glycogen compared to fat oxidizers. Therefore, in the postprandial conditions, although changes in substrate utilization were the same between the two groups, CHO oxidizers may have had an impaired ability to store CHO as glycogen, and as a result, they did not experience a similar drop in

plasma glucose concentration to that of fat oxidizers. Additionally, differences between the two groups in how TG concentration changed from fasting to postprandial conditions may be a consequence of CHO oxidizers utilizing a greater proportion of CHO for energy, which may have resulted in an a greater amount of TG being transported to adipose tissue for storage.

5.2 The relationship between 24 Hour Respiratory Quotient and Study Variables

The second aim of the present study was to determine what metabolic and biochemical variables were associated with RQ_{24} measured in individuals with T2D. No significant association was found between RQ_{24} and age, measures of body weight and composition, resting systolic BP, and years with T2D. Twenty-four hour RQ also did not differ between males and females. No other studies have examined RQ_{24}in relation to these variables in individuals with T2D. Studies that have been done in healthy normal weight and overweight individuals have produced inconsistent results. A relationship between RQ_{24} and age has been reported in some but not all studies, and while Ravussin and Bogardus (1998) reported RQ_{24} to differ between genders, others have not supported these findings. Weyer et al. (1999) reported a positive correlation between RQ_{24} and percent FM, however no other study has replicated these results. A limitation of many studies that have examined RQ_{24}, including the present study, is that sample size is often small. This may affect the ability to detect significant associations between $RQ_{24}$and certain variables.

Twenty-four hour RQ was associated with both $RQ_{Exercise}$ and RQ_{Sleep}; but there was no relationship between $RQ_{24}$and $RQ_{Fasting}$ or $RQ_{Postprandial}$. These results indicate that RQ_{24} may be useful in predicting how nutrients are used during exercise and sleep, however, it may not be appropriate to use RQ_{24} to predict substrate oxidation during fasting or postprandial conditions. Due to the high cost and space requirements of whole body calorimetry units, measurements of substrate oxidation are often taken over the course of only a few hours using metabolic carts that are smaller in size and more affordable. While measurements are often taken at rest during fasting conditions and during insulin stimulated or postprandial conditions, results from the current study suggest that these measures may not be an accurate estimation of how substrates are oxidized over the 24 hour period.

An inverse relationship was found between RQ_{24} and fasting plasma glucose concentration. This indicates that an increase in the proportion of CHO oxidized for energy is associated with a decrease in fasting glucose concentration. A relationship between RQ_{24} and fasting glucose concentration has not been previously reported by other researchers in healthy normal weight or overweight participants (Zurlo, et al., 1990). Those with T2D have previously been reported to have a decreased capacity to store glycogen (Borghouts, et al., 2002). As a consequence, in the fasting condition when there is typically mobilization of glucose from glycogen for energy, less endogenous glucose may be available in those with T2D. Therefore, in these individuals an increased utilization of CHO over the 24 hour period in combination with decreased endogenous glucose

availability may result in decreased glucose concentration in fasting conditions. Although 24 hour glucose concentration was measured in this study, data were not available for the current analysis. As a result, the relationship between daily substrate utilization and 24 hour glucose concentration remains unknown. Future analysis of this data is therefore required to better understand the relationship between RQ_{24} and plasma glucose concentration (Tereda, et al., unpublished data).

5.3 The relationship between Fasting Respiratory Quotient and Study Variables

The final aim of this thesis was to examine the relationship between $RQ_{Fasting}$ and participant characteristics, metabolic and biochemical variables. In the present study, $RQ_{Fasting}$ was not found to be associated with age, measures of body weight and composition, resting systolic BP or years with T2D. Fasting RQ was significantly higher in males than females. In a previous study performed by van de Weijer, et al. (2013) in a group of obese individuals with and without T2D (n = 103), fasting RQ was also reported to be higher in men than women. Mechanisms involved in the possible differences in substrate utilization between genders remain unknown. Although it is commonly accepted that body composition differs between males and females, neither FFM nor FM were found to be associated with $RQ_{Fasting}$in the present study. Only one previous study has reported FM to be a significant predictor of fasting fat oxidation (Blaak, et al.,

2006). Hormonal differences between male and females in relation to substrate utilization have not yet been examined.

Upon investigation of the biochemical variables, an inverse relationship was found between $RQ_{Fasting}$ and fasting serum FFA concentration. Thus as fasting FFA concentration increased there was an increase in the proportion of fat oxidized for energy and a concurrent decrease in CHO oxidation. These results are consistent with those of Blaak, et al. (2006) who demonstrated that fasting FFA concentration were positively associated with fasting fat oxidation in a group of lean and obese individuals with varying insulin sensitivity. Typically, the secretion of insulin in response to a meal results in the suppression FFA mobilization and oxidation (Storlien, Oakes, & Kelley, 2004). In the present study, a lower $RQ_{Fasting}$ was associated with a greater reduction in the concentration of serum FFAs from fasting to postprandial conditions. Fasting RQ was also inversely associated with metabolic flexibility from fasting to postprandial conditions. These results suggest that ability to oxidize a greater proportion of fat in the fasting state may be related to a greater insulin mediated suppression of the mobilization and oxidation of FFAs. Therefore, those that have a lower $RQ_{Fasting}$ may have a greater ability to switch from high rates of fat oxidation during fasting conditions, to the suppression of fat mobilization and oxidation and an increase in CHO oxidation in postprandial conditions. It therefore appears that in response to the ingestion of a meal, individuals with T2D that have a lower $RQ_{Fasting}$ have a greater capacity to adjust fuel oxidation to fuel availability than those with a higher $RQ_{Fasting}$. These results support others that

have reported $RQ_{Fasting}$ to be associated with metabolic flexibility from fasting to postprandial conditions (Blaak, et al., 2006; Stull, et al., 2010).

5.4 Strengths

One important strength of this research project was that it examined substrate oxidation over the 24 hour period in individuals with T2D. Although RQ has been examined up to several hours in this population during feeding studies, hyperinsulinemic euglycemic clamp studies and exercise studies, there has been a lack of research done over the 24 hour period. Studies that have examined 24 hour substrate oxidation have been completed primarily in healthy lean, overweight and obese individuals.

A second strength of this project was the highly controlled environment in which participants were studied. Participants were fed meals with the same foods and macronutrient composition and they followed a specific schedule of daily activities. Since substrate oxidation can be influenced by both diet composition and physical activity, this controlled environment allowed for the comparison of inter-individual results. The method used to estimate daily energy requirements also minimized differences in energy balance, another factor previously shown to influence RQ_{24} (Zurlo, et al., 1990; Astrup, et al., 1992; Snitker, et al., 1998; Toubro, et al., 1998).

Prior to their stay in the unit, all participants completed a fitness test to determine their VT. Results were used to standardize the intensity at which participants walked on the treadmill while inside the calorimetry unit. Since there

were differences in fitness levels between participants, a standardized test allowed for the comparison of exercise RQ results, and also ensured RQ_{24} would not be affected by potential differences in exercise intensity performed by participants.

5.5 Limitations

The mean age of the participants in the present study was similar to the mean age reported by others that have examined substrate oxidation in individuals with T2D in the short-term. This age is reflective of those in the general population diagnosed with T2D as reported by the Public Health Agency of Canada (PHAC; 2011). Weight, BMI, and fat mass however were lower in the present study compared to others (Despres, 1991; Galgani, et al., 2008; Meex, et al., 2010; van de Weijer, et al., 2013). Although 24.4% of individuals with T2D are not overweight or obese (PHAC, 2011), many researchers have only included overweight and/or obese individuals in their studies (Galgani, et al., 2008; Meex, et al., 2010; van de Weijer, et al., 2013). In the current study, individuals were recruited with varying body weights. Although including both normal weight and overweight individuals may better represent individuals with T2D in the Canadian population, it limited the ability to compare results of the current study to previous research.

The use of antihyperglycemic medications by participants while in the calorimetry study also made it difficult to compare results to other studies. Often, participants will discontinue the use of their medications prior to the measurement of substrate oxidation. The length of time they discontinue their medication prior

to testing, however, varies from 48 hours up to seven days (Golay, et al., 1984; Borghouts, et al., 2002; Meex, et al., 2010; van de Weijer, et al., 2013). The lack of a standardized protocol for the discontinuation of medications may result in different effects on substrate utilization (Blaak, et al., 2006). Due to concerns about participant health and safety, all participants remained on their current medications throughout the current study. Despite the use of medications by participants, inter-individual differences in substrate utilization still existed. Data on medication use were collected from all study participants and all medications used were approved prior to the calorimetry testing. The consideration of medications used by study participants is important because as previously stated in section 5.1, antihyperglycemic medications can have an impact on RQ and glucose oxidation. There was no clear difference in the pattern of medication use between the fat and CHO oxidizers in the current study.

In previous studies, run-in diets have often been prescribed to individuals who participate in research examining substrate oxidation. The length of the run-in diets has ranged from one to four days prior to calorimetry testing (Rising, et al., 1996; Snitker, Tatarani, & Ravussin, 1998), and the studies have differed in methodology from simply requesting that participants consume a certain amount of CHO per day (Paolisso, et al., 1994; Rigalleau, et al., 1997) to supplying all meals while participants stayed on a metabolic ward (Zurlo, et al., 1990; Weyer, et al., 1999). A limitation to any run-in diet is that unless food consumption is monitored, compliance to the diet may vary. Research has shown that the RQ may only be affected by foods consumed up to two days prior to the measurement of

substrate oxidation (Toubro, et al., 1998). While many studies include a run-in diet in their study protocol, some studies have not (Mandarino, et al., 1996; Blaak, et al., 2006). Due to financial constraints, a run-in diet was not feasible in the current study. Participant burden was also a concern since participants were required to visit the research unit five times over the course of the study including the two 24 hour calorimetry measurements. Anecdotally, many participants in the current study expressed that they made efforts to follow a diet recommended for individuals with T2D. The Canadian Diabetes Association Clinical Practice Guidelines suggests that people with diabetes consume a diet with a macronutrient composition of 45 – 60% CHO, 20 – 35% fat and 15 – 20% protein (Dworatzek, et al., 2013). These guidelines are similar to the diet consumed by participants during the current calorimetry testing. Additionally, in the present study, participants were required to have maintained a stable body weight for at least 6 months prior to the study. These factors may have therefore minimized the effects of a lack of a run-in diet in the current study.

The size of the sample used in this project limited the ability to define fat and CHO oxidizers. Although differences in RQ_{24} were found among participants in this study, a larger sample would have provided more evidence towards the values currently used to classify individuals. In order to use the methodology of Zurlo, et al. (1990) to classify participants as fat and CHO oxidizers, a considerably larger sample would be required. In their study, Zurlo et al. had a sample of 152 participants. Financial constraints limited the number of participants used in the current study. Although the RQ cutoff points in the

current study were considered a working definition, the mean RQ_{24} of the fat and CHO oxidizers was significantly different. Additionally, RQ_{24} of fat and CHO oxidizers illustrated in **Figure 4.2** demonstrated that there was a pattern of elevated RQ in CHO oxidizers compared to fat oxidizers throughout the entire 24 hour period. The significantly higher mean RQ of this group was not attributed to an elevated RQ during one or two specific periods of the day. It is important to recognize that the definition of fat and CHO oxidizers established in this study was done so with a group of individuals with T2D. This definition cannot therefore be generalized to the healthy population. The small sample size used in the current study may have also increased the likelihood of failing to reject the null hypothesis, and therefore committing type 2 error. In other words, the small sample size may have affected the ability to detect significant differences between fat and CHO oxidizers, and correlations between RQ and measured variables. In addition, a small sample size is more vulnerable to the effects of outliers. In the current study, one participant had fasting glucose concentrations distinctively higher than those of the other study participants. Additionally, another participant had fasting FFA concentrations higher than the rest of the sample. Although these values may be considered outliers by some, they were not excluded from the analysis because they were values conceivable in people with T2D. Caution should be taken however, when interpreting results relating to these variables as more extreme values such as these can increase the risk of falsely rejecting the null hypothesis (type 1 error). A small sample size was chosen for the current study because it was a pilot project, and the cost of determining 24 hour substrate

oxidation in a calorimetry unit is high. Although, there was an increased risk of committing type 2 error, this pilot study was successful at providing a preliminary examination of 24 hour substrate oxidation in people with T2D.

5.6 Future Direction

This thesis provided a preliminary look at substrate oxidation in people with T2D over the 24 hour period, and it has laid a foundation for future research. An important limitation of the current study was the small sample size used. Increasing the size of the sample by recruiting more participants for this project would increase statistical power, and therefore reduce the chances of committing both type 1 and 2 error. A larger sample would help to increase the understanding of the physical, metabolic and biochemical differences of fat and CHO oxidizers, as well as the relationship between RQ_{24} and these variables. In addition, adding a healthy, age and weight matched control group to this project would provide information about how RQ_{24} differs between people with T2D and those without the disease.

Research into the mechanisms involved in the inter-individual differences in RQ_{24}in people with T2D is still needed. Understanding these mechanisms will help to identify whether these differences precede the development of T2D or occur after its onset. Further knowledge about the mechanisms involved in the oxidative differences between individuals may help in establishing individualized diet and exercise programs for fat and CHO oxidizers that may aid in the prevention and treatment of T2D.

5.7 Conclusion

Literature describing substrate oxidation in individuals with T2D has focused on nutrient use over the short-term; and research examining substrate oxidation over the 24 hour period is lacking. The aim of present study was therefore to provide a better understanding of daily nutrient utilization in people with T2D. Results from this study demonstrated that within a group of people with T2D inter-individual differences exist in 24 hour substrate oxidation. Results also suggest that people with T2D might be more likely to oxidize CHO versus fat for energy over the 24 hour period. Additionally, those who oxidized predominantly more CHO for energy had a greater central fat distribution and higher resting systolic blood pressure, two risk factors for metabolic syndrome. The classification of fat and CHO oxidizers, however, was based on a working definition. In order to develop a strong understanding of 24 hour RQ in people with T2D and to create a clear definition of fat and CHO oxidizers, more data needs be collected on substrate oxidation. This should be done under the same experimental conditions and ideally within the same calorimetry unit in individuals with T2D, as well as in healthy age and weight matched controls.

An inverse relationship between RQ_{24} and fasting glucose concentration was also present in the current study. A reduced availability of endogenous glucose during fasting conditions caused by a reduced capacity to store glycogen in individuals with T2D in combination with an increased 24 hour CHO oxidation may be one possible mechanism involved in this relationship. Although 24 hour glucose concentration was measured in this study, data were not available for

analysis in this thesis. Examining the relationship between RQ_{24} and 24 hour glucose concentration may be important in understanding how glucose concentration impacts daily fat and CHO oxidation in people with T2D.

The examination of 24 hour macronutrients oxidation provides a further measure in understanding T2D. Identifying individuals with T2D as those that predominantly use CHO or fat for energy may have implications in prescribing individualized higher fat or higher CHO diets. Additionally, examining the relationships between substrate oxidation and metabolic and biochemical variables may identity factors that may help in the prevention and treatment of T2D. The present study provided a preliminary examination of daily substrate oxidation in people with T2D, and data obtained from this study provides important groundwork for future research.

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Appendices

Appendix A: Ethics Approval

Notification of Ethics Approval

Study ID:	Pro00021556
Study Title:	Calorimetry Assessment in Diabetes
Study Investigator:	Linda McCargar
Funding/Sponsor (validated):	Alberta Diabetes Institute
Approval Expiry Date:	April 16, 2012

I have received your application for research ethics review and conclude that your proposed research, including revisions to the study materials received April 12, 2011, meet the University of Alberta standards for research involving human participants (GFC Policy Section 66). On behalf of the Physical Education and Recreation, Agricultural, Life & Environmental Sciences and Native Studies Research Ethics Board (PER-ALES-NS REB), I am providing **research ethics approval** for your proposed research.

The research ethics approval is valid for one year and will expire on April 16, 2012.

A renewal report must be submitted prior to the expiry of this approval if your study still requires ethics approval at that time. If you do not renew before the renewal expiry date, you will have to re-submit an ethics application. If there are changes to the project that need to be reviewed, please file an amendment. If any adverse effects to human participants are encountered in your research, please contact the undersigned immediately.

Sincerely,

Kelvin Jones, Ph.D. Chair, Physical Education and Recreation (PER), Agricultural Life & Environmental Sciences (ALES) and Native Studies (NS)

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Appendix B: Study Information Sheet

STUDY INFORMATION SHEET

TITLE OF STUDY: Calorimetry Assessment in Diabetes

INVESTIGATORS: Principal Investigator: Linda McCargar PhD RD
Co-investigators: Peter Senior MD PhD, Gordon Bell PhD, Vicki Harber PhD

Contact Information

Study Coordinator: Sarah Loehr BSc	(780) 492-4182
Study Coordinator: Emmanuel Guigard BSc	(780) 492-7820
Principal Investigator: Linda McCargar PhD RD	(780) 492-9287

WHAT IS THE BACKGROUND AND PURPOSE OF THIS STUDY?

Background:
A new facility has been built at the Alberta Diabetes Institute, called a Whole Body Calorimetry Unit. It is like a small hotel room. It is the only unit of its kind in Western Canada and only the second unit in the country. Through various monitoring systems it has the capability to measure how many calories an individual burns in a day; how efficiently they can use carbohydrate and fat for energy, and if carbohydrate or fat is a preferred source of energy during exercise. These are calculated based on the amount of oxygen consumed and carbon dioxide exhaled by an individual. In Type 2 Diabetes (T2D), this is important because glucose and fat use can be significantly altered. We propose information about the ability to use carbohydrate and fat, and to readily switch between these fuels as availability changes is needed to better understand T2D. This information may have implications for diet and exercise prescriptions for people with T2D.

Purpose and Objectives:
The purpose of this study is to test the Whole Body Calorimetry Suite and to determine the similarity of a person's measurements on two different occasions. This study will include people with Type 2 Diabetes, consuming a regular diet, able to walk on a treadmill; and thus will establish standard procedures and baseline test results for comparison with future studies. This study will describe the assessment of ***24 hour energy metabolism*** of people with Type 2 Diabetes; whereby all "**energy in**" (food intake) and "**energy out**" (activity at rest, during exercise and sleeping) will be monitored and recorded which can then be compared to healthy controls.

WHAT WILL I BE ASKED TO DO?
You will be asked to spend a 24 hour period (8:00 am to 8:00 am the following day) in the Whole Body Calorimetry Suite on two separate occasions to test the reproducibility of the measurements. The suite is a self-contained unit comprised of a bed, chair, table, sink, toilet, television, computer and treadmill. All meals will be provided for you while you are in the unit.

Participating in this study will involve completing some **pre-test** requirements and some **test-day** requirements.

Pre-test: Prior to the test day, two meetings (~1.0 – 2 hours each) will be held with you. The first meeting will be to explain the unit, obtain consent and personal information, to complete height and weight measurements, and to spend one hour in the Calorimetry Suite to see how it feels to be inside. At this time you can practice walking on the treadmill.
The second meeting will be to assess your fitness so we will know what a safe level of activity is for you during your time in the Calorimetry Suite. We will ask you to complete two physical activity questionnaires before the fitness test.

The fitness test will be done while walking on a treadmill. Your blood pressure and heart rate will be measured before you begin and during a 5 minute warm-up walk on the treadmill. Then there will be a 5 minute break to drink water and attach headgear and a mouthpiece to collect the air you exhale out during the test. As the test begins, the speed and incline of the treadmill will be increased slowly every 3 minutes until you reach a level where your exhaled breath changes. This change should happen when you are at less than 60% of your ability. Your blood pressure and heart rate will be measured every 3 minutes during the test and again after 5 minutes of rest at the end. Water will be provided while you rest.

We will also ask you to complete a 3-day food record and wear an accelerometer (like a pedometer) for 3 consecutive days. This will help us to determine eating patterns, energy requirements and usual activity. This will also allow us to individualize food preparation for you. You will be asked to carefully record all food and beverages consumed over this time, noting specific portion sizes, brands, cooking methods and restaurant meals. Accelerometers will be worn on the waist-band and total energy expenditure will be estimated for the pre-tests days. Accelerometers will also be worn on the test days to compare results between a "normal" day and a calorimetry unit day.

Test Days (~25 hours total x 2 occasions = 50 hours):You will be asked to report to the research unit at 7:30 am, the morning that you begin the 24 hour test day. You should refrain from exercise 24 hours before the test and should be fasted for 12 hours (water only), taking no food, no caffeine, and not smoking. A fasting blood sample will be collected before you enter the Calorimetry Suite. Total blood required at this time will be 5-10mL (1-2 teaspoons). A continuous glucose monitor (CGM) will be worn while you are in the Calorimetry Suite. It is similar in size to a pager and requires that a glucose sensor be inserted just under your skin and secured with a bandaid. When it is first turned on we will need to double check that it is giving a correct reading by having you check your blood sugar using your own handheld glucose monitor. The CGM will measure your blood glucose every few minutes for entire time that you are in the Calorimetry Suite. This means after comparing to your own meter in the morning you will not need to check your blood sugar with a finger prick for the rest of the day.

Diet: A standardized healthy diet protocol and menu will be developed to keep carbohydrate, protein and fat proportions similar for all participants. All meals and snacks will be prepared on site at the research centre kitchen. The same menu will be used for all participants, and all food and beverage servings will be weighed and measured such

that calorie levels are appropriate to meet your personal energy requirements to maintain weight. Three meals and two snacks will be provided for you at 09:00, 12:00, 15:00, 18:00, and 21:00. All food choices and amounts eaten will be carefully documented.

Exercise: You will walk at a comfortable pace on the treadmill (BH T10 Pro Treadmill) for 30 minutes. The speed of the treadmill will be determined from the results of the fitness test completed before the study began. This will be completed between 1:30 and 3:30 in the afternoon. The purpose of this activity is to assess how you react to a moderate exercise bout in terms of fuel use and energy expenditure.
Diabetes Medications: You will be asked to take oral diabetes agents as prescribed in relationship to meals and at the same time each visit. You will not need to check your blood glucose while in the Calorimetry Suite because it will be monitored throughout the day with the CGM. Episodes of hypoglycemia will be treated according to Canadian Diabetes Association guidelines.

Urine Test: Urine must be collected for the entire time you are in the unit. This is necessary to determine total nitrogen excretion and protein use by your body during the test day. This is then used further in calculations for energy expended and fat and carbohydrate utilization. Four litre plastic containers (with a small amount of acidic preservative) will be provided to you, with guidance on how to appropriately collect the sample. There are blinds on the windows of the unit to provide privacy during collection times.

Key Measurements:
a) Calories consumed from food and beverage intake.
b) Calories expended: at rest, after eating, during and after exercise and total amount for the day.
c) Energy balance (energy in – energy out) and body weight.

Note: There will always be two staff people present for every 24 hour test period that will monitor unit instrumentation and your comfort.

WHAT ARE THE POSSIBLE BENEFITS OF PARTICIPATING IN THIS STUDY?
You will learn how many calories you burn in a 24 hour day; what your blood glucose levels are throughout a 24 hour day; how your body metabolizes fat at rest and during exercise; and information about your metabolic rate.You will also receive a body composition assessment (Dual-Energy X-Ray Absorptiometry (DXA) Scan or BODPOD) and explanation of your results.

WHAT ARE THE POSSIBLE RISKS OF PARTICIPATING IN THIS STUDY?
It is possible, but unlikely that you may feel some mild temporary soreness in your muscles after walking on the treadmill. However the activity will be moderate in nature. Both the CGM sensors and finger prick can cause bruising and a small risk of infection, which will be minimized with proper procedures. You may also feel somewhat uncomfortable being alone in the suite. However there will always be two people very close by, and there is an intercom system to talk to them.

WILL MY RECORDS BE KEPT CONFIDENTIAL?

Any personal information relating to this study will be kept strictly confidential; they will be kept in locked cabinets or on secure password protected computers in locked offices at the University of Alberta. Any research data collected about you during this study will not identify you by name, only by a coded number. Your name will not be disclosed to anyone outside of the research project. Any report published as a result of this study will not identify you by name; only group data will be presented.

IS MY PARTICIPATION VOLUNTARY?

You are free to withdraw from the research study at any time. You can withdraw from the study at anytime by contacting the study coordinator at (780) 492-4182 (Sarah).

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS?

If you have concerns about your rights as a study participant, you may contact the Research Ethics Office at the University of Alberta at (780) 492-2615. This office has no affiliation with the study investigators.

Please contact any of the individuals identified below if you have any questions or concerns specifically related to this study:

	Phone Number
Sarah Loehr BSc RD (Study Coordinator)	(780) 492-4182
Emmanuel Guigard BSc (Study Coordinator)	(780) 492-7820
Dr. Linda McCargar PhD RD (Principal Investigator)	(780) 492-9287

Appendix C: Consent Form

Part 1 (to be completed by the Principal Investigator):

Title of Project: Calorimetry Assessment in Diabetes

Principal Investigator	**Phone Number**
Dr. Linda McCargar PhD RD	(780) 492-9287
Study Coordinators	
Sarah Loehr BSc	(780) 492-4182
Emmanuel Guigard BSc	(780) 492-7820

Part 2 (to be completed by the research participant):	**YesNo**
Do you understand that you have been asked to be in a research study?	☐☐
Have you read and received a copy of the attached Information Sheet?	☐☐
Do you understand the benefits and risks involved in taking part in this research study?	☐☐
Have you had an opportunity to ask questions and discuss this study?	☐☐
Do you understand that you are free to withdraw from the study at any time?	☐☐
Has the issue of confidentiality been explained to you?	☐☐
Do you understand who will have access to your information?	☐☐
Do you want the investigator(s) to inform your family doctor that you are participating in this research study? If so, give his/her name _____	☐☐

Who explained this study to you?
_____

I agree to take part in this study:	YES	☐	NO	☐
I agree to be contacted for future research related to this unit:	YES	☐	NO	☐
I agree to allow researchers to use my body composition data, if I choose to have this test done (for my own information)	YES	☐	NO	☐

Signature of Research Participant_____

(Printed Name)_____

Date:_____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator or Designee _____ Date _____

Appendix D: Personal Information Sheet

D-Cal Study
Contact Information Sheet

Personal Information

Name: _____ (First) _____ (Middle) _____ (Last)

Address:_____ (Street)

_____ (City) _____ (Province)

_____-_____ (Postal Code)

Phone Numbers:

_____ (Home)

_____ (Cell Phone)

_____ (Work)

E-mail address:

_____

_____

Date of Birth: _____ (date) _____ (month) _____(year)

Emergency Contact Information:

Name: _____

Relationship: _____

Phone numbers:

_____ (Home)

_____ (Cell Phone)

_____ (Work)

Emergency Contact Information (Alternate):

Name: _____

Phone numbers:

_____ (Home)

_____ (Cell Phone)

_____ (Work)

Family Doctor Information:

Name: _____

Phone number: _____

D-Cal Study
Participant Information Sheet

Age: _____ years

In the following table, please tell us about any medical conditions, including diabetes, that you have. Please write down all prescription medications you currently take for the condition.

Medical Condition	**How long have you had this condition?**	**Medication(s) you are currently taking for this condition**	**How long have you been on this medication?**	**What time of day do you typically take this medication?**

Please list any other medications (ex. birth control pills) and/or supplements (including herbal) you are taking:

_____

_____

Anything else that we should know about:

_____

_____

_____

** For female participants only:

Day of start of your last menstrual period: _____

Measured height: _____cm Waist Circumference: _____ / _____ / _____

Average: _____ cm

Measured weight when entering and leaving the calorimeter:

Test Day 1: (1)_____kg /(2) _____kg

Test Day 2: (1)_____kg /(2) _____kg

Prescribed treadmill settings:

Speed: _____mph Incline: _____% grade

Appendix E: Participant Instructions for Fitness Testing

UNIVERSITY OF ALBERTA

Participant Instructions for Fitness Testing

Please adhere to the following conditions for the fitness appraisal. Note that you should be in good general health, not injured at all and under good glucose control prior to and on the day of the fitness appraisal. You should be cleared by your physician for general physical activity.

What should I do the day before my fitness test?

- **Physical Activity:** Please avoid any strenuous physical activity the day before, light exercise is fine (e.g. a short walk or low intensity workout) but avoid any evening exercise.
- **Food and Beverages:** Please make sure you eat and drink "normally" the day before (e.g. do not skip any meals or snacks that you regularly eat) and avoid any alcohol the day before the appraisal.
- **Sleep:** Try to have a good sleep the night before.
- **Medication:** Make sure you take any medication that you are prescribed by your physician.

What should I do the day of my fitness test?

- **Dress Requirements:** Please bring exercise clothes like shorts or sweat pants, preferably a short-sleeve shirt and running shoes that are appropriate for activity. There is a washroom available for changing and there are shower facilities so bring a towel if you wish to shower after your appraisal.
- **Food and Beverages:** Depending on the time of day of your assessment, eat as you normally would during the day, but do not eat a large "heavy" meal for at least two hours before your fitness appraisal. However, it is

important that you do eat a light meal of fruit, granola bar, or a small sandwich as examples about 2 hours before. Make sure you drink about 500 ml of water (2 cups) 1 hour before your fitness test. Also refrain from drinking caffeinated beverages (coffee, tea, pop, energy drinks) for two hours before and no alcohol at all on the day of testing**.**

- **Smoking:** Do not smoke at least two hours prior to the appraisal.
- **Medication:** Please take any medication as prescribed by your physician on the day of your fitness appraisal. Also, follow any instructions your physician has given you about taking your medication before exercise.
- **Blood Sugar:** Check your blood sugar prior to your fitness test to ensure that you are under good glucose control.

What should I do after my fitness test?

- **Recovery:** Make sure you cool-down properly and be sure to drink small amounts of water to rehydrate. Have your blood pressure and heart rate monitored and check your blood sugar levels after exercise and periodically for several hours after to ensure that you are under good glucose control. You may need an additional nutritional snack after exercise.

Appendix F: Calorimetry Unit Test Days Schedule

7:15 -	Arrival Void Participant gets weighed (in hospital gown). Fasting blood sample taken Continuous glucose monitor inserted Participant waits comfortably in patient lounge
7:50 -	Move toward unit…
8:00 -	Test in unit begins. 60 minutes of rest on bed.
9:00 – 9:30	Breakfast is provided (must be eaten within 30 minutes).
9:30 – 11:30	Participants leisure/work time (TV, computer, reading)
11:30 –	2 Hour post-meal blood draw
12:00 – 12:30	Lunch provided (must be eaten within 30 minutes).
12:30 – 1:30	Participants leisure/work time (TV, computer, reading)
1:30-2:00	Prepare for exercise session
2:00-2:35	Exercise session on treadmill.
2:35-3:00	Participants leisure/work time (TV, computer, reading)
3:00	Afternoon snack (must be eaten within 30 minutes).
3:00 – 6:00	Participants leisure/work time (TV, computer, reading)
6:00 – 6:30	Dinner provided (must be eaten within 30 minutes).
6:30 – 9:00	Participants leisure/work time (TV, computer, reading)
9:00	Evening snack (must be eaten within 30 minutes).
10:00	Get ready for bed.
10:30 – 6:30	Sleep
6:30	Wake-up call
7:00	Be ready to leave
7:15	Out of unit/get weighed in hospital gown

Appendix G: Calorimetry Unit Sample Menu

***CALORIMETRY MENU – 2200 kcal** (50% CHO, 21% Pro, 31% Fat)*

MEAL	DESCRIPTION	QUANTITY
Breakfast	Scrambled eggs	2 large egg and 1 Tbsp 1% milk
71.08g carb (26%)	Toast	2 slices of w/w bread
	Peanut butter	18g peanut butter
	Orange slices	1 oranges (6.7cm each)
	Activia Vanilla Yogurt*	100g
Lunch	Turkey wrap	1 flour tortilla (17.8 cm)
70.30g carb (25%)		1 Tbsp Ranch dressing regular
		100g turkey deli cut
		21g cheddar cheese shredded
		0.5 cup shredded romaine lettuce
		20g of red tomato
	Peaches, canned in juice	1 can (142mL)
	Tomato soup	200 ml
Afternoon snack	Apple	1 each (7 cm)
29.79g carb (11%)	Crackers	18g (Garden vegetable - Breton)
	Mozzarella cheese	21g (16.8 % M.F.)
Dinner	Chicken stir-fry	110g chicken breast (boneless/skinless)
72.52g carb (26%)		2 tsp soy sauce
		2 tsp corn starch
		0.5 tsp ground ginger

		0.25 tsp garlic powder 1.5 tsp Canola oil 25g chopped celery 32g chopped carrot 30g chopped onion 1 tsp bouillon cubes
	Rice	1.25 cup, brown, medium grain
Evening snack 34.89g carb (12.5%)	1% Milk Cheerios Almonds	1 cup 1.25 cup 10g, dry roasted

Appendix H: DXA Information Sheet and Consent Form

DXA Scan: Body Composition Testing

Information Sheet

Test Background:
Dual Energy X-Ray Absorptiometry (DXA) is a simple test that provides a very accurate measurement of bone density, lean tissue mass, and total and regional body fat (ie. abdominal body fat). This test uses very low dose x-rays of two different levels to distinguish between bone and soft tissue.

DXA is a painless, non-invasive test. The test requires that you put on a hospital gown and lie on an x-ray bed. The scan takes about 5 minutes and is very low dose radiation (equivalent to approximately 1 day of natural background radiation). This dosage is 1000 times less than the limit for trivial exposure, and is classified as a negligible individual dose according to the standards of the National Council of Radiation Protection and Measurements.

Preparation for the Test:
No special preparation is necessary. Pregnant women and individuals who have recently undergone barium tests/exams (within 2 weeks), or who have had a nuclear medicine scan or been injected with an X-ray dye (within 1 week) cannot have a DXA scan. We ask that you do not wear anything metal (metal may affect bone density values). We will ask you to remove all jewellery.

PREGNANT WOMEN CANNOT PARTICIPATE IN A DXA SCAN. Prior to taking part in the scan, women will be asked to provide a urine sample to verify that they are not pregnant. The pregnancy test that we are using meets WHO guidelines for pregnancy testing, and can detect pregnancy within 1 week after conception. No pregnancy test is, however, 100% accurate, and there is always the possibility of an incorrect result. All results should be confirmed by your physician. You may choose not to undergo this test if you are pre-pubertal (no regular menstrual cycle), taking oral/injection contraceptives, post-menopausal (no menstrual cycle for $\geq$ 6 months), or if you have had a hysterectomy. All other women must undergo a pregnancy test.

Purpose and Time Commitment:
The purpose of the DXA scan is to assess body composition by quantifying bone, muscle, and fat mass. This information helps researchers to monitor changes in body composition over time. An experienced certified Medical X-Ray Technologist will be conducting the scan. The total time required to complete a total body scan is 20 minutes, including the time required to change into the gown, get positioned on the table and complete the scan. Women will be asked to provide a urine sample for a pregnancy test prior to the DXA scan, and thus the test may take up to 30 minutes.

Potential Benefits
After participating in this DXA scan, you will find out information about your body composition; that is – details about your lean body mass, fat mass and/or bone mass.

Potential Risks
The x-ray dose associated with a total body scan is very low and safe for repeated measurements. With the exception of pregnant women, there are no known risks associated with a DXA scan. The potential risks associated with radiation exposure to an unborn fetus are not known, and therefore we ask that you undergo a pregnancy test to verify that you are not pregnant. Having a DXA scan does not make it unsafe for you to have other x-rays taken in the near future.

Stopping the Test
You may ask the technologist to stop the test at any time without jeopardy to you.

Confidentiality
Your scan will be saved in our database using an identification number known only to the researcher for your study. The results of your scan will only be disclosed to the researcher for your study and will be saved in our database for one year.

Appendix I: DXA Consent Form

DXA Scan: Body Composition Testing
Consent Form

Consent*:(Please circle your answers)*

Sex **M F**

Females: Are you pregnant?
Yes No

Females: Do you agree to undergo a pregnancy test? **Yes No**

If No, circle reason: Pre-pubertal (no regular menstrual cycle)
Taking oral/injection contraceptives
Post-menopausal (no menstrual cycle for ≥ 6 months)
Hysterectomy

Have you had a barium test/exam in the last 2 weeks? **Yes No**

Have you had a nuclear medicine scan or injection of an X-ray dye in the past week?
Yes No

Have you read and received a copy of the Information Sheet? **Yes No**

Do you understand the benefits and risks involved in taking part in this test?
Yes No

Have you had an opportunity to ask questions and discuss testing procedures?
Yes No

Do you understand that you can stop the DXA testing at any time and that you do not have to say why?
Yes No

Has confidentiality been explained to you? **Yes No**

Date **(If applicable)**	**Date of Last Menstrual Period**
Name of Participant	**Signature of Participant**
Name of Witness	**Signature of Witness**
Name of Investigator	**Signature of Investigator**

Appendix J: Supplementary Data

Table J.1 Pearson's Product-Moment Correlations between 24 Hour Carbohydrate Oxidation and Study Variables

(n = 10)	***r***	**95% CI**	***P* value**
Age (years)	0.333	-3.6, 9.1	0.347
Weight (kg)[1]	0.716	0.6, 5.7	0.020*
BMI (kg/m^2)[1]	0.618	-0.3, 16.7	0.057
Fat Mass (kg)[2]	0.087	-6.2, 7.5	0.824
Fat Mass (%)[2]	-0.167	-6.9, 4.7	0.668
Fat Free Mass (kg) [2]	0.587	-0.6, 5.6	0.096
Distribution of Central Fat Mass[2,3]	0.737	29.8, 299.9	0.023*
Resting Systolic Blood Pressure (mmHg)	0.462	-1.7, 7.8	0.178
Years with T2D[2]	-0.128	-104.1, 77.8	0.742
REE/kg Body Weight	-0.020	-15.3, 14.6	0.958
Fasting CHO Oxidation (g/min)	0.871	510.6, 1382.6	0.001*
Postprandial CHO Oxidation (g/min)	0.595	-114.4, 2392.5	0.069
Exercise CHO Oxidation (g/min)	0.371	-89.6, 261.5	0.292
Sleep CHO Oxidation (g/min)	0.908	985.3, 2172.8	< 0.000*
Fasting Plasma Glucose (mmol/L)[2]	-0.287	-470.8, 234.6	0.455
Postprandial Plasma Glucose (mmol/L)[2]	0.014	-313.4, 323.5	0.971
ΔPlasma Glucose (mmol/L)[2,4]	0.559	-7.0, 49.7	0.118
Fasting Serum Insulin (µIU/ml)[2]	0.329	-97.7, 222.5	0.387
Postprandial Serum Insulin (µIU/ml)[2]	0.296	-140.8, 290.2	0.439
ΔSerum Insulin (µIU/ml)[2,4]	-0.032	-2.5, 2.3	0.936
Fasting Serum TG (mmol/L)[2]	0.000	-280.7, 282.7	0.993
Postprandial Serum TG (mmol/L)[2]	-0.048	-331.9, 297.9	0.902
ΔSerum TG (mmol/L)[2,4]	-0.162	-323.4, 223.0	0.677
Fasting Serum FFA (mmol/L)[2]	-0.710	-510.4, -31.0	0.032*
Postprandial Serum FFA (mmol/L)[2]	0.037	-419.8, 457.0	0.923
ΔSerum FFA (mmol/L)[2,4]	0.669	2.4, 816.9	0.049*
HOMA-IR[3]	0.158	-110.3, 158.6	0.684

*Independent t-test significant at $P< 0.05$

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), T2D (type 2 diabetes), REE (resting energy expenditure), CHO (carbohydrate), g (grams), min (minute), mmol (millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)

Table J.2 Pearson's Product-Moment Correlations between 24 Hour Fat Oxidation and Study Variables

(n = 10)	***r***	**95% CI**	***P* value**
Age (years)	0.098	-1.1, 1.3	0.787
Weight (kg)[1]	0.396	-0.3, 0.9	0.257
BMI (kg/m^2)[1]	0.405	-0.8, 2.7	0.246
Fat Mass (kg)[2]	0.179	-1.3, 2.0	0.645
Fat Mass (%)[2]	-0.053	-1.5, 1.4	0.893
Fat Free Mass (kg) [2]	0.314	-0.6, 1.2	0.411
Distribution of Central Fat Mass[2,3]	-0.214	-59.8, 36.2	0.576
Resting Systolic Blood Pressure (mmHg)	-0.178	-1.2, 0.7	0.622
Years with T2D[2]	0.090	-20.2, 24.7	0.818
REE/kg Body Weight	0.174	-2.1, 3.2	0.630
Fasting Fat Oxidation (g/min)	0.303	-500.9, 1140.0	0.395
Postprandial Fat Oxidation (g/min)	0.205	-457.5, 774.0	0.570
Exercise Fat Oxidation (g/min)	0.809	110.4, 431.3	0.005*
Sleep Fat Oxidation (g/min)	0.817	446.9, 1654.8	0.004*
Fasting Plasma Glucose (mmol/L)[2]	0.834	29.0, 112.7	0.005*
Postprandial Plasma Glucose (mmol/L)[2]	0.642	-3.1, 97.5	0.062
ΔPlasma Glucose (mmol/L)[2,4]	-0.217	-8.6, 5.2	0.576
Fasting Serum Insulin (µIU/ml)[2]	-0.114	-39.2, 30.3	0.770
Postprandial Serum Insulin (µIU/ml)[2]	-0.294	-59.8, 29.1	0.442
ΔSerum Insulin (µIU/ml)[2,4]	-0.384	-0.7, 0.2	0.308
Fasting Serum TG (mmol/L)[2]	-0.263	-73.1, 39.0	0.494
Postprandial Serum TG (mmol/L)[2]	0.294	-59.8, 29.1	0.442
ΔSerum TG (mmol/L)[2,4]	0.618	-5.4, 84.4	0.076
Fasting Serum FFA (mmol/L)[2]	0.188	-0.542, 83.7	0.628
Postprandial Serum FFA (mmol/L)[2]	0.306	-55.2, 117.1	0.424
ΔSerum FFA (mmol/L)[2,4]	-0.157	-131.4, 91.6	0.686
HOMA-IR[3]	0.217	-20.6, 34.2	0.575

*Independent t-test significant at $P < 0.05$;

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), T2D (type 2 diabetes), REE (resting energy expenditure), g (grams), min (minute), mmol (millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)

Table J.3 Pearson's Product-Moment Correlations between the Rate of Fasting Carbohydrate Oxidation and Study Variables

(n = 10)	***r***	***95%CI***	***P* value**
Age (years)	0.532	-0.00, 0.01	0.113
Weight (kg)[1]	0.720	0.00, 0.01	0.019*
BMI (kg/m^2)[1]	0.680	0.00, 0.02	0.030*
Fat Mass (kg)[2]	-0.010	-0.01, 0.00	0.798
Fat Mass (%)[2]	-0.425	-0.01, 0.00	0.254
Fat Free Mass (kg) [2]	0.717	0.00, 0.00	0.030*
Distribution of Central Fat Mass[2,3]	0.500	-0.04, 0.19	0.170
Resting Systolic Blood Pressure (mmHg)	0.240	-0.00, 0.01	0.505
Years with T2D[2]	-0.255	-0.08, 0.04	0.509
REE/kg Body Weight	-0.014	-0.01, 0.01	0.969
Postprandial CHO Oxidation (g/min)	0.503	-0.4, 2.1	0.138
Exercise CHO Oxidation (g/min)	0.174	-0.1, 0.2	0.630
Sleep CHO Oxidation (g/min)	0.732	0.3, 2.1	0.016*
Fasting Plasma Glucose (mmol/L)[2]	-0.047	-0.4, 0.4	0.904
Postprandial Plasma Glucose (mmol/L)[2]	0.083	-0.3, 0.4	0.832
ΔPlasma Glucose (mmol/L)[2,4]	0.297	-0.02, 0.05	0.437
Fasting Serum Insulin (μIU/ml)[2]	0.263	-0.1, 0.2	0.494
Postprandial Serum Insulin (μIU/ml)[2]	0.072	-0.2, 0.2	0.853
ΔSerum Insulin (μIU/ml)[2,4]	-0.223	-0.00, 0.00	0.565
Fasting Serum TG (mmol/L)[2]	-0.092	-0.3, 0.3	0.814
Postprandial Serum TG (mmol/L)[2]	0.086	-0.3, 0.4	0.825
ΔSerum TG (mmol/L)[2,4]	0.224	-0.2, 0.4	0.561
Fasting Serum FFA (mmol/L)[2]	-0.833	-0.5, -0.1	0.005*
Postprandial Serum FFA (mmol/L)[2]	-0.048	-0.5, 0.4	0.904
ΔSerum FFA (mmol/L)[2,4]	0.718	0.06, 0.85	0.029*
HOMA-IR[3]	0.194	-0.1, 0.2	0.618

*Independent t-test significant at $P < 0.05$; **Independent t-test shows trend for significance

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), T2D (type 2 diabetes), REE (resting energy expenditure), CHO (carbohydrate), (grams), min (minute), mmol (millimoles), L (litre), μIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)

Table J.4 Pearson's Product-Moment Correlations between the Rate of Fasting Fat Oxidation and Study Variables

(n = 10)	***r***	***95%CI***	***P* value**
Age (years)	-0.644	-0.00, 0.00	0.044*
Weight (kg)[1]	-0.193	-0.00, 0.00	0.594
BMI (kg/m^2)[1]	-0.097	-0.00, 0.00	0.790
Fat Mass (kg)[2]	0.594	-0.00, 0.00	0.092
Fat Mass (%)[2]	0.596	-0.00, 0.00	0.090
Fat Free Mass (kg) [2]	-0.191	-0.00, 0.00	0.623
Distribution of Central Fat Mass[2,3]	0.101	-0.04, 0.04	0.795
Resting Systolic Blood Pressure (mmHg)	0.098	-0.00, 0.00	0.787
Years with T2D[2]	0.017	-0.02, 0.02	0.965
REE/kg Body Weight	0.086	-0.00, 0.00	0.814
Postprandial Fat Oxidation (g/min)	0.054	-0.6, 0.6	0.882
Exercise Fat Oxidation (g/min)	0.000	-0.3, 0.3	0.994
Sleep Fat Oxidation (g/min)	0.142	-0.8, 1.2	0.695
Fasting Plasma Glucose (mmol/L)[2]	0.475	-0.02, 0.09	0.197
Postprandial Plasma Glucose (mmol/L)[2]	0.296	-0.04, 0.07	0.439
ΔPlasma Glucose (mmol/L)[2,4]	-0.371	-0.01, 0.00	0.325
Fasting Serum Insulin (µIU/ml)[2]	0.020	-0.03, 0.03	0.960
Postprandial Serum Insulin (µIU/ml)[2]	0.000	-0.04, 0.04	0.997
ΔSerum Insulin (µIU/ml)[2,4]	0.063	-0.00, 0.00	0.872
Fasting Serum TG (mmol/L)[2]	0.251	-0.03, 0.06	0.514
Postprandial Serum TG (mmol/L)[2]	0.199	-0.04, 0.07	0.607
ΔSerum TG (mmol/L)[2,4]	0.104	-0.04, 0.06	0.789
Fasting Serum FFA (mmol/L)[2]	0.736	0.01, 0.09	0.024*
Postprandial Serum FFA (mmol/L)[2]	0.117	-0.1, 0.1	0.764
ΔSerum FFA (mmol/L)[2,4]	-0.664	-0.15, 0.00	0.051
HOMA-IR[3]	0.191	-0.02, 0.03	0.622

*Independent t-test significant at $P< 0.05$

[1]Body weight and BMI are from the morning measurement on test day 1

[2] n = 9

[3] Calculated as the ratio of android to gynoid fat mass

[4] Change from fasting to postprandial conditions

Abbreviations: *r* (Pearson's Product-Moment Correlation Coefficients), CI (confidence interval), kg (kilogram), BMI (body mass index), m (meters), mmHg (millimeter of mercury), T2D (type 2 diabetes), REE (resting energy expenditure), g (grams), min (minute), mmol (millimoles), L (litre), µIU (microinternational units), ml (millilitre), TG (triglycerides), FFA (free fatty acids), HOMA-IR (homeostasis model assessment-estimated insulin resistance)