The effect of fitness on energy metabolism in lean and obese healthy men

By

© Alicia Ricketts

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of

Master of Science in Kinesiology

School of Human Kinetics and Recreation

Memorial University of Newfoundland

May 2017

St. John's, Newfoundland

ABSTRACT

This study was designed to assess the independent contributions of cardiorespiratory fitness (CRF) and adiposity to the metabolic health of obese individuals. It specifically focused on how body composition and CRF affect the relative contribution of glucose, fat and protein to energy production (EP) in resting state as well as during and after exercise. Eight obese fit (OF – 30±12 yrs., 178±6 cm, 105±8 kg) and eight lean fit (LF – 36±9 yrs., 181±5 cm, 82±5 kg) were screened for medical conditions and food/health supplement use and partook in two sessions – separated by 72 hr – that consisted of the following measurements: (1) a basal metabolic rate (BMR) in a fasting state for the determination of substrate oxidation (baseline) followed by an incremental running test for the determination of $\dot{V}O_{2max}$ and of exercise intensity that elicits maximal fat oxidation (MFO); (2) a BMR, an iso-caloric (300 kcal expenditure) treadmill exercise at MFO, a post-exercise metabolic rate (PEMR) in fasting state (30 min after exercise), and two PEMR in the fed state at 90 and 150 min after exercise. The fed state was achieved by a 300 kcal meal provided immediately after the first PEMR. Food intake and physical activity were monitored throughout the study. Although statistical significance was found in %FAT (p<0.001) between both groups (35±4% and 18±4 % for OF and LF, respectively), no differences were detected in $\dot{V}O_{2max}$ (OF:4.1±0.5 L·min^{-1} and LF:4.2±0.5 L·min-1) and $\dot{V}O_{2MFO}$ (OF:1.6±0.4 L·min^{-1} and LF:1.8±0.5 L·min^{-1}) confirming CRF between-groups homogeneity. Further to these results, participants showed no substrate oxidation differences before, during, and after exercise. In conclusion, obese fit individuals display a pattern of substrate partitioning comparable to that observed in lean fit individuals,

confirming that fitness can modulate the metabolic impacts of obesity and could reduce the risks of chronic diseases associated with obesity.

ACKNOWLEDGEMENTS

Completion of this thesis would not have been possible without the help of some very important people. Firstly, I would like to express my heartfelt gratitude to someone who is not only my graduate supervisor but who has become a dear friend, Dr. Fabien Basset, whose patience and understanding has helped me complete this paper with ease. Furthermore, I would like to say a special thank you to Dr. Denis Joanisse (Laval University) who assisted in guiding me throughout my program and research; Dr. Thamir (Tim) Alkanani for his kindness and assistance in the exercise physiology laboratory; and to my colleagues: Jason, Adebayo, and Mohammad. Finally, I would like to express my deepest appreciation to my best friend and husband, Philip, who has done nothing but support and carry me throughout all my years of study. Without his sacrifices, this paper would not be possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BF: breathing frequency

BM: body mass

BMI: body mass index

BMR: basal metabolic rate

BS: blood sample

CHO: carbohydrate

EE: energy expenditure

EP: energy production

FBG: fasting blood glucose

FFA: free fatty acids

FFM: fat free mass

G_{ox}: glucose oxidation

HR: heart rate

IC: indirect calorimetry

IR: insulin resistance

kcal: kilocalorie

LM: lean mass

L_{ox}: lipid oxidation

MetS: metabolic syndrome

MFO: maximal fat oxidation

MHO: metabolically healthy but obese

MR: metabolic rate

NEAT: non-exercise activity thermogenesis

NIDDM: non-insulin dependent diabetes mellitus

PEMR: post-exercise metabolic rate (30 min post exercise)

PMMR1: post-meal metabolic rate (30 min post-meal)

PMMR2: post-meal metabolic rate (90 min post-meal)

PRO: protein

P_{ox}: protein oxidation

RER: respiratory exchange ratio

RMR: resting metabolic rate

RQ: respiratory quotient

SBP: systolic blood pressure

TAG: triacylglycerol

$\dot{V}CO_2$: carbon dioxide production

$\dot{V}O_2$: oxygen uptake

$\dot{V}O_{2max}$: maximal rate of oxygen uptake

VE: ventilation

VS: vital signs

VT: tidal volume

CHAPTER 1 INTRODUCTION

1.1 Background of study

Rates of obesity have been steadily increasing over the past few years. This condition is characterized by an excess of body fat and is clinically defined by a body mass index (BMI) greater than 30 $kg \cdot m^{-2}$ (World Health Organization, 2015). Obesity is a risk factor for many adverse health conditions such as metabolic syndrome (MetS), insulin resistance (IR), noninsulin dependent diabetes mellitus (NIDDM), increased blood pressure (BP), cardiovascular disease and can lead to premature death (WHO, 2015). Physical inactivity along with obesity are independent risk factors for NIDDM (Lee, Sui, Church, Lee, & Blair, 2009). Despite the fact that obesity is associated with several chronic diseases, obesity itself is a condition, not a disease.

It has been well-established that obesity is associated with impaired lipid oxidation (L_{ox}) during exercise (Blaak & Saris, 2002). During post-absorptive conditions and beta-adrenergic stimulation, the skeletal muscle of obese individuals exhibits a reduced utilization of free fatty acids (FFA). This decreased reliance on fats as an energy source is one of the many reasons behind the positive fat balance and increased fat storage in obesity (Blaak & Saris, 2002). Physical activity may contribute to reducing the risks of chronic diseases associated with obesity because it stimulates L_{ox} not only during the activity itself but in the post-exercise state as well (Gaesser & Brooks, 1984). The health benefits produced by exercise are numerous and include increases in insulin sensitivity, improved blood lipid profile, decreased BP and weight maintenance (Blaak & Saris, 2002) to name a few.

In the last few years, a new concept has emerged from the literature, the notion of "fat fit". Fat fit should not be confused with "metabolically healthy but obese" (MHO). Similar to the idea that not all normal weight individuals are "metabolically healthy", not all obese individuals are metabolically unhealthy (Karelis, St-Pierre, Conus, Rabasa-Lhoret, & Poehlman, 2004). As mentioned previously, although obesity correlates with NIDDM and IR, and increases the likelihood of CVD, not all obese persons have these risk factors (Després & Lemieux, 2006). MHO individuals are a unique subset of obese individuals who, despite having excess body fat, display a favorable metabolic profile characterized by high insulin sensitivity levels, a favorable lipid profile, and no sign of hypertension (Karelis et al., 2005).

Many studies have assessed the effects of exercise on L_{ox} in lean persons and the impairment of L_{ox} in obese persons. However, to the best knowledge of the author, no studies in the current literature have compared substrate partitioning between lean fit and obese fit men. Thus, the purpose of this research is to examine the contribution of substrates to EP of OF and LF healthy men.

1.2 Purpose of study

The purpose of this study was to assess the independent effect of fitness level and body composition on whole-body lipid oxidation (L_{ox}) in lean fit and obese fit, healthy men to determine resting and post-exercise substrate partitioning. The extent of differences in whole body L_{ox} and glucose oxidation (G_{ox}) between the two groups were assessed after an isoenergetic maximal fat oxidation treadmill exercise was performed. Another objective of this study was to describe the independent effect of cardiorespiratory fitness on substrate partitioning post-exercise and in a fed state in lean and obese, fit men. It was hypothesized that a high fitness level, regardless of body composition, will alter L_{ox}. More specifically, it was predicted that L_{ox} will be higher in lean, fit men compared to obese, fit individuals.

1.3 Significance of study

Rates of obesity have been steadily rising over the past several years. Obesity is well-known to be associated with many adverse health conditions. It has also been well-established that obesity is associated with a diminished capacity to use fats as fuel. However, a few studies have shown contradictions with substrate partitioning, risk of cardiovascular disease, and all-cause mortality in obese, fit individuals. These studies led to the idea that perhaps impaired L_{ox} is not associated with anthropometric factors. “Fat-fit”, a relatively new concept, implies that individuals can be fit but fat with no metabolic disorders commonly associated with obesity. Exercise training has a positive impact on L_{ox}, insulin insensitivity, and BP in multiple studies. Physical activity, for instance, stimulates L_{ox} during as well as after exercise. Where exercise has been shown to have such a profound effect on L_{ox} in terms of

up-regulation and impaired L_{ox} has been shown to have adverse effects on metabolic functioning, promotion of regular exercise should be emphasized to the population as opposed to weight loss itself. Shifting the perspective from weight loss to cardiorespiratory fitness to enhance metabolic health may be more appealing to the population and subsequently more sustainable than fad diets and extreme exercise regimens. Therefore, understanding the mechanisms that affect substrate partitioning of lean and obese, fit men in resting and post-exercise states may provide insight into the role of CRF, independent of body composition, on metabolic health and chronic disease.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Metabolism, heat production, and calorimetry

According to Brooks et al., metabolism can be defined as the sum total of processes occurring in a living organism while metabolic rate indicates the rate of heat production as these processes produce heat (1996). All the energy intake of humans derives from potential chemical energy contained in the nutrients absorbed from the diet. Much of this energy is stored in the form of hydrocarbon bonds found in carbohydrates (CHO), lipids, and protein. The potential energy stored within the chemical bonds are released in the body through oxidative metabolism (Simonson & DeFronzo, 1990). Since all processes of metabolism ultimately depend on biological oxidation, measuring the rate of oxygen consumption yields a good estimate of the rate of heat production. The basic unit of heat measurement is the Joule (SI) but physiologists opt for kilocalorie (1000 calories) to express heat change (Brooks, Fahey, White, & Baldwin, 1996).

The procedure to measure heat is coined calorimetry as heat must be measured to determine metabolic rate. There are two types of calorimetry – direct (the measurement of convection and radiation) and indirect (the measurement of fraction of gases) (Brooks et al., 1996). Direct calorimetry measures heat dissipated by the body whereas indirect calorimetry (IC) measures heat released by oxidative processes (Jequier & Felber, 1987). IC measures BMR, resting metabolic rate (RMR) and exercise metabolic rate (EMR) through O_2 and CO_2 cell technology (da Rocha, Alves, & da Fonseca, 2006). Specifically, it measures the amount of fraction of O_2 inspired and CO_2 expired that correspond to the heat generated from

substrate catabolism (Haugen, Chan, & Li, 2007). Healthcare professionals such as nutritionists and physiologists utilize non-invasive IC methods to measure metabolic rate change (Simonson & DeFronzo, 1990).

The relationship between heat production and O_2 uptake began with the eighteenth century French chemist, Antoine Lavoisier, who recognized that living animals give off heat and breathe while dead animals do not. One of Lavoisier's inventions, the respirometer, was able to establish that something in air (O_2) was consumed by animals and that something else (CO_2) was produced in approximately equal amounts. This respirometer device is known as an indirect calorimeter because it estimates heat production by determining either O_2 uptake or CO_2 production (Brooks et al., 1996). Some of the first indirect calorimeters used closed-circuits designs however this is not practical for humans only small animals. Most of the indirect calorimeters used today have an open-circuit design, which means both ends of the system are open to atmospheric pressure, however; this design is not without limitations. For instance, unfamiliarized subjects tend to hyperventilate when they are required to breathe through a mask or mouthpiece – this in turn yields high O_2 consumption and CO_2 production rates, and there is always the risk of leaks when using a mask which may not have an airtight seal (Simonson & DeFronzo, 1990).

Over a century ago, Zuntz and Schumburg (1901) developed tables to demonstrate the relationship between metabolic rate to O_2 uptake and CO_2 production, and the combustion of various substrates (CHOs and lipids) as determined by all forms of calorimetry – direct, indirect and bomb, as cited by Brooks et al. (1996). Except for protein, the caloric equivalents for the combustion of substrates inside and outside the body are the same. Protein

has a different caloric and combustion equivalent as its component amino acids are only partially oxidized. The non-oxidized component is excreted from the body, mainly in urine, in the form of urea. In 1928, Lusk developed the non-protein caloric equivalents chart based on the RQ by removing the caloric release of basal protein catabolism to better determine the oxidation of glucose and fat. However, this chart is not valid in cases of increased protein catabolism (i.e. increased duration and intensity of exercise, low CHO diet, and hyperventilation).

Since for each liter of oxygen consumed there is a known amount of heat released, the measurement of a subject's oxygen uptake and carbon dioxide production is the principle on which IC is based (Jequier & Felber, 1987). Under well-controlled conditions IC also provides a way to estimate, through stoichiometry equations, the contribution of fuels to EP at rest and during light-to-moderate intensity exercise. In addition, during steady-state conditions the respiratory exchange ratio (RER), defined as the ratio of $\dot{V}CO_2$ over $\dot{V}O_2$, mirrors the respiratory quotient (RQ), an indicator of cellular respiration. (Brooks et al., 1996). A mathematical model states that when RER=1, 100% of energy produced is derived from CHOs while at an RER of 0.7, 100% of energy comes from fat. Above and below this range, IC loses validity and reliability in estimating substrate partitioning. Additionally, RER is less responsive to changes in rapid gas exchange such as during exercise (Headley, 2003). Furthermore, stoichiometry of the oxidative reactions can be written with slightly different coefficients (Ferrannini, 1988). Jequier and Felber (1987) state that oxygen consumption values for nutrient oxidation depend upon the type of substrate being oxidized. For example, $\dot{V}O_2$ per gram of protein will vary depending upon whether the protein is from meat, milk or

cereal origin while $\dot{V}O_2$ per gram of CHO depends whether the sugar is a mono-, di-, or polysaccharide. If free glucose as opposed to glycogen is the main form of CHO being oxidized but the glycogen value is used in the calculations, the rate of CHO oxidation will be underestimated by 11% (Jequier & Felber, 1987). Another problem occurs when subjects are acutely studied in both a fasted state and after an infusion of glucose since the exogenous glucose load will not necessarily be oxidized immediately (Jequier & Felber, 1987). This is the case for the current study as subjects are studied while in both fed and fasted states.

Despite certain disadvantages, IC has many advantages. Compared with direct calorimetry, heat released by oxidative processes can be immediately assessed by IC but is not accompanied by similar increases in heat losses. IC also has an advantageous short time response compared to the poor time response of direct calorimetry. The response time of IC is short because the body's oxygen stores are very small and the capacity of substrate level phosphorylation for synthesis of ATP is limited (Jequier & Felber, 1987). Non-invasive IC methods provide quantitative information about the type of substrates that are oxidized, the energy output, and therefore the choice to collect metabolic measurements in humans.

2.2 Relationship between glucose and lipid: a physiological perspective

The Randle Cycle

To truly understand the use of substrates in the body, it is important to first understand the relationship between substrates from a more in-depth, physiological perspective. The glucose-fatty acid cycle, also known as the Randle Cycle (so named after

the person who first introduced it, Sir Philip Randle) describes the relationship between glucose and lipid metabolism (Randle, Garland, Hales, & Newsholme, 1963). Proposed in 1963, the original idea was that lipids drive the metabolic pathways in the human body, i.e., the availability of fatty acids controls the glucose-fatty acid relationship. An increase in the oxidation of fatty acids increases the mitochondrial ratio of [acetyl CoA:CoA] which in turn suppresses the pyruvate dehydrogenase (PDH) complex directly. Suppression of this complex also inhibits phosphofructokinase-1 and hexokinase indirectly via a build-up of citrate and glucose-6-phosphate (G6P), respectively (Randle et al., 1963). Ultimately, glycolysis is inhibited as well as glucose transport which results in an accumulation of glucose in the blood. The traditional glucose-fatty acid cycle may serve as a link between high FFA concentration and IR as a high FFA concentration will lead to high L_{ox} which would inhibit glucose uptake and oxidation (Sidossis & Wolfe, 1996).

Although the Randle cycle has been essential for the understanding of the relationship between glucose and L_{ox} and is nonetheless accepted among many scientists, concrete evidence for its existence in humans is limited. When Randle and his associates produced the concept of the glucose-fatty acid cycle, the experiment was done *in vitro* on rat heart and diaphragm muscles. The whole idea of the glucose-fatty acid cycle depends on increased L_{ox} inhibiting G_{ox} due to an accumulation of intracellular citrate and G6P concentrations. However, studies performed in humans have failed to demonstrate a change in either citrate or G6P concentrations. In a study that examined the effect of increased plasma FFA concentrations on muscle metabolism in exercising men, glucose uptake was decreased by intralipid infusions (dietary fats administered by an IV drip) but G6P levels in the muscle

were unaffected (Hargreaves, Kiens, & Richter, 1991). In the same year, Boden and colleagues (1991) looked at the effects of fat on insulin-stimulated CHO metabolism in men. Glycogen synthase activities were measured along with concentrations of citrate, G6P, acetyl CoA and free CoA-SH before and after insulin/lipid and insulin/saline infusions. Although lipid infusions resulted in an increase in L_{ox} and a decrease in G_{ox}, muscle concentrations of citrate and G6P did not change. In another study examining fatty-acid induced inhibition of glucose uptake, there was a reduction in glycogen synthesis. This was explained by two scenarios. First was an impairment of muscle glycogen synthase that only occurred with a very high FFA concentration (~750uM) which was in fact associated with an *increase* in G6P. However, the second defect which preceded the glycogen synthase defect was seen at a medium chain FFA concentration (~550 μM) and was associated with a *decrease* in G6P (Boden, Chen, Ruiz, White, & Rossetti, 1994). In the latter two studies mentioned, apparently G_{ox} had decreased due to the increase in plasma FFA concentration by lipid and heparin infusions. However, as pointed out by Sidossis & Wolfe (1996), the hyperinsulinemic conditions may have elicited the response of a decreased G_{ox} due to an inhibition of glucose transport. It remains unclear whether the increase in FFA concentration impaired the intracellular oxidation of glucose.

Evidence of the glucose-fatty acid cycle has been demonstrated in the obese population as reviewed by Elks (1990). Studies have indicated that since serum FFA levels are elevated in obesity, fat is the preferred fuel for oxidation. Ample evidence suggests that the excessive amount of FFA in the obese may lead to impaired muscle G_{ox} and storage and consequently, to glucose intolerance and diabetes (Elks, 1990).

The Reverse Randle Cycle

The results of the previous studies mentioned do not necessarily coincide with Randle's theory which leaves room for interpretation. For many years, due to Randle's theory that high FFA concentrations are linked with glucose intolerance, high fat foods were to blame for the obesity pandemic; ironically enough, the rates of obesity did not decrease when dietary fat was replaced with CHO. According to Willett (1998), evidence of the relationship between dietary fat and obesity is weak. In recent years, new light has been shed on the idea of a reversed glucose-fatty acid cycle. This Randle cycle reciprocal theorizes that glucose is the driver of metabolic pathways in the body, i.e. high levels of glucose and insulin in the blood inhibit fatty acid oxidation in adipocytes (Hue & Taegtmeyer, 2009). In this scenario, an increase in circulating glucose produces an abundance of acetyl-CoA which condenses with oxaloacetate to form citrate in the TCA cycle. However, not all the citrate stays in the TCA cycle, some travels back to the cytosol to be regenerated into acetyl-CoA. Acetyl-CoA in the cytosol may then be carboxylated to malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl CoA then inhibits carnitine palmitoyl transferase I (CPT I) which controls the entry and oxidation of long-chain fatty acids (LCFAs) in the mitochondria (Hue & Taegtmeyer, 2009).

Although it remains a debate about whether glucose inhibits fatty acid oxidation, a study conducted on different rates of glucose infusion and its effect on lipolysis and re-esterification in humans revealed that glucose did in fact decrease fat mobilization by inhibiting lipolysis and stimulating re-esterification but it was dose-dependent (Wolfe &

Peters, 1987). Some years later, a study was conducted to assess the utilization of FFA by skeletal muscle in individuals with NIDDM and found that during fasting and postprandial conditions, NIDDM patients had reduced rates of L_{ox} compared with non-diabetic patients (Kelley & Simoneau, 1994). Finally, Sidossis and colleagues (1996) examined the effects of an acute elevation of glucose availability and oxidation on the oxidation of fatty acids. It was found that even when fatty acid availability remained constant with infusions of glucose plus insulin, there was a decrease in plasma long-chain fatty acids (LCFA). However, this had no effect on medium-chain fatty acids (MCFA) as they can diffuse freely across the inner mitochondrial membrane. The studies described above provide evidence for a reversed Randle cycle; high glucose levels inhibit fatty acid oxidation.

The importance of altered glycogen levels has been generally overlooked and through a review of the literature and using his two-compartment computer model, Flatt suggests that if glycogen reserves in the body (which are small in comparison to fat reserves) are never depleted, consumption of CHO in the diet will promote heavily insulin secretion and G_{ox} while minimizing L_{ox} (Flatt, 2004). Since blood glucose levels are typically higher in the obese population, the reversed glucose-fatty acid cycle may provide an explanation for the metabolism behind obesity.

2.3 Obesity

Introduction to obesity

Obesity, in combination with several chronic diseases (cardiovascular disease, NIDDM, MetS and cancer) has become one of the main killers in Westernized society with its prevalence growing rapidly in developing countries (Roberts & Barnard, 2005). This condition is characterized by an excess of body fat and clinically defined by a BMI greater than 30 $kg \cdot m^{-2}$ (WHO, 2015). Obesity is a complex health condition related to energy imbalance, that is energy intake constantly exceeds energy expenditure (EE) (Bouchard & Blair, 1999). Individuals who are of the obese stature tend to store greater amounts of fat in the abdominal region (visceral fat) with impaired use of fat as a fuel source (Ross et al., 2000; Ross et al., 2004). In terms of bodyweight regulation and obesity, the energy balance equation (energy intake=EE) has provided a universal explanation. This equation coincides with what Bouchard and Blair (1999) stated, that obesity is indeed due to excessive energy intake or insufficient EE. This statement is rebutted by Flatt who claims that this is not the way to approach the obesity problem and that it is essential to recognize that food intake should be approached relative to EE and vice versa (Flatt, 1997). He then theorizes that weight maintenance is achieved by regulation of substrate balance, independent of energy balance (Flatt, 2011). Tremblay gives a thorough review of studies that have looked at energy balance versus substrate balance. Past research has concluded that short-term lipid balance does not have the potential to induce a metabolic adjustment which would allow a rapid return to energy and lipid balance. Lipid imbalance resulting from an excess lipid intake over

L_{ox} can be progressively attenuated by an increase in adipose tissue mass until a new equilibrium is reached. Coinciding with Flatt, Tremblay (1992) concludes that spontaneous energy balance cannot be achieved without lipid balance.

Metabolism of obesity

An inability to oxidize lipids appears to be an important factor in the etiology of obesity. Obesity is associated with increased IR and elevated levels of intramuscular triacylglycerol, in which resting levels of L_{ox} is disturbed (Kelley, Goodpaster, Wing, & Simoneau, 1999). A study of Pima Indians from Arizona, a population with a high prevalence of obesity and type II diabetes, has shown that a high 24 hr RER, indicative of relatively low L_{ox}, is predictive of future weight gain (Zurlo et al., 1990).

Although L_{ox} within muscle fibres will not be studied in the current study, it is important to understand that obesity affects muscles fibres at the cellular level in addition to whole-body L_{ox}. In a study conducted by Malenfant et al. (2001) researchers attempted to determine whether obese participants were more likely to store lipids within each of the major types of skeletal muscle fibers and to differentiate the intramyocellular distribution of fat between lean and obese individuals. There are three main types of muscle fibres in the human body – type I (slow-twitch or slow-oxidative) and type II muscle fibres. The latter can be broken into two categories: type IIa (fast oxidative) fibres and type IIx (fast glycolytic) fibres (Fitts, 1994). Fourteen obese and seven lean individuals participated in this study and none of the participants were engaged or previously engaged in any fitness program. Muscle biopsies were taken from the right *vastus lateralis*. The main findings obtained from this

study were that the size of fat aggregates was not affected by obesity but the number of lipid droplets within muscle fibers was twice as abundant in obese compared to lean individuals. This was seen in type I and both type II muscle fibres. Additionally, they found that the distribution of lipid droplets was more central in muscle fibres of obese compared to lean participants (Malenfant et al., 2001). In a muscle cell, most mitochondria are located directly beneath the membrane (also known as the sarcolemma) (Weber et al., 1996). Because oxidation of lipids occurs in the mitochondria and the localization of lipid stores in obese muscle fibres are further away from the sarcolemma (and thus the mitochondria), the ability of the cell to oxidize fat may be negatively impacted.

Obesity not only affects whole-body and muscle cell metabolism but beta-adrenergic stimulation also. The sympathetic nervous system (SNS) plays a major role in the regulation of EE and fuel metabolism (Blaak et al., 1994). The SNS along with the parasympathetic nervous system make up the autonomic nervous system (ANS). SNS activity is high during exercise and its activity stimulates secretion of norepinephrine and epinephrine (which are known as blood catecholamines). Epinephrine affects both α and β receptors and β receptors can further be divided into two groups (β_1 and β_2). In fact, the β_2 receptor affects tissue metabolism (Brooks et al., 1996). In one study, researchers examined the effect of beta-adrenergic stimulation on whole-body EE and skeletal muscle substrate fluxes in lean and obese men. Whole body EE was determined during rest and during intravenous (IV) infusion of increasing doses of the nonselective β-agonist isoprenaline. Metabolism of forearm skeletal muscle was also investigated with isoprenaline infusion with and without simultaneous infusion of the β-blocker atenolol (AT). The increase in whole body EE with

isoprenaline was similar in lean and obese participants. With isoprenaline infusion, the increase in arterial or arterialized glycerol and nonesterified fatty acids (NEFA) was lower in obese than lean participants. The increased [glycerol] indicates a lipolytic response and since it did not increase much in the obese group, it could signify a lower β-adrenergically mediated lipolysis in obesity. During infusion of increasing doses of isoprenaline, the respiratory exchange ratio (RER) decreased significantly in lean participants but not in the obese participants. This shows a more distinct increase in L_{ox} in lean participants (Blaak et al., 1994). This decreased reliance on fats as an energy source is one of the many reasons behind the positive fat balance and increased fat storage in obesity (Blaak & Saris, 2002).

Conversely, in an earlier study conducted by Golay and associates (1984), it was found that L_{ox} was elevated in the nondiabetic and diabetic obese groups compared to the control groups. Participants were divided into four groups based on their level of glucose intolerance, group A (obese, normal glucose tolerance); group B (obese, impaired glucose tolerance); group C (obese, diabetes with hyperinsulinemic response); and group D (obese, diabetes with impaired insulin response). Controls groups consisted of ideal body weight individuals and were assigned based on age. All four groups presented with an increase in L_{ox}, both in the fasting state and during the 3 hr glucose tolerance test, when compared to the controls (Golay et al., 1984). It was suggested that L_{ox} rates were higher due to increased fat stores and as a result may cause a decrease in G_{ox}; this is supportive of Randle's theory.

Beta-oxidation is clearly impaired in obese individuals but the reason for this impairment remains unknown and clearly does not pertain to one specific mechanism. One

possibility for beta-oxidation impairment relates to the reduction of the CPT-1 activity. CPT-1 regulates the transport of LCFA across the mitochondrial membrane. This metabolic response occurred in obese human skeletal muscle from which homogenates were examined and decrements in CPT-1 activity were observed (Kim, Hickner, Cortright, Dohm, & Houmard, 2000). Researchers hypothesized that the decrease in CPT-1 activity may be linked to the presence of malonyl-CoA, an outcome supporting the reversed Randle cycle as it suggests that increased levels of malonyl-CoA (due to high blood glucose levels) inhibit the CPT-1 enzyme.

Physical activity and benefits of exercise

Physical activity and exercise are sometimes used interchangeably so for clarification purposes, both will be defined. As stated in the *ACSM's guidelines for exercise testing and prescription* (American College of Sports Medicine, 2014), physical activity is defined as bodily movement produced by the contraction of skeletal muscle that increases EE above the basal level and refers to daily activities such as occupational, household, leisure time, and transportation (i.e. walking, cycling, running). Exercise, on the other hand, is physical activity that is planned, structured, and repetitive for the purpose of conditioning any part of the body. It is utilized to improve health, maintain fitness and is important as a means of physical rehabilitation. Physical activity induces metabolic stress that challenges all physiological systems of the human body. Physical activity triggers the activation and control of the musculoskeletal system, which, in turn, depends on the respiratory, and cardiovascular systems to sustain movements over extended periods. Exercise training has been proven to

have many health benefits. As a matter of fact, physical activity may contribute to reducing the risks of chronic diseases associated with obesity because it has been shown to stimulate L_{ox} not only during the activity itself but in the post-exercise state as well (Gaesser & Brooks, 1984). Additionally, exercise was shown to decrease concentrations of malonyl-CoA during a single bout of glycogen-depleting bicycling (Saha et al., 2005). Because malonyl-CoA is an inhibitor of CPT-1 in the mitochondria, a decrease in malonyl-CoA concentration from rest-to-moderate intensity exercise in humans may contribute to the increase in L_{ox} that exercise causes.

In his thorough review of literature, Bouchard highlights the works of several authors reporting that regular exercise could be an important factor in the development of sustained negative energy balance, provided the volume of activity is high enough. He then concludes that in order to induce significant weight and fat losses and to treat overweight and obese patients, several years of compliance to the program is required (Bouchard, Després, & Tremblay, 1993). Exercise causes acute metabolic changes that match the energy demand of the task. These physiological responses help to maintain a constant internal environment by delivering important substances such as gases and fuel to the active tissues. Acute effects of exercise on resting metabolic rate are well noted (increases in insulin sensitivity, improved blood lipid profile, decreased BP and weight maintenance), but long-term effects of exercise training are small and quickly diminish when exercise training is ceased (Bouchard, Després, & Tremblay, 1993). Specifically, when exercise is terminated, the body returns to a resting metabolic state and if not repeated over time, the acute response to exercise will not induce

metabolic acclimations. In other words, to maintain the benefits exercise provides, individuals must continually train.

2.4 Lipid Oxidation and Exercise Intensity

Lean individuals

With the impairment of L_{ox} in the obese population, understanding the effect of exercise intensity in relation to fat metabolism regulation becomes of importance (Colberg, Simoneau, Thaete, & Kelley, 1995). Exercise physiologists proposed that exercise stimulating L_{ox} promotes weight loss and maintenance. A handful of studies have shown that from low- to moderate-exercise intensities, the absolute rate of L_{ox} increases steadily, reaching a nadir between 35 and 50% $\dot{V}O_{2max}$. It then decreases rapidly as a function of intensity (Jones et al., 1980; Romijn et al., 1993). It confirms that in the post-absorptive state, EP derives mainly from lipids; a metabolic response that fits with the "crossover concept" (Brooks & Mercier, 1994). The contribution of each substrate (i.e., CHO or lipid) as a fuel for ATP synthesis during exercise highly depends on exercise intensity (van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001), and exercise physiologists have attempted to determine at which intensity L_{ox} reaches a nadir.

Regarding the up-regulation of L_{ox} during exercise, FAs from the plasma non-esterified fatty acid pool are the main source of energy at rest and during low-to moderate-intensity exercise (Frayn, Arner, & Yki-Jarvinen, 2006). According to Romijn and associates (1993), from rest to low- to moderate-intensity exercise, the L_{ox} rates are elevated because of

the increased plasma FA availability and increased transport of FA away from adipose tissue and toward the exercising muscle. The increase in FA availability and therefore, L_{ox}, decreases G_{ox} by suppressing the pyruvate dehydrogenase complex activity and reducing glycolytic flux (Randle, Garland, Newsholme, & Hales, 1965).

Literature regarding exercise intensity and L_{ox} has been contradictory. Researchers have shown an increase in resting L_{ox} after endurance training at a moderate intensity in young fit individuals and elderly (Calles-Escandon, Goran, O'Connell, Nair, & Danforth, 1996; Romijn et al., 1993). Conversely, researchers found no effect of exercise intensity on resting lipid metabolism in lean and obese men and women (Friedlander, Casazza, Horning, Usaj, & Brooks, 1999; Friedlander, Casazza, Horning, Buddinger, & Brooks, 1998; van Aggel-Leijssen, Saris, Wagenmakers, Hul, & van Baak, 2001; van Aggel-Leijssen, Saris, Wagenmakers, Senden, & van Baak, 2002). Other studies have attempted to determine the intensity at which the rate of L_{ox} is at its maximum by comparing three or four different exercise intensities. In one study, researchers studied eight endurance-trained women at rest and during 25, 65 and 85% $\dot{V}O_{2max}$. They found that G_{ox} increased steadily with exercise intensity while L_{ox} peaked during exercise at 65% $\dot{V}O_{2max}$ (Romijn, Coyle, Sidossis, Rosenblatt, & Wolfe, 2000). On the other hand, eight male cyclists were studied at rest and during three consecutive 30 min stages of exercise at varying intensities of 40, 55, and 75% of maximal work load (W_{max}). Researchers concluded that whole-body peak L_{ox} occurred at 55% of W_{max} (equal to 57% of $\dot{V}O_{2max}$) (van Loon et al., 2001).

Despite conflicting research, Achten, Gleeson, and Jeukendrup (2002) developed a continuous graded cycling exercise test to determine the maximal fat oxidation (MFO). The graded exercise test to exhaustion on a cycle ergometer consisted of 5 min stages with 35-W increments. Authors have reported a MFO corresponding to 64±4% $\dot{V}O_{2max}$. Although this value for MFO is higher than those from the studies described above, it is worth noting that exercise intensities lower than 3.35 $kJ \cdot L \cdot O_2$ were not included in the MFO calculation.

An interesting note to make is that absolute L_{ox} rates are dependent on CHO intake (Achten, Gleeson, & Jeukendrup, 2002). This was demonstrated in a study by Bergman and Brooks (1999) who investigated seven trained and untrained men who exercised at target power outputs of 22% and 40% $\dot{V}O_{2max}$ for 2 hr, 59% $\dot{V}O_{2max}$ for 1.5 hr, and 75% $\dot{V}O_{2max}$ for a minimum of 30 min after either a 12 hr overnight fast or a 3 hr after a standardized breakfast. Absolute $\dot{V}O_{2max}$ for trained individuals was found to be 4.27±0.21 $L \cdot min^{-1}$ and 2.40±0.13 $L \cdot min^{-1}$ for untrained individuals. In the fasted state, MFO occurred at 40% $\dot{V}O_{2max}$ for trained participants compared to 59% $\dot{V}O_{2max}$ for untrained individuals (Bergman & Brooks, 1999) while in the fed state, the highest absolute rates of L_{ox} were observed at 40% $\dot{V}O_{2max}$ in both trained and untrained individuals. After an overnight fast of 12 hr, it appears that MFO occurs at a lower % $\dot{V}O_{2max}$ for trained individuals which is close to the MFO point of the current study.

The studies mentioned above clearly demonstrate that low- to moderate-intensity exercises result in higher rates of L_{ox} than high-intensity exercise. Since lipid utilization is

impaired in obese individuals, it is logical to assume that low- to moderate-intensity exercises are best suited to up-regulate L_{ox} in the obese person.

Obese individuals

As previously discussed, research suggests that training within a low- to moderate-intensity range at a relative MFO point may be advantageous to the weight loss process, especially for the obese persons. According to Blaak and Saris (2002), obesity is associated with a diminished capacity to use fat as fuel during exercise. This is reported in a study conducted by Perez-Martin and associates (2001) that looked at substrate oxidation during submaximal exercise between lean and obese individuals. The experimental groups consisted of 32 and 26 overweight and normal weight individuals, respectively. Both groups had a mix of men and women. All volunteers were sedentary and *untrained* and no dietary restrictions were made. Participants underwent a test with the same relative workload and compared at the same percentage of maximal aerobic power (W_{max}). The main findings were that the RER was similar in both groups at rest but was increased during exercise in the overweight group which indicates that the rates of L_{ox} during exercise were significantly decreased in the overweight group. Other interesting results from this study is that the crossover point of substrate utilization was decreased in the overweight group and the MFO point was also much lower in this group as well (Perez-Martin et al., 2001). Normally, at low- to moderate-intensity exercise, there is a larger contribution of lipid as the fuel source but as aerobic power output reaches 60-65%, the body switches to deriving more of its energy from CHO than from lipids. In the previous study discussed, the overweight group had a lower crossover

point than the lean group – the crossover point for the overweight group was at about 33% of their W_{max} and for the lean group the point was at approximately 50% of their W_{max}.

In a study by Dumortier and colleagues (2003), 28 obese individuals trained for 40 min, 3 days per week for 8 wk at MFO. Along with significant reductions in body weight and waist and hip circumferences, significant increases in $\dot{V}O_{2max}$ and the rate of L_{ox} at MFO were detected. However, it should be noted that the exercise training was supervised only at the beginning of the program with the rest of the sessions completed as home-based exercises. Also, there was no comparison to another group completing a high-intensity interval-based exercise (Dumortier et al., 2003).

Although training at generic or individually-based exercise intensities have been shown to enhance skeletal muscle capacity to utilize fat during exercise in lean individuals (Talanian, Galloway, Heigenhauser, Bonen, & Spriet, 2007) and some obese individuals (Bruce et al., 2006), evidence is scarce as to the impact of exercise intensity on substrate oxidation in overweight and obese men. In one study that examined 24 healthy obese males, subjects were divided into one of three groups: low-intensity (40% $\dot{V}O_{2max}$), high-intensity (70% $\dot{V}O_{2max}$) or a non-exercising control group. Exercising groups trained three times a week for 12 wk where sessions lasted 57.1±8.0 and 32.8±2.5 min, for the LI and HI groups, respectively. Researchers found an increase in L_{ox} during the LI exercise while finding no effect on L_{ox} after the HI exercise for the same duration and energy cost of exercise (van Aggel-Leijssen et al., 2002). Conversely, Saris and Schrauwen found no difference in substrate oxidation over 24 hr in eight obese men who cycled at either high intensity (three x

30 min alternative bouts of 2.5 min at 80% and 50% W_{max}) or low intensity (three x 60 min at 38% W_{max}) in a respiration chamber (Saris & Schrauwen, 2004). The major difference between these two studies lies in its experimental design; one study randomly divided 24 obese men into one of three groups (van Aggel-Leijssen et al., 2002) while a randomized crossover design was followed in Saris and Schrauwen's study (2004). The design followed for each study may be the reason for the difference in results. However, in another counterbalanced, crossover design, Venables and Jeukendrup studied eight sedentary, obese healthy men who underwent two 4 wk blocks of endurance training at either a predetermined intensity eliciting MFO (TP_{CON}) or at 5 min intervals of ± 20% MFO (TP_{INT}) (Venables & Jeukendrup, 2008). TP_{CON} was 44% ± 6% $\dot{V}O_{2max}$ with the low and high intensities for TP_{INT} being 25 ± 6% and 65 ± 6% $\dot{V}O_{2max}$. EE did not differ between the two exercise protocols and there was a significant reduction in RER for the TP_{CON} group compared to the TP_{INT}. Rates of L_{ox} increased by 44% after TP_{CON}, but no change was observed after $TP_{INT.}$ Further, there was a 27% increase in the insulin sensitivity index after TP_{CON} but no change was observed after $TP_{INT.}$

Furthermore, studies that have examined substrate partitioning in obese individuals have concluded that obese individuals have a higher RER thus lower rate of L_{ox} during exercise. However, one study conducted by Goodpaster et al. (2002) resulted in an opposite conclusion. These researchers examined the effects of obesity on plasma and non-plasma fatty acid oxidation during exercise. Seven lean (BMI 20 to 24 kg m^{-2}) and seven obese (BMI ≥ 30 kg m^{-2}) healthy, sedentary men matched for age (25 to 45 years) performed 60 min of moderately intense cycle ergometry exercise at approximately 50% of their previously

determined $\dot{V}O_{2max}$. Participants were given a standardized meal the night before and then fasted until completion of the study. Researchers found that fasting plasma triacylglycerol (TAG) and FFA levels before exercise did not differ significantly between lean and obese men. Mean plasma palmitate and plasma glucose concentrations increased during exercise in the obese group. What is most interesting about this study is that at rest there was no difference in the RER between both groups but RER at all time-points and the mean RER during exercise was *lower* in obese men compared to lean men (Goodpaster, Wolfe, & Kelley, 2002). This indicates that obese men were deriving more of their energy from fat than from CHO also indicating that total G_{ox} was lower in obese men during the exercise. In addition, obese men had a 10% higher fasting plasma glucose level and a 3-fold higher post-absorptive insulin levels than lean men. It was also concluded that rates of glycogen use were 50% lower in obese men as well. Overall, this study established that rates of fatty acids oxidation derived from non-plasma sources were increased in obese men but both the obese and lean groups had similar rates of plasma FA oxidation (Goodpaster et al., 2002). Although participants recruited were sedentary, it was not specified if they have been physically inactive for a certain length of time therefore, the fitness level of the obese participants could have had an impact on the substrate partitioning during exercise.

2.5 The "Fat-Fit" Concept

As indicated from most of the above studies briefly described, obesity has been associated with impaired L_{ox} during exercise. However, it may be possible that impaired L_{ox} is not associated with all obese people. Like the idea that not all normal weight individuals are

"metabolically healthy", not all obese individuals are metabolically unhealthy (Karelis et al., 2004). MHO individuals are a unique subset of obese persons who, despite having excess body fat, display a favorable metabolic profile characterized by high insulin sensitivity levels, a favorable lipid profile, and no sign of hypertension (Karelis et al., 2005).

Metabolic response in fit, lean individuals

To understand the "fat-fit" concept, one must first understand a normal metabolic response. BMR is the minimal amount of energy required to sustain the body's vital functions in the waking state. According to Simonson & DeFronzo (1990), it is the largest contributor to daily basal EE and is dependent on age, sex and body size. Total EE (TEE) consists of BMR, thermic effect of food (TEF), non-exercise activity thermogenesis (NEAT) (i.e. daily activities such as cooking, housework, and walking to the car) and physical activity. RMR consists of BMR and NEAT and is the energy expended at rest in the fasted state. RMR, in normally active healthy individual, accounts for 60-75% of TEE and correlates strongly with fat free mass (FFM) and body weight (Ravussin, Burnand, Schutz, & Jequier, 1982). In the early 1980s, Tremblay and associates (1983) examined the influence of exercise training on dietary-induced thermogenesis (DIT) or TEF. Eight trained and non-trained males participated in the study. During their second session, after determination of $\dot{V}O_{2max}$, fasted subjects were given a mixed-meal (244 kcal PRO, 629 kcal CHO, and 763 kcal FAT). A significant correlation was observed between postprandial RER and TEF indicating that the reduced EE observed in trained subjects is related to greater L_{ox} (Tremblay, Cote, & LeBlanc, 1983). Later in the same decade, Tremblay and associates (1986) conducted a study

to establish whether exercise-training has an influence on RMR. In a cross-sectional design, RMR was measured between 7 and 8 am after an overnight fast of at least 10 hr in 20 trained and 39 non-trained lean, male subjects and it was found that trained subjects exhibited a significantly higher RMR than untrained individuals. When expressed per unit of FFM, a significant correlation between RMR and FFM was observed for both groups along with a trend toward significance between groups on RER (Tremblay et al., 1986).

The topic of food utilization efficiency has been gaining popularity with the study of human energy balance and weight regulation (Poehlman, Melby, & Badylak, 1988). Poehlman and colleagues compared RMR and TEF in trained and untrained individuals (18 lean, men aged 18-37 yr). At least 24 hr after $\dot{V}O_{2max}$ was determined, RMR and TEM were established for each individual by IC using a ventilated hood system. They concluded that highly trained subjects ($\dot{V}O_{2max}$ 70.5±1.8 $ml \cdot min^{-1} \cdot kg^{-1}$) demonstrated a higher RMR when expressed in kilocalories per minute and per kg fat-free mass than untrained subjects. Interestingly, TEF was lower in trained than untrained men which signifies an increased efficiency in energy utilization after meal consumption which confirms what Tremblay and colleagues (1983) had observed in their study.

Since RMR represents such a large fraction of TEE, exercise which significantly alters RMR may have a substantial impact on energy balance. Several studies have examined the effects of exercise on substrate partitioning in lean subjects. Interestingly, the metabolism of lean individuals seems to differ in terms of substrate partitioning depending on fitness status. For example, G_{ox} has been shown to be significantly higher in lean, sedentary men

compared to lean, endurance-trained men when performing a cycle ergometer exercise (Sidossis, Wolfe, & Coggan, 1998). In this study, all experiments were performed in the morning after a 12-h overnight fast. The sedentary group and the trained group exercised on a cycle ergometer at a $\dot{V}O_2$ of ~2.0 $L \cdot min^{-1}$, representing 80 and 40% peak $\dot{V}O_2$, respectively. Additionally, researchers found that the oxidation of oleate, a long-chain fatty acid, was higher in the endurance-trained group compared with sedentary men (Sidossis et al., 1998). The results of this study are pertinent to the current study as they suggest that endurance training may increase L_{ox} during exercise in part by enhancing FA entry into the mitochondria. In 1999, Bergman and Brooks showed that during a cycle ergometer exercise, trained men showed a significantly decreased RER when compared to untrained men in both fed and fasted state. Trained, fasted subjects exhibited a greater contribution of lipid to TEE and greater absolute rates of L_{ox} at 22 and 40% $\dot{V}O_{2peak}$, respectively, while increased rates of absolute G_{ox} were observed at 59 and 75% $\dot{V}O_{2peak}$. It was concluded that training increases relative L_{ox} during mild-to-moderate exercise only when subjects exercise in the fasted state (Bergman & Brooks, 1999). Furthermore, recent literature has further expanded on the idea of nutritional impact on RER – the notion of food quotient – and L_{ox} in male athletes. Exercise in a post-absorptive state was performed with a lower RER compared with that in the post-prandial state. More importantly, overall 24 hr $L_{ox}$was significantly higher when exercise was performed in a post-absorptive state (Shimada et al., 2013).

In 2005, using lean, moderately active men and women, researchers determined that while CHO was the major fuel source during moderate-to-high-intensity exercise, there was substantial post-exercise L_{ox} augmentation. Additionally, L_{ox} was the same during post-

exercise recovery whether the relative power output was 45% or 65% of $\dot{V}O_{2peak}$ when EE of exercise was matched by energy intake (Kuo, Fattor, Henderson, & Brooks, 2005).

Evidently, rates of L_{ox} appear to differ between lean individuals who are endurance-trained (fit) compared with those who are sedentary. This may relate to the limiting mitochondrial fatty acid uptake and oxidation during high-intensity exercise in sedentary individuals, as suggested by Sidossis et al. (1998). During exercise, RER was significantly higher in lean, sedentary men and women than lean, trained men and women (Melanson et al., 2009). Although there was nothing of significance the lean, trained group tended to have a greater L_{ox} during rest and exercise when compared to the lean, sedentary group (Melanson et al., 2009). It is highly probable that there were no significant differences between the groups for L_{ox} as each group contained a small number and a mix of men and women.

The studies described above demonstrate that lean, trained individuals have a lower RER and therefore increased L_{ox} during rest and exercise when compared to lean, sedentary individuals. The fact that L_{ox} differs between fit and unfit persons even though they are lean suggests that bodyweight may not negatively impact L_{ox} but rather the fitness status of the individual.

Cardiorespiratory fitness

As previously mentioned, although obesity is a risk factor for NIDDM, IR and increases the likelihood of cardiovascular disease (CVD), not all obese persons have these risk factors (Després & Lemieux, 2006). CRF is the ability of the body's circulatory and respiratory systems to supply fuel and oxygen to active tissues during sustained physical activity. It

represents a very good indicator of physical activity performance. This should not be confused with physical fitness which is defined as a set of attributes a person has regarding a person's ability to perform physical activities that require endurance, strength, or flexibility and is determined by a combination of regular activity and genetically inherited ability. CRF is another important factor when considering body composition and mortality. A low level of CRF is a strong and independent predictor of CVD and all-cause mortality (Lee, Blair, & Jackson, 1999) and is of considerable importance with that of diabetes mellitus and other CVD risk factors such as smoking, hypertension and high cholesterol (Wei et al., 1999). It has been shown that physically unfit men with low waist girths (<87 cm) have greater risk of all-cause and CVD mortality than fit men with high waist girths (>99 cm). Church and Blair (2004) observed, among the fat-fit individuals, an association between physical activity and reduced risk of chronic disease. In a previous study involving 3,217 men who were fat, fit and active compared with 2,182 men who were fat, unfit and inactive, they determined a lower risk of CVD in the men who were fat, fit and active. CRF was determined by $\dot{V}O_{2max}$ using a graded maximal treadmill test. For all subjects, the treadmill speed, was held constant. The initial grade was set at 0% for the 1st and 2nd min, was 2% for the 3rd min, and was increased by 1% every 2 min thereafter. Participants were placed into either a low-, moderate-, or high-CRF group ($\dot{V}O_{2max}$ 29 ± 3.7, 36.2 ± 3.7, 44.3 ± 4.6 ml·min·kg^{-1}, respectively) (Lee et al., 2005). Researchers concluded that a high CRF status is linked with a significant reduction in health risks for a given level of visceral and subcutaneous fat when examining the association between CRF levels to MetS and all-cause mortality. (Lee et al.,

1999). The results from these studies indicate that increasing CRF alone may serve to reduce the complications normally associated with obesity.

The location of fat in an obese person is also important in the determination of metabolic alterations. For example, visceral (deep-abdominal or central) fat is more often associated with increased risks for conditions such as diabetes, hyperlipidemia, and hypertension than subcutaneous fat, mainly the subcutaneous fat that is stored in the lower body (Després et al., 1990). In one study examining skeletal muscle utilization of FFA among women with visceral obesity, it was observed that women with a higher visceral fat content had a decreased uptake of FFA across the leg muscle during basal conditions (Colberg et al., 1995). Also, visceral fat was lower in men who were fat and fit and higher in men who were fat and unfit (Lee et al., 2005). Fit men had lower triacylglycerol levels and higher high-density lipoprotein (HDL) cholesterol levels for a certain amount of visceral fat (O'Donovan et al., 2009). Interestingly, the ratio of visceral fat to subcutaneous abdominal adipose tissue (SAAT) was lower in slim-fit and fat-fit than slim-unfit and fat-unfit individuals. Visceral adipose tissue more closely associated with higher risks of chronic diseases than SAAT (O'Donovan et. al, 2009). Obese individuals (fat-unfit) with an excess of abdominal fat displayed a diminished capacity of subcutaneous fat storage that results in accumulation of fat in undesired locations such as the liver, pancreas (ectopic fat), the heart and skeletal muscle (Després & Lemieux, 2006). These above-cited scientific reports point to the importance of fitness in general and more specifically, cardiorespiratory fitness on health status of overweight and obese individuals.

2.6 Metabolic syndrome

Metabolic syndrome is a multifactorial condition that includes obesity (mainly central abdominal obesity), hypertension, dyslipidemia, and type 2 diabetes (Després & Lemieux, 2006). IR is a core component of the MetS. Whole-body IR may be caused by an oversupply of lipid to insulin-sensitive tissues and an accumulation of fat in the abdominal region. Fat in this region is easily altered and fatty acids can drain directly into the portal system (Greenfield & Campbell, 2004). According to Lewis and associates, lipolysis, or the breakdown of triacylglycerol, in the central abdominal adipocytes is less well suppressed by insulin (Lewis, Carpentier, Adeli, & Giacca, 2002). There is also a great amount of interest in how altered lipid metabolism contributes to muscle IR. A build-up of FAs in insulin-sensitive non-adipose tissues (i.e. muscle and liver), can impair glucose uptake via insulin depend mechanisms (Hegarty, Furler, Ye, Cooney, & Kraegen, 2003).

Obesity and physical training

Several studies have shown improvement in glucose tolerance and insulin action in insulin-resistant humans. Almost 50 years ago, researchers subjected 10 obese patients to physical training that consisted of a 8 wk period, 4 hr a day, five days a week during the first two weeks and thereafter 3 hr a day three days a week. After the training period, no change in body fat was observed, therefore; the increase in insulin sensitivity is not due to adipose tissue factors (Bjorntorp, De Jounge, Sjostrom, & Sullivan, 1970). Furthermore, the increase in insulin sensitivity did not coincide with the increase in body cell mass. In other words, increased insulin sensitivity was unrelated to the increase in muscle mass. When studying an

obese population, it appears that muscle may be an important determining factor for insulin sensitivity (Bjorntorp et al., 1970). In 1982, researchers concluded that aerobic endurance training resulted in an increase in the oxidative capacity of skeletal muscle (Gollnick & Saltin, 1982). In a randomized controlled study, 154 sedentary, overweight/obese subjects were assigned to either a control or an exercise group for 6 mo. Exercise groups consisted of either a low-volume/moderate intensity (12 miles walking per week at 40-55% $\dot{V}O_{2max}$), a low-volume/high-intensity (12 miles jogging per week at 65-80% $\dot{V}O_{2max}$) or a high-volume/high-intensity (20 miles jogging per week at 65-80% $\dot{V}O_{2max}$). Volume consisted of 115 min per week for the low-volume/high-intensity group and 170 min per week for the other two groups. Researchers examined the effect volume and intensity of exercise training on insulin sensitivity and found that insulin sensitivity improved substantially in groups that exercised for 170 min per week than the group that exercised for only 115 min per week, regardless of exercise intensity and volume. This leads to the conclusion that exercise duration is more important than volume and exercise intensity when trying to improve insulin sensitivity (Houmard et al., 2004).

DeFronzo and colleagues (1987) evaluated insulin secretion and action with a hyperglycemic and euglycemic insulin clamps in 7 moderately obese subjects before and after a 6 wk training exercise training program; controls consisted of 39 normal-weight, age-matched subjects. After the 6 wk training period, fasting plasma insulin concentrations fell by 26% and total plasma insulin response during the hyperglycemic clamp declined by a similar percentage. Before the training program, obese subjects were insulin-resistant but afterwards, a significant improvement in tissue sensitivity to insulin was observed, however,

insulin sensitivity remained less than the control group. Researchers concluded that a moderate-intensity physical training program is capable of partially ameliorating the IR observed in obese individuals; both peripheral and hepatic tissues contribute to the improved tissue sensitivity to insulin (DeFronzo, Sherwin, & Kraemer, 1987). It is worthy to note that all obese subjects were women and controls consisted of both men and women. Researchers did not mention if they controlled for the menstrual cycle of women which could have had an impact on hormone secretion and substrate partitioning.

In 1993, Romijn and associates studied the regulation of endogenous fat and CHO metabolism in relation to exercise intensity and duration. Testing trained cyclists at different exercise intensities, researchers found an increase in plasma glucose concentrations after 30 min of exercise at 25, 65, and 85% $\dot{V}O_{2max}$; however, during subsequent 90 min of exercise, glucose concentrations did not change at 25% $\dot{V}O_{2max}$ and decreased to pre-exercise levels at 65% $\dot{V}O_{2max}$ (Romijn et al., 1993). On the other hand, researchers who concluded that exercise increases L_{ox} at rest unrelated to changes in energy balance or lipolysis, found no change in plasma glucose concentrations after an intervention of exercise (Calles-Escandon et al., 1996). Participants were randomly assigned to either a control group (C), an over-fed group (OF), an over-fed + exercise group (OF-EX) or an exercise group (EX). Plasma glucose and serum insulin did not differ significantly between groups at baseline but it was found that serum insulin decreased in both the OF-EX and EX groups (Calles-Escandon et al., 1996). It appears that regardless of diet, exercise has an impact on insulin levels within the body.

Additionally, studies have also investigated the effect of exercise intensity on insulin sensitivity in individuals with and without NIDDM. In a study conducted by Kang et al. (1996), 6 obese men and 6 obese men with NIDDM exercised for 7-days on two separate occasions, cycling at a power output of 50% $\dot{V}O_{2max}$ for 70 min or 70% $\dot{V}O_{2max}$ for 50 min. Researchers found that exercising at 70% $\dot{V}O_{2max}$ for 50 min improved exercise-induced insulin sensitivity in the obese men only while the intensity had no significant effect on insulin sensitivity in the obese men with NIDDM. However, this could be because the latter group were relatively hypoinsulinemic (Kang et al., 1996). When examining women with NIDDM, researchers found that low-intensity exercise was just as effective as high-intensity exercise in enhancing insulin sensitivity when EE was matched (Braun, Zimmermann, & Kretchmer, 1995).

Furthermore, the effect of exercise training at difference intensities on fat metabolism in obese men was investigated. Plasma samples of total FFA, glycerol, TAG, and insulin were taken. After a 12 wk exercise intervention, at rest, researchers found that plasma FFA oxidation was decreased significantly in the high-intensity (HI) group after training but found no change in the low-intensity (LI) or control groups. Plasma glycerol levels were not significantly different after the intervention from baseline and while plasma TAG concentrations were reduced in the HI group ($p<0.05$). Plasma insulin did not change at rest after the intervention. However, during the exercise intervention, plasma FFA, glycerol, and TAG concentrations after training were decreased significantly from before in the HI group while levels of plasma insulin did not change (van Aggel-Leijssen et al., 2002). A few years later, Bruce and associates (2006) found that endurance training in obese humans improved

glucose tolerance and mitochondrial fatty acid oxidation and altered muscle lipid content. Over an 8 wk period, obese individuals exercised at a moderate intensity. After training, researchers found a significant improvement in glucose tolerance, with a reduction under the area of the curve for glucose and insulin during an oral glucose tolerance test (OGTT) (Bruce et al., 2006).

2.7 Conclusive remarks

In summary, current literature has indicated that being obese can have an adverse impact on substrate oxidation during rest and exercise. Previous research has additionally shown that exercise training can be an effective strategy for improving metabolic and physiological health. The fitness level of individuals regardless of body composition seems to also have an impact on metabolic health which is highlighted through the "fat-fit" concept. To extend the work of our predecessors, this project will examine the effect that fitness has on energy metabolism in obese fit (OF) and lean fit (LF) healthy men at baseline, during and after exercise. More specifically, the intention of this research is to examine the contribution of substrates to energy production of OF and LF individuals. It is hypothesized that a high fitness level, regardless of body composition, would alter L_{ox}. More specifically, it is predicted that L_{ox} would be higher in lean, fit men compared to obese, fit individuals.

CHAPTER 3 METHODOLOGY

3.1 Study participants

Sixteen men aged 19-50 years old were recruited for this study. Participants were divided into two groups which consisted of eight physically fit lean men and eight physically fit obese men. Participants pool consisted of individuals from St. John's, Newfoundland and the surrounding area; participants were recruited by posters and investigators. During the familiarization session [first], participants completed a modified version of Baecke's Habitual Physical Activity Questionnaire to determine level of activity and a Physical Activity Readiness Questionnaire (PAR-Q) to screen for lifestyle and medical conditions including smoking, hypertension, cardiorespiratory disease, diabetes, musculoskeletal injuries or a family history of any of the above-mentioned conditions. Individuals were excluded from the study if they failed the PAR-Q, had less than a fair $\dot{V}O_{2max}$ score for their age group according to ACSM criteria (American College of Sports Medicine, 2014) took prescribed medications of any kind, were smokers, had a fasting blood sugar level $\geq$6.0mmol·L^{-1}, or diagnosed as having respiratory problems, heart disease, hypertension, chronic or acute illness, anxiety disorders, and drug or alcohol abuse. Each participant signed a written informed consent in compliance with the declaration of Helsinki and with Memorial University's ethics committee regulations. Screened participants were then given information about the equipment used in the study and the experimental design. Additionally, participants underwent anthropometric measurements [height, weight, and waist circumference], a fasting

blood glucose (FBG) test, and had their vital signs [BP, heart rate, and body temperature] monitored.

Participants must also have been involved in a training program consisting of a minimum of 4 hr a week of structured activity for at least the past 6 months. Participants were instructed to manually record their food intake and to wear a physical activity tracker in order to monitor their physical activity level. Anthropometric and training and CRF characteristics of the study participants are reported in Tables 4-1 and 4-2, respectively.

3.2 Study Timeline

Each participant came to the lab for a total of 3 sessions on separate days. A familiarization session was the first session in which all participants were informed of the study procedure and received instructions regarding the completion of the food and physical activity log. Anthropometric measurements, vital signs (VS) and a FBG test were taken during this session and at the beginning of the testing and experimental sessions.

During the testing session, BMR was collected between 07:00 and 08:00. Participants were required to void their bladder right before this measurement. Following completion of BMR, the first urine sample (US) was collected from each participant. Subjects then completed an incremental test on a treadmill to determine $\dot{V}O_{2max}$ and the maximal fat oxidation (MFO).

During the experimental [and final] session, BMR was collected between 07:00 and 08:00. At 08:00 subjects performed a treadmill exercise at their previously determined MFO point until 300 kcal were expended. Thirty minutes after the end of exercise, participants

underwent a post-exercise metabolic rate (PEMR) measurement. At 10:00, participants were given a meal. Two post-meal metabolic rate (PMMR) measurements were taken 30 min and 90 min after the meal. Blood samples (BS) were collected at 07:00, 09:00, and 10:00, and every 30 min after this time point; urine was voided at 07:00 a.m. [before BMR], and urine samples were collected at 07:45, 09:00, and after each metabolic rate measurement. See study timeline in figure 3-1.

Anthropometrics. Height, weight, waist circumference and body composition (i.e. % body fat and lean mass) measurements were obtained from all participants. Height (using stadiometer, ± 0.1 cm – Perspective Enterprises, Portage, Michigan, USA) and body weight (using a weight scale, ± 50.00g or 0.02% of the maximum scale – LifeSource, model UC-321, A&D Company, Tokyo, Japan) values were recorded. Waist circumference was measured using a tape measure and the abdominal area was free of clothing. Starting at the hip bone, the tape measure was wrapped all the way around the waist at level with the belly button. Body composition was determined using DXA (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI).

DXA. Body composition measurements for each participant were determined by Dual-Energy X-Ray Absorptiometry (DXA) on a day chosen by the participant if it occurred before the experimental session. DXA was collected at the Health Sciences Centre (St. John's, NL). For this measurement, all participants were asked to remove any metal or jewellery from their body and change into a hospital gown to ensure accuracy of the measurement. The subjects were then weighed on a platform manual scale balance (Health O Meter Inc, Bridgeview, IL). DXA (Lunar Prodigy; GE Medical Systems, Madison, WI) was

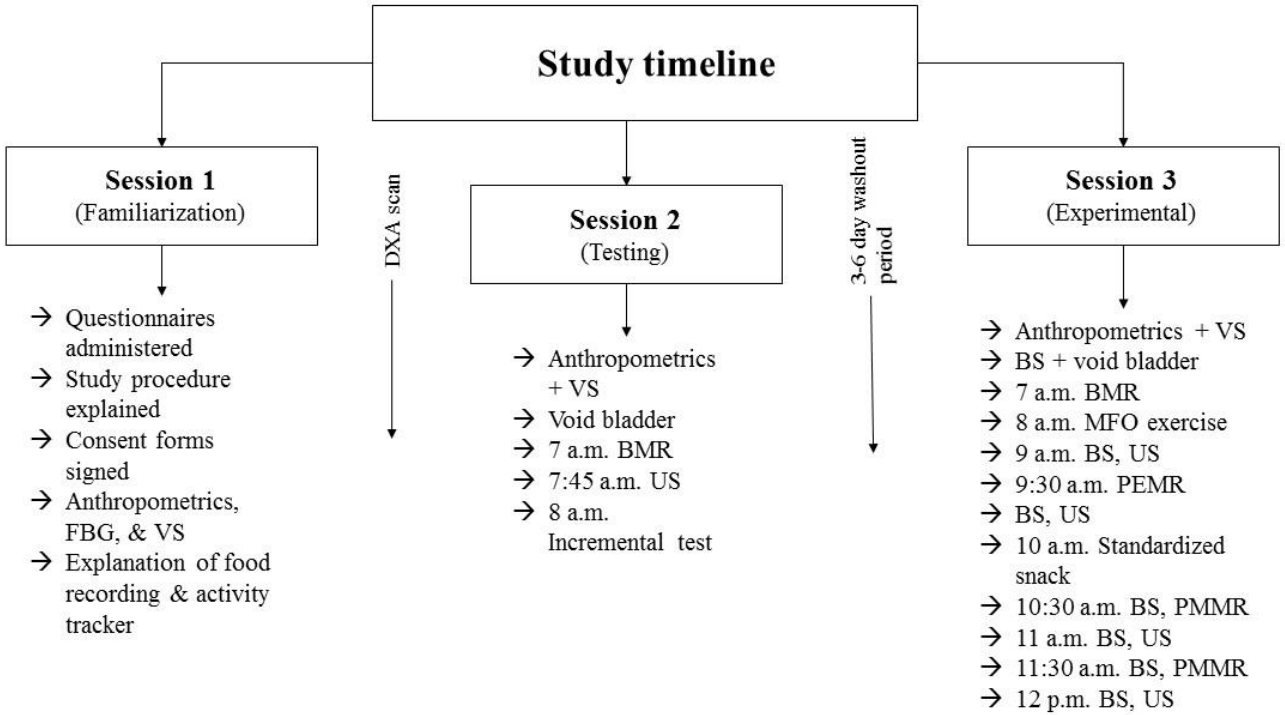

Figure 3-1. Experimental design

used for the measurement of whole-body composition, including fat mass, lean body mass (which comprised of muscle, internal organs, and body water), and bone mineral mass and density. Percent body fat was calculated from entire BM (including bone mineral densities) by using the manufacturer's software (version 4.0). DXA measurements were performed while the subject was lying in a supine position. (Sun et al., 2005)

Questionnaire. Each participant was given a PAR-Q to fill out along with a detailed physical activity history questionnaire to ensure that individuals were routinely active within the last six months. The PAR-Q was provided by the Canadian Society for Exercise Physiology (CSEP) and the detailed physical activity questionnaire was a modified version of Baecke's habitual physical activity questionnaire (Baecke, Burema, & Frijters, 1982). This questionnaire was developed to evaluate a person's habitual physical activity and was separated into three distinct dimensions: work activity, sports activity and leisure time activity. However, we were not interested in NEAT so we excluded the work and leisure time activity sections (see appendix A).

Food intake and physical activity logs. The experimental design also required control of energy balance and monitoring of physical activity. In doing so, participants were given an explanation on how to record their food intake and how to use the Garmin Vivofit activity tracker. Throughout the experiment, participants were required to keep a record of their food intake. Depending on the participant's schedule, this ranged from 3-6 days. A minimum of three days (one weekend day and two weekdays) of food recording was required to obtain a more accurate idea of each participant's diet. All participants were given blank copies of food

logs and a short tutorial on how to record their food intake. Participants were also given standard measuring cups to assist in quantifying their intake. Food logs were collected by the research team at the beginning of the last session. Food data was then entered on a web-based program, TotalCoaching.com, which calculated the total number of calories and the amount of CHOs, lipids, and protein the individual consumed [based on the Canadian Nutrient File, (Health Canada, 2015)]. Physical activity was monitored using the Vivofit activity tracker that well-estimates daily EE and physical activity in the number of steps and distance travelled (Alsubheen, George, Baker, Rohr, & Basset, 2016)

***Standardized meal*.** After the MFO treadmill exercise, participants were provided with a standardized meal (300 kcal; 12g fat, 42 g CHO, and 10 g protein) to nullify the energy deficit created by exercise. The intent of this procedure was to examine the metabolic response (substrate partitioning) of participants independent of exercise.

Blood samples. An 18G polyethylene catheter was inserted into the antecubital vein from where background blood draws were taken. Seven 4-ml BS were collected from each participant. All BS were kept frozen at -20^{o}C until further analyses. Analyses of BS included assays for insulin, glucose, non-esterified fatty acids (NEFAs), TAGs, and glycerol. Plasma glucose and TAG were measured using kits from Abbott Diagnostics and automated on a clinical chemistry analyzer (Architect c16000, Abbott Laboratories, Abbott Park, Illinois, USA). Plasma insulin was measured by a chemiluminescence immunoassay technique and automated on an immunoassay analyzer (Architect i2000sr, Abbott Laboratories, Abbott

Park, Illinois, USA). Note that NEFA and glycerol values were not reported due to technical issues.

Urine samples. Participants were required to provide urine samples throughout the study to determine protein oxidation through analysis of urinary urea nitrogen. All urine samples were kept frozen at -20^{o}C until further analyses. Prior to analysis, however; samples were thawed once and were then aliquoted to avoid constant freezing and thawing. Urinary urea nitrogen was determined using an enzyme-based colorimetric assay (Bio Scientific Corporation, Cat. No. 5602-01, Austin, TX). The dilution factor used was 1:26 where 10 µl of urine was diluted in 250 µl of 0.9% sodium chloride (N^{o} 04888, DIN 00037796). All assays were performed in duplicate.

3.3 Respirometry measurements

An indirect calorimetry system (Sable Systems International, Las Vegas NV, USA) collected oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) simultaneously through a canopy during BMR, PEMR, and PMMR. The system was set to record the fractional amount of oxygen and carbon dioxide, mixing chamber temperature, water vapor pressure, barometric pressure, subsample flow rate, and mass flow rate in a negative pressure design. The mass flow generator and controller (FK-500) was set at a rate of 75 L•min^{-1} during all metabolic rates. A subsample of that flow (Sub-sampler, SS4) was then pulled at 130 ml•min^{-1} through a water vapor analyzer (RH-300), a dual infrared carbon dioxide analyzer, and a paramagnetic oxygen analyzer (CA-10 Carbon Dioxide and PA-10 Oxygen Analyzers). Fractions of gases in the room were recorded before and after each measurement

for baseline references. Prior to testing, the oxygen and carbon dioxide analyzers were calibrated with room air and reference gases (100% nitrogen and 1% carbon dioxide gases). Water vapor pressure was zeroed after drying by passing through a column of magnesium percolate and the sub-sampler pump was calibrated using a flow meter (Gilmont Rotameter).

Expired air during both exercise protocols was continuously analyzed with an automated breath-by-breath system (Jaeger Oxycon Pro, Hochberg, Germany) equipped with a nafion filter tube and a turbine flow meter (optoelectric) to record $\dot{V}O_2$, $\dot{V}CO_2$, V_T, BF, and $\dot{V}E$. The system was configured in a push set-up for both the incremental and the fat oxidation test. During exercise, participants' expired air was collected breath-by-breath through a facemask. This experimental set-up was chosen during exercise to obtain real time respirometric measurements to monitor energy expenditure of exercise. Additionally, to ensure accurate performance of both metabolic systems, a propane gas verification was performed with a gas mass flow meter set at 200, 300 and 400 ml min^{-1} and measured $\dot{V}O_2$ and $\dot{V}CO_2$ were compared against theoretical stoichiometric values.

3.4 Incremental treadmill and maximal fat oxidation tests

Participants were first subjected to a modified (incremental) $\dot{V}O_{2max}$ test to determine MFO and $\dot{V}O_{2max}$ scores. Participants began walking at 3 km hr^{-1} on a 3-degree incline. Speed increased by 1.5 km hr^{-1} every 3 min with a constant incline up to 8.5 km hr^{-1}. Afterward, the speed increased by 1.0 km hr^{-1} every 30 sec until participants' volitional exhaustion. Three to seven days after the $\dot{V}O_{2max}$ determination, participants performed an isoenergetic exercise

(300 kcal) on the treadmill at MFO. Speed and duration of the exercise varied depending on the individuals' relative MFO value.

3.5 Basal and post-exercise metabolic rates

Two basal metabolic rates were measured in this study (one during the testing session and the other during the experimental session) along with one PEMR and two PMMRs during the experimental session. Before each BMR, participants were instructed to not consume food or caloric beverages for 12 hr prior but could drink water *ad libitum*. They were also instructed not to engage in moderate-to-high intensity exercise for 36 hr prior to the BMR sessions. Measurements of BMR were recorded in the early morning in a supine position with the participants' head supported by a single pillow; room temperature was maintained at 22ºC and lights were dimmed; participants were instructed to lie motionless but awake and not to talk. Both PEMRs and PMMRs followed the same standardized procedure. Thirty minutes following the maximal fat oxidation treadmill exercise, participants were transferred from the treadmill to the bed where they lay again in the supine position for 30 min while PEMR was recorded. Two PMMRs were, then, recorded 30 min and 90 min after consumption of the standardized meal.

3.6 Substrate oxidation and MFO exercise calculations

Glucose and lipid oxidation rates were calculated according to the following equations from Simonson and DeFronzo (1990) during rest and exercise:

$$G_{ox}\ (g\ min^{-1}) = 4.57\dot{V}CO_2 - 3.23\dot{V}O_2 - 2.60N \qquad (eq.1)$$

L_{ox} (g min^{-1}) = 1.69$\dot{V}O_2$ – 1.69$\dot{V}CO_2$ – 2.03N (eq.2)

P_{ox} (g min^{-1}) = 6.25N (eq.3)

Where $\dot{V}O_2$ and $\dot{V}CO_2$ were expressed in l·min^{-1} and protein oxidation rates were determined through urinary urea nitrogen analysis. EP was then calculated from individual contributions of each substrate to the fuel mixture (Simonson & DeFronzo, 1990) as follows:

EP (kcal·min^{-1}) = 3.74 G_{ox} + 9.46 L_{ox} + 4.32 P_{ox} (eq.4)

3.7 Data reduction

Basal metabolic rate measurements were truncated by 10 min out of 45 min data collection. This procedure discarded the first 5 min and the last 5 min to nullify any signal instability due to familiarization with the ventilated hood and the expected termination of data collection. All metabolic parameters of interest collected during the incremental test were smoothed using polynomial regression models (absolute $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}E$, BF, HR). Ventilatory threshold was determined by plotting ventilatory equivalent of O_2 and CO_2 over absolute $\dot{V}O_2$ to determine the ventilatory threshold. MFO was determined by computing substrate oxidation from equations 1 to 3 and plotting L_{ox} over time to detect its nadir. In addition, the latter time point was used to determine the speed at MFO as well as the duration to expend 300 kcal. Post-exercise and post-meal metabolic rates were truncated by 10 min out of 30 min data collection. This procedure discarded the first 5 min and the last 5 min for the same reasons mentioned above. Respirometry data were then integrated, normalized over time, and used for substrate oxidation calculations and ultimately, EP. Data collected during the MFO exercise test were integrated over time to determine absolute $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{V}E$, BF, HR, substrate oxidation, and exercise duration. Data from the incremental test were used

to determine the speed and length of time the participant exercised during the maximal fat oxidation exercise. Data collection during the latter exercise were also integrated over time and a mean value representing the entire exercise time for each participant was recorded and used for calculations of substrate oxidation and EE.

3.8 Statistical analysis

Power statistics has been calculated using G*Power software. The analysis was performed by computing the alpha and beta error probability, the effect size (0.75) for 2 groups and 4 measurements. The sample size of 8 participants per group was set with a λ of 21.60, a Critical F of 3.098 for a Power of 0.955 (G*Power software). Tests for statistical assumptions (i.e., normality and homogeneity of variance) were performed, that is, the homogeneity of variance was tested using Levene's test and normality was tested using Kolmogorov-Smirnov test. Two-tailed unpaired t-tests were used to compare groups for the anthropometric characteristics, training profile, CRF, nutritional data, physical activity, exercise metabolic and substrate rates, and HOMA-IR. In addition, differences between groups were determined by a 2-way ANOVA with treatment as the independent variable and time (BMR, PEMR, 2 PMMR) as the repeated measure for MR, substrate oxidation, and blood markers. Statistical significance was set at $p < 0.05$. Statistical Package for the Social Sciences (SPSS) for Windows will be used to evaluate data.

CHAPTER 4 RESULTS

4.1 Anthropometric, training and CRF characteristics

Anthropometrics

Anthropometric data for OF and LF are shown in Table 4-1. The statistical analysis shows that there was a significant difference between BM, BMI, waist circumference, fat mass, and body fat percentage ($p<0.001$) as well as a significant difference between visceral fat among the two groups ($p<0.01$). However, no significant difference in lean mass between the two groups was detected. There was no significant difference observed between OF and LF on systolic (129 ± 10 mmHg, 122 ± 9 mmHg, respectively), diastolic (75 ± 6 mmHg, 71 ± 5 mmHg, respectively) BP, or FBG (5.1 ± 0.5 mmol L^{-1}, 4.8 ± 0.4 mmol L^{-1}).

Training profile and CRF scores

Training profile and cardiorespiratory fitness characteristic data are displayed in Table 4-2. There was no statistical difference observed between OF and LF in terms of training. Training intensity, hours of training per week (hr wk^{-1}) and number of months of training per year (mo yr^{-1}) were self-reported by each participant through a physical activity history questionnaire and were not statistically different between the two groups. CRF was assessed using an incremental treadmill test. Absolute $\dot{V}O_{2max}$ did not differ significantly between OF and LF participants. However, a statistical difference was observed between the groups for relative $\dot{V}O_{2max}$ ($p<0.001$) but when expressed in terms of FFM, no statistical difference was found.

Table 4-1. Anthropometric characteristics of participants

Variables	OF (n=8)	LF (n=8)	*p*-value
Age (yrs)	30 ± 12	36 ± 9	NS
Body mass (kg)	105 ± 8	82 ± 5	#
Height (cm)	178 ± 6	181 ± 5	
BMI ($kg \cdot m^{-2}$)	32 ± 4	24 ± 2	#
Waist Circumference (cm)	105 ± 8	82 ± 5	#
Fat mass (kg)	34 ± 7	13 ± 3	#
Fat free mass (kg)	63 ± 10	60 ± 6	NS
Visceral fat (kg)	1.4 ± 0.7	0.2 ± 0.1	**
Body fat (%)	35 ± 4	18 ± 4	#

Mean ± SD, where NS = not significant, and strength of significance is quantified by p<0.05=*, p<0.01=**, and *p*<0.001=#

Table 4-2. Training profile and CRF of OF and LF participants

Variables	OF (n=8)	LF (n=8)	*p*-value
Intensity	2.8± 0.5	2.8± 0.5	NS
hr wk^{-1}	3.8± 1.5	4.4± 1.1	NS
mo yr^{-1}	4.9±0.4	4.9±0.4	NS
VO_2 ($L \cdot min^{-1}$)	4.12 ± 0.55	4.21 ± 0.47	NS
VO_2 ($ml \cdot min^{-1} \cdot kg^{-1}$)	41 ± 5	54 ± 3	#
VO_2 ($ml \cdot min^{-1} \cdot kg^{-1}$ FFM)	66 ± 7	70 ± 3	NS

Mean ± SD, where intensity was quantified as low (=1), medium (=2), and high (=3); hours per week was quantified as <1 hr (=1), 1-2 hr (=2), 2-3 hr (=3), 3-4 hr (=4), and >4 hr (=5); and months per year was quantified as <1 mo (=1), 1-3 mo (=2), 4-6 mo (=3), 7-9 mo (=4), and >9 mo (=5). NS = not significant, and strength of significance is quantified by *p*<0.05=*, *p*<0.01=**, and *p*<0.001=#

4.2 Nutritional and Physical Activity Data

Participants self-reported food intake throughout the study and physical activity was tracked using an activity tracker. Nutritional and physical activity data are displayed in Table 4-3. A statistical difference was observed between OF and LF on total caloric intake ($p<0.05$), fat ($p<0.05$) and CHO ($p<0.05$) intake; however, between the groups, PRO intake, total daily steps, and distance travelled were not statistically significant between the groups.

4.3 Metabolic Data and Substrate Partitioning

BMR Data

BMR was collected during the testing and experimental sessions. $\dot{V}O_2$, $\dot{V}CO_2$, and RER values are shown in Table 4-4. Two BMRs were collected to ensure MR values were within measurement error of 5% (2% instrumentation and 3% biological response). No significant interaction and/or significant main effect was observed between OF and LF in either of the three parameters for the two BMRs. These outcomes confirmed the validity and the reliability of the IC system.

Resting metabolic and substrate partitioning data

Table 4-5 displays the respirometry data for BMR, PEMR, PMMR1, and PMMR2. No statistical differences were observed between OF and LF on $\dot{V}O_2$, $\dot{V}CO_2$, or RER during any of the metabolic rate measurements. However, a significant main effect of time was observed on $\dot{V}CO_2$ and the pairwise comparison showed that $\dot{V}CO_2$ after the meal (PMMR1) differed from all other time points ($p=0.008$). Rates of substrate oxidation can be seen in Figure 4-1.

Table 4-3. Nutritional and physical activity data for OF and LF participants

	OF	LF	*p*-value
Food intake (kcal/day)	1624 ± 291	2604 ± 624	*
Fat intake (g/day)	65 ± 15	103 ± 23	*
CHO intake (g/day)	165 ± 29	310 ± 91	*
PRO intake (g/day)	92 ± 24	122 ± 32	NS
Steps (#/day)	6362 ± 2380	9907 ± 3506	NS
Distance (km/day)	5.1 ± 1.8	7.8 ± 3.1	NS

Mean ± SD, where NS = not significant, and strength of significance is quantified by $p<0.05$=*, $p<0.01$=**, and $p<0.001$=#

Table 4-4. BMR data from two sessions

	BMR1		BMR2		*p*-value
Parameters	**OF**	**LF**	**OF**	**LF**	
$\dot{V}O_2$ (ml·min^{-1})	254 ± 76	239 ± 29	276 ± 85	250 ± 42	NS
$\dot{V}CO_2$ (ml·min^{-1})	211 ± 70	195 ± 41	230 ± 0.081	199 ± 39	NS
RER	0.83 ± 0.06	0.81 ± 0.097	0.83 ± 0.06	0.79 ± 0.07	NS

Table 4-5. Resting metabolic data for OF and LF groups

	BMR		PEMR		PMMR1		PMMR2	
Parameters	**OF**	**LF**	**OF**	**LF**	**OF**	**LF**	**OF**	**LF**
$\dot{V}O_2$ (ml·min^{-1})	276 ± 85	250 ± 42	278 ± 60	242 ± 41	293 ± 87	289 ± 40	294 ± 72	268 ± 55
$\dot{V}CO_2$ (ml·min^{-1})	230 ± 81	199 ± 39	234 ± 65	194 ± 26	243 ± 92	256 ± 43	228 ± 58	219 ± 38
RER	0.83 ± 0.06	0.79 ± 0.07	0.83 ± 0.06	0.81 ± 0.06	0.82 ± 0.11	0.89 ± 0.08	0.78 ± 0.08	0.83 ± 0.08

Mean ± SD, no group effects observed

No significant difference was observed between OF and LF on glucose, lipid, or PRO during any of the metabolic measurements. However, it should be noted that a significant main effect of time on glucose was observed between BMR and PMMR1 (p=0.032) and PMMR1 and PMMR2 (p=0.023). As well, a significant main effect of time was also detected on PRO. Pairwise comparisons showed that PEMR differed from both PMMR1 and PMMR2 (p=0.011).

MFO Exercise

Data from the MFO exercise are displayed in table 4-6. BF, $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, and HR did not differ significantly between the two groups as well as for the rate of substrate oxidation. Furthermore, average length of the exercise was not statistically different between the groups (with a delta of 2 min±5 sec).

4.4 Blood Analysis

Figure 4-2 shows blood glucose, triacylglycerol, and insulin levels as a function of time. The statistical analyses performed on blood markers revealed a significant main effect of time. Since sphericity was not met for the latter blood marker, the Greenhouse Geisser adjustment was applied. The pairwise comparisons showed that blood glucose (p<0.012), triacylglycerol (p<0.001), and insulin (p<0.001) were lower prior PMMR1 compared to BMR as well as lower than PMMR2 (p<0.001 and p<0.007 for triacylglycerol and insulin, respectively). In addition, there was a significant main effect of group on blood insulin level with OF displaying a higher value than LF (p<0.04). Finally, as displayed on Figure 4-3 HOMA-IR score at baseline (or fasting state) was significantly higher for OF than for LF (p<0.04).

Table 4-6. Metabolic parameters during MFO exercise

Parameters	OF	LF	*p*-value
BF (breath·min^{-1})	26 ± 4	25 ± 4	NS
VE (L·min^{-1})	41 ± 8	40 ± 13	NS
$\dot{V}O_2$ (ml·min^{-1})	1612 ± 415	1757 ± 508	NS
$\dot{V}CO_2$ (ml·min^{-1})	1432 ± 377	1550 ± 446	NS
RER	0.89 ± .04	0.89 ± 0.04	NS
HR (bpm)	111 ± 20	97 ± 19	NS
CHO (mg·min^{-1})	1267.8 ± 450	1323.5 ± 569.0	NS
LIPID (mg·min^{-1})	339.0 ± 74.9	393.9 ± 202.0	NS
PRO (mg·min^{-1})	88.5 ± 58.3	84.7 ± 43.6	NS
Time (min)	40 ± 8	42 ± 13	NS

Mean ± SD, where NS = not significant, and strength of significance is quantified by $p<0.05$=*, $p<0.01$=**, and $p<0.001$=#

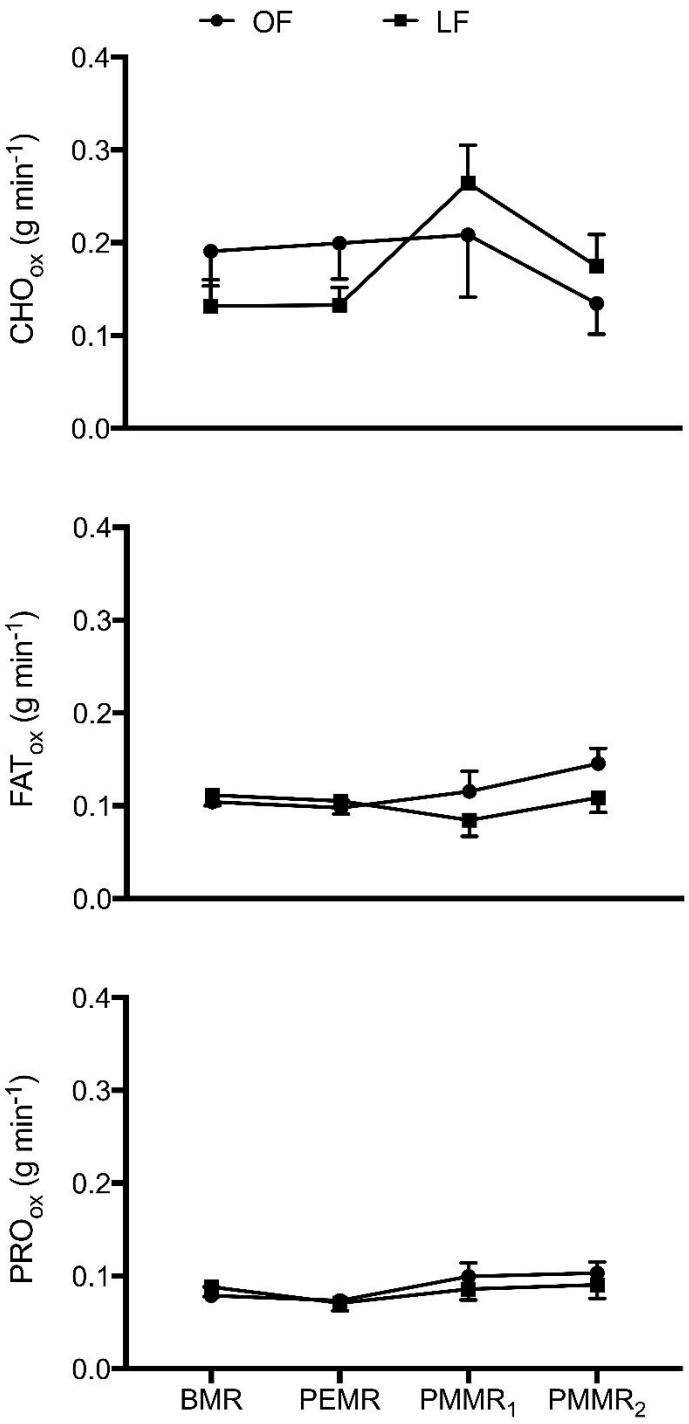

Figure 4-1. Substrate oxidation pre- and post-exercise where BMR=baseline after 12 hr fast, PEMR=30 min post-exercise, PMMR1=30 min post-meal, and PMMR2=90 min post-meal

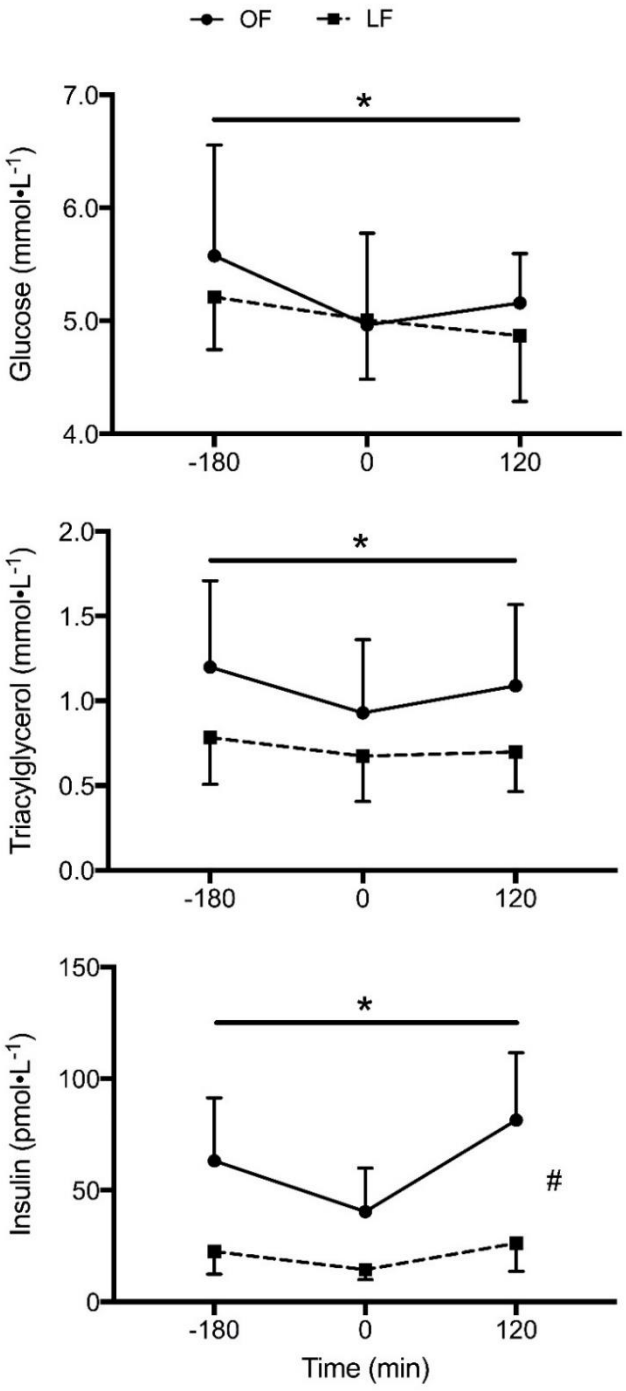

Figure 4-2. Blood glucose (top), triacylglycerols (middle), insulin (bottom). * main effect of time, $p<0.05$; # main effect of groups, $p<0.05$. Pre-BMR, pre-exercise (t=-180 min), 60 min post-exercise, before meal (t=0 min), and 2 hr after meal intake (t=120 min)

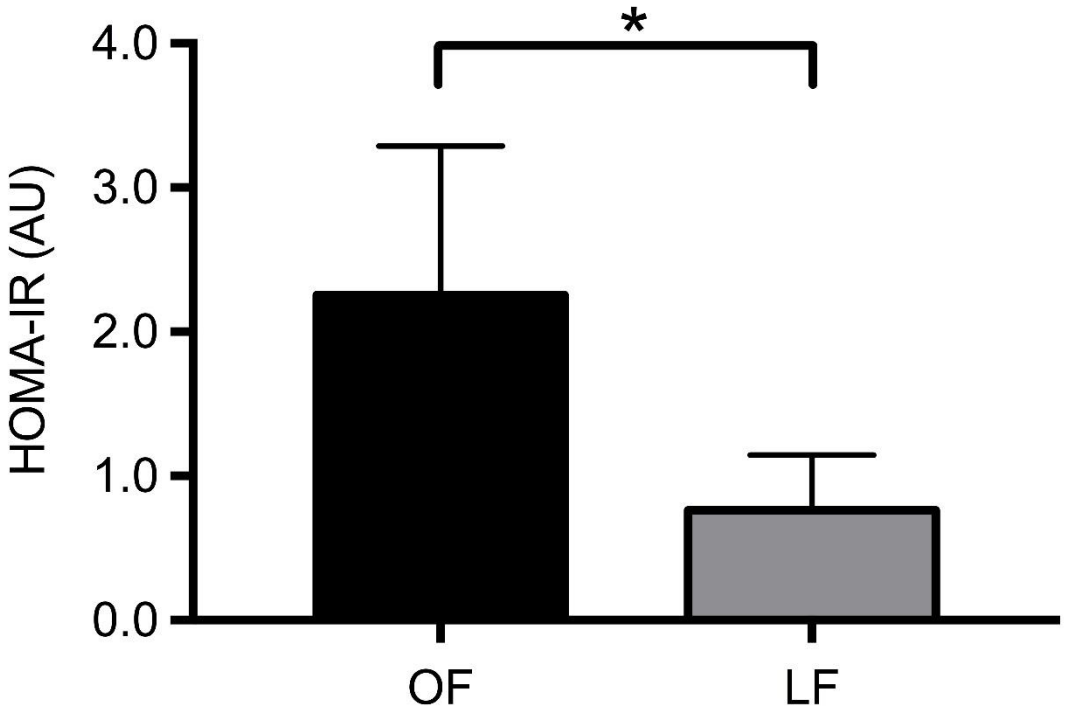

Figure 4-3. HOMA-IR, measured after a 12 hr fast. * main effect of groups, $p<0.05$

CHAPTER 5 DISCUSSION

This study was designed to assess the contribution of CRF, independent of adiposity, to the metabolic health of obese individuals. The project focused on how body composition and CRF affect the relative contribution of glucose, fat and protein to energy production in the basal (resting) state as well as during and after exercise. The aim of the study was to determine how CRF, independent of body composition, alters substrate partitioning of lean and obese, fit men in resting, during exercise and in post-exercise states. The primary finding of the current study revealed that, although obese fit individuals display a pattern of substrate partitioning comparable to that observed in lean fit individuals, confirming the impact of fitness on metabolic profile of obesity, obese fit individuals showed a lower insulin response than lean fit individuals.

5.1 Anthropometric characteristics, CRF scores, and training profile of participants

Obesity is defined as an accumulation of excess body fat, to the extent that health and well-being is adversely affected (WHO, 2000); the most commonly used method to classify relative fatness is BMI. A BMI of 25.0 – 29.9 $kg \cdot m^{-2}$ is referred to as overweight while a BMI of 30.0 – 34.9 $kg \cdot m^{-2}$ is categorized as class I obese (WHO, 2000). The obese fit participants in the current study had a BMI of 32 ± 4 $kg \cdot m^{-2}$ indicating that participants had a moderately increased risk of co-morbid conditions (Field et al., 2001) while lean fit participants had a BMI of 24±2 $kg \cdot m^{-2}$ with a percent body fat of 18±4. The mean BMI for the OF group and the standard deviation indicate that some of the participants were below a BMI of 30.0 $kg \cdot m^{-2}$ and therefore, not classified as "obese". However, OF participants were only initially recruited by BMI. To confirm whether participants were in fact obese, body fat

was measured using DXA. Men are considered obese when body fat exceeds 24% (Jeukendrup & Gleeson, 2010) and OF participants in the current study had a mean body fat of 35±4%. The body composition was an important controlled variable since a large significant effect between groups strengthens the conclusions drawn from CRF and substrate partitioning outcomes.

Since the current study revolves around the relatively new topic of "fat but fit", it is imperative to discuss the fitness characteristics of the participants as there is limited research on what defines an obese person as fit. Firstly, CRF status of participants was based on individual $\dot{V}O_{2max}$ values which were expressed in absolute, relative, and FFM terms. Absolute $\dot{V}O_{2max}$ is a good indicator of the capacity of the cardiorespiratory system. Difference between groups for absolute $\dot{V}O_{2max}$ scores was negligible (0.09 $L \cdot min^{-1}$). However, the gold standard to express $\dot{V}O_{2max}$ is in relative terms (i.e. divided by bodyweight), in this case an invalid approach as the OF group have a higher fat mass than the LF group. When expressed relative to body weight, $\dot{V}O_{2max}$ of the LF group was 31.7% higher than the OF group ($p<0.001$) but fat tissue not being an oxygen consumer during exercise, therefore, relative $\dot{V}O_{2max}$ does not represent best fitness of our OF participants. FFM, however, is the active tissue involved in O_2 extraction and when scores were expressed in terms of FFM, the difference between groups was again, negligible (see table 4-2). As the literature regarding obese fit persons is scarce, it is important to compare fitness scores of our OF group to other research that has focused on unfit obese and lean subjects. In one study that investigated predictors of CRF in sedentary men, unfit obese persons were found to have a $\dot{V}O_{2max}$ value of 31.6±1.0 $ml \cdot min^{-1} \cdot kg^{-1}$ (Riou et al., 2009), 24.4% lower than that of our OF

group. Moreover, Lee et al. (2005) assigned sedentary men based on relative $\dot{V}O_{2max}$ to either a low-, moderate-, or high-CRF group (29 ± 3.7, 36.2 ± 3.7, 44.3 ± 4.6, respectively) where the low-CRF group consisted of obese men (BMI 32.1± 3.5 $kg \cdot m^{-2}$) and the moderate-CRF group consisted of overweight men (BMI 29.1±3.4 $kg \cdot m^{-2}$). As one can see, the relative $\dot{V}O_{2max}$ of our OF participants is much higher than that of both the low- and moderate- CRF groups (refer to table 4-2). It is also higher when compared to scores for unfit, lean men. In two separate studies, unfit, lean men were found to have a $\dot{V}O_{2max}$ score of 39.9±5.1 and 38.2±1.6 $ml \cdot min^{-1} \cdot kg^{-1}$ (Melanson et al., 2009; Riou et al., 2009); 2.7 and 6.8% lower than that of our OF group, respectively. Absolute $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ expressed in terms of FFM, combined with the results from the above-mentioned studies provide ample evidence to support that our OF group was in fact, fit. Additionally, training profile between OF and LF groups were the same (see table 4-2); the training completed by both groups induced the same chronic response as shown by the CRF scores through absolute and FFM means. Finally, LF participants are easily classified as fit according to the standards set by the ACSM (American College of Sports Medicine, 2014).

5.2 Nutritional and physical activity data

Nutritional data indicated that the LF group consumed 60.3% more calories on a daily basis compared to the OF group ($p<0.05$). LF participants also consumed significantly higher amounts of CHO and lipids when compared to OF (87.8 and 58.5%). Groups did not, however, differ in the amount of protein they consumed. Daily EE of OF was 2,937.6 kcal, however, participants reported a daily caloric intake of only 1624 ± 291 kcal. On the other

hand, daily EE for LF was 2,779.2 kcal and caloric intake was recorded at 2604 ± 624 kcal per day. All participants have had a stable body mass for the past six months and were not currently in the process of losing weight, therefore, it is possible that the OF group may have under-reported food intake which research has shown is not uncommon for overweight and obese individuals (Macdiarmid & Blundell, 1998). Daily steps and distance travelled recorded by the physical activity tracker revealed no significant differences between OF and LF groups as shown in table 4-3. However, LF had a higher number of steps taken in a day and subsequently, a greater distance travelled. Although these results are not significant they seem to correlate with the fact that the LF group also consumed a higher number of calories in a day compared with the OF group.

5.3 Metabolic profile and substrate partitioning

Basal metabolic rate

BMR reflects the minimum amount of energy required to maintain vital functions and is an indicator of a person's metabolic profile. The downside of using RER resides in the fact that RER does not necessarily reflect metabolic rate (i.e. EE). For instance, a person's RER at rest could be 0.83 with a $\dot{V}O_2$ of 250 ml·min^{-1} but while walking, the same RER could correspond to a $\dot{V}O_2$ of 1000 ml·min^{-1}. Therefore, substrate partitioning is a better indicator of an individual's metabolic profile (MP). Much of the literature compares obese and lean individuals in terms of RER and indicates that it is lower in lean than obese persons (Colberg et al., 1995). During BMR, $\dot{V}O_2$, $\dot{V}CO_2$ and RER exhibited negligible differences between groups (as shown in table 4-5). Similarly, Poehlman (1988) also did not find any significant

differences in resting RER between highly trained and untrained, lean men (RER=0.81). In this case, it appears as though fitness level did not have any effect on RER, however; unfit participants in this study were actually quite fit (53 ml·min·kg^{-1}). In another study involving lean and obese, unfit persons, researchers determined that RER was 0.75 and 0.80, respectively, and did not differ significantly (Perez-Martin et al., 2001). Likewise, RER amongst our two groups did not differ significantly but values were slightly higher (refer to table 4-5). The most likely explanation for the lower RER values in the former study is that more than half of the participants of both groups consisted of women which are known to be more lipolytic than men (Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). Furthermore, in a study involving 16 healthy, lean, moderately active men and women, RER was determined to be 0.86 at rest, 8.9% and 3.6% higher than the RER of our LF and OF participants (Melanson et al., 2002). Compared to the current study, groups consisted of both men and women and RER was measured over 24 hr by whole room calorimetry; differences in methodology could have had an impact on RER values. Different results from these studies emphasize how difficult it is to use RER to describe the metabolic profile of obese and lean individuals as it does not provide any details of substrate oxidation and does not indicate an individual's fitness status.

As mentioned above, RER does not necessarily reflect metabolic rate so substrate partitioning is a better indicator. In the current study, although not significantly different, G_{ox} rates were found to be higher in OF compared to LF participants by 0.22 kcal·min^{-1}, while L_{ox} and P_{ox} rates were only slightly different between groups (0.07 kcal·min^{-1} and 0.04 kcal·min^{-1}, respectively). Previous studies have found similar results in L_{ox} rates. For

instance, data presented by Perez-Martin et al. (2001) showed L_{ox} rates to be ~110 mg·min^{-1} (for an equivalent of 1.04 kcal·min^{-1}) for obese, sedentary volunteers, only 5.8 mg·min^{-1} (or 0.05 kcal·min^{-1}) higher than the L_{ox} rate for our OF group. Differences in L_{ox} rates may be due to a variety of factors. In the former study, both men and women were involved as opposed to the current study which only included men. On the other hand, another study revealed much lower rates of L_{ox} compared with the current study. Golay et al. (1984) presented data in which basal L_{ox} rates for sedentary, obese participants was 0.72 kcal·min^{-1}, 27% lower than the rate of our OF group. In addition, G_{ox} rates for the same group were found to be 0.36 kcal·min^{-1} (Golay et al., 1984) compared to 0.71 kcal·min^{-1} for the OF group. Rates of substrate partitioning for the current study were higher than the previous study and may be explained by the fitness status of our participants. Higher levels of L_{ox} and G_{ox} coincide with the fact that our OF group also have a higher BMR compared to the obese, sedentary group in the former study. A higher BMR can be attributed to the anthropometric characteristics (i.e. weight and height). Resting rates of substrate partitioning for the LF group are shown in figure 4-1. Melanson and colleagues (2009) determined resting levels of L_{ox} in obese sedentary, lean trained (LT) and untrained men and women. LT subjects had L_{ox} rates of 36.8 mg·min^{-1}, lower than the L_{ox} rate of our LF group. Again, the large difference in rates between the two groups may be attributed to fitness. Although relative $\dot{V}O_{2max}$ values were the same between the LT and our LF group, when expressed in terms of FFM, our group's score was 13.1% higher. This indicates that our LF group was more trained than the LT group from the previous study.

Exercising metabolic rate

Determining an exercise intensity that can elicit the highest rate of L_{ox} has been the focus for the obese population as it has been suggested that lipid utilization is impaired during exercise. As cited by Achten et al. (2002), studies suggest that MFO occurs at low to moderate exercise intensities (or 33-65% $\dot{V}O_{2max}$). In the current study, MFO was determined to be 39 and 32% $\dot{V}O_{2max}$ for OF and LF. Compared to a handful of studies (Romijn et al., 2000 & van Loon et al., 2001), our MFO occurred at a much lower point for our participants. Achten, Gleeson, and Jeukendrup (2002) developed a continuous graded cycling exercise test to determine the MFO and reported MFO corresponding to 64±4% $\dot{V}O_{2max}$. However, authors excluded exercise intensities in the MFO calculation if they were lower than 3.35 $kJ \cdot L \cdot O_2$. Conversely, MFO occurred at 40% $\dot{V}O_{2max}$ for trained participants compared to 59% for untrained participants in another study (Bergman & Brooks, 1999). Trained individuals were cyclists and had a $\dot{V}O_{2max}$ of 58 $ml \cdot min \cdot kg^{-1}$, slightly higher than that of our LF and much higher than that of our OF, however, when expressed in terms of FFM, $\dot{V}O_{2max}$ was 64.1, lower than both the OF and LF groups. Therefore, we can exclude fitness as a possible reason our MFO was lower. Another important point to consider is that EE was approximately the same for both groups during the exercise and was not statistically different (323.3 ± 14.9 kcal for OF and 337.9 ± 44.8 kcal for LF) which ensures the same metabolic stress was induced for both groups. Additionally, during exercise, RER and substrate partitioning were measured to see the effects of exercise on metabolic profile. RER was the same for OF and LF participants during exercise (see table 4-6); a 7.2 and 12.7% increase from baseline for each group. From baseline to exercise, L_{ox} increased by a factor of 2.3 and

2.5 for OF and LF, respectively while G_{ox} increased by a factor of 5.6 and 9.0 for OF and LF. More importantly, there were no significant differences between groups for substrate partitioning which indicates homogeneity. Other studies have also shown similar increases in RER from baseline to an exercising state (Perez-Martin et al., 2001 & Goodpaster et al., 2002). However, during exercise RER was shown to be significantly higher in obese, sedentary participants compared to lean, sedentary participants at all exercise intensities (Perez-Martin et al., 2001). Surprisingly, RER was significantly lower in obese, untrained men when compared to lean, untrained men in data presented by Goodpaster and colleagues (2002). Researchers concluded that during moderate exercise (50% $\dot{V}O_{2max}$), obese sedentary men have increased rates of FA oxidation from non-plasma sources and reduced rates of G_{ox}, particularly muscle glycogen, compared with lean sedentary men (Goodpaster et al., 2002). Essentially, obese men in this study had higher rates of L_{ox} and lower rates of G_{ox} compared with the lean men, however; it is hard to make a comparison to the current study as the experimental protocol was very different. For instance, isotope infusions were used to determine non-plasma and plasma FA oxidation and glucose levels whereas IC was used to determine whole-body L_{ox} and G_{ox} rates in the current study. Melanson et al. (2009) determined that RER was significantly higher in lean, sedentary men and women than lean, trained men and women during exercise. Also, although not significant, the lean, trained group tended to have a greater L_{ox} during the exercise when compared to the lean, sedentary group (Melanson et al., 2009). In this case, both groups were lean (i.e. no difference in body composition) but metabolic profile differed, possible due to fitness. Compared with the

current study, groups differed in body composition but exhibited the same metabolic profile, most likely because both groups were fit.

Post-exercise metabolic rate

At one time, measurements of PEMR were recorded to document the increase in post-exercise metabolic response. In the early 70s and 80s, researchers interpreted the increases in EP post-exercise as the result of an O_2 debt that corresponded to the substrate-level phosphorylation for ATP production during metabolic inertia (Brooks, Brauner, & Cassens, 1973; Gaesser & Brooks, 1984). However, O_2 debt hypothesis has been replaced by the more appropriate term, excess post-exercise oxygen consumption (EPOC), as many other factors are associated with elevated $\dot{V}O_2$ (Gaesser & Brooks, 1984). According to LaForgia, Withers, & Gore (2006), the magnitude of EPOC is dependent on the exercise intensity and duration. As reviewed by LaForgia et al. (2006), work at moderate exercise intensities (≥ 50 – 60% $\dot{V}O_{2max}$) stimulate a linear increase in EPOC as exercise duration increases. Furthermore, prolonged EPOC (3 – 24 hr) may result from exercising for ≥ 50 min and at ≥ 70% $\dot{V}O_{2max}$, or for ≥ 6 min and at ≥ 105% $\dot{V}O_{2max}$. In the current study, changes in $\dot{V}O_2$ between groups from baseline to post-exercise were negligible. This is not surprising as years of research on EPOC show that the intensity and duration at which our participants exercised was not enough to alter substrate partitioning and EP.

Post-meal metabolic rate

For the purpose of this study, a standardized meal was given to participants' post-exercise in order to negate the energy deficit created by exercise. A secondary aim was to see if groups

differ in terms of substrate partitioning when given food. During PMMR1, although not significantly different, the following trends are worth noting. For instance, LF participants had a much greater increase (101%) from baseline in G_{ox} than OF (9.2%) while L_{ox} decreased 24.2% from baseline in LF but increased 10.7% in OF. Interestingly, during the second postprandial MR measurement, G_{ox} in OF participants dropped 29.6% below baseline values while L_{ox} increased 39.8% above baseline levels. One possible reason for this may be that OF were not oxidizing glucose but rather using glucose to replenish glycogen stores as after 1 hr of acute, submaximal exercise, glycogen in working muscle is nearly completely utilized (Ahlborg, Bergstrom, Ekelund, & Hultman, 1967). This would correspond to the increase in L_{ox} for OF as TAGs would be broken down to FFA to provide ATP for glycogen replenishment. In contrast, G_{ox} in LF participants increased 32.2% compared to baseline measurements while L_{ox} remained approximately the same. A conclusion that could be drawn from these data is that OF deplete more muscle glycogen during exercise than LF individuals resulting is less G_{ox} 2 hr post-meal. Although it is unclear why OF use more muscle glycogen than LF (as both groups were fasted for more than 12 hr), it could be due to the greater BM of the OF participants. On a separate matter, another study provided different reasoning to explain their results. Researchers assessed the effect of an acute exercise on energy metabolism during a 17-hr recovery phase under strictly controlled conditions in young, fit men and found a significantly greater CHO and lower fat storage post-exercise, post-meal compared to a control day; G_{ox} decreased by 32.6% while L_{ox} increased by 60% (Bielinski, Schutz, & Jequier, 1985). As cited by Bielinski et al., studies have shown that consumption of a glucose load during the recovery period following severe exercise is characterized by

two changes: 1) a large fraction of the exogenous glucose escapes hepatic retention and 2) muscle glycogen stores, which have been diminished during exercise, are gradually reconstituted. In other words, repletion of muscle glycogen takes priority over hepatic glycogen repletion. Although severe exercise was not performed in the present study, participants were fasted for a minimum of 12 hr before the start of exercise which may have had some impact on muscle glycogen stores, explaining the decrease in G_{ox} and L_{ox} increase that was seen for the OF group.

5.4 Blood analysis

Obesity has long been associated with a variety of health risks including elevated levels of blood lipids, insulin, and glucose (Hubert, Feinleib, McNamara, & Castelli, 1983) which, consequently, have been shown to be contributing factors in cardiovascular disease, MetS, and NIDDM (Roberts & Barnard, 2005). According to the National Institute for Clinical Excellence (NICE) target FBG levels are between 4.0 and 5.9 $mmol \cdot L^{-1}$ (The Global Diabetes Community, 2016); target fasting insulin levels are <174 $pmol \cdot L^{-1}$ (Melmed, Polonsky, Larsen, & Kronenberg, 2011), and normal TAG levels are < 1.7 $mmol \cdot L^{-1}$(Mayo Clinic Staff, 2015). In the present study, fasting insulin, glucose, and TAG levels in both groups were within target values (refer to figure 4-2). This suggests that despite a mean BMI of 32 ± 4 $kg \cdot m^{-2}$, and an excess accumulation of body fat, the current study obese cohort was metabolically healthy at baseline. Blood was drawn from each participant and analyzed for insulin, glucose, and TAG at baseline (-180 min), post-exercise pre-meal (0 min), and 2 hr post-meal (120 min). Recall that NEFA and glycerol analyses were not reported due to technical issues. Although group differences for glucose and TAG levels were negligible,

data from figure 4-2 revealed some trends. For instance, baseline levels of glucose and TAG levels were 7.1 and 51.8% higher in the OF than the LF group, however, levels decreased in both groups at time 0 min (just before PMMR1). Two hours post-meal (just after PMMR2), compared to baseline, glucose levels for OF and LF decreased similarly (7.5 and 6.5%) while TAG levels dropped below baseline in OF and increased slightly for LF. Bielinski et al. (1985) found that postprandial plasma glucose peaked 60 min post-meal and had a delayed return to baseline values while in the current study, blood glucose levels decreased below baseline values 60 min post-exercise and 120 min post-meal. The glucose load given in the current study was most likely not enough to stimulate a surge in blood glucose levels as participants consumed only 42 g of CHO while in the latter study participants consumed roughly 180 g of CHO. The decrease in TAG levels seen with OF is consistent with the increase in L_{ox} during PMMR2; TAGs may have been catabolized into FFA to supply ATP to replenish glycogen. Statistical analysis showed a significant main effect of group for insulin levels. Although no significant interaction was observed between group and time points (-180, 0, 120 min), insulin levels were consistently higher in the OF group compared with the LF group. At baseline (before BMR), insulin levels were 1.8 times greater in OF than LF while a slight decrease was observed in both groups before PMMR1 (at point 0 min). The small decrease in insulin levels is consistent with the slight decrease that was seen for glucose levels. After exercise, insulin levels fall partly due to a higher insulin binding activity and cellular responsiveness to insulin and an elevated level of epinephrine that suppresses insulin secretion (Brooks et al., 1996). Two hours post-meal, insulin levels increased 29.1% in OF and 16.9% in LF when compared to baseline levels. Similar fluctuations in insulin

levels can be seen in another study that examined the relation of plasma catecholamine and insulin levels to the blood pressure response during and after submaximal exercise (Landry et al., 1992). Nine lean, fit young men performed a cycle ergometer exercise at 55% of their $\dot{V}O_{2max}$ for 60 min. Insulin levels were measured every 15 min before, during, and after the exercise session. At baseline, insulin levels were ~53 pmol·L^{-1}. Fifteen minutes after the end of exercise, insulin levels were ~50 pmol·L^{-1} while 30 min after exercise, insulin levels decreased to ~43 pmol·L^{-1}. In the current study, baseline levels of insulin were 63.1 ± 28.3 and 22.5 ± 10.1 pmol·L^{-1} for the OF and LF groups, respectively. Insulin levels were measured 60 min after the end of exercise and decreased to 40.5 ± 19.4 and 14.5 ± 4.5 pmol·L^{-1} for OF and LF. Our LF participants had a lower baseline insulin level than the latter study cohort which indicates that our participants were more insulin-sensitive. Although participants in both studies showed similar responses to exercise, our participants appeared to have a greater insulin decrease post-exercise, however, insulin was measured 60 min post-exercise while insulin levels in the other study were measured only up to 30 min post-exercise. In a different study, researchers investigated the metabolic and hormonal response to exhaustive exercise in obese subjects (Vettor, Macor, Rossi, Piemonte, & Federspil, 1997). Nine healthy, obese and ten lean control participants, both untrained, performed a cycle ergometer exercise to exhaustion with blood drawn 30 min post-exercise. At baseline, obese subjects had an insulin level of 119.1 pmol·L^{-1} while insulin in lean control group measured 58.14 pmol·L^{-1}. Although exercise induced a small but significant decrease in insulin (119.1 vs 90.83 pmol·L^{-1}) in obese subjects, 30 min after the end of exercise, insulin levels rose to ~130 pmol·L^{-1}, higher than baseline (Vettor et al., 1997). Furthermore, plasma insulin levels

remained significantly higher than the lean control group during the test. In the present study, insulin levels were not measured during exercise but 60 min after and had decreased below baseline values. In support of the data presented by Vettor et al., during acute bouts of high-intensity exercise, cortisol secretion is increased while plasma insulin is decreased (Hartley et al., 1972). Immediately after exercise, plasma insulin levels increase rapidly (Wahren, Felig, Hendler, & Ahlborg, 1973). The results from Vettor' study mentioned above most likely differed from the present study for two reasons: (1) the exercise protocol in the current study was a low-to-moderate intensity as opposed to an exhaustive exercise and (2) the fitness level of our participants may have made our OF group more insulin-sensitive than the untrained obese group. Interestingly, the lean control group in the other study was more insulin-resistant than our LF group (58.14 vs. 22.5 $pmol \cdot L^{-1}$ at baseline). In addition, no interaction was found between groups for insulin during any time points in the current study which means that obesity, with a slightly higher IR at baseline, does not affect the insulin response to exercise. Lastly, Stiegler et al. showed that after a 20 min cycle ergometer exercise at ~65% of their $\dot{V}O_{2max}$, male subjects displayed insulin levels of ~318 $pmol \cdot L^{-1}$ 1 hr after a 400 kcal liquid meal (27% fat, 10% protein, 63% CHO) and 258 $pmol \cdot L^{-1}$ 2 hr post-meal (Stiegler, Sparks, & Cunliffe, 2008). Unfortunately, researchers in this study did not test lean and obese men separately but rather placed them in the same group. Baseline levels of insulin were provided for lean and obese subjects within the group (49.2 $pmol \cdot L^{-1}$ and 94.8 $pmol \cdot L^{-1}$) but the same breakdown of insulin levels within the group was not provided during the post-exercise, post-meal phase. Researchers report that plasma insulin levels were significantly higher in the obese cohort than the lean. Post-meal insulin levels listed above are those for

the whole group which makes it difficult to compare the hormonal responses between lean and obese participants. Insulin levels of participants in the latter study are a lot higher at baseline and during all time points of recovery than our participants. This could be because participants in the other study were: (1) in a positive energy balance (i.e. expended 200 kcal during exercise but consumed a 400-kcal liquid meal), (2) consumed a higher amount of CHO (63 g vs. 42 g) and (3) were unfit which may explain why they were more insulin resistant than the current study's participants.

Another parameter determined through BS was homeostasis model assessment of IR (HOMA-IR) which is the product of glucose and insulin concentrations divided by a factor (Matthews et al., 1985). IR is characterized by a decreased ability of insulin-mediated glucose disposal into muscles and the liver; and is closely associated with a sedentary lifestyle, overweight, and abdominal obesity (Vogeser et al., 2007). HOMA is an assessment of fasting plasma insulin and glucose concentrations to determine IR and β-cell deficiency in humans. Lower values of the HOMA-IR index correlate to a higher degree of insulin sensitivity (Vogeser et al., 2007). As IR measured this way should be treated as a continuum, there is no consensus on what clinical cut-off values should be. However, it has been suggested by several authors that a value of 2.7 or greater be the new defining level (Karelis & Rabasa-Lhoret, 2008). In the current study, HOMA-IR values were significantly different with levels in OF being 197% higher than the LF group (refer to figure 4-3). Values greater than 2.7 could potentially indicate a level of IR that may be considered worrisome. Although HOMA-IR values were higher for OF, mean values did not surpass 2.7. These data indicate that OF participants have a lower insulin response than the LF but they are not clinically

insulin resistant. Overall, despite no differences in MR and substrate partitioning, blood analysis revealed that OF individuals require a higher rate of insulin production to achieve the same metabolic response compared to LF. Consequently, over time this may lead to obese fit individuals developing IR at faster rate. These observations suggest that fitness is not the only factor in the determination of one's metabolic health; body composition also matters to a certain extent.

5.5 Methodological Considerations

Some limitations are inherent in the current study design. Firstly, according to Ferrannini (1988), substrate oxidation calculations using respirometry measurements are based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ recorded at the mouth reflect that at the tissue level. Secondly, the present findings are limited to a small sample size of lean fit and obese fit men living in the St. John's area, Newfoundland (Canada). Furthermore, this study is representative of a healthy population; these findings cannot be applied to those individuals who suffer from chronic diseases such as NIDDM or CVD. Thirdly, food logs completed by participants were most likely underestimated which would explain the discrepancy between what was recorded and BMR measurements of participants.

5.6 Future Studies

The findings from this study are very promising but further research is needed in the area of "fat but fit". For instance, results from this study would benefit with the addition of two control groups, lean and obese unfit men, in order to make comparisons with lean and obese fit individuals. The study should also be completed using female participants to see if CRF

has the same effect on the metabolic health of women. Additionally, the type of exercise could be altered to see if the same results are produced. As shown in a study conducted by Miller et al. (2014), a short-term high-intensity circuit training program could have positive effects on several health markers in obese men. Lastly, although more intricate, the study protocol could be completed using a cross-sectional design where researchers look at obese participants of different age cohorts, where subjects within each age group are matched for weight, height, and fitness level. The purpose of this would be to see if HOMA-IR levels increase as a function of age.

CHAPTER 6 CONCLUSION

As hypothesized, RER values and rates of substrate oxidation did not differ significantly between OF and LF groups. OF individuals display a pattern of substrate partitioning comparable to that observed in LF individuals, confirming that fitness can modulate the metabolic impacts of obesity and could reduce the risks of chronic diseases associated with obesity. To the researchers' surprise, blood analyses indicated OF individuals require a higher rate of insulin release to achieve the same metabolic control as the lean individuals; consequently, this may lead to inevitable IR in the future for obese persons as shown by HOMA-IR difference between the two groups. This implies that fitness is not the only factor in the determination of one's metabolic health; body composition also matters to a certain extent.

REFERENCES

Achten, J., Gleeson, M., & Jeukendrup, A. E. (2002). Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine and Science in Sports and Exercise, 34*(1), 92-97. doi:10.1097/00005768-200201000-00015

Ahlborg, B., Bergstrom, J., Ekelund, L., & Hultman, E. (1967). Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiol.Scand., 70,* 129-142.

Alsubheen, S., George, A., Baker, A., Rohr, L., & Basset, F. (2016). Accuracy of the vivofit activity tracker. *Journal of Medical Engineering and Technology, 40*(4), 298-306.

American College of Sports Medicine (2014). *ACSM's guidelines for exercise testing and prescription* (9th ed.), edited by Pescatello, L.S., Arena, R., Riebe, D., Thompson, P.D. Philadelphia, PA: Lippincott Williams & Wilkins.

Baecke, J. A., Burema, J., & Frijters, J. E. (1982). A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *The American Journal of Clinical Nutrition, 36*(5), 936-942.

Bergman, B. C., & Brooks, G. A. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *Journal of Applied Physiology (Bethesda, Md.: 1985), 86*(2), 479-487.

Bielinski, R., Schutz, Y., & Jequier, E. (1985). Energy metabolism during the postexercise recovery in man. *The American Journal of Clinical Nutrition, 42*(1), 69-82.

Bjorntorp, P., De Jounge, K., Sjostrom, L., & Sullivan, L. (1970). The effect of physical training on insulin production in obesity. *Metabolism: Clinical and Experimental, 19*(8), 631-638. doi:0026-0495(70)90020-X [pii]

Blaak, E. E., M. A. Van Baak, Kemerink, G. J., Pakbiers, M. T., Heidendal, G. A., & Saris, W. H. (1994). Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. *American Journal of Physiology - Endocrinology and Metabolism, 267*(2), 306-315.

Blaak, E. E., & Saris, W. H. (2002). Substrate oxidation, obesity and exercise training. *Best Practice & Research.Clinical Endocrinology & Metabolism, 16*(4), 667-678.

Boden, G., Jadali, F., White, J., Liang, Y., Mozzoli, M., Chen, X., . . . Smith, C. (1991). Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. The Journal of Clinical Investigation, 88(3), 960-966. doi:10.1172/JCI115399

Boden, G., Chen, X., Ruiz, J., White, J. V., & Rossetti, L. (1994). Mechanisms of fatty acid-induced inhibition of glucose uptake. *The Journal of Clinical Investigation, 93*(6), 2438-2446. doi:10.1172/JCI117252

Bouchard, C., & Blair, S. N. (1999). Introductory comments for the consensus on physical activity and obesity. *Medicine and Science in Sports and Exercise, 31*(11 Suppl), S498-501.

Bouchard, C., Depres, J. P., & Tremblay, A. (1993). Exercise and obesity. *Obesity Research, 1*(2), 133-147.

Braun, B., Zimmermann, M. B., & Kretchmer, N. (1995). Effects of exercise intensity on insulin sensitivity in women with non-insulin-dependent diabetes mellitus. *Journal of Applied Physiology (Bethesda, Md.: 1985), 78*(1), 300-306.

Brooks, G. A., Brauner, K. E., & Cassens, R. G. (1973). Glycogen synthesis and metabolism of lactic acid after exercise. *The American Journal of Physiology, 224*(5), 1162-1166.

Brooks, G. A., Fahey, T. D., White, T. P., & Baldwin, K. M. (1996). *Exercise physiology: Human bioenergetics and its applications*. Mountain View, CA: Mayfield Publishing Company.

Brooks, G. A., & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: The "crossover" concept. *Journal of Applied Physiology (Bethesda, Md.: 1985), 76*(6), 2253-2261.

Bruce, C. R., Thrush, A. B., Mertz, V. A., Bezaire, V., Chabowski, A., Heigenhauser, G. J., & Dyck, D. J. (2006). Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *American Journal of Physiology - Endocrinology and Metabolism, 291*(1), E99-E107. doi:00587.2005 [pii]

Calles-Escandon, J., Goran, M. I., O'Connell, M., Nair, K. S., & Danforth, E., Jr. (1996). Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. *The American Journal of Physiology, 270*(6 Pt 1), E1009-14.

Church, T. S., & Blair, S. N. (2004). The fitness, obesity, and health equation: Is physical activity the common denominator? *The Journal of the American Medical Association, 292*(10), 1232-1234. doi:10.1001/*Jama*.292.10.1232

Colberg, S. R., Simoneau, J. A., Thaete, F. L., & Kelley, D. E. (1995). Skeletal muscle utilization of free fatty acids in women with visceral obesity. *The Journal of Clinical Investigation, 95*(4), 1846-1853. doi:10.1172/JCI117864

da Rocha, E. E., Alves, V. G., & da Fonseca, R. B. (2006). Indirect calorimetry: Methodology, instruments and clinical application. *Current Opinion in Clinical*

Nutrition and Metabolic Care, 9(3), 247-256. doi:10.1097/01.mco.0000222107.15548.f5 [doi]

DeFronzo, R. A., Sherwin, R. S., & Kraemer, N. (1987). Effect of physical training on insulin action in obesity. *Diabetes, 36*(12), 1379-1385.

Després, J., & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature, 444*(7121), 881-887. doi:10.1038/nature05488

Després, J., Moorjani, S., Lupien, P. J., Tremblay, A., Nadeau, A., & Bouchard, C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 10*(4), 497-511. doi:10.1161/01.ATV.10.4.497

Dumortier, M., Brandou, F., Perez-Martin, A., Fedou, C., Mercier, J., & Brun, J. F. (2003). Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. *Diabetes & Metabolism, 29*(5), 509-518. doi:MDOI-DM-11-2003-29-5-1262-3636-101019-ART7 [pii]

Elks, M. L. (1990). Fat oxidation and diabetes of obesity: The randle hypothesis revisited. *Medical Hypotheses, 33*(4), 257-260. doi:0306-9877(90)90138-5 [pii]

Ferrannini, E. (1988). The theoretical bases of indirect calorimetry: A review. *Metabolism: Clinical and Experimental, 37*(3), 287-301. doi:0026-0495(88)90110-2 [pii]

Field, A. E., Coakley, E. H., Must, A., Spadano, J. L., Laird, N., Dietz, W. H., . . . Colditz, G. A. (2001). Impact of overweight on the risk of developing common chronic diseases

during a 10-year period. *Archives of Internal Medicine, 161*(13), 1581-1586. doi:ioi00652 [pii]

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiological Reviews, 74*(1), 49-94.

Flatt, J. P. (2011). Issues and misconceptions about obesity. *Obesity, 19*(4), 676-686. doi:10.1038/oby.2011.7

Flatt, J. P. (1997). How NOT to approach the obesity problem. *Obesity Research, 5*(6), 632-633.

Flatt, J. P. (2004). Carbohydrate-fat interactions and obesity examined by a two-compartment computer model. *Obesity Research, 12*(12), 2013-2022. doi:12/12/2013 [pii]

Frayn, K. N., Arner, P., & Yki-Jarvinen, H. (2006). Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. *Essays in Biochemistry, 42*, 89-103. doi:bse0420089 [pii]

Friedlander, A. L., Casazza, G. A., Horning, M. A., Buddinger, T. F., & Brooks, G. A. (1998). Effects of exercise intensity and training on lipid metabolism in young women. *The American Journal of Physiology, 275*(5 Pt 1), E853-63.

Friedlander, A. L., Casazza, G. A., Horning, M. A., Usaj, A., & Brooks, G. A. (1999). Endurance training increases fatty acid turnover, but not fat oxidation, in young men. *Journal of Applied Physiology, 86*(6), 2097-2105.

Gaesser, G. A., & Brooks, G. A. (1984). Metabolic bases of excess post-exercise oxygen consumption: A review. *Medicine and Science in Sports and Exercise, 16*(1), 29-43. doi:10.1249/00005768-198401000-00008

Golay, A., Felber, J. P., Meyer, H. U., Curchod, B., Maeder, E., & Jequier, E. (1984). Study on lipid metabolism in obesity diabetes. *Metabolism: Clinical and Experimental, 33*(2), 111-116. doi:0026-0495(84)90121-5 [pii]

Gollnick, P. D., & Saltin, B. (1982). Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clinical Physiology (Oxford, England), 2*(1), 1-12.

Goodpaster, B. H., Wolfe, R. R., & Kelley, D. E. (2002). Effects of obesity on substrate utilization during exercise. *Obesity Research, 10*(7), 575-584. doi:10.1038/oby.2002.78

Greenfield, J. R., & Campbell, L. V. (2004). Insulin resistance and obesity. *Clinics in Dermatology, 22*(4), 289-295. doi:S0738081X04000124 [pii]

Hargreaves, M., Kiens, B., & Richter, E. A. (1991). Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. *Journal of Applied Physiology, 70*(1), 194-201.

Hartley, L. H., Mason, J. W., Hogan, R. P., Jones, L. G., Kotchen, T. A., Mougey, E. H., . . . Ricketts, P. T. (1972). Multiple hormonal responses to graded exercise in relation to physical training. *Journal of Applied Physiology, 33*(5), 602-606.

Haugen, H. A., Chan, L. N., & Li, F. (2007). Indirect calorimetry: A practical guide for clinicians. *Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition, 22*(4), 377-388. doi:22/4/377 [pii]

Headley, J. M. (2003). Indirect calorimetry: A trend towards continuous metabolic assessment. *American Association of Critical Care Nurses, 14*(2), 155-167.

Health Canada. (2015). The canadian nutrient file. Retrieved from http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/cnf_aboutus-aproposdenous_fcen-eng.php

Hegarty, B. D., Furler, S. M., Ye, J., Cooney, G. J., & Kraegen, E. W. (2003). The role of intramuscular lipid in insulin resistance. *Acta Physiologica Scandinavica, 178*(4), 373-383. doi:1162 [pii]

Houmard, J. A., Tanner, C. J., Slentz, C. A., Duscha, B. D., McCartney, J. S., & Kraus, W. E. (2004). Effect of the volume and intensity of exercise training on insulin sensitivity. *Journal of Applied Physiology (Bethesda, Md.: 1985), 96*(1), 101-106. doi:10.1152/japplphysiol.00707.2003 [doi]

Hubert, H. B., Feinleib, M., McNamara, P. M., & Castelli, W. P. (1983). Obesity as an independent risk factor for cardiovascular disease: A 26-year follow-up of participants in the framingham heart study. *Circulation, 67*(5), 968-977.

Hue, L., & Taegtmeyer, H. (2009). The randle cycle revisited: A new head for an old hat. *American Journal of Physiology - Endocrinology and Metabolism, 297*(3), 578-591. doi:10.1152/ajpendo.00093.2009

Jequier, E., & Felber, J. P. (1987). Indirect calorimetry. *Bailliere's Clinical Endocrinology and Metabolism, 1*(4), 911-935.

Jeukendrup, A. E., & Gleeson, M. (2010). *Sport nutrition: An introduction to energy production and performance*. Champaign, US: Human Kinetics Publishers.

Jones, N. L., Heigenhauser, G. J., Kuksis, A., Matsos, C. G., Sutton, J. R., & Toews, C. J. (1980). Fat metabolism in heavy exercise. *Clinical Science (London, England:1979), 59*(6), 469-478.

Kang, J., Robertson, R. J., Hagberg, J. M., Kelley, D. E., Goss, F. L., DaSilva, S. G., . . . Utter, A. C. (1996). Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care, 19*(4), 341-349.

Karelis, A.D. & Rabasa-Lhoret, R. (2008). Inclusion of C-reactive protein in the identification of metabolically healthy but obese (MHO) individuals. *Diabetes & Metabolism, 34*, 182-184.

Karelis, A. D., Faraj, M., Bastard, J. P., St-Pierre, D. H., Brochu, M., Prud'homme, D., & Rabasa-Lhoret, R. (2005). The metabolically healthy but obese individual presents a favorable inflammation profile. *The Journal of Clinical Endocrinology and Metabolism, 90*(7), 4145-4150. doi:jc.2005-0482 [pii]

Karelis, A. D., St-Pierre, D. H., Conus, F., Rabasa-Lhoret, R., & Poehlman, E. T. (2004). Metabolic and body composition factors in subgroups of obesity: What do we know? *The Journal of Clinical Endocrinology and Metabolism, 89*(6), 2569-2575. doi:10.1210/jc.2004-0165 [doi]

Kelley, D. E., & Simoneau, J. A. (1994). Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *The Journal of Clinical Investigation, 94*(6), 2349-2356. doi:10.1172/JCI117600

Kelley, D. E., Goodpaster, B., Wing, R. R., & Simoneau, J. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology - Endocrinology and Metabolism, 277*(6), 1130-1141.

Kim, J., Hickner, R. C., Cortright, R. L., Dohm, G. L., & Houmard, J. A. (2000). Lipid oxidation is reduced in obese human skeletal muscle. *American Journal of Physiology - Endocrinology and Metabolism, 279*(5), 1039-1044.

Kuo, C. C., Fattor, J. A., Henderson, G. C., & Brooks, G. A. (2005). Lipid oxidation in fit young adults during postexercise recovery. *Journal of Applied Physiology (Bethesda, Md.: 1985), 99*(1), 349-356. doi:00997.2004 [pii]

LaForgia, J., Withers, R. T., & Gore, C. J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of Sports Sciences, 24*(12), 1247-1264. doi:L4GQ464GU1772M70 [pii]

Landry, J. F., Depres, J. P., Prud'homme, D., Lamarche, B., Tremblay, A., Nadeau, A., & Bouchard, C. (1992). A study of some potential correlates of the hypotensive effects of prolonged submaximal exercise in normotensive men. *Canadian Journal of Physiology and Pharmacology, 70*(1), 53-59.

Lee, C. D., Blair, S. N., & Jackson, A. S. (1999). Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *The American Journal of Clinical Nutrition, 69*(3), 373.

Lee, D., Sui, X., Church, T. S., Lee, I. -., & Blair, S. N. (2009). Associations of cardiorespiratory fitness and obesity with risks of impaired fasting glucose and type 2 diabetes in men. *Diabetes Care, 32*(2), 257-262. doi:10.2337/dc08-1377

Lee, S., Kuk, J. L., Katzmarzyk, P. T., Blair, S. N., Church, T. S., & Ross, R. (2005). Cardiorespiratory fitness attenuates metabolic risk independent of abdominal

subcutaneous and visceral fat in men. *Diabetes Care, 28*(4), 895-901. doi:10.2337/diacare.28.4.895

Lewis, G. F., Carpentier, A., Adeli, K., & Giacca, A. (2002). Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocrine Reviews, 23*(2), 201-229. doi:10.1210/edrv.23.2.0461 [doi]

Macdiarmid, J., & Blundell, J. (1998). Assessing dietary intake: Who, what and why of under-reporting. *Nutrition Research Reviews, 11*(2), 231-253. doi:10.1079/NRR19980017 [doi]

Malenfant, P., Joanisse, D. R., Thériault, R., Goodpaster, B. H., Kelley, D. E., & Simoneau, J. (2001). Fat content in individual muscle fibers of lean and obese subjects. *International Journal of Obesity, 25*(9), 1316-1321. doi:10.1038/sj.ijo.0801733

Matthews, D., Hosker, J., Rudenski, A., Naylor, B., Treacher, D., & Turner, R. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*, 412-419.

Mayo Clinic Staff. (2015). Triglycerides: Why do they matter? Retrieved from http://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/triglycerides/art-20048186

Melanson, E. L., Gozansky, W. S., Barry, D. W., Maclean, P. S., Grunwald, G. K., & Hill, J. O. (2009). When energy balance is maintained, exercise does not induce negative fat balance in lean sedentary, obese sedentary, or lean endurance-trained individuals. *Journal of Applied Physiology (Bethesda, Md.: 1985), 107*(6), 1847-1856. doi:10.1152/japplphysiol.00958.2009 [doi]

Melanson, E. L., Sharp, T. A., Seagle, H. M., Horton, T. J., Donahoo, W. T., Grunwald, G. K., . . . Hill, J. O. (2002). Effect of exercise intensity on 24-h energy expenditure and nutrient oxidation. *Journal of Applied Physiology (Bethesda, Md.: 1985), 92*(3), 1045-1052. doi:10.1152/japplphysiol.00706.2001 [doi]

Melmed, S., Polonsky, K., Larsen, P. R., & Kronenberg, H. M. (2011). *Williams textbook of endocrinology* (12th ed.). Philadelphia: Elsevier Saunders.

Miller, M. B., Pearcey, G. E., Cahill, F., McCarthy, H., Stratton, S. B., Noftall, J. C., . . . Button, D. C. (2014). The effect of a short-term high-intensity circuit training program on work capacity, body composition, and blood profiles in sedentary obese men: A pilot study. *BioMed Research International, 2014,* 191797. doi:10.1155/2014/191797 [doi]

O'Donovan, G., Thomas, E. L., McCarthy, J. P., Fitzpatrick, J., Durighel, G., Mehta, S., . . . Bell, J. D. (2009). Fat distribution in men of different waist girth, fitness level and exercise habit. *International Journal of Obesity, 33*(12), 1356-1362. doi:10.1038/ijo.2009.189

Perez-Martin, A., Dumortier, M., Raynaud, E., Brun, J., Fedou, C., Bringer, J., & Mercier, J. (2001). Balance of substrate oxidation during submaximal exercise in lean and obese people. *Diabetes & Metabolism, 27*(4), 466-474.

Poehlman, E. T., Melby, C. L., & Badylak, S. F. (1988). Resting metabolic rate and postprandial thermogenesis in highly trained and untrained males. *The American Journal of Clinical Nutrition, 47*(5), 793-798.

Randle, P. J., Garland, P. B., Hales, C. N., & Newsholme, E. (1963). The glucose-fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *The Lancet, 281*(7285)

Randle, P. J., Garland, P. B., Newsholme, E. A., & Hales, C. N. (1965). The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. *Annals of the New York Academy of Sciences, 131*(1), 324-333. doi:10.1111/j.1749-6632.1965.tb34800.x

Ravussin, E., Burnand, B., Schutz, Y., & Jequier, E. (1982). Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. *The American Journal of Clinical Nutrition, 35*(3), 566-573.

Riou, M. E., Pigeon, E., St-Onge, J., Tremblay, A., Marette, A., Weisnagel, S. J., & Joanisse, D. R. (2009). Predictors of cardiovascular fitness in sedentary men. *Applied Physiology, Nutrition, and Metabolism, 34*(2), 99-106. doi:10.1139/H08-144 [doi]

Roberts, C. K., & Barnard, R. J. (2005). Effects of exercise and diet on chronic disease. *Journal of Applied Physiology (Bethesda, Md.: 1985), 98*(1), 3-30. doi:98/1/3 [pii]

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *The American Journal of Physiology, 265*(3 Pt 1), E380-91.

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Rosenblatt, J., & Wolfe, R. R. (2000). Substrate metabolism during different exercise intensities in endurance-trained women. *Journal of Applied Physiology (Bethesda, Md.: 1985), 88*(5), 1707-1714.

Ross, R., Dagnone, D., Jones, P. J., Smith, H., Paddags, A., Hudson, R., & Janssen, I. (2000). Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Annals of Internal Medicine, 133*(2), 92-103. doi:200007180-00008 [pii]

Ross, R., Janssen, I., Dawson, J., Kungl, A. M., Kuk, J. L., Wong, S. L., . . . Hudson, R. (2004). Exercise-induced reduction in obesity and insulin resistance in women: A randomized controlled trial. *Obesity Research, 12*(5), 789-798. doi:12/5/789 [pii]

Saha, A. K., Halberg, N., Kiens, B., Ruderman, N. B., Roepstorff, C., Richter, E. A., . . . Wojtaszewski, J. F. P. (2005). Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. *The American Journal of Physiology, 288*(1), E133.

Saris, W. H., & Schrauwen, P. (2004). Substrate oxidation differences between high- and low-intensity exercise are compensated over 24 hours in obese men. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 28*(6), 759-765. doi:10.1038/sj.ijo.0802631 [doi]

Shimada, K., Yamamoto, Y., Iwayama, K., Nakamura, K., Yamaguchi, S., Hibi, M., . . . Tokuyama, K. (2013). Effects of post-absorptive and postprandial exercise on 24 h fat oxidation. *Metabolism: Clinical and Experimental, 62*(6), 793-800. doi:10.1016/j.metabol.2012.12.008 [doi]

Sidossis, L. S., Stuart, C. A., Shulman, G. I., Lopaschuk, G. D., & Wolfe, R. R. (1996). Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into

the mitochondria. *The Journal of Clinical Investigation, 98*(10), 2244-2250. doi:10.1172/JCI119034

Sidossis, L. S., & Wolfe, R. R. (1996). Glucose and insulin-induced inhibition of fatty acid oxidation: The glucose-fatty acid cycle reversed. *The American Journal of Physiology, 270*(4 Pt 1), E733-8.

Sidossis, L. S., Wolfe, R. R., & Coggan, A. R. (1998). Regulation of fatty acid oxidation in untrained vs. trained men during exercise. *The American Journal of Physiology, 274*(3 Pt 1), E510-5.

Simonson, D. C., & DeFronzo, R. A. (1990). Indirect calorimetry: Methodological and interpretative problems. *The American Journal of Physiology, 258*(3 Pt 1), E399-412.

Stiegler, P., Sparks, S. A., & Cunliffe, A. (2008). Moderate exercise, postprandial energy expenditure, and substrate use in varying meals in lean and obese men. *International Journal of Sport Nutrition and Exercise Metabolism, 18*(1), 66-78.

Sun, G., French, C. R., Martin, G. R., Younghusband, B., Green, R. C., Xie, Y. G., . . . Zhang, H. (2005). Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *The American Journal of Clinical Nutrition, 81*(1), 74-78. doi:81/1/74 [pii]

Talanian, J. L., Galloway, S. D., Heigenhauser, G. J., Bonen, A., & Spriet, L. L. (2007). Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *Journal of Applied Physiology (Bethesda, Md.: 1985), 102*(4), 1439-1447. doi:01098.2006 [pii]

Tarnopolsky, L. J., MacDougall, J. D., Atkinson, S. A., Tarnopolsky, M. A., & Sutton, J. R. (1990). Gender differences in substrate for endurance exercise. *Journal of Applied Physiology (Bethesda, Md.: 1985), 68*(1), 302-308.

The Global Diabetes Community (2016). Blood sugar level ranges. Retrieved from http://www.diabetes.co.uk/diabetes_care/blood-sugar-level-ranges.html

Tremblay, A. (1992). Human obesity: A defect in lipid oxidation or in thermogenesis? *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 16*(12), 953-957.

Tremblay, A., Cote, J., & LeBlanc, J. (1983). Diminished dietary thermogenesis in exercise-trained human subjects. *European Journal of Applied Physiology and Occupational Physiology, 52*(1), 1-4.

Tremblay, A., Fontaine, E., Poehlman, E. T., Mitchell, D., Perron, L., & Bouchard, C. (1986). The effect of exercise-training on resting metabolic rate in lean and moderately obese individuals. *International Journal of Obesity, 10*(6), 511-517.

van Aggel-Leijssen, D. P., Saris, W. H., Wagenmakers, A. J., Hul, G. B., & van Baak, M. A. (2001). The effect of low-intensity exercise training on fat metabolism of obese women. *Obesity Research, 9*(2), 86-96. doi:10.1038/oby.2001.11 [doi]

van Aggel-Leijssen, D. P., Saris, W. H., Wagenmakers, A. J., Senden, J. M., & van Baak, M. A. (2002). Effect of exercise training at different intensities on fat metabolism of obese men. *Journal of Applied Physiology (Bethesda, Md.: 1985), 92*(3), 1300-1309. doi:10.1152/japplphysiol.00030.2001 [doi]

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H., & Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, *536*(Pt 1), 295-304. doi: PHY_12382 [pii]

Venables, M. C., & Jeukendrup, A. E. (2008). Endurance training and obesity: Effect on substrate metabolism and insulin sensitivity. *Medicine and Science in Sports and Exercise*, *40*(3), 495-502. doi:10.1249/MSS.0b013e31815f256f [doi]

Vettor, R., Macor, C., Rossi, E., Piemonte, G., & Federspil, G. (1997). Impaired counterregulatory hormonal and metabolic response to exhaustive exercise in obese subjects. *Acta Diabetologica*, *34*(2), 61-66.

Vogeser, M., Konig, D., Frey, I., Predel, H. G., Parhofer, K. G., & Berg, A. (2007). Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. *Clinical Biochemistry*, *40*(13-14), 964-968. doi:S0009-9120(07)00207-X [pii]

Wahren, J., Felig, P., Hendler, R., & Ahlborg, G. (1973). Glucose and amino acid metabolism during recovery after exercise. *Journal of Applied Physiology*, *34*(6), 838-845.

Weber, J., Brichon, G., Zwingelstein, G., McClelland, G., Saucedo, C., & Weibel, E. (1996). Design of the oxygen and substrate pathways. IV. partitioning energy provision from fatty acids. *Journal of Experimental Biology*, *199*(8), 1667-1674.

Wei, M., Kampert, J. B., Barlow, C. E., Nichaman, M. Z., Gibbons, L. W., Paffenbarger, R. S.,Jr, & Blair, S. N. (1999). Relationship between low cardiorespiratory fitness and

mortality in normal-weight, overweight, and obese men. *The Journal of the American Medical Association, 282*(16), 1547-1553. doi:joc90699 [pii]

World Health Organization (2000). Obesity: Preventing and managing the global epidemic. report of a WHO consultation. *World Health Organization Technical Report Series, 894*, i-xii, 1-253.

Willett, W. (1998). Is dietary fat a major determinant of body fat? *The American Journal of Clinical Nutrition, 67*, 556S-62S.

Wolfe, R. R., & Peters, E. J. (1987). Lipolytic response to glucose infusion in human subjects. *American Journal of Physiology - Endocrinology and Metabolism, 252*(2), 218-223.

World Health Organization. (2015). Obesity. Retrieved from http://www.who.int/topics/obesity/en/

Zurlo, F., Lillioja, S., Esposito-Del Puente, A., Nyomba, B. L., Raz, I., Saad, M. F., . . . Ravussin, E. (1990). Low ratio of fat to carbohydrate oxidation as predictor of weight gain: Study of 24-h RQ. *AJP - Endocrinology and Metabolism, 259*(5), E650-E657.

APPENDICES

Appendix A: Habitual Physical Activity Questionnaire

This questionnaire was developed to evaluate a person's habitual physical activity and was separated into three distinct dimensions:

(1) Work activity
(2) Sports activity
(3) Leisure time activity

However, we were not interested in NEAT so we excluded the work and leisure time activity sections. The questionnaire below was graded as follows: intensity was quantified as low (=1), medium (=2), and high (=3); hours per week was quantified as <1 (=1), 1-2 hr (=2), 2-3 hr (=3), 3-4 hr (=4), and >4 hr (=5); and months per year was quantified as <1 mo (=1), 1-3 mo (=2), 4-6 mo (=3), 7-9 mo (=4), and >9 mo (=5).

Section A: Work Index

Question	Response
What is your main occupation?	low activity
	moderate activity
	high activity
At work I sit	Never
	Seldom
	Sometimes
	Often
	Always
At work I stand	Never
	Seldom
	Sometimes
	Often
	Always
At work I walk	Never
	Seldom

	Sometimes
	Often
	Always

Work Index (continued)

At work I lift heavy loads	Never
	Seldom
	Sometimes
	Often
	Always
After working I am tired	very often
	Often
	Sometimes
	Seldom
	Never
At work I sweat	very often
	Often
	Sometimes
	Seldom
	Never
In comparison of others of my own age I think my work is physically	much heavier
	Heavier
	as heavy
	Lighter
	much lighter

Sports Index

What sport do you play most frequently?	Response
At which intensity	low intensity
	medium intensity
	high intensity
How many hours do you play a week?	< 1 hour
	1-2 hours
	2-3 hours
	3-4 hours
	> 4 hours
How many months do you play in a year?	< 1 month
	1-3 months
	4-6 months
	7-9 months
	> 9 months

Leisure Index

Question	**Response**
During leisure time I watch television	never
	seldom
	sometimes
	Often
	very often
During leisure time I walk	never
	seldom

	sometimes
	Often
	very often
During leisure time I cycle	never
	seldom
	sometimes
	Often
	very often
How many minutes do you walk and/or cycle per day to and from work school and shopping?	< 5 minutes
	5-15 minutes
	15-30 minutes
	30-45 minutes
	> 45 minutes

Appendix B: Food log

A blank food log was given to each participant along with a brief explanation on how to record food intake. Participants were required to record intake for at least one weekend day and two weekdays. The following provides an example of participant's food log.

The effect of fitness level vs body composition on the body's fat burning process in healthy men

Food Diary

Session date & Time: Participant code

Please record your food intake for at least 3 days (include one weekend day and two weekdays)

Date	Time	Food	Quantity
Feb 8	6 am	Honey Nut Cheerios	1 cup
		1% milk	1 cup
	11:15am	Turkey and cheddar sandwich	
		Blueberry muffin	
	7 pm	Chicken breast	200 g
		Mashed potatoes	1.5 cups
		Pepsi	375 ml
Feb 10	6 am	Honey Nut Cheerios	1 cup
		1% milk	1 cup
	11:20 am	Chicken breast	210 g
		Mashed potatoes	1.5 cups
	6 pm	Caesar salad	250 g
		Kraft balsamic vinaigrette	30 ml
		Rougement apple juice	350 ml
Feb 12	6 am	Kellogg's frosted flakes	2 cups
		Milk 1%	1.5 cups
	11:20 am	Pillsbury mini pizza	2; 190 g
		Apple	
	6 pm	Chicken breast	200 g
		Fries, Cavendish, flavour crisp	130 g