Advancing the Use of Exercise Testing as a Tool to Assess Whole-Body Substrate Selectivity and Metabolic Function in Individuals at Risk for Developing Type 2 Diabetes

Avigdor Dori Arad

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ABSTRACT

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Avigdor Dori Arad

Type 2 diabetes is a metabolic disease marked by an abnormally high level of glucose (sugar) in the blood. Type 2 diabetes is now reaching an epidemic level with more than 30 million adults in the United States afflicted and 1.5 million new cases documented every year. Type 2 diabetes is linked with obesity, heart disease, hypertension, and liver disease, and individuals with type 2 diabetes are at an increased risk for heart failure, stroke, blindness, kidney failure, and amputation. According to the Centers for Disease Control and Prevention, more than $245 billion was spent in the United States in 2012 on medical expenses related to diabetes and despite that, nearly a quarter of a million Americans are losing their lives due to this disease each year. Indeed, type 2 diabetes is one of the leading causes of death in the United States and worldwide; its prevalence has almost doubled in the last 35 years, from 4.7% of the total population in 1980 to 8.5% in 2014. Consequently, more than 400 million people are at high risk for severe health problems and complications, poor quality of life, and early death.

Research such as the Diabetes Prevention Program (DPP), the Finnish Diabetes Prevention Study (DPS), the Vesterbotten Intervention Program (VIP), and the Diabetes Prevention Program Outcome Study (DPPOS) suggests that type 2 diabetes can be delayed, and even prevented, with a lifestyle behavioral modification program that includes healthy eating and/or exercise. Therefore, focus has been shifted from management to prevention. An early manifestation of dysfunction in the progression of type 2 diabetes is insulin resistance, a metabolic impairment associated with obesity. Indeed, it is estimated that ~90% of people with type 2 diabetes also are obese. The link between insulin resistance and obesity is well-established; however, the mechanistic basis(es) underpinning this link is/are still debated with multiple candidate molecules, systems, and pathways potentially involved. One

theory that has gained traction in recent years suggests that type 2 diabetes, and the insulin-resistant state that predates it, are rooted in dysfunctional lipid metabolism (i.e., a reduced capacity to use lipid for energy production in circumstances where lipid would be preferred, such as in the basal fasting condition, after a high-fat meal, and during light- and moderate-intensity exercise). However, there are conflicting findings regarding the degree to which the ability to oxidize lipid during these circumstances is compromised for individuals with the overweight/obesity that is associated with the disease progression. The reason(s) for this ambiguity is/are unclear but might have to do with a number of factors that were poorly controlled when substrate selectivity (i.e., lipid vs. carbohydrate oxidation rates) were compared between normal-weight individuals and those with the overweight/obese condition. These include: (a) acute energy balance and macronutrient composition of the diet; (b) the intensity and duration of the exercise bout; and (c) subject characteristics including the amount of muscle tissue they possess, their cardiorespiratory fitness level, and, perhaps most importantly, their insulin-sensitivity state. The purpose of this dissertational work is to: (a) help to resolve this ambiguity by identifying the degree to which conflicting results that have been reported might be explained by factors that were left unaccounted for and/or inadequately controlled in previous research; and (b) compare substrate selectivity in normal-weight individuals and those with the overweight/obesity condition during a physiologically-equivalent exercise challenge with the aforementioned factors rigidly controlled.

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CHAPTER 1 – INTRODUCTION

1.1 Purpose

An early manifestation of dysfunction in the progression to hyperglycemia (i.e., elevated blood glucose levels) and type 2 diabetes is insulin resistance. Recent studies link insulin resistance with the accumulation of lipids in skeletal muscle and, therefore, type 2 diabetes has been "redefined" as a disease of dysfunctional lipid metabolism (i.e., lipid storage in muscle that exceeds lipid oxidation resulting in lipid accumulation). Excess body fat is associated with an increased risk of developing insulin resistance and the chronic positive energy balance that facilitates weight gain results in excess lipid accumulation throughout the body. Consequently, insulin resistance and an impaired capacity for lipid metabolism have been suggested to form the basis for the overweight/obese condition. The link between insulin resistance and the overweight/obese condition is well established, and overweight/obesity accounts for 80-85% of the likelihood of developing type 2 diabetes. However, it is interesting to note that not all individuals with the overweight/obese condition demonstrate insulin resistance and, heretofore, there are conflicting findings regarding the degree to which the ability to oxidize lipid during functional circumstances where lipid use is preferred (e.g., during fasting and in response to a high-fat diet/meal) is compromised for individuals with overweight/obesity. The reason(s) for this ambiguity is/are unclear but might have to do with measurement bias and/or a number of important factors that were not well controlled in these conditions (e.g., low metabolic demand, day-to-day variability in energy balance, weight imbalance, and plasma glucose and free-fatty acid concentration). Citing these concerns, scientists are now recommending assessing substrate selectivity (i.e., rates of lipid vs. carbohydrate oxidation) during exercise as a way to explore the link between impaired lipid metabolism and insulin resistance. Unlike the resting or feeding state, metabolic demand is significantly higher during exercise and therefore, if a defect exists, it is more likely to show. However, conflicting findings also exist regarding the degree to which excess fat and/or insulin resistance are associated with impaired lipid metabolism during exercise. Once again, the ambiguity is likely related to a number of key factors that were poorly controlled when substrate selectivity was compared between individuals with and without the overweight/obesity. These include: (a) acute energy balance and macronutrient composition of the diet; (b) the intensity and duration of the exercise bout; and (c) subject characteristics including the amount of

muscle tissue they possess, their cardiorespiratory fitness level, and, perhaps most importantly, their insulin-sensitivity.

The purpose of this dissertational work is to: (a) study the influence of the overweight/obese condition on exercise lipid oxidation from the existing data; (c) inform future research by determining the degree to which conflicting results that have been reported might be explained by factors left unaccounted for and/or inadequately controlled in previous research; and (c) resolve this ambiguity by comparing substrate selectivity in individuals with and without overweight/obesity during both fasting and exercise with the aforementioned factors rigidly controlled.

1.2 Background and Rationale

1.2.1 Diabetes—Definition, Prevalence, and Impact

Diabetes is a complex disease marked by a high level of glucose (sugar) in the blood (e.g., fasting glucose, $\geq$126mg·dl^{-1}; 2-hr post 75g glucose drink, $\geq$200mg·dl^{-1}; HbA1c, $\geq$6.5%). The vast majority (>90%) of individuals with diabetes possess type 2 diabetes, a condition associated with insulin resistance and obesity. Recent reports from the Centers for Disease Control and Prevention (CDC) suggest that diabetes has reached an epidemic level with more than 1.5 million new cases documented every year and, at present, nearly 30 million people in the United States have diabetes. Individuals with diabetes are at increased risk for various complications and co-morbidities, including blindness, heart disease, stroke, hypertension, kidney failure, liver disease, obesity, infertility, and amputation. In 2012, the economic cost of diabetes in the United States was $245 billion (a 41% increase from 2007), which indicates the significant burden that diabetes has on our society (American Diabetes Association, 2013). In addition, individuals with diabetes have a 50% greater risk for premature death compared with individuals without diabetes, and more people in the United States are dying from diabetes than from AIDS and breast cancer combined. In 2010, diabetes was mentioned as a cause of death in more than 234,000 cases, and together with heart disease and stroke, diabetes is among the seven leading causes of death in the United States (CDC, 2014). Given the devastating health consequences and the substantial economic burden, research and clinical work has been shifted, to some degree, from diabetes management to diabetes prevention.

1.2.2 Obesity—Definition, Prevalence, and Impact

The state of overweight/obesity is characterized by excess body fatness. Typically, overweight and/or obesity are classified using the body-mass index (BMI) scale, which is a mathematical computation using body weight (in kilograms) and body height (in meters squared). A normal BMI is between 18.5 and 24.9 $kg \cdot m^{-2}$, which suggests an appropriate level of body fat relative to body weight and height. Conversely, a BMI greater than 25.0 $kg \cdot m^{-2}$ is considered abnormally high ("overweight") and, at present, more than 70% of U.S. adults fall in to this category. Moreover, a BMI greater than 30.0 $kg \cdot m^{-2}$ indicates a state of excess fat accumulation labeled "obesity." The prevalence of obesity in the United States has increased significantly in recent years, from 11.1% in 1970 to 14% in 1990 to nearly 40% in 2017, with more than a quarter of the people with this condition falling into the morbidly-obese (BMI >35 $kg \cdot m^{-2}$) and extremely-obese (BMI >40 $kg \cdot m^{-2}$) categories (Flegal, Carroll, Ogden, & Curtin, 2010). From a public health perspective, this is alarming because epidemiological studies have demonstrated that a BMI greater than 30 $kg \cdot m^{-2}$ is a major risk factor for various neurological, psychological, circulatory, physiological, and metabolic disturbances, including heart disease, high blood pressure, fatty liver, stroke, heart failure, sleep apnea, depression, and osteoporosis (Mitchell, Catenacci, Wyatt, & Hill, 2011). Interestingly, the overweight/obese state exerts its greatest wrath via metabolic repercussion with 44% of the burden of diabetes attributable to this condition (Press, 2009). It is therefore not surprising that overweight/obesity and type 2 diabetes are linked at epidemic level. The primary cause(s) for the rise in the prevalence and incidences of obesity is/are not fully understood; however, experimental and epidemiological studies suggest that the root cause is multifactorial (e.g., poor nutrition, lack of physical activity, socioeconomic status, genetics, hormonal dysfunction, medication, smoking, education level, and other medical conditions) (Herpertz, 2008). One specific theory posits a relationship between weight gain and dysfunctional lipid metabolism based on the notion that excess accumulation of lipid in skeletal muscle (i.e., muscle fat) becomes toxic ("lipotoxic") and breeds insulin resistance when it is not turned over on a regular basis (McGarry, 1992; Moro, Bajpeyi, & Smith, 2008). In support of this view, there is evidence to suggest that individuals with obesity demonstrate an impaired ability to rely on lipid oxidation for the production of energy in circumstances where lipid would be the preferred fuel, such as after an overnight fast, in response to a high-fat meal, and during moderately-intense exercise. However,

evidence also exists suggesting the contrary—specifically, that lipid oxidation is not impaired and might even be enhanced with obesity. The reason(s) for this ambiguity is/are not understood but might reflect a number of factors that are typically poorly controlled when lipid oxidation is compared between individuals with normal weight and overweight/obesity. Specifically, the insulin-resistant status of the participant with the obesity condition is often not considered and it stands to reason that substrate shifts associated with insulin desensitization could exert an effect independent of body fatness. Therefore, it remains unknown if the metabolic change is due to the excess accumulation of fat or the insulin-resistant state that often accompanies it; heretofore, no research has compared lipid oxidation at rest and during a physiological-equivalent exercise challenge in individuals with normal weight and overweigh/obesity, with insulin-resistant status well controlled.

1.2.3 Insulin Resistance and Dysfunctional Lipid Oxidation

Insulin resistance characterizes a metabolic state for which muscle tissue, adipose tissue, and the liver do not respond in the appropriate manner to the hormone insulin. The result is elevated blood glucose (Wilcox, 2005). It is well recognized that insulin resistance is a primary defect in the progression to type 2 diabetes that shares a strong association with obesity (Kahn & Flier, 2000). For example, more than 90% of people with type 2 diabetes are overweight or obese. The link between insulin resistance and the overweight/obesity state is well documented (Kahn & Flier, 2000); however, the mechanistic basis(es) is/are still being debated with multiple candidates potentially involved. One theory, proposed by Dennis J. McGarry in 1992, suggests that type 2 diabetes is rooted in the dysfunctional metabolism of lipid (fat), not sugar. In support of this postulation, a growing body of evidence suggests that insulin resistance is associated with excess accumulation of lipid in skeletal muscle and that these elevated intramyocellular lipids (i.e., fat stored in droplets in muscle cells) become “lipotoxic” when it is not “turned over” on a regular basis. Insulin-stimulated glucose metabolism occurs predominantly in the muscle; hence, excess lipid accumulation interferes with normal glucose processing. Within that schema, chronic hypercaloric intake and a sedentary lifestyle would provide the conditions for pathological elevation of intramyocellular lipids that leads to insulin resistance and the diabetes progression.

A number of studies support this contention. For example, Krssak et al. (1999) reported an inverse relationship between insulin sensitivity and both fasting plasma free-fatty acid concentration (FFA)

and intramyocellular triglyceride content (IMTG) in normal-weight non-diabetic adults. A similar relationship was also found in non-diabetic Pima Indian men, a group that is at high risk for obesity and diabetes, independent of body fat percentage (Pan et al., 1997). It is interesting to note that endurance athletes also demonstrate markedly high levels of intramyocellular lipids; however, they also demonstrate high insulin-sensitivity levels (Goodpaster, He, Watkins, & Kelley, 2001). Moreover, a higher content of intramyocellular lipids has been reported in individuals post exercise intervention (Dubé et al., 2008). These findings appear to suggest that lipid accumulation in skeletal muscle per se is not pathological. Since day-to-day energy metabolism and lipid use are typically greater in those who are physically active versus sedentary, one possible explanation is that lipid accumulation in muscle becomes toxic, or pathological, when it is not consistently and sufficiently used. An important point in this regard is that energy expenditure should be, on average, lower than energy intake (i.e., a negative energy balance should be in effect) for this protectivity.

The accumulation of lipid in skeletal muscle can be the result of excess FFA uptake, inadequate lipid utilization, or both. In 1999, Kelley, Goodpaster, Wing, and Simoneau et al. measured glucose and FFA uptake in the basal state together with indirect calorimetry across the leg in lean and obese subjects. They found that FFA uptake exceeded FFA oxidation in both groups, which means that lipid accumulation was taking place in the muscle regardless of body weight. Moreover, the rate of FFA uptake by skeletal muscle was similar between the two groups. However, lipid oxidation was significantly lower in those with obesity, which suggests that the net accumulation of lipid in muscle was greater. It also implies that it is the ability to use the fat stored in the muscle (i.e., intramyocellular lipid oxidation) that dictates whether storage is benign or harmful (Kelley et al., 1999).

If the line of reasoning advanced by Kelley et al. (1999) is correct, individuals with insulin resistance and/or obesity should demonstrate dysfunctional lipid metabolism. Specifically, this should manifest as an impaired capacity to rely on lipids as the main fuel source in circumstances where lipid would be the preferred fuel for energy production (e.g., fasting, in response to a high-fat meal and during exercise performed at or below the lactate threshold; i.e., moderate-intensity exercise). With regard to exercise, it is important to note that compared to untrained subjects, endurance-trained individuals oxidize more lipid at the same absolute and relative work rate (Holloszy & Coyle, 1984; Van Loon, Jeukendrup,

Saris, & Wagenmakers, 1999) and this increase in total lipid oxidation is almost completely derived from IMTG- and/or very-low-density lipoproteins (Schrauwen et al., 2002). Furthermore, the increased capacity for IMTG storage and breakdown in subjects after a 6-week endurance training regimen (see above) is correlated and mediated via expression of perilipins 2 and 5 (Shepherd et al., 2013). Importantly, these lipid-droplet proteins attenuate lipotoxic damage (Bosma et al., 2012) and, indeed, as is typically the case after an endurance-training intervention, the protocols employed in that study improved insulin sensitivity despite a ~2-fold increase in resting IMTG content (Shepherd et al., 2013). Collectively, these findings suggest that it is intramyocellular lipid *oxidation and turnover*, as opposed to FFA uptake and accumulation per se, that determine their influence on insulin's regulatory prowess. With this in mind, it is important to clarify existing ambiguity regarding the degree to which lipid oxidation is compromised for individual with obesity and/or insulin resistance in circumstances where lipid oxidation is the preferred fuel (see above).

1.2.4 Assessing Fuel Metabolism and Dysfunctional Lipid Oxidation During Basal Conditions

It is well established that during the basal state (e.g., immediately after a 12-hr overnight fast), skeletal muscle of a normal-weight healthy individual relies on lipids to supply the majority of energy production (Andres, Cader, & Zierler, 1956; Baltzan, Andres, Cader, & Zierler, 1962). The ability to predominantly rely on lipids during basal conditions is related to muscle-fiber type (Kelley et al., 1999; Mogensen et al., 2007); mitochondrial content and function (Kelley et al., 1999); enzymatic activity (He, Watkins, & Kelley, 2001); hormonal state (Randle, Kerbey, & Espinal, 1988); and substrate availability (Randle et al., 1988). Based on this observation, researchers have measured energy metabolism and fuel selectivity (i.e., rates of lipid versus carbohydrate oxidation) in the basal condition as a way to determine if impairment is present. For example, in a seminal study, Kelly et al. (1999) confirmed a lower rate of lipid oxidation for individuals with obesity compared with normal-weight subjects after a 12-hr overnight fast. This suggests that individuals with obesity may indeed have an impaired capacity to oxidize lipid for energy. Filzof et al. (2000) reported a lower lipid-oxidation rate in post-obese subjects compared with age- and BMI-matched never-obese controls, despite similar rates of energy production. Solomon et al. (2008) reported a greater reliance on lipid oxidation during sleep following a weight-loss intervention (induced by diet or diet and exercise) for healthy obese women. Goodpaster, Katsiaras, and Kelley (2003)

reported increased lipid oxidation during fasting in pre-diabetic obese subjects after a weight loss induced by exercise or exercise and diet. Collectively, these findings support the notion that dysfunctional lipid metabolism in the basal state could be the primary defect that links insulin resistance and the overweight/obesity conditions. However, other studies provide contradictory findings; specifically, that lipid oxidation in the basal state is not reduced in the overweight/obese state (Balcı, 2012; Blaak et al., 2006; Colberg et al., 1996; Ekelund et al., 2007; Ezell et al., 1999; Guesbeck et al., 2001; Helge, Watt, Richter, Rennie, & Kiens, 2001; Kanaley, Weatherup-Dentes, Alvarado, & Whitehead, 2001; Perez-Martin et al., 2001; Steffan, Elliott, Miller, & Fernhall, 1999), and might even be enhanced (Nicklas, Rogus, & Goldberg, 1997; Schutz, Tremblay, Weinsier, & Nelson, 1992; van Aggel-Leijssen, Saris, Hul, & van Baak, 2001). The reason(s) for the ambiguity is/are not fully clear but might be related to the fact that during basal conditions, metabolic demand is low and absolute lipid-oxidation rate is minimal. Moreover, acute energy balance and dietary macronutrient composition of the diet in the days prior markedly influence the measurement.

1.2.5 Assessing Substrate Selectivity in Response to Insulin-simulation

The role of insulin in regulating oxidative and non-oxidative pathways related to energy metabolism, substrate utilization, and glucose homeostasis is well documented (Felber et al., 197; Saltiel & Kahn, 2001). In a healthy person, increasing the blood insulin level either via insulin stimulation (e.g., post intake of an oral glucose drink or high-carbohydrate meal) and/or insulin infusion (e.g., post insulin clamp) leads to up regulation of glucose uptake and oxidation in skeletal muscle and down regulation of lipid metabolism and oxidation. Based on these effects, the ability to switch from predominantly lipid oxidation in the basal condition to predominantly carbohydrate oxidation post insulin stimulation/infusion has been used to assess skeletal muscle “metabolic flexibility,” which is defined as the capacity of skeletal muscle to adjust fuel use based on fuel availability. In 1999, Kelley et al. were the first to demonstrate impaired substrate switching in overweight/obese subjects. By comparing fuel utilization and substrate selectivity immediately during insulin infusion with that collected during basal conditions, they demonstrated that normal-weight subjects have a robust capacity to shift fuel use from predominant lipid oxidation in the basal state to predominant glucose oxidation post infusion. In contrast, obese subjects demonstrated a lower level of lipid oxidation during the basal condition and impaired switching to glucose

oxidation when infused with insulin. This observation suggested that individuals with obesity lack the metabolic flexibility that characterized healthy function, and since insulin resistance can manifest in both a lower level of lipid oxidation in the basal state and blunted suppression in the insulin-induced state, the authors reasoned that this might reflect a primary defect associated with obesity that links it with insulin resistance. However, using a similar protocol (i.e., hyperinsulinemic clamp), Galgani, Moro, and Ravussin (2008) found similar metabolic flexibility in individuals with obesity and type 2 diabetes compared with individuals with obesity but no type 2 diabetes after adjusting for the rate of glucose disposal which, by nature, is reduced when insulin resistance is present. This may suggest that the metabolic inflexibility to glucose in patients with type 2 diabetes is related to a defect in glucose transport as opposed to fuel selectivity (i.e., carbohydrate and/or lipid oxidation) (Galgani, Moro, & Ravussin, 2008). In addition, the insulin-infused conditions, which have been employed by Kelley el al. and others to assess metabolic flexibility and functional fuel switching, have raised concerns because of their “supra-physiological” nature (Bergouignan et al., 2012).

1.2.6 Assessing Substrate Selectivity in Response to High-fat Meal

The capacity to increase fuel oxidation based on substrate availability is another hallmark of normal metabolic function (Storlien, Oakes, & Kelley, 2004). In normal-weight healthy individuals, increasing the proportion of dietary fat increases FFA availability and oxidation, whereas a high-carbohydrate meal/drink or glucose infusion suppresses fat oxidation and promotes carbohydrate oxidation. Based on these effects, researchers started using a high-fat meal (acute) or diet (chronic) as a way to assess metabolic function/flexibility based on the capacity to up-regulate fat oxidation in accordance with substrate availability. For example, Heilbronn, Gregersen, Shirkhedkar, Hu, and Campbell (2007) found a significant difference in metabolic flexibility toward dietary fat in individuals with a family history of diabetes compared with age- and fatness-matched controls, despite similar level of insulin sensitivity, fasting fat oxidation, and postprandial FFA level in the blood. Importantly, there was no difference between the groups in metabolic flexibility toward carbohydrate. Kelley et al. (1999) found an impaired capacity to increase reliance on lipid oxidation that lasted more than 6 hours after eating a high-fat meal in individuals with diabetes compared with weight-match healthy controls, despite similar levels of oxidative enzymes. Berk, Kovera, Boozer, Pi-Sunyer, and Albu (2006) demonstrated a significantly

lower rate of lipid oxidation after 7 days of a high-fat (>50% of total calories) feeding protocol in African American women (i.e., a group predisposed to insulin resistance and type 2 diabetes) compared with their Caucasians counterparts, despite similar age, wait-to-hip ratio, BMI, percent body fat and fat-free mass. Together, these observations suggest a link between insulin action and dysfunctional lipid metabolism, and the presence of metabolic inflexibility to dietary lipid in individuals with a family history of diabetes may indicate that impaired lipid oxidation may precede insulin resistance. However, using postprandial fuel use as a way to assess impairments related to lipid metabolism has been questioned after Blaak et al. (2006) found increased fat oxidation in a large cohort of individuals with obesity and insulin resistance compared with normal-weight healthy subjects after high-fat meals. This difference remained significant even after adjusting for fat mass, fasting fat oxidation, sex, and physical activity (Blaak et al., 2006). One possible factor that may explain the discrepancy is the relative low level of metabolic challenge that exists in the basal and/or postprandial state (Galgani, Moro, & Ravussin, 2008).

1.2.7 Assessing Substrate Selectivity in Response to Exercise Stimulation

Citing the various limitations and conflicting findings regarding the degree to which dysfunctional lipid metabolism can or should be assessed in the basal, insulin-stimulated, and postprandial states, Galgani, Moro, and Ravussin (2008) proposed using exercise as a tool to explore the link between insulin resistance and dysfunctional lipid metabolism and the role it may play in obesity. The authors based this contention on the fact that energy expenditure and metabolic demand are significantly higher during exercise compared to basal or postprandial conditions. Therefore, if a defect exists, it is more likely to manifest during an activity that requires highly coordinated regulation between fuel supply and the oxidative machinery. It is well established that fit individuals are able to up regulate lipid oxidation in response to an exercise challenge, in part, to conserve precious sources of muscle glycogen (Storlien et al., 2004). Indeed, Nordby, Saltin, and Helge (2006) found that lipid oxidation is higher, and occurs at higher exercise intensities, in fit compared to unfit individuals. While exercise can, in theory, provide a consistent medium for assessing lipid use, conflicting findings exist regarding the degree to which exercise lipid oxidation is impaired in individuals with overweight/obesity with evidence to suggest greater (Goodpaster, Wolfe, & Kelley, 2002; Horowitz & Klein, 2000; Kanaley et al., 2001; Lanzi et al., 2014), lesser (Hickner, Privette, McIver, & Barakat, 2001; Keim, Belko, & Barbieri, 1996; Perez-Martin et al.,

2001), and similar (Balcı, 2012; Colberg et al., 1996; Ezell et al., 1999) rates of lipid oxidation in individuals with obesity and/or insulin resistance compared with normal-weight healthy subjects. This lack of consistency might be related to a number of factors that were poorly controlled when substrate selectivity (i.e., lipid vs. carbohydrate oxidation rates) was compared between overweight/obese and normal weight subjects. These include: (a) acute energy balance and macronutrient composition of the diet; (b) the intensity and duration of the exercise bout; and (c) subject characteristics including the amount of muscle mass they possess, their cardiorespiratory fitness level, and, perhaps most importantly, their state of insulin sensitivity. The research study proposed for this dissertation work is the first to date to assess substrate selectivity in normal-weight and overweight individuals during physiologically-equivalent exercise challenges, while rigidly controlling for various factors that could, in theory, affect the comparison.

1.3 Statement of Research Questions

1.3.1 Systematic Review

1.3.1.1. Do individuals with the overweight/obese condition rely on lipid oxidation to a greater, lesser or the same extent during exercise compared to normal-weight subjects?

1.3.2 Clinical Trial

1.3.2.1 Do individuals with the overweight/obese condition rely on lipid oxidation to a greater, lesser or the same extent during a physiologically-equivalent exercise challenge compared to age-, gender-, and fitness-matched normal-weight subjects?

1.3.2.2 *Hypothesis #1:* Rates of lipid oxidation during exercise, adjusted for fat-free mass, will be lower in individuals with the overweight/obese conditions.

1.3.2.3 Hypothesis #2: The lower rates of lipid oxidation will be related to changes associated with the loss of metabolic health (e.g., insulin resistance) rather than the overweight/obese state per se.

1.4 Significance and Implications

The overlying objective of this dissertation work was to resolve ambiguity regarding the capacity for lipid oxidation in individuals with the overweight/obese condition. This is important because the degree to which the overweight/obese condition affects substrate selectivity and the influence it exerts are

important factors to consider when designing an exercise intervention to “treat” this disease. For example, if lipid use is impaired in individuals with the overweight/obese condition, it supports the contention that a mitochondrial defect exists; hence, exercise that is specific for improving mitochondrial capacity (e.g., high-intensity interval training) would be warranted. Conversely, if the capacity for lipid use is similar, or enhanced, with the overweight/obese condition, mitochondrial function would, apparently, be sufficient; hence, a training work rate situated closer to the one at which lipid use is maximized might be preferred as a way to perturb the intramyocellular lipid pool and reduce its pathogenic influence.

1.5 Scope and Delimitation

This clinical research is a cross-sectional comparison between four groups designed to determine whether dysfunctional lipid oxidation is related to the overweight/obese condition (e.g., due to excessive fat accumulation throughout the body and, specifically, in areas other than adipose tissue; i.e., “ectopic fat deposition”) or loss of metabolic health (e.g., insulin desensitization). Due to the nature of the design, the application is not equipped to establish a causality relationship (e.g., dysfunctional lipid oxidation leads to insulin resistance or vice versa) but may be able to establish an associative relationship (e.g., dysfunctional lipid oxidation is associated with insulin resistance, but not to ectopic fat accumulation).

1.6 Definitions of Terms

Type 2 diabetes (T2D): A metabolic disorder associated with insulin resistance and/or insulin production characterized by an abnormal level of blood sugar.

Metabolic flexibility: The ability to shift between carbohydrate and fat as the predominant fuel based on physiological needs and circumstances. The lack thereof is known as metabolic inflexibility.

Metabolic syndrome: A cluster of conditions, including elevated blood pressure, high blood sugar, excess abdominal fat, and suboptimal blood lipids, that occur together and increase risk of heart disease, diabetes, stroke, and other illnesses.

Family history of diabetes: The presence of an affected first-degree relative.

High-intensity interval training: Repeated bouts of high-intensity effort separated by recovery intervals.

Endurance training: Exercise that is rhythmic, continuous, sustainable, and performed using large muscle groups.

$\dot{V}O_{2max}$: The highest rate of oxygen consumption that can be achieved by the individual.

Gas exchange threshold (GET): A breakpoint in the production of carbon dioxide ($\dot{V}CO_2$) compared to the consumption of oxygen ($\dot{V}O_2$) obtain during a progressive exercise test.

Constant-work-rate exercise: An exercise bout where the external power output does not change.

High-fat diet: A diet pattern characterized by dietary fat as the greatest contributor to total energy consumption relative to carbohydrate or protein; for example, a dietary pattern with 50% of the daily caloric intake as dietary fat.

Heart-healthy diet: As per the American Heart Association and the USDA, a heart-healthy diet is one that provides 45–55% of total calories from carbohydrates, 25–35% from fat, and 12–20% from protein.

CHAPTER 2 – REVIEW OF THE LITERATURE

2.1 Insulin Resistance and Dysfunctional Lipid Metabolism

According to the World Health Organization (WHO, 2018), over 1.9 billion adults worldwide were classified as “overweight” in 2014 with more than 600 million considered “obese.” This “obesity epidemic” conveys a considerable health risk; for example, a raised BMI is a major risk factor for heart disease, stroke, musculoskeletal disorders, and some cancers (WHO, 2018). However, overweight/obesity exerts its greatest wrath via metabolic repercussion, with 44% of the burden of diabetes attributable to this condition (Press, 2009). It is, therefore, not surprising that a “diabetic epidemic” (Zimmet, Alberti, & Shaw, 2001) is also in full swing with 23 million U.S. citizens diagnosed and another 7.2 million undiagnosed, but afflicted (CDC, 2014). The vast majority possess type 2 diabetes, which is a disorder associated with insulin resistance that is often a characteristic of overweight/obesity (Ludvik, Nolan, Baloga, Sacks, & Olefsky, 1995). The link between insulin resistance and the overweight/obese state is well established; however, mechanistic basis(es) is/are still debated with multiple candidate molecules, systems, and pathways potentially involved (Qatanani & Lazar, 2007).

In the late 19th century, Dr. Oskar Minkowski, who was a professor of medicine at the University of Breslau (Germany) and a pioneer in the area of diabetes and endocrinology research, was questioning the assertion that pancreatic enzymes were needed to break down fatty acids in the gut. A complete removal of the pancreas was required to test his concerns; therefore, it was impossible to assess for a human subject. Consequently, Dr. Minkowski proceeded with his experiment using his dog. After a complete removal of the pancreas, Dr. Minkowski noticed that the urine from his pancreactomized dog attracted an unusually high number of flies. Curious, he decided to taste the urine and was struck by its sweetness. Based on this observation, he concluded that the pancreas produces some entity essential for controlling blood-sugar level which, when absent, resulted in an abnormally-high level of sugar in the blood and urine. Thirty years later, when Fredrick Benting discovered insulin, the concept of an insulin-glucose relationship as a central component of fuel metabolism came into being, and diabetes has, therefore, been viewed as a disorder primarily associated with abnormal glucose metabolism. Indeed, diabetes, or impaired glucose tolerance (i.e. “pre-diabetes”), which is an early state in the progression to type 2 diabetes, is typically diagnosed via fasting plasma glucose and/or HbA1c measurement, which are

tests that identify elevated plasma glucose levels. However, for diagnostic and treatment purposes, it might be short-sighted to consider diabetes as a disease of glucose metabolism alone because the other primary fuel source, lipids, might also play a role (McGarry, 1992). In support of this view is the work of Reaven and colleagues in the late 1980s which confirmed a significantly higher plasma FFA concentration at hourly intervals for 24 hours in patients with diabetes compared to non-diabetic counterparts, despite similar levels of insulin (Reaven, Hollenbeck, Jeng, Wu, & Chen, 1988). Based on the principles of the Randle glucose-fatty acid cycle (DeFronzo, Bonadonna, & Ferrannini, 1992), abnormally-high levels of plasma FFA will drive the conversion of pyruvate (i.e., the end product of glycolysis) toward lactate (reduction), as opposed to acetyl CoA (oxidation), which will further enhance the rate of glucose production and resultant hyperglycemia. In this study, both FFA and lactate were significantly and chronically higher in the diabetes group which further supports this contention (Reaven et al., 1988). In the same cohort, when plasma FFA levels were compared during a 5-hour hyperglycemic clamp at low (10 $mU \cdot ml^{-1}$, 0–150 min) and high (60 $mU \cdot ml^{-1}$, 150–300 min) insulin-infusion rates, the afflicted subjects had significantly higher plasma FFA levels at baseline that remained high despite infusion of insulin at either rate (Chen, Golay, Swislocki, & Reaven, 1987). Furthermore, in both animal and human models, treatment with an antilipolytic agent (e.g., nicotinc acid) results in a rapid decrease in blood-glucose concentration in afflicted subjects (Reaven et al., 1988; Saloranta, Franssila-Kallunki, Ekstrand, Taskinen, & Groop, 1991). Collectively, these findings suggest that the insulin-resistant state, which is central to type 2 diabetes, is characterized by the inability of insulin (stimulated or infused) to affect the liver (i.e., suppress liver gluconeogenesis and glucose output), skeletal muscles (i.e., increase glucose uptake), *and* fat cells (i.e., suppress adipose tissue lipolysis and the release of FFA).

In 1992, citing some of the observations mentioned above, Dr. Denis McGarry, who was a professor of internal medicine at the University of Texas Southwestern Medical Center and a world-leading expert on metabolism and biochemistry, wrote an interesting essay entitled “What If Minkowsky Had Been Ageusic? An Alternative Angle on Diabetes.” McGarry explored the possibility that traditional thinking about diabetes as a disease of glucose metabolism might carry the wrong emphasis. Instead, he speculated that the pathophysiological mechanisms of insulin resistance (and the resultant hyperglycemia) are offshoots of dysfunctional lipid metabolism. Indeed, if Dr. Minkowski had lacked a

sense of taste, McGarry postulated that instead of tasting the urine and appreciating its sweetness, he would have smelled the acetone (i.e., a direct byproduct of acetoacetic acid). In his opinion piece, McGarry outlined a series of events based on Randle's substrate-competition model (DeFronzo et al., 1992) whereby an increase in lipid oxidation would inhibit glucose use. Within this schema, the abnormally-high level of insulin in the blood that results from insulin resistance (hyperinsulinemia) and resultant liver lipogenesis and synthesis of very low-density lipoproteins (VLDL) would serve to increase the flux of triglycerides into storage sites including adipose tissue and muscle (intramyocellular lipid; IMCL), thereby interfering with normal glucose processing (i.e., glycogen synthesis and glucose oxidation). The end result would be an elevation of plasma glucose that completes a vicious cycle of aberrant fuel partitioning (McGarry, 1992) that would be more likely to lead to overweight/obesity if a positive energy balance is in effect because, compared to carbohydrate storage, lipid storage is a more energetically-efficient way to increase body energy stores.

It is interesting to note that when Dr. McGarry wrote his piece 25 years ago, diabetes only affected about ~1-6% of the adult population in the United States with type 2 diabetes accounting for ~75% of the cases. Today, diabetes affects ~10% of the total population, with type 2 diabetes accounting for more than 90%. It is interesting to speculate that our current obesogenic environment, which is characterized by a chronic calorie overconsumption (and consequent excess lipid storage throughout the body) and a sedentary lifestyle, plays a central role. Moreover, the intake of dietary fat in the United States has also increased in the past few decades (Vadiveloo, Scott, Quatromoni, Jacques, & Parekh, 2014) and, while speculative, this may also play a role.

2.2 The Role of Insulin Resistance and β-cell Function in the Progression to Type 2 Diabetes

The progression from a healthy state to full-blown diabetes likely occurs on a continuum that has been suggested to include five unique stages. Each of these stages is characterized by distinguishable changes in pancreatic β-cell mass and/or phenotype and/or function (Weir & Bonner-Weir, 2004). In the first stage (i.e., "compensation"), β-cells increase insulin secretion to maintain blood glucose within a normal range (i.e., 70-99 $mg \cdot dL^{-1}$) in the face of insulin resistance. The cause of insulin resistance is still unclear, but might be related to genetic and/or environmental factors including obesity, poor diet, smoking, lack of physical activity, defective mitochondrial structure/function, impaired fuel metabolism,

inflammation, or family predisposition (ATTVALL, Fowelin, Lager, Schenck, & Smith, 1993; Turner & Heilbronn, 2008; Weir & Bonner-Weir, 2004). Despite the insulin-resistant glucose metabolic state, the ability of the β-cells to increase insulin production results in seemingly normal clinical presentation (i.e., glucose tolerance) (DeFronzo et al., 1992). The second stage (i.e., "adaptation") is characterized by the inability of the β-cells to continue to compensate for the loss of insulin sensitivity by producing more insulin to keep blood glucose in the normal range. However, β-cells are still able to produce enough to keep blood glucose level from reaching a state of diabetes (>125 mg·dL^{-1}). Individuals with impaired glucose tolerance ("pre-diabetes") evidenced by fasting blood sugar of 100–125 mg·dL^{-1} and/or HbA1c 5.7–6.4% and/or OGTT 140–199 mg·dL^{-1} are said to be at that stage in terms of insulin resistance and β-cell function. Despite being a significant risk factor, not all individuals with impaired glucose tolerance (stage 2) will progress to stage 3 ("unstable early decompensation") and/or frank diabetes. Individual variability depends on genetics, β-cell function and resilience, environment, eating habits, physical activity, and other factors (Weir & Bonner-Weir, 2004). However, it is now clear that overt diabetes develops in those whose pancreatic β-cells cannot continue to compensate. One of the prominent changes in β-cell phenotype and function that is often a prominent feature of this stage is loss of the acute phase of insulin secretion that occurs 3–10 min after glucose infusion (glucose-stimulated insulin secretion; GSIS) or early insulin release, which is completely absent when blood glucose reaches levels characteristic of the diabetic state (Weir & Bonner-Weir, 2004). The exact cause of GSIS is not fully understood, but might be related in full or part to elevated plasma FFA and lipotoxicity (Weir & Bonner-Weir, 2004). Stage 3 of the progression of insulin resistance is characterized by a relatively rapid and transient increase in blood glucose from a stable adaptation at ~100–125 mg·dL^{-1} (stage 2) to ~250–350 mg·dL^{-1} (stage 4). This pathologic progression signifies the point at which β-cell mass and function become inadequate to compensate for the insulin-resistant state and, at this point, the progression to frank diabetes is inevitable. The transition from a stable stage 2 to a stable stage 4 via the relatively short but rapid unstable stage 3 may or may not be coupled with classic symptoms associated with diabetes, such as weight loss, frequent urination, extreme fatigue, hunger, thirst, and dry mouth and skin. Stage 4 ("stable decompensation") is marked by a number of significant changes in β-cell mass and function, including a 50% reduction in mass and a severe decreased efficiency of insulin secretion (Weir & Bonner-

Weir, 2004). Despite markedly-high glucose in the blood (e.g., 250–350 mg·dL^{-1}), individuals in stage 4 still produce enough insulin to keep blood sugar "abnormally" stable and to avoid progression to ketoacidosis. Individuals with diabetes might stay in stage 4 for a lifetime; however, once the reduction in β-cell mass and function is severe enough (stage 5; "severe reduction"), a pathological increase in the production of ketone bodies is present (severe ketoacidosis) as is severe hyperglycemia, and the result is that they must inject insulin to live their next day.

2.3 Quantifying Insulin Action and Fuel Metabolism Using Glucose Clamp Technique

In 1979, De Fronzo and co-workers developed and validated a technique for assessing insulin resistance by measuring whole-body tissue sensitivity to insulin. The procedure is called the euglycemic-hyperinsulinemic clamp (DeFronzo, Tobin, & Andres, 1979). For this method, plasma insulin concentration is acutely raised to a higher plateau to induce physiological hyperinsulinemia (e.g., 100 mU·mL^{-1} above baseline), which is maintained for the entire procedure via constant insulin infusion. This level of circulating insulin would rapidly induce a very low blood-glucose concentration characteristic of severe hypoglycemia if glucose was not kept at its basal euglycemic levels (e.g. 70–100 ml·dL^{-1}). Therefore, the technique involves the simultaneous constant infusion of a predetermined fixed dose of insulin (e.g. 40 mU·mL^{-1}·min^{-1}) to achieve hyperinsulinemia in conjunction with a variable rate of glucose infusion to maintain basal arterial plasma glucose. When a steady state is achieved, glucose-infusion rate must equal glucose uptake by all tissues in the body. Of particular note in this regard is skeletal muscle, which is a major site for insulin-stimulated glucose disposal and, therefore, an important determinant of whole-body-tissue insulin sensitivity (DeFronzo et al., 1979).

The euglycemic-hyperinsulinemic clamp was used to establish the link between insulin sensitivity/resistance and dysfunctional lipid metabolism in 1999, when Kelley et al. (1999) described the impaired substrate switching that is present with obesity. These researchers compared leg respiratory quotient (RQ; i.e., rate of carbon dioxide production divided by rate of oxygen consumption with values ranging from 0.7 to 1.0 reflecting exclusive lipid and carbohydrate oxidation, respectively) during a euglycemic-hyperinsulinemic clamp with fasting values and found that for lean subjects, RQ increased significantly from the fasting to insulin-stimulated condition, whereas for obese subjects, it did not (Kelley et al., 1999). Indeed, for obese individuals, RQ remained relatively fixed at a value that was greater than

the fasting value for lean subjects and less than the insulin-stimulated one (Kelley et al., 1999). This impaired capacity to match fuel oxidation to fuel availability (i.e., predominantly lipid oxidation during fasting and a high rate of glucose oxidation during insulin stimulation) suggests that obese individuals lack the "metabolic flexibility" that characterizes healthy function. In accordance with these findings, researchers began using the difference in RQ across the extremes established during the clamp procedure ($\Delta RQ_{clamp\text{-}fast}$ or $\Delta NPRER_{clamp\text{-}fast}$ when non-protein respiratory exchange ratio measured at the mouth is used for the measurement) as a way to assess metabolic health and/or explore the link between impaired insulin action and the overweight/obese state (Adamska et al., 2013, 2014; van de Weijer et al., 2013; Wohl, Wohl, Girman, & Pelikánová, 2004).

2.4 Substrate Switching in Response to Insulin Infusion

Insulin resistance can manifest in both an elevated fasting RQ/NPRER ($RQ/NPRER_{fast}$) and a blunted elevation in insulin-stimulated RQ/NPRER ($RQ/NPRER_{clamp}$), which is why $\Delta RQ/NPRER_{clamp\text{-}fast}$ can be markedly reduced when overweight/obesity is present. Indeed, it has been suggested that the reduction in $\Delta RQ/NPRER_{clamp\text{-}fast}$ that has been reported for obese subjects could reflect a "primary defect" associated with obesity (Kelley et al., 1999). However, Galgani et al. report that the reduced $\Delta RQ/NPRER_{clamp\text{-}fast}$ they observed in individuals with type 2 diabetes, compared to non-afflicted subjects matched for obesity, was abolished after adjusting for glucose disposal rate which would, by nature, be reduced when insulin resistance is present (Galgani, Heilbronn, et al., 2008). This suggests that the decreased $RQ/NPRER_{clamp}$ observed with obesity reflects the insulin resistance-related inability for glucose transport as opposed to an inherent inability to oxidize glucose per se (Galgani, Heilbronn, et al., 2008). The insulin-infused condition that establishes the "ceiling" of the range has also raised concerns because of its "supra-physiological" nature (Bergouignan et al., 2012).

2.5 Substrate Switching to Lipid During Fasting

Unlike the insulin-stimulated state, the inability to up regulate lipid oxidation adequately during fasting, which elevates the "floor" by increasing $RQ/NPRER_{fast}$, is not affected by type 2 diabetes (Galgani, Heilbronn, et al., 2008) and could, therefore, reflect an inherent defect that links insulin resistance with the overweight/obese state (Kelley et al., 1999). A number of findings support this contention. Zurlo et al. reported that independent of 24-hour energy expenditure, non-diabetic Pima

Indian men (a group that is predisposed to obesity and type 2 diabetes) with a higher 24-hour RQ were at 2.5-times higher risk of gaining weight, compared to counterparts with lower 24-hour RQ (90th and 10th percentile, respectively) (Zurlo et al., 1990). Post-obese subjects also demonstrate a higher fasting RQ despite a similar resting metabolic rate, compared to age- and BMI-matched never-obese controls (Filozof et al., 2000). These findings cohere with reports of decreased sleep RQ following a weight-loss intervention (severe caloric restriction induced by diet alone or diet plus exercise) for healthy obese women (Kempen, Saris, & Westerterp, 1995) and increased fasting lipid oxidation for obese subjects with impaired glucose tolerance after weight loss due to exercise or exercise with caloric restriction (Solomon et al., 2008). Finally, for nondiabetic subjects with obesity, the enhanced rate of fasting lipid oxidation that occurred due to weight loss following 16 weeks of exercise training with caloric restriction was the strongest predictor of the improvement in insulin sensitivity that was also observed (Goodpaster et al., 2003). Collectively, these observations support the notion that impaired substrate switching to lipid, which manifests in the fasted state, could be a “primary defect” that links insulin resistance with the overweight/obese condition. However, other findings suggest that fasting lipid oxidation is not reduced in overweight/obese subjects and might even be enhanced. For example, in a cross-sectional analysis, Blaak et al. (2006) found that fasting RQ does not increase with increasing BMI (it decreased or was unaffected when the value was stated in absolute terms or relative to fat mass, respectively), and there are numerous reports of a similar fasting NPRER/rate of lipid oxidation for obese compared to normal-weight subjects (Balcı, 2012; Colberg et al., 1996; Ekelund et al., 2007; Ezell et al., 1999; Guesbeck et al., 2001; Kanaley et al., 2001, Perez-Martin et al., 2001; Steffan et al., 1999). Furthermore, no correlation was found between percent body fat and substrate oxidation in the fasted state for untrained healthy males (Helge et al., 1999) and longitudinal analyses indicate that fasting lipid oxidation for obese subjects is unaffected (Goodpaster et al., 2003) or, in some cases, reduced (Nicklas et al., 1997; Schutz et al., 1992; van Aggel-Leijssen et al., 2001) by weight loss if exercise is not part of the intervention (Goodpaster et al., 2003; Nicklas et al., 1997; van Aggel-Leijssen et al., 2001). Consistent with the latter observation, Schutz et al. (1992) combined the results from cross-sectional and longitudinal analyses of women with obesity to conclude that for each 10-kg increase in fat mass that occurs, fasting lipid oxidation increases by 0.8 g/hr. Felber et al. (1987) also reported a greater rate of lipid oxidation in the

fasting state for subjects with obesity regardless of metabolic health (i.e., normal glucose tolerance, impaired glucose tolerance or diabetic), compared to healthy subjects without obesity. Finally, Poynten et al. (2003) found that compared to pre-diet level, fasting lipid oxidation was reduced for subjects with the overweight/obese condition with both type 2 diabetes and normal glucose tolerance 5 years after losing/regaining ~5% of body weight. Importantly, in both groups, progressive increases in adiposity and hyperinsulinemia also occurred during the 5-year period, which suggests that even if fasting lipid oxidation is impaired in subjects with the overweight/obese condition, it is an acquired defect that develops longitudinally in concert with obesity-related metabolic decline (Poynten et al., 2003).

As illustrated above, conflicting findings exist regarding the degree to which individuals with the overweight/obesity condition demonstrate impaired substrate selectivity towards lipid in circumstances where lipid is the preferred fuel (e.g., after an overnight fast). This ambiguity may reflect day-to-day variability in energy balance, macronutrients composition of the diet, body weight and plasma glucose and free-fatty acids if these factors are not controlled. Support for this comes from a number of studies demonstrating that day-to-day variations in energy balance can profoundly affect fasting RQ/RER levels (Schutz et al., 1992; Weyer, Vozarova, Ravussin, & Tataranni, 2001). For example, Schutz and co-workers demonstrated that 1-day increases in energy intake followed by 1-day decreases produced significantly different RQ values. Furthermore, Hill and colleagues (1991) showed that a consumption of a eucaloric diet with fat or carbohydrate being the major fuel source modified the fasting RQ value according to the diet FQ (food quotient) value. Evidence also exists to suggest that under energy balance, 24-hour RQ/RER will, eventually, equal 24-hour FQ in both normal-weight and obese subjects (Davy, Horton, Davy, Bessessen, & Hill, 2001; Hill et al., 1991; Kim, Hickner, Cortright, Dohm, & Houmard, 2000; Prentice, 2005; Weyer et al., 2001), although the time it takes for this alignment may vary depending on weight, health status, physical activity level, and other factors (Galgani, Heilbronn, et al., 2008). Regardless of this distinction, however, increasing availability of carbohydrate in the diet will result in a greater propensity for glucose oxidation (RER >0.86), whereas increasing fat availability in the diet will drive metabolism toward lipid (RER <0.82). While the speed of adaptation may vary between individuals, evidence suggests that adaptation to a higher carbohydrate or fat diet occurs within 2–3 for a high-carbohydrate diet and within 3–5 days for a high-fat diet) (Galgani, Heilbronn, et al., 2008).

In addition to energy balance and macronutrient composition of the diet, rate of carbohydrate and lipid oxidation is also greatly influenced by the availability and concentration of plasma glucose and FFA. The chronic elevated blood-glucose concentration characteristic of hyperglycemia may lead to substrate-level regulation and a propensity toward glucose oxidation. Similarly, elevated blood FFA can lead to increased lipid oxidation (Galgani, Heilbronn, et al., 2008). Finally, the relatively low metabolic demand during the fasting/resting condition calls assessment of substrate selectivity during "challenges" like these into question (Galgani, Heilbronn, et al., 2008). In addition, absolute lipid-oxidation rate in the fasting/resting state is minimal compared with other circumstances. Given the extensive capacity of skeletal muscle to increase metabolic rate and energy production in circumstances where a rapid and large energy supply is required during physical activity, it has been suggested that it is exercise and not the resting state that provides the appropriate criterion challenge with which to evaluate the capacity for healthy substrate selectivity (Galgani, Heilbronn, et al., 2008).

Table 2.1

Study Comparison Table: Fasting Lipid Oxidation in Individual With Obesity vs. Normal Weight

First Author	Year	Participants	Design	Results
Kelley	1999	Normal weight (n = 16) vs. obesity (n = 40)	Cross-sectional	Significantly higher RQ in the group with obesity during fasting Significantly higher RQ in the normal-weight group after insulin-stimulation. RQ did not change in the group with obesity. Significantly smaller delta RQ (fasting vs. insulin stimulation) in the group with obesity
Filozof	2000	Post-obese (n = 8) vs. BMI-matched never-obese adults	Cross-sectional	After adjusting for fat and fat-free mass, significantly higher RQ in the post-obese group
Blaak	2006	Normal weight (n = 113) vs. obese/morbidly obese (n=701)	Cross-sectional	After adjusting for differences in fat and fat-free mass, fasting lipid oxidation rate significantly higher for individuals with obesity Lipid oxidation rate increased with increasing BMI category

Table 2.1 (continued)

Schutz	1992	Sedentary females with obesity but otherwise healthy (n = 106)	Cross-sectional	Rate of lipid oxidation during basal condition increases with the degree of obesity ($20g \cdot d^{-1}$ for every additional 10 kg)
Ekelund	2007	Normal-weight (n = 15) vs. obese (n = 13)	Cross-sectional	No differences in resting lipid oxidation rate and RQ
Galgani	2008	Sex- and race-matched obese with (n = 59) and without (n = 42) type 2 diabetes	Cross-sectional	No significant difference in fasting RQ Significantly lower RQ in the diabetes group after insulin stimulation, which resulted in a significantly smaller delta RQ (i.e., fasting vs. insulin-stimulated). Differences in insulin-stimulated and delta RQ were completely abolished after adjusting for the rate of glucose disposal, which is, by definition, reduced in type 2 diabetes
Perez-Martin	2001	Normal weight (n = 26) vs. overweight/obesity (n = 32)	Cross-sectional	No significant differences in fasting lipid oxidation rates
Kanaley	2001	Non-obese (n = 8) vs. obese (n = 23)	Cross-sectional	No significant differences in fasting lipid oxidation rates
Colbert	1996	Normal weight (n = 7) vs. obese (n = 7) vs. Type 2 diabetes (n = 7)	Cross-sectional	Fasting RER and lipid oxidation rate were similar in all three groups Lipid oxidation accounted for more than 66% of total energy production in the basal fasting state in all three groups
Ezell	1999	Never obese (n = 5) vs. obese (n = 5) vs. post-obese (n = 5)	Cross-sectional	Similar lipid oxidation rates in all three groups during fasting conditions
Steffan	1999	Normal weight (n = 15) vs. obese (n = 20) sedentary women	Cross-sectional	Similar RER during fasting in both groups
Goodpaster	2002	Normal weight (n = 7) vs. obese (n = 7)	Cross-sectional	No difference in fasting RER between the two groups
Weyer	2001	Normal weight (n = 6) vs. morbidly obese (n = 8)	Cross-sectional	No differences in fasting RER and lipid oxidation rates between the two groups

2.6 "Metabolic Flexibility" During Exercise

As described above, ambiguous findings exist regarding the degree to which impaired fasting lipid oxidation predates and/or accompanies overweight/obesity, and the use of the fasting state to assess substrate selectivity with respect to lipid has been questioned because of the low metabolic demand associated with the condition and degree to which the value is influenced by acute energy balance and dietary macronutrient consumption (Galgani, Heilbronn, et al., 2008). Citing these concerns, Galgani, Heilbronn et al. (2008) recommended assessing fuel selection during exercise as a better way to explore metabolic flexibility to lipid to elucidate the link between dysfunctional lipid metabolism and the insulin-resistant state. However, it is not clear what constitutes "metabolic flexibility" in response to an exercise challenge**.** For example, Prior, Ryan, Stevenson, and Goldberg (2014) assessed subjects with the overweight/obesity condition and found that during 10 minutes of treadmill exercise at ~50% and ~60% $\dot{V}O_2$ reserve, subjects with impaired glucose tolerance had a significantly lower non-protein RER (hence, propensity toward lipid oxidation) due to a blunted increase in non-protein RER in relation to the pre-test resting condition. The authors interpreted this as an inability to up regulate carbohydrate oxidation during exercise and, therefore, a metabolically-inflexible response compared to a glucose tolerant group, and this was supported by the lower $\Delta NPRER_{clamp\text{-}fast}$ that they reported for the glucose-intolerant subjects (Prior et al., 2014). In contrast, Russell, Kraemer, and Nelson (2013) believed that a greater increase in carbohydrate use from rest to peak "exercise" (passive stretching) for subjects with type 2 diabetes compared to healthy controls indicated a metabolically-inflexible response with intermediate values for glucose-tolerant subjects with a family history of type 2 diabetes (FH+), which is suggested to have diagnostic value for early detection. These authors found a lesser increase in non-protein RER following glucose loading in FH+ and type 2 diabetes subjects that appears to support their interpretation (Russell et al., 2013). This lack of congruence exemplifies the danger of using imprecise terminology (i.e., "metabolic flexibility") when referring to substrate selectivity in circumstances where different responses are "functional" (e.g., a transition from fasting to insulin stimulation where a complete switch is optimal, compared to a transition from lower to higher exercise intensity where the ability to up regulate lipid oxidation to spare carbohydrate use is the "healthy" response).

2.7 Lipid Oxidation During Exercise

With respect to substrate selectivity during exercise, it has long been known that the ability to up regulate lipid use in response to an exercise challenge is a hallmark of the endurance-trained state (Holloszy & Coyle, 1984). For example, in a cross-sectional analysis, Nordby et al. (2006) found that the peak rate of whole-body lipid oxidation during a graded cycling test is higher and occurs at a higher relative work rate in endurance-trained compared to untrained subjects. Moreover, Jansson and Kaijser (1987) reported that when cycling at 65% $\dot{V}O_{2max}$, endurance-trained individuals satisfied ~53% of the energy demands with lipid oxidation compared to ~33% for untrained controls and this difference appeared exclusive to greater utilization of non-plasma lipid sources. Collectively, these findings suggest that an increased reliance on lipids during exercise might explain why endurance-trained individuals possess elevated IMCL without pathological repercussion (i.e., the so-called “athlete’s paradox”) (Amati et al., 2011; Goodpaster et al., 2001) and how endurance training can increase intramyocellular lipids while also improving insulin sensitivity in overweight/obese individuals with insulin resistance (Dubé et al., 2008). Conversely, if Intramyocellular lipid accumulation consequent to dysfunctional lipid metabolism underpins the link between insulin resistance and the overweight/obese state, overweight/obese subjects would be expected to demonstrate an impaired capacity for lipid oxidation during exercise compared to their normal-weight counterparts. A number of studies support this contention. For example, Wade, Marbut, and Round (1990) found a positive correlation between percent body fat and NPRER during cycling at 100 W for a group of predominantly-sedentary men with relatively low body-fat percentage (12.7 +/- 7.3%), and subsequent research confirmed lower rates of lipid oxidation at submaximal work rates in healthy sedentary overweight/obese adults (Perez-Martin et al., 2001) and at high- but not low-intensity work rates (i.e., 40-60% but not 0-30% $\dot{V}O_{2peak}$) in obese pubescent children (Zunquin, Theunynck, Sesboüé, Arhan, & Bouglé, 2009). Similarly, Lanzi et al. (2014) found that obese subjects relied less on lipid oxidation at higher exercise intensities (e.g., 65-85% $\dot{V}O_{2peak}$) compared to lean subjects during an incremental cycling test with 6-minute stages. Collectively, these findings suggest that increased body fatness is associated with an impaired capacity for lipid metabolism during exercise. Further insight comes from Geusbeck et al. (2001), who found that subjects who were previously morbidly obese oxidized more carbohydrate and less lipid during 10 minutes of submaximal cycling (work rates of

both 15 W and 65% $\dot{V}O_{2peak}$) compared to weight-matched never-obese controls. Interestingly, lean children with a severely-obese parent also exhibit a reduced reliance on lipid oxidation during 10 minutes of 15-W cycling compared to children with non-obese parents (Eaves, Colon, DuBose, Collier, & Houmard, 2012). Consequently, these findings cohere with the notion that an impaired capacity for lipid oxidation during exercise is a function of an inherent defect that predisposes obesity as opposed to the presence of obesity per se (Guesbeck et al., 2001). Indeed, impaired mitochondrial lipid oxidation has been reported in vitro for myotubes donated by obese humans (Boyle, Zheng, Anderson, Neufer, & Houmard, 2012). However, similar studies using longer exercise bouts (e.g., 60 minutes) found no difference in substrate oxidation rates between formerly-obese subjects and similarly-matched controls (Ezell et al., 1999; Ranneries et al., 1998), which suggests that the duration of the criterion bout might play a role (Kanaley, Cryer, & Jensen, 1993). Moreover, despite similar elevation in fatty Acyl-CoA (a lipid intermediate) in muscle strips of both overweight/obese and extremely-obese individuals, only the latter demonstrate impaired palmitate oxidation, which suggests that degree of obesity might also be important to consider (Hulver et al., 2003). In-vivo measurements also suggest that exercise-stimulated whole-body lipid oxidation is not impaired by a decrease in mitochondrial density in normal-weight healthy men (Eaves et al., 2012) or by the reduction in mitochondrial content (Boushel et al., 2007; Larsen et al., 2009) and/or function (Mogensen et al., 2007; Phielix et al., 2008) that is present in subjects with type 2 diabetes (Blaak et al., 2006; Boon et al., 2007; Colberg et al., 1996; Ghanassia, Brun, Fedou, Raynaud, & Mercier, 2006; Kang et al., 1996; Larsen et al., 2009). Finally, much like the fasting state, decreased lipid oxidation during exercise for overweight/obese subjects is not a universal finding, with the preponderance of evidence suggesting that body fatness exerts no influence (Balcı, 2012; Colberg et al., 1996; Croci et al., 2014; Devries et al., 2013; Ezell et al., 1999; Geerling et al., 1994; Helge et al., 1999; Kanaley et al., 2001; Larsen et al., 2009; Mittendorfer, Fields, & Klein, 2004; Steffan et al., 1999; Thyfault et al., 2004; Venables, Achten, & Jeukendrup, 2005) or might even convey an enhancement (Ara et al., 2011; Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 2001; Lanzi et al., 2014).

2.8 Obesity and Enhanced Lipid Oxidation During Exercise

In the aforementioned study, Lanzi et al. (2014) found a similar capacity for lipid oxidation at work rates between 35 and 60% $\dot{V}O_{2peak}$ for obese compared to lean subjects with obese subjects

demonstrating higher rates from 20-30%. Similarly, Ara et al. (2011) found a higher peak rate of lipid oxidation for obese subjects compared to age-matched controls during incremental arm and leg cycling, and obese subjects had higher circulating FFA and augmented intramyocellular lipid concentrations that might have contributed to the enhancement. However, Numao et al. (2006) found a similar NPRER during 60 minutes of cycling at 50% $\dot{V}_{O2peak}$ for subjects with visceral compared to abdominal-subcutaneous obesity despite ~59% higher plasma concentration of FFA for the former group, which suggests that if exercise lipid oxidation is, indeed, enhanced due to increased lipid availability in obese subjects (Ara et al., 2011; Goodpaster et al., 2002), the enhanced capacity is finite (i.e., a plateau exists). Alternatively, the enhancement might not be related to lipid availability per se, at least with respect to plasma sources (see below). Ara et al. (2011) also found that post-obese subjects that were BMI-matched to the control group demonstrated an enhanced capacity for lipid use during exercise; however, this was only the case for leg cycling. This means that regional differences can play a role. In another study, Goodpaster et al. (2002) compared obese men with lean subjects matched for age and $\dot{V}O_{2max}$ and found that obese subjects derived a greater proportion of their energy from fatty acids during 60 minutes of cycling at 50% $\dot{V}O_{2max}$. Specifically, this difference reflected a ~50% greater oxidation of non-plasma fatty acids and ~50% lower oxidation of muscle glycogen as oxidation of plasma fatty acids and glucose was similar between groups (Goodpaster et al., 2002). Horowitz et al. (2000) also found greater (~25%) whole-body fatty-acid oxidation for abdominally-obese compared to lean women during prolonged cycling at 54% $\dot{V}O_{2max}$ despite similar rates of plasma fatty acid appearance, uptake, and oxidation. Similarly, Kanaly et al. (2001) reported that compared to lean women, obese women oxidized 30% more lipid while expending a similar amount of energy during 30 minutes of walking at 70% $\dot{V}O_{2peak}$ despite no difference in circulating FFA between groups. Collectively, these findings suggest that obese subjects might use lipid more predominantly during exercise in particular with respect to non-plasma sources (i.e., presumably, intramyocellular lipids) (Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 2001).

Interestingly, Thyfault et al. (2004) found that compared to normal-weight women, severely-obese women and women who were previously severely obese (post gastric bypass surgery) demonstrated a decreased ability to oxidize plasma FFA (assessed using tracer methodology) during both fasting and exercise, despite the fact that neither fasting nor exercise total lipid oxidation (assessed using indirect

calorimetry) was different between groups. Similarly, Mittendorfer et al. (2004) found no difference in total lipid oxidation during the final 30 minutes of a 90-minute cycling bout for lean, overweight and obese subjects; however, lean subjects derived ~50% of the fatty acids from non-plasma sources with that percentage increasing with increasing adiposity. An attractive interpretation of these findings is that the ability to maintain plasma FFA uptake/oxidation in the presence of elevated intramyocellular lipid accumulation/oxidation determines the "net effect" on total exercise lipid oxidation (i.e., increase, decrease or unaltered) consequent to the overweight/obese state. Interestingly, an "offsetting" relationship like this has been reported for obese diabetic subjects who demonstrate similar total lipid oxidation when cycling at 50% $\dot{V}O_{2max}$ compared to obese normoglycemic controls even though oxidation of plasma-derived fatty acids is markedly reduced (Blaak, van Aggel-Leijssen, Wagenmakers, Saris, & van Baak, 2000).

2.9 Methodological Concerns for Assessing the Capacity for Lipid Oxidation During Exercise

In addition to the dynamic balance between utilization of plasma FFA and intramyocellular lipids, discrepant findings in the literature regarding the influence of body fatness on exercise lipid oxidation might be related to characteristics of the criterion exercise bout. For example, in the aforementioned studies, the comparison between lean and obese subjects was made during exercise at either the same absolute work rate (Croci et al., 2014; Geerling et al., 1994; Guesbeck et al., 2001; Horowitz & Klein, 2000; Lanzi et al., 2014; Wade et al., 1990) and/or a work rate calculated relative to the subject's individual $\dot{V}O_{2max}$ (Ara et al., 2011; Colberg et al., 1996; Croci et al., 2014; Devries et al., 2013; Ezell et al., 1999; Geerling et al., 1994; Goodpaster et al., 2002; Guesbeck et al., 2001; Helge et al., 1999; Horowitz & Klein, 2000; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009; Mittendorfer et al., 2004; Perez-Martin et al., 2001; Steffan et al., 1999; Thyfault et al., 2004; Zunquin et al., 2009). While the latter is the "traditional" way to normalize exercise intensity across subjects, it is now well established that the $\dot{V}O_2$ response exhibits non-linear characteristics, such that subjects working at the same percentage of $\dot{V}O_{2max}$ will encounter considerable intersubject variability with respect to the physiological challenge at hand (DiMenna & Jones, 2009; Katch, Weltman, Sady, & Freedson, 1978; Lansley, Dimenna, Bailey, & Jones, 2011). Furthermore, as previously mentioned, the duration of the criterion bout might also play a role. For example, the ~30% greater lipid oxidation observed by Kanaley et al. (2001) after 30 minutes of

walking for obese compared to normal-weight women was present, despite no significant difference between groups during the first 15 minutes. Consequently, a minimum duration of exercise might be required to unveil overweight/obesity-related differences. Finally, in addition to characteristics of the exercise bout, differences in subject characteristics might also explain the lack of consensus regarding the effect of overweight/obesity on the capacity for exercise lipid oxidation. Specifically, age and/or gender might play a role (Balcı, 2012), and it is also important to recognize that fat-free mass exerts a positive impact on the capacity to oxidize lipids. For example, Venables et al. (2005) reported that the positive correlation they observed between the maximal rate of lipid oxidation and fat mass in a large group of healthy subjects was no longer present when lipid oxidation was corrected for fat-free mass. Consequently, it is important to match fat-free mass between groups and/or express lipid oxidation relative to fat-free mass instead of total body weight, so that a valid comparison between lean and obese subjects can be made (Devries et al., 2013). Another important factor to consider is the cardiorespiratory fitness of the subjects because the sedentary lifestyle that is associated with overweight/obesity would be predicted to lead to lower cardiorespiratory fitness and, therefore, a reduced capacity for lipid oxidation regardless of overweight/obesity per se. Goodpaster et al. (2002) were the first to account for this factor by matching groups according to $\dot{V}O_{2max}$ and, indeed, this important aspect of control might explain their unexpected finding (i.e., increased capacity for lipid oxidation in overweight/obese subjects). However, Croci et al. (2014) also matched overweight/obese and normal-weight subjects by $\dot{V}O_{2max}$ and observed no difference between groups for exercise lipid oxidation. One important distinction between these studies is that in the former, no mention is made of confirming that the observed $\dot{V}O_{2peak}$ was, indeed, the $\dot{V}O_{2max}$, while in the latter, four of the five criteria used for this purpose were either ineffective (heart rate, RER and blood lactate concentration) or often not present (a "$\dot{V}O_2$ plateau") (Poole, Wilkerson, & Jones, 2008). This is important because if some subjects had stopped their test prior to attainment of $\dot{V}O_{2max}$, their cardiorespiratory fitness stratification would have been underpredicted. It is now well established that in lieu of the $\dot{V}O_2$ plateau that is often lacking at attainment of $\dot{V}O_{2max}$ during incremental exercise (Day, Rossiter, Coats, Skasick, & Whipp, 2003), a "severe-intensity" constant-work-rate bout can be performed shortly after completion of the $\dot{V}O_{2max}$ test to "create" such a plateau to confirm $\dot{V}O_{2max}$ or reveal a further substantial increase that refutes it (Midgley & Carroll, 2009; Midgley, McNaughton, & Carroll, 2006; Poole

et al., 2008). Interestingly, a "verification bout" of this nature has recently been established for sedentary adults with obesity (Midgley & Carroll, 2009); hence, heretofore, it is reasonable to suggest that attempts to match cardiorespiratory fitness between overweight/obese subjects should include this critical aspect of control. Other important differences between the two studies that might explain the discrepancy include the duration of the exercise bout (one 60-minute bout for the former vs. multiple 4-minute bouts for the latter) and the cardiorespiratory fitness of the subjects. Specifically, Croci et al. (2014) recruited subjects who were "recreationally trained," while Goodpaster et al. (2002) assessed "sedentary" individuals (i.e., $\dot{V}O_{2max}$ of ~50 compared to ~40 $mL \cdot kgFFM^{-1} \cdot min^{-1}$). Unfortunately, Croci et al. did not determine the metabolic profile of their subjects; hence, they could only speculate that they possessed greater insulin sensitivity, compared to that which would be present for the average overweight/obese phenotype (CROC*2014). If this was true, their subjects' "metabolic health" was likely much different (e.g., "metabolically-healthy obese;" MHO), compared to the overweight/obese individuals assessed by Goodpaster et al. (2002), who possessed 10% higher fasting blood glucose and threefold higher postabsorptive insulin levels compared to the normal-weight controls. Although exercise lipid oxidation was not associated with fasting insulin levels in that study, this raises the interesting possibility that the counterintuitive finding of an enhanced capacity for lipid oxidation in overweight/obese, compared to normal-weight subjects matched for cardiorespiratory fitness, might represent a "downstream" influence of the insulin resistance associated with overweight/obesity, as opposed to the effect of overweight/obesity per se (Braun, Sharoff, Chipkin, & Beaudoin, 2004; Goodpaster et al., 2002).

2.10 "Functional" Insulin Resistance

On the surface, the suggestion that an "unhealthy" condition like insulin resistance might facilitate what would be considered the preferred exercise response (i.e., increased reliance on lipid oxidation and consequent glucose/glycogen sparing) is difficult to reconcile. However, insulin resistance has recently been "redefined" based on the observation that it is well preserved from an evolutionary perspective (Soeters & Soeters, 2012). Within this schema, insulin resistance and elevated intermyocellular lipids are "functional" responses because they provide a "survival advantage" during the periods of prolonged food deprivation that characterize the feast-or-famine circumstances of a hunting/gathering lifestyle (Stannard & Johnson, 2004). Under such conditions, insulin resistance effectively prevents glucose flow "upstream,"

thereby protecting blood stores by “opening the door” for increased lipid oxidation from easy-to-access intramuscular sources (Stannard & Johnson, 2004). The dramatic change in insulin sensitivity that characterizes the circ-annual cycle of mammalian hibernation exemplifies this concept (Martin, 2008). However, once prolonged energetic deficit is removed from the equation (e.g., due to the advent of an agricultural society and/or the modern-day energetic prosperity that begets the obesogenic lifestyle), the phenotype becomes dysfunctional and, ultimately, pathogenic.

2.11 Insulin Resistance and Enhanced Lipid Oxidation During Exercise

Recognizing the possible influence of insulin resistance on exercise substrate utilization with respect to the findings of Goodpaster et al. (2002), Braun et al. (2004) stress that in addition to age, maximal aerobic capacity, and habitual level of physical activity, the insulin-resistant status of obese and normal-weight subjects should be controlled to conclude that a shift toward less carbohydrate/more lipid use during exercise is attributable to body fatness per se. Accordingly, these authors compared exercise substrate use in a group of untrained, overweight/obese women with insulin resistance to that which was present for insulin-sensitive women of similar age, weight, body composition, habitual physical activity, and maximal aerobic capacity. Importantly, Braun et al. chose insulin-resistant subjects who were normoglycemic to ensure that the mass action effect of hyperglycemia would not contribute to differences in glucose uptake/oxidation they observed in the insulin-resistant group. The authors found that during 50 minutes of treadmill walking at 45% $\dot{V}O_{2peak}$, blood-glucose oxidation was similar between groups; however, total carbohydrate oxidation and the estimated proportion of energy derived from muscle glycogen were less for the subjects with insulin resistance. Importantly, the end result was that subjects with insulin resistance satisfied a greater proportion of the energetic cost of exercise with lipid (~56% vs. ~44% for the insulin sensitive controls). Although the mechanism(s) of action could not be determined, the authors concluded that independent of body fatness, insulin resistance spares muscle glycogen by shifting substrate oxidation away from carbohydrate, despite similar blood-glucose uptake during exercise (Braun et al., 2004).

2.12 The Effect of Insulin Resistance on Exercise Lipid Oxidation Independent of Body Fatness

The findings of Braun et al. (2004) might help to explain equivocal reports regarding exercise substrate selectivity for overweight/obese subjects based on the notion that the subjects’ insulin-resistant

status was not controlled in those investigations. However, a number of limitations to their findings must be considered. Much like the predominance of research that has addressed this topic (Ara et al., 2011; Colberg et al., 1996; Crozi et al., 2014; Devries et al., 2013; Ezell et al., 1999; Geerling et al., 1994; Goodpaster et al., 2002; Guesbeck et al., 2001; Helge et al., 1999; Horowitz & Klein, 2000; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009; Mittendorfer et al., 2004; Perez-Martin et al., 2001; Steffan et al., 1999; Thyfault et al., 2004; Zunquin et al., 2009), exercise intensity was prescribed relative to $\dot{V}O_{2peak}$ in that study (Braun et al., 2004). This means that subjects were likely working at different intensities relative to their maximal capacity for lipid oxidation, which generally occurs at or slightly below the individual lactate threshold (Achten & Jeukendrup, 2004; Rynders, Angadi, Weltman, Gaesser, & Weltman, 2011), as there is considerable intersubject variability with respect to where lactate threshold is situated relative to $\dot{V}O_{2peak}$ (Meyer, Gabriel, & Kindermann, 1999; Weltman et al., 1990). Conversely, a proper comparison of exercise-stimulated substrate selectivity across subjects should assess lipid oxidation at a given percentage of lactate threshold. Moreover, if it is the insulin-resistant status of the obese individual that alters exercise substrate selectivity independent of obesity, one would expect insulin-resistant individuals who were not overweight/obese to also express this phenotype. However, normal-weight insulin resistant and insulin-sensitive subjects were not included in that study; hence, that determination could not be made (Braun et al., 2004).

2.13 Summary

Equivocal findings exist regarding the influence of overweight/obesity on substrate selectivity, especially towards lipid, in the fasting/resting and exercise-stimulated states. This likely reflects methodological differences between studies and, specifically, a lack of control for key variables that can confound a comparison between overweight/obese and normal-weight subjects. These include: (a) acute energy balance and macronutrient composition of the diet, and (b) fat-free mass, cardiorespiratory fitness and insulin-resistant status of the subjects. In additional, no study to date has compared lipid oxidation during a physiological-equivalent exercise challenge in individuals with normal weight and overweigh/obesity with these factors well controlled.

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Chapter 3 – Systematic Review

3.1 The Influence of Overweight/Obesity on Exercise Lipid Oxidation: A Systematic Review

3.1.1. Introduction

Obesity (World Health Organization [WHO], 2018) and diabetes (Zimmet, Alberti, & Shaw, 2001) epidemics are in full swing and a link between the insulin resistance (IR) that predates type 2 diabetes (T2D) and the overweight/obese condition is well established. However, the mechanistic basis(es) underpinning this link is/are still debated with multiple candidate molecules, systems, and pathways potentially involved (Qatanani & Lazar, 2007). One theory posits a relationship with dysfunctional lipid metabolism based on the notion that excess accumulation of lipid in skeletal muscle (intramuscular triglyceride; IMTG) becomes “lipotoxic” and begets IR when it is not “turned over” on a regular basis (McGarry, 1992; Moro, Bajpeyi, & Smith, 2008; Pan et al., 1997; Perseghin et al., 1999). In support of this contention, evidence suggests that obese individuals demonstrate an impaired capacity to up regulate lipid oxidation during fasting, which represents a condition with an energetic demand that should be satisfied predominantly by lipid as substrate (Goodpaster, Katsiaras, & Kelley, 2003;Kelley, Goodpaster, Wing, & Simoneau, 1999; Kim, Hickner, Cortright, Dohm, & Houmard, 2000). However, use of the fasting state to assess the capacity to oxidize lipid has been questioned because of the low metabolic demand and degree to which the value is influenced by acute energy balance and dietary macronutrient consumption (Galgani, Moro, & Ravussin, 2008). A better alternative might be to investigate the capacity to use lipid during exercise when metabolic demand is higher, yet lipid can still contribute significantly (Galgani, Heilbronn, et al., 2008). In this regard, obese individuals possess reduced mitochondrial content (Kim et al., 2000) and lower oxidative enzyme capacity (Simoneau, Veerkamp, Turcotte, & Kelley, 1999), which are “downstream” factors that could limit the ability to oxidize available lipid during exercise. Moreover, “upstream” limitations in lipid availability during exercise might be present with overweight/ obesity due to a greater cortisol response (Wong & Harber, 2006), impaired growth hormone (Wong & Harber, 2006), and plasma catecholamine (Gustafson, Farrell, & Kalkhoff, 1990; Salvadori, Fanari, Mazza, & Baudo, 1993) responses and/or reduced lipolytic effect of a given level of circulating catecholamines (Connacher et al., 1991; Mittendorfer, Fields, & Klein, 2004). There is also evidence of a blunted reduction in insulin concentration from the resting to exercise condition in association with the

obese condition (Thyfault et al., 2004). Each of these could contribute independently or in concert to a reduced capacity for exercise lipid reliance for overweight/obese individuals.

Despite this strong theoretical basis, studies carried out to test the hypothesis that exercise lipid use is reduced with obesity have returned equivocal findings with some reporting a decrement and others finding no difference. Interestingly, there is even evidence of enhanced exercise lipid oxidation with obesity, which might reflect an increase in the availability of stored lipid in muscle (Goodpaster, Wolfe, & Kelley, 2002; Horowitz & Klein, 2000) and/or substrate selection that is influenced by the IR that often accompanies the overweight/obese state (Braun, Sharoff, Chipkin, & Beaudoin, 2004). The reason(s) for these conflicting findings is/are unclear but might reflect methodological differences regarding a number of important factors that should be controlled to isolate the influence of overweight/obesity on lipid use during exercise. For example, the fat-free mass (FFM) and cardiorespiratory fitness (CRF) of the subject (Goodpaster et al., 2002) and the energy balance and macronutrient composition of the subject's diet (Gregory et al., 2011; Helge, Watt, Richter, Rennie, & Kiens, 2001) are factors that can affect exercise lipid use irrespective of body-fat stores. Furthermore, the ability to use lipid as fuel during exercise is profoundly influenced by both the intensity (Brooks & Mercier, 1994; Venables, Achten, & Jeukendrup, 2005) and duration (van de Weijer et al., 2013) of the criterion exercise bout.

Resolving ambiguity regarding the influence of overweight/obesity on the capacity to oxidize lipid is important because understanding this influence can provide insight into the etiology of the disease. For example, if a reduced capacity for lipid oxidation is present in overweight/obese subjects, it is consistent with the contention that an inherent defect (e.g., compromised lipolytic activity that limits lipid availability and/or a mitochondrial defect that restricts oxidation of available lipid) predisposes the overweight/obese condition (Guesbeck et al., 2001; Thyfault et al., 2004). According to this schema, a shift in substrate selectivity away from lipid in favor of carbohydrate metabolism during physical activity would be accompanied by a reciprocal shift toward lipid storage, which represents the more energetically-efficient way to increase energy stores when a positive energy balance is present. Consequently, a reduced capacity to oxidize lipid would be associated with a greater propensity to gain body fat (Hickner, Privette, McIver, & Barakat, 2001). Moreover, the degree to which overweight/obesity affects lipid oxidation and the influence it exerts might be important factors to consider when designing an exercise intervention to

manage and/or treat the disease (Goodpaster et al., 2002). For example, if an impaired capacity for lipid utilization predisposes the overweight/obese state, exercise specific for improving mitochondrial function (e.g., high-intensity interval training) would be warranted (Gibala, Little, MacDonald, & Hawley, 2012; (Little et al., 2011). A higher-intensity approach (e.g., sustained exercise at a metabolic rate that exceeds that which allows for the maximal rate of lipid oxidation) would also be advantageous for overweight/ obese subjects because the "target energy expenditure" to ensure adequate rate of weight loss (e.g., 300 kcals per session) can be achieved with reduced time commitment (Bogdanis, Vangelakoudi, & Maridaki, 2008). Conversely, if exercise lipid use is not reduced with overweight/obesity, mitochondrial function would, apparently, be sufficient (Fisher-Wellman et al., 2014; Holloszy, 2008). In this case, an exercise metabolic rate at which lipid oxidation is maximal (i.e., at the so-called "FATmax") might be preferred to improve metabolic health irrespective of body-fat loss by optimally perturbing the IMTG pool (Dumortier et al., 2003; Thompson, Townsend, Boughey, Patterson, & Bassett, 1998; Venables & Jeukendrup, 2008) and/or changing its relationship with muscle mitochondria (Devries et al., 2013).

The primary aim of this systematic review was to formulate a consensus opinion regarding the influence of overweight/obesity on exercise lipid oxidation from the existing research. A secondary aim was to inform future research by determining the degree to which conflicting results might be explained by factors that were unaccounted for and/or inadequately controlled in prior investigations.

3.2 Methods

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and is registered in the PROSPERO database (PROSPERO 2015:CRD42015027519).

3.2.1 Search Strategy

Two researchers independently conducted a search and selected the articles included in this review. The search, which was undertaken on June 30, 2017, was performed using the databases PubMed, ProQuest, ISI Web of Science/, and the Cochrane Library. The general search strategy was employed within the PubMed database and subsequently applied to the other three sources. Search terms were: (a) "obes" or "overweight"; (b) "substrate utilization" or "lipid oxidation" or "fat oxidation"; and (c) "exercise" or "fatmax" or "fatomax" or "lipomax." Limits were set to include articles that: (a) appeared in

a peer-reviewed journal published in English, and (b) reported findings from an IRB-approved clinical trial of human subjects. Following extraction, reference lists of remaining studies were examined so that relevant articles not flagged by the original search could be considered.

3.2.2 Study Selection: Inclusion and Exclusion Criteria

Articles were included if they presented original research that provided information regarding the influence of overweight/obesity on fuel utilization during exercise in generally-healthy (i.e., without metabolic, cardiopulmonary, neurological, or musculoskeletal disease and/or not taking medication that might affect fuel metabolism) sedentary adults (>18 years). Accordingly, for a study to be included, subjects had to have been stratified into one of at least two distinct groups defined according to either Body Mass Index (BMI) or body-fat percentage. Articles were excluded if they assessed: (a) older adults (>65 years), (b) individuals with chronic disease and/or exercise limitations, and (c) physically-active subjects (e.g., recreational or competitive athlete).

3.2.3 Data Extraction and Synthesis

Once the selection process was complete, one author extracted the following information from included articles: author(s), journal, date of publication, sample size, subject characteristics (e.g., age, sex, BMI, percent body fat, maximal/peak rate of oxygen consumption [$\dot{V}O_{2max}$/$\dot{V}O_{2peak}$]), criterion-exercise characteristics (e.g., mode, intensity and duration), aim(s), hypothesis(es), methodological design, primary outcomes and additional relevant findings.

3.2.4 Quality Assessment

Methodological quality assessment was performed on two levels. We combined relevant questions from three widely-accepted scales (PEDro, MINORS, and NIH) to assess general methodological quality while also utilizing a custom-designed tool to assess the degree to which 12 factors that can affect exercise lipid use regardless of body fatness were adequately controlled. In each case, two researchers provided independent assessments by judging items as either absent/insufficient, present/insufficient or present/sufficient (score = 0, 1 or 2, respectively). Scores were subsequently averaged to provide a single rating per study for each level of assessment. Regardless of assessed quality, all studies satisfying the inclusion criteria were included in this review with scores revealed to provide perspective.

3.3 Results

Figure 1 illustrates the flow diagram for this systematic review. A total of 1,144 articles were identified during the database search (1,141) and through additional sources (3), and after duplicates were removed, 685 articles were considered for inclusion. Once titles, abstracts, and/or manuscripts were assessed, it was determined that 24 satisfied the inclusion criteria. The investigations detailed in these 24 articles were all cross-sectional; however, three also included longitudinal components. Specifically, two involved 16 weeks of energy restriction for the obese group (Kanaley, Cryer, & Jensen, 1993; Kanaley, Weatherup-Dentes, Alvarado, & Whitehead, 2001), while one included 12 weeks of endurance training for both groups (Devries et al., 2013). For the former two, the lipid-oxidizing comparison between lean and obese subjects was made for the baseline measurement only (Kanaley et al., 1993; Kanaley et al., 2001), while the latter involved comparisons between groups both before and after the training intervention (Devries et al., 2013).

In 17 of the 24 included studies, a comparison of the capacity for whole-body (Ardevol et al., 1998; Balcı, 2012; Chatzinikolaou et al., 2008; Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Grams, Kueck, Haufe, Tegtbur, & Nelius, 2017; Horowitz & Klein, 2000; Keim, Belko & Barbieri, 1996; Lanzi et al., 2014; Mittendorfer et al., 2004; Perez-Martin et al., 2001; Santiworakul, Chuaychoo, Kriengsinyos, Saengsirisuwan, & Jalayondeja 2014; Slusher et al., 2015; Steffan, Elliott, Miller, & Fernhall, 1999; Thyfault et al., 2004; Wong & Harber, 2006), or whole-body and source-specific (Goodpaster et al., 2002; Horowitz & Klein, 2000; Mittendorfer et al., 2004; Thyfault et al., 2004) lipid oxidation during exercise between non-overweight/obese (most often called “lean,” but also referred to as “control,” “leaner,” “never obese,” “nonobese,” and “normal weight”) and overweight/obese subjects was a primary purpose. In two of these studies, the effect of weight loss was also investigated by comparing lean subjects with obese subjects who had lost weight; however, a third group comprising obese subjects who had not lost weight was also included for comparison (Ezell et al., 1999; Thyfault et al., 2004).

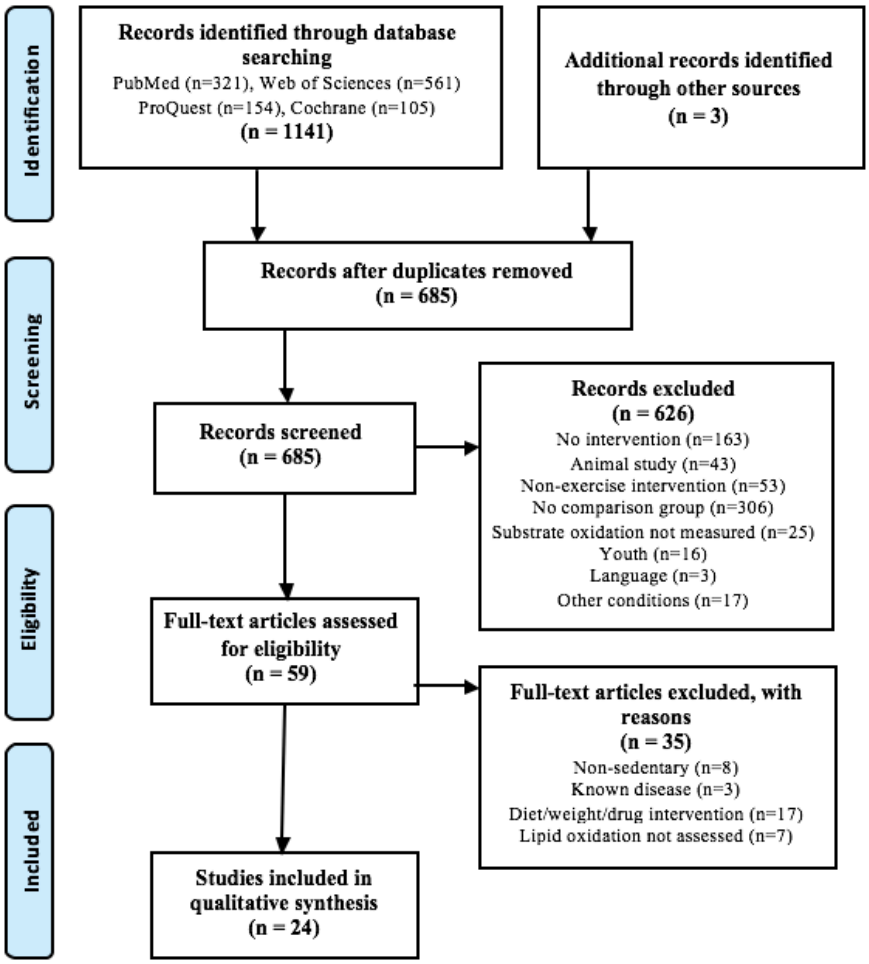

Figure 3.1. PRISMA flow diagram

Another study investigated the effect of time of day on exercise lipid use; however, normal and obese subjects were included and stratified into separate groups with a comparison between groups at each time of day provided (Mohebbi & Azizi, 2011). In the other six studies, the primary purpose was to investigate differences due to subject characteristics other than overweight/obesity; however, a comparison between normal-weight and overweight/obese subjects was also included. For example, in one investigation, the objective was to determine whether differences in exercise lipid oxidation exist between lean and obese African American and lean and obese Caucasian women (Hickner et al., 2001). However, results of a two-way (i.e., race × body composition) ANOVA were presented so that the independent effect of obesity both within and across race could be determined (Hickner et al., 2001).

Similarly, in two studies, the authors' objective was to determine whether substrate utilization during exercise differs in subjects with T2D compared to nondiseased controls (Colberg et al., 1996; Larsen et al., 2009). However, the methodology included both a lean and obese control group so that a comparison of the effect of obesity without the potential confounding influence of the disease was also reported (Colberg et al., 1996; Larsen et al., 2009). In two other studies, the purpose was to determine whether the relationship between free fatty acid (FFA) availability and lipid oxidation is different for obese subjects with different types of body-fat distribution (Kanaley et al., 1993; Kanaley et al., 2001). However, non-obese subjects were also assessed and a comparison across the three groups was provided (Kanaley et al., 1993; Kanaley et al., 2001). Finally, in one study, a group of endurance-trained subjects was included to assess 24-hour substrate use in trained individuals compared to untrained obese individuals on exercise and non-exercise days (Melanson et al., 2009). However, a group of untrained lean subjects was also included and a comparison of substrate use exclusive to the exercise bout was provided (Melanson et al., 2009).

3.3.1 Subject Characteristics

3.1.1 Sex and Race

Table 3.1 presents the characteristics of the subjects assessed in the 24 included studies. In nine studies, males were assessed exclusively (Balcı, 2012; Chatzinikolaou et al., 2008; Goodpaster et al., 2002; Lanzi et al., 2014; Larsen et al., 2009; Mittendorfer et al., 2004; Mohebbi & Azizi, 2011; Santiworakul et al., 2014; Wong & Harber, 2006), while females were studied exclusively in nine other investigations (Ardevol et al., 1998; Devries et al., 2013; Ezell et al., 1999; Hickner et al., 2001; Horowitz & Klein, 2000; Kanaley et al., 1993; Kanaley et al., 2001; Steffan et al., 1999; Thyfault et al., 2004). In six studies, both male and female subjects were included (Clberg et al., 1996; Grams et al., 2017; Keim et al., 1996; Melanson et al, 2009; Perez-Martin et al., 2001; Slusher et al., 2015); however, only three of these included a report of results according to sex either exclusively (Grams et al., 2017) or in addition to collectively (Keim et al., 1996; Perez-Martin et al., 2001). Only one study included mention of race with both African Americans and Caucasians included as subjects (Hickner et al., 2001).

3.3.2 Stratification Criteria for Body Fatness

In 13 studies, BMI was used exclusively to stratify subjects into groups (Ardevol et al., 1998; Balcı, 2012; Devries et al., 2013; Goodpaster et al., 2002; Hickner et al., 2001; Kanaley et al., 2001; Melanson et al., 2009; Mittendorfer et al., 2004; Mohebbi & Azizi, 2011; Perez-Martin et al., 2001; Santiworakul et al., 2014; Slusher et al., 2015; Thyfault et al., 2004). In two of these studies, the classification system endorsed by the World Health Organization (WHO) was used to define "normal" and "overweight" groups (BMI, <25 and ≥25 $kg \cdot m^{-2}$, respectively) (Balcı, 2012; Perez-Martin et al., 2001), while in another, the cut-off point was arbitrary (28 $kg \cdot m^{-2}$) (Hickner et al., 2001). Additionally, in one study, the WHO system was used to stratify three groups by differentiating obese (BMI, ≥30 $kg \cdot m^{-2}$) from overweight subjects (Mittendorfer et al., 2004), while in another, the authors used a BMI of 30 $kg \cdot m^{-2}$ as a cut-off point to differentiate an "obese" from "control" group (Ardevol et al., 1998). We chose to include the latter study because the mean ± SD for the control group was 20.9 ± 0.5 $kg \cdot m^{-2}$ (n = 8) (Ardevol et al., 1998). In the other eight studies that used BMI exclusively, the authors chose to classify in zones to provide separation; for example, obese (Devries et al., 2013; Melanson et al., 2009; Mohebbi & Azizi, 2011; Slusher et al., 2015), or extremely-obese (Thyfault et al., 2004), compared to normal-weight subjects using the WHO criterion ("extremely obese" BMI, ≥35 $kg \cdot m^{-2}$) or obese subjects compared to control subjects with BMI <23 (Santiworakul et al., 2014) or <24 (Goodpaster et al., 2002; Kanaley et al., 2001) $kg \cdot m^{-2}$.

In four studies, body-fat percentage was used exclusively to stratify subjects with various criteria employed; for example, obese and lean designations as >25% and <16%, respectively (Wong & Harber, 2006), an obese/lean cut-off point of 30% for women (Keim et al., 1996; Steffan et al., 1999), and 20% for men (Keim et al., 1996) or age-specific (20–39 years or 40–59 years) overweight/normal-weight cut-off points for women (32.5 and 34.5%) and men (20.5 and 22.5%) (Grams et al., 2017). In two other studies, body-fat percentage was used in conjunction with BMI; for example, obese subjects possessing a body-fat percentage >30% with BMI >29 $kg \cdot m^{-2}$ compared to "never-obese" controls with body-fat percentage no more than 'slightly above 30%' and BMI <27 $kg \cdot m^{-2}$ (mean ± SD, 20.6 ± 0.9 $kg \cdot m^{-2}$; n = 5) (Ezell et al., 1999) or obese subjects with body-fat percentage >40% and BMI of 35.0–39.9 $kg \cdot m^{-2}$ compared to lean subjects with body-fat percentage <30% and BMI ≤23 $kg \cdot m^{-2}$ (Horowitz & Klein, 2000). Finally, in five

other studies, the mean ± SD for BMI (Colberg et al., 1996) or both BMI and body-fat percentage (Chatzinikolaou et al., 2008; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009) for each group was provided to illustrate the difference(s); however, no mention was made of a specific criterion that was employed to establish the separation.

3.3.3 Fat-free Mass

In eight of the 24 included studies, FFM was either not measured (Chatzinikolaou et al., 2008; Slusher et al., 2015; Thyfault et al., 2004) or measured, but not reported (Ezell et al., 1999; Melanson et al., 2009; Perez-Martin et al., 2001; Santiworakul et al., 2014; Steffan et al., 1999). In the other 16 studies, the overweight/obese group possessed a higher FFM in all but two cases (Grams et al., 2017; Kanaley et al., 1993). However, in some studies, this difference was only present for overweight/obese subjects with specific characteristics; for example, female, but not male (Keim et al., 1996), obese, but not overweight (Mittendorfer et al., 2004) and upper- but not lower-body situated obesity (Kanaley et al., 2001).

3.3.4 Aerobic Fitness

In three of the 24 included studies, $\dot{V}O_{2peak/max}$ was either not measured (Perez-Martin et al., 2001) or measured, but not reported (Ardevol et al., 1998; Santiworakul et al., 2014). For the other 21 studies, 12 included a report of $\dot{V}O_{2peak/max}$ expressed relative to FFM and in 11 of those investigations, values did not differ between groups (Devries et al., 2013; Goodpaster et al., 2002; Hickner et al., 2001; Horowitz & Klein, 2000; Kanaley et al., 1993; Kanaley et al., 2001; Keim et al., 1996; Mittendorfer et al., 2004; Mohebbi & Azizi, 2011; Steffan et al., 1999; Wong & Harber, 2006), although in one of these studies, this was the case for African American women only (Hickner et al., 2001). In the other investigation (Lanzi et al., 2014) and for the Caucasian women in the aforementioned study (Hickner et al., 2001), overweight/obese subjects demonstrated lower values. In contrast, overweight/obese subjects demonstrated lower values in 11 of the 14 studies that included a report of $\dot{V}O_{2peak/max}$ expressed relative to BM (Ardevol et al., 1998; Balcı, 2012; Grams et al., 2017; Lanzi et al., 2014; Larsen et al., 2009; Melanson et al., 2009; Mohebbi & Azizi, 2011; Slusher et al., 2015; Steffan et al., 1999; Thyfault et al., 2004; Wong & Harber, 2006), with no difference present in the other three studies (Chatzinikolaou et al., 2008; Colberg et al., 1996; Ezell et al., 1999). Moreover, when a report of $\dot{V}O_{2peak/max}$ expressed in

absolute terms was also included, no difference (Ezell et al., 1999; Goodpaster et al., 2002; Grams et al., 2017; Hickner et al., 2001; Horowitz & Klein, 2000; Keim et al., 1996; Lanzi et al., 2014; Mittendorfer et al., 2004; Slusher et al., 2015; Steffan et al., 1999; Thyfault et al., 2004), or a greater value (Keim et al., 1996; Wong & Harber, 2006) was observed for the overweight/obese group. Finally, in seven of the 24 included studies, the word "matched" was used to describe how the groups compared with respect to CRF (Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 1993; Keim et al., 1996; Mittendorfer et al., 2004); however, specific mention of how the matching was accomplished was only provided in one case (i.e., pair-wise range matching for $\dot{V}O_{2peak}$ per FFM; range, ±3 $ml \cdot kg^{-1} \cdot min^{-1}$) (Keim et al., 1996). One other study included an analysis of lean and obese subgroups (*n* = 8 each) matched for $\dot{V}O_{2peak}$ per FFM to support the primary study's findings for the entire group (*n* = 16 each) (Lanzi et al., 2014).

3.3.5 Insulin Responsiveness

In 14 studies, fasting plasma insulin concentration was used to infer insulin responsiveness (Ardevol et al., 1998; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009; Melanson et al., 2009; Mittendorfer et al., 2004; Perez-Martin et al., 2001; Slusher et al., 2015; Thyfault et al., 2004). In all but two cases where no difference was detected between groups (Melanson et al., 2009; Mittendorfer et al., 2004), the obese group possessed a higher value suggesting lower insulin sensitivity (IS). Furthermore, in six studies, insulin responsiveness was assessed using HOMA-IR either exclusively (Chatzinikolaou et al., 2008) or in addition to fasting plasma insulin concentration (Devries et al., 2013; Lanzi et al., 2014; Larsen et al., 2009, Slusher et al., 2015; Thyfault et al., 2004. In these six studies, HOMA-IR confirmed lower IS in the overweight/obese group in all but two cases (Chatzinikolaou et al., 2008; Larsen et al., 2009). Conversely, in nine studies, no assessment of insulin responsiveness was reported (Balcı, 2012; Grams et al., 2017; Hickner et al., 2001; Kanaley et al., 2001; Keim et al., 1996; Mohebbi & Azizi, 2011; Santiworakul et al., 2014; Steffan et al., 1999; Wong & Harber, 2006).

3.3.6 Menstrual Phase

Four of the 15 studies that included females as subjects involved control of the subject's menstrual phase (Ezell et al., 1999; Horowitz & Klein, 2000; Kanaley et al., 1993; Kanaley et al., 2001). In

all four cases, the researchers chose to test during the follicular phase with three providing more detail; for example, days 1–14 (Horowitz & Klein, 2000), days 3–9 (Kanaley et al., 2001), or days 6–11 (Ezell et al., 1999).

3.3.7 Diet

In 14 studies, participants performed exercise in the fasted state with no longer-term dietary control (Ardevol et al., 1998; Balcı, 2012; Chatzinikolaou et al., 2008; Devries et al, 2013; Grams et al., 2017; Hickner et al., 2001; Keim et al., 1996; Larsen et al., 2009; Mohebbi & Azizi, 2011; Perez-Martin et al., 2001; Slusher et al., 2015; Steffan et al., 1999; Thyfault et al., 2004; Wong & Harber, 2006). A greater degree of short-term dietary control was established in other included studies; for example, in addition to an overnight fast, in two studies, subjects performed exercise after a standardized meal (Horowitz & Klein, 2000) or meal plus snack (Mittendorfer et al., 2004) the evening before, while in three others, a standardized breakfast was provided prior to the test (Ezell et al., 1999; Melanson et al., 2009; (Santiworakul et al., 2014). Finally, in seven studies, some form of longer-term dietary control was in place prior to testing. For example, subject-specific diets were prescribed in two of these investigations; specifically, a eucaloric diet with set macronutrient proportions for 3 (energy content based on 3-day recall) (Ezell et al., 1999) or 4 (energy content estimated based on FFM with activity factor) (Melanson et al., 2009) days plus breakfast prior to the test. The other five studies with prolonged dietary control included the same generic diet for all subjects; for example, a “balanced diet” comprising the same macronutrient and energy content for 21 (obese subjects) or 4 (lean subjects) days (Lanzi et al., 2014) or a diet containing ≥200 g of carbohydrate daily for 3 days (Colberg ete al., 1996; Goodpaster et al., 2002; Kanaley et al., 2001), or “at least two weeks” (Kanaley et al., 1993) prior to an overnight fast before testing.

With respect to the “fasted state,” it was defined in four studies in a general manner as the condition during the morning following an overnight fast (Ardevol et al., 1998; Colberg et al., 1996; Goodpaster et al., 2002; Horowitz & Klein, 2000), while in other cases, a specific timeframe was provided; for example, 4 (Wong & Harber, 2006); 4–6 (Steffan et al., 1999); 5–6 (evening session) (Mohebbi & Azizi, 2011); 8 (Hickner et al., 2001; Slusher et al., 2015); 8–12 (morning session) (Mohebbi & Azizi, 2011); 10–12 (Kanaley et al., 1993; Larsen et al., 2009; Mittendorfer et al., 2004); 12 (Devries et al.,

2013; Grams et al., 2017; Kanaley et al., 2001; Lanzi et al., 2014; Perez-Martin et al., 2001; Thyfault et al., 2004); or 15 (Balcı, 2012)) hours. In two other studies, the “fasted” (Chatzinikolaou et al., 2008) or “post-absorptive” (Keim et al., 1996) state was not defined. Finally, in the three studies that required exercise to be performed following breakfast, the time interspersed was 60 (Melanson et al., 2009), 90 (Ezell et al., 1999), or 180 (Santiworakul et al., 2014) minutes.

3.3.8 Criterion Exercise Challenge

3.3.8.1 Exercise Mode

Table 3.1 presents the characteristics of the criterion exercise bout(s) that was/were used to assess substrate selectivity. In 23 of the 24 studies, endurance exercise was studied—specifically, conventional leg cycle ergometry (Ardevol et al., 1998; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Grams et al., 2017; Hickner et al., 2001; Kanaley et al., 1993; Keim et al., 1996; Lanzi et al., 2014; Larsen et al., 2009; Melanson et al., 2009; Perez-Martin et al., 2001; Thyfault et al., 2004; Wong & Harber, 2006); recumbent cycling (Horowitz & Klein, 2000; Mittendorfer et al., 2004); treadmill exercise (Balcı, 2012; Kanaley et al., 2001; Mohebbi & Azizi, 2011; Slusher et al., 2015; Steffan et al., 1999); vacuuming (MET level >3; hence, included) (Grams et al., 2017); floor walking (Grams et al., 2017); platform stepping (Grams et al., 2017); or mode not stated (Santiworakul et al., 2014). In one of these studies, in addition to leg ergometry, subjects performed arm cranking so that a comparison across different modes of exercise could be made (Larsen et al., 2009). In the one included study that did not involve what would be considered endurance exercise, 30 minutes of circuit resistance training with sets performed intermittently for 10-12 repetitions at 70-75% of the one-repetition-maximum weight was used (Chatzinikolaou et al., 2008).

3.3.8.2 Exercise Duration

Thirteen of the 23 studies that involved endurance exercise to assess substrate selectivity required one testing session during which a single continuous bout at a fixed work rate (i.e., constant-work-rate exercise; CWR) was performed with duration set at 30 (Kanaley et al., 2001; Slusher et al., 2015; Wong & Harber, 2006); 40 (Colberg et al., 1996); 60 (Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Melanson et al., 2009; Thyfault et al., 2004); 90 (Horowitz & Klein, 2000; (Mittendorfer et al., 2004); or 150 (Kanaley et al., 1993) minutes, or that which was required so that total

energy expenditure would reach 300 kilocalories for each subject (~30 and ~28 minutes for lean and obese subjects, respectively) (Santiworakul et al., 2014). Another study also involved CWR during a single exercise session; however, in this case, two 10-minute bouts at different work rates were performed in succession (Hickner et al., 2001). Six "activities of daily living" were performed in succession with 2 minutes of recovery between in another study (Grams et al., 2017). For the purpose of this review, only the final four (vacuuming, floor walking, platform stepping and leg cycling) were considered "exercise" (MET level reported as >3) and they were performed in a CWR manner for 12, 6, 12, and 12 minutes, respectively (Grams et al., 2017). In two other studies, subjects were required to perform two CWR bouts on separate days with duration set at 15 (Steffan et al., 1999) or 45 (Balcı, 2012) minutes. In these two studies, substrate selectivity was also assessed during a third testing session using an incremental "Bruce protocol" (3-minute stages performed in succession) that was "open ended" (i.e., continued until termination criteria were achieved; specifically, heart rate of 220 minus age, ventilatory equivalent for O_2 "close to 30 $l \cdot min^{-1}$" and RER >1.15 (Balcı, 2012) or at least two of the following: a plateau in $\dot{V}O_2$, RER ≥1.1, rating of perceived exertion ≥18 and/or "volitional fatigue" (Steffan et al., 1999). Incremental protocols were used exclusively in the other six investigations that involved endurance exercise; however, in two of these studies, close-ended paradigms were employed. Specifically, one study required subjects to perform four 6-minute bouts in succession (Perez-Martin et al., 2001) while another involved four (women) or five (men) 5-minute bouts performed intermittently with 5 minutes of rest interspersed (Keim et al., 1996). The other four investigations involved open-ended tests with two utilizing 3-minute stages until an RER of 1.0 (Mohebbi & Azizi, 2011) or limit of tolerance (Ardevol et al., 1998) was reached, and another comprising 6-minute stages performed until a criterion RER (>1.0) or power output (>65% of the previously-determined peak) was achieved (Lanzi et al., 2014). The fourth investigation that involved the assessment of substrate selectivity during open-ended incremental testing included both a leg and arm protocol performed until limit of tolerance on separate days. For the leg test, stage length was set at 5 minutes until an RER of 1.0 was achieved, after which 2-minute stages were employed (Larsen et al., 2009). For the arm test, three 6-minute stages were completed and a 5-minute rest period allowed before 1-minute stages were performed until limit of tolerance (Larsen et al., 2009).

3.3.8.3 Exercise Intensity

In 14 of the 17 studies that included assessment of substrate use during CWR (see above), work rate was calculated relative to the $\dot{V}O_{2peak/max}$ achieved during a maximal incremental test; for example, 40% (Colberg et al., 1996); 45% (Kanaley et al., 1993); 50% (Devries et al., 2013; Grams et al., 2017; Goodpaster et al., 2002; Horowitz & Klein, 2000; Mittendorfer et al., 2004; Steffan et al., 1999; Thyfault et al., 2004); 55% (Melanson et al., 2009); 60–65% (Ezell et al., 1999); 65% (Hickner et al., 2001); 70% (Kanaley et al., 2001); or 75% (Slusher et al., 2015; Steffan et al., 1999). In one of these studies, a two-bout series was performed with the first-bout work rate set at the same absolute power output (15 W) for all subjects instead (Hickner et al., 2001). In one of the other three CWR studies, walking and running bouts were performed at 1.0 $km \cdot hr^{-1}$ below and above the preferred walk/run transition speed (Balcı, 2012), while in another, vacuuming, floor walking and platform stepping were regulated by subjects at their "usual pace" (Grams et al., 2017). In the other two investigations, the imposed work rate was that which was aligned with the "ventilatory threshold" estimated by assessing the $\dot{V}CO_2/\dot{V}O_2$ relationship ("V-slope method") (Santiworakul et al., 2014) or ventilatory-equivalent-for-CO_2 response (Wong & Harber, 2006) during an initial incremental-exercise test.

A number of different intensity-loading paradigms were used in the eight included studies that assessed substrate utilization during incremental exercise. In six studies, work-rate increments were prescribed in the same absolute terms for all subjects. For example, in two studies that used treadmill exercise, the modified Bruce Protocol, which starts at 2.7 $km \cdot hr^{-1}$ with 0% (Balcı, 2012) or 5% (Steffan et al., 1999) grade and progresses to 2.7 $km \cdot hr^{-1}$/10% grade with grade increases of 2% per stage thereafter (stage speeds at 4.0, 5.5, 6.8, 8.1, 8.9, 9.7, 10.5, 11.3 and 12.1 $km \cdot hr^{-1}$), was used. In another treadmill study, substrate oxidation was assessed at 3.5 $km \cdot hr^{-1}$/1% grade, followed by speed increases of 1.0 $km \cdot hr^{-1}$ for four stages and grade increases of 2% thereafter (Mohebbi & Azizi, 2011). For three other studies, stages at the same absolute work rate were performed during cycle ergometry—specifically, 0-W leg cycling followed by 30-W work-rate increments (Ardevol et al., 1998; Keim et al., 1996) or exercise at 95 or 20 W followed by work-rate increments of 35 or 15 W for leg and arm cycling, respectively (Larsen et al., 2009). Conversely, the other two of the eight studies that included incremental exercise involved work-rate increments established relative to each subject's capacity. For example, in one study, subjects

cycled at 20% of their peak work rate measured previously on an incremental test followed by work-rate increments of 7.5% per stage (Lanzi et al., 2014), while in the other, the subject's initial work rate was set at 20% of their estimated peak work rate with four subsequent increases of 10% per stage (Perez-Martin et al., 2001).

3.3.9 The Effect of Overweight/Obesity on Exercise Lipid Use

Table 3.2 presents the findings regarding exercise lipid use for the included studies and the outcome measure(s) that was/were cited to draw the conclusions. Thirteen of the 24 studies provided no evidence that the null hypothesis regarding the capacity for exercise lipid use in overweight/obese compared to normal-weight subjects should be rejected (Ardevol et al., 1998; Balcı, 2012; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Melanson et al., 2009; Mittendorfer et al., 2004; Santiworakul et al., 2014; Slusher et al., 2015; Steffan et al., 1999; Thyfault et al., 2004; Wong & Harber, 2006). Outcome measures used to support this conclusion were: (a) RQ/RER (Ardevol et al., 1998; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Melanson et al., 2009; Steffan et al., 1999; Thyfault et al., 2004; Wong & Harber, 2006); (b) total lipid oxidation in absolute terms (Ezell et al., 1999; Kanaley et al., 1993), relative to BM (Ezell et al., 1999) and FFM (Ezell et al., 1999), in kilocalories (Santiworakul et al., 2014) and/or as a percentage of total energy expenditure (Colberg et al., 1996; Santiworakul et al., 2014); (c) lipid oxidation rate in absolute terms (Balcı, 2012; J. A. Kanaley et al., 1993; Slusher et al., 2015) or relative to BM (Colberg et al., 1996; Devries et al., 2013; Thyfault et al., 2004) and/or FFM (Devries et al., 2013; Mittendorfer et al., 2004); and (d) maximal lipid oxidation rate in absolute terms (Balcı, 2012), relative to FFM (Balcı, 2012) or as an RER (Balcı, 2012), heart rate (Balcı, 2012), $\dot{V}O_2$ relative to BM (Balcı, 2012) and/or percentage of $\dot{V}O_{2max}$ (Balcı, 2012).

This conclusion was drawn from four of the nine studies on men exclusively (Balcı, 2012; (Mittendorfer et al., 2004; Santiworakul et al., 2014; Wong & Harber, 2006), six of the nine on women exclusively (Ardevol et al., 1998; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Steffan et al., 1999; Thyfault et al., 2004) and three of the six on a mixed group (Colberg et al., 1996; Melanson et al., 2009; Slusher et al., 2015). The conclusion was supported by 12 of the 17 studies that involved CWR endurance exercise (Balcı, 2012; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Melanson et al., 2009; Mittendorfer et al., 2004; Santiworakul et al., 2014; Slusher et al., 2015;

Steffan et al., 1999; Thyfault et al., 2004; Wong & Harber, 2006), and three of the eight for which incremental endurance exercise was assessed (Ardevol et al., 1998; Balcı, 2012; Steffan et al., 1999). With respect to mode, the conclusion was supported by 9 of 17 studies that involved leg cycling (Ardevol et al., 1998; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Melanson et al., 2009; Mittendorfer et al., 2004; Thyfault et al., 2004; Wong & Harber, 2006), three of five that involved treadmill exercise (Balcı, 2012; Slusher et al., 2015; Steffan et al., 1999) and one study for which the mode was not stated (Santiworakul et al., 2014).

Seven of the 24 studies included a report of a reduced capacity for lipid oxidation in overweight/obese compared to normal-weight subjects for some (Grams et al., 2017; Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Mohebbi & Azizi, 2011) or all (Chatzinikolaou et al., 2008; Perez-Martin et al., 2001) of the outcome measures, subject populations and/or modes that were assessed. Outcome measures used to support this conclusion were: (a) RER (Chatzinikolaou et al., 2008; Hickner et al., 2001; Keim et al., 1996; Perez-Martin et al., 2001); (b) total lipid oxidation as a percentage of total energy expenditure (Grams et al., 2017); (c) lipid oxidation rate in absolute terms (Grams et al., 2017; (Hickner et al., 2001; Lanzi et al., 2014; Perez-Martin et al., 2001) and/or relative to FFM (Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Mohebbi & Azizi, 2011; Perez-Martin et al., 2001); (d) maximal lipid oxidation rate expressed as a power output (Perez-Martin et al., 2001), percentage of $\dot{V}O_{2peak/max}$ (Lanzi et al., 2014; Mohebbi & Azizi, 2011), WR_{max} (Lanzi et al., 2014) or HR_{max} (Lanzi et al., 2014) and/or as a zone (Lanzi et al., 2014); and (e) crossover point in absolute and relative terms as a work rate and percentage of WR_{max}, respectively (Perez-Martin et al., 2001).

This conclusion was drawn from three of the nine studies on men exclusively (Chatzinikolaou et al., 2008; Lanzi et al., 2014; Mohebbi & Azizi, 2011), one of the nine on women exclusively (Hickner et al., 2001), and three of the six on a mixed group (Grams et al., 2017; Keim et al., 1996; Perez-Martin et al., 2001). The conclusion was supported by two of the 17 studies that involved CWR endurance exercise (Grams et al., 2017; Hickner et al., 2001) and four of the eight that assessed incremental endurance exercise (Keim et al., 1996; Lanzi et al., 2014; Mohebbi & Azizi, 2011; Perez-Martin et al., 2001). With respect to mode, the conclusion was supported by four of 17 studies that involved leg cycling (Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Perez-Martin et al., 2001), one of five that involved

treadmill exercise (Mohebbi & Azizi, 2011) and the single studies that involved resistance training (Chatzinikolaou et al., 2008) and activities of daily living (Grams et al., 2017). Finally, two studies indicated subject-population specificity for drawing this conclusion as a reduced capacity for lipid oxidation with overweight/obesity was present for female, but not male subjects (Keim et al., 1996) and for female subjects when compared to Caucasian, but not African American normal-weight controls (Hickner et al., 2001).

Five of the 23 studies included a report of an increased capacity for lipid oxidation in overweight/obese compared to normal-weight subjects for some (Goodpaster et al., 2002; Kanaley et al., 2001; Lanzi et al., 2014; Larsen et al., 2009) or all (Horowitz & Klein, 2000) of the outcome measures and/or subject populations that were assessed. Outcome measures used to support this conclusion were: (a) RER (Goodpaster et al., 2002; Kanaley et al., 2001; Lanzi et al., 2014); (b) lipid energy expenditure as a percentage of total energy expenditure (Goodpaster et al., 2002; Horowitz & Klein, 2000); (c) lipid oxidation rate in absolute terms (Lanzi et al., 2014) and/or relative to FFM (Horowitz & Klein, 2000; Kanaley et al., 2001; Lanzi et al., 2014); and (d) maximal lipid oxidation rate as a percentage of $\dot{V}O_{2peak/max}$ (Larsen et al., 2009) and as an RER (Lanzi et al., 2014).

This conclusion was drawn from three of the nine studies on men exclusively (Goodpaster et al., 2002; Lanzi et al., 2014; Larsen et al., 2009) and two of the nine on women exclusively (Horowitz & Klein, 2000; Kanaley et al., 2001). The conclusion was supported by three of the 17 studies that involved CWR endurance exercise (Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 2001) and two of the eight for which incremental endurance exercise was assessed (Lanzi et al., 2014; Larsen et al., 2009). With respect to mode, the conclusion was supported by three of 17 studies that involved leg cycling (Goodpaster et al., 2002; Horowitz & Klein, 2000; Lanzi et al., 2014), one of five that involved treadmill exercise (Kanaley et al., 2001) and the only study that involved arm cranking (Larsen et al., 2009).

Eight of the 24 included studies returned equivocal findings based on multiple outcome measures, subject populations and/or exercise characteristics that were included for assessment (Goodpaster et al., 2002; Grams et al., 2017; Hickner et al., 2001; Kanaley et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Larsen et al., 2009; Mohebbi & Azizi, 2011) (see Table 3.2). Seven of the 17 studies that included a report of multiple outcome measures provided evidence that choice of outcome measure

can influence whether the null hypothesis regarding the comparison of exercise lipid use for overweight/obese compared to normal-weight subjects should be accepted or rejected (Goodpaster et al., 2002; Grams et al., 2017; Hickner et al., 2001; Kanaley et al., 2001; Lanzi et al., 2014; Larsen et al., 2009; Mohebbi & Azizi, 2011). Interestingly, two of three studies that included a report of sex-specific results confirmed that an overweight/obesity-related decrement was present for male, but not female subjects (Grams et al., 2017; Keim et al., 1996; *cf*. Perez-Martin et al., 2001) and a similar disparity was identified for African American compared to Caucasian women in the only study to provide comparisons based on race (Hickner et al., 2001). Furthermore, findings from one of two studies that included two distinct groups of obese subjects based on fat-deposition pattern suggested that this factor can influence the conclusion at least based on one of two outcome measures that was reported (Kanaley et al., 2001; *cf.* Kanaley et al., 1993). Finally, findings from one of three studies that included assessment of lipid oxidation at different time points during CWR exercise (Kanaley et al., 2001; *cf.* Ezell et al., 1999; Goodpaster et al., 2002); four of nine that included assessment at different exercise intensities/work rates (Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Mohebbi & Azizi, 2011; *cf.* Ardevol et al., 1998; Balcı, 2012; Larsen et al., 2009; Perez-Martin et al., 2001; Steffan et al., 1999); and both studies that included assessment of different exercise modes (Grams et al., 2017; Larsen et al., 2009) showed that each of these characteristics of the exercise session can exert an influence that affects the conclusion that is drawn.

3.4 Quality Assessment of Included Studies

The quality-assessment scores of the included studies are provided in Tables 3.3 and 3.4. The mean ± SD score for general methodological quality was 1.51 ± 0.14 (median, 1.46; range: 1.29–1.79) while our custom-designed tool revealed an average score of 0.93 ± 0.27 (median, 0.83; range: 0.58–1.42) for the degree to which important factors that can affect exercise lipid utilization, regardless of body fatness were adequately controlled.

Table 3.1

Characteristics of Subjects

Study	Subjects		Exercise for Assessment	Result(s) and Relevant Outcome Measure(s)
Ardévol et al. [1998]	Female Obese: $n = 8$ age 30.0 years BMI 32.3 ± 0.8 kg/m^2 body fat 35.7 ± 1.1%	Control: $n = 8$ age 25.4 years BMI 20.9 ± 0.5 kg/m^2 body fat 23.4 ± 1.3%	Leg cycling Incremental 0 W + 30 W↑/3-min stage → T_{lim}	Obese = Lean RQ
Balci [2012]	Male Overweight/Obese: $n = 9$ age 21.4 ± 0.6 years BMI 31.6 ± 1.1 kg/m^2 body fat 24.2 ± 1.3%	Normal Weight: $n = 10$ age 21.9 ± 0.7 years BMI 22.6 ± 0.4 kg/m^2 body fat 13.8 ± 0.5%	Treadmill CWR (1/session) 45 min at 1 km below PTS 45 min at 1 km above PTS Incremental Modified Bruce Protocol	Overweight/Obese = Normal Weight Lipid oxidation rate (g·min^{-1}) Maximal lipid oxidation rate (g·min^{-1}, mg·kgFFM^{-1}·min^{-1}, RER, $\dot{V}O_2$·kg^{-1}BM, %$\dot{V}O_{2max}$, HR)
Chatzinikolaou et al. [2008]	Male Obese: $n = 8$ age 23.4 ± 0.8 years BMI 31.2 ± 1.0 kg/m^2 body fat 27.7 ± 1.2%	Lean: $n = 9$ age 23.8 ± 1.2 years BMI 23.7 ± 0.5 kg/m^2 body fat 11.1 ± 1.4%	Resistance Circuit 3 cycles × 10 exercises w/ 10-12 reps/set separated by 30-s rest	Obese < Lean RER
Colberg et al. [1996]	Male/Female Obese: $n = 4/3$ age 50 ± 3 years BMI 35.0 ± 1.4 kg/m^2	Lean: $n = 3/4$ age 48 ± 3 years BMI 23.4 ± 1.0 kg/m^2	Leg cycling CWR 40 min at 40% $\dot{V}O_{2peak}$	Obese = Lean RER Lipid oxidation rate (mg·BM^{-1}·min^{-1}) Lipid energy expenditure (% total)
Devries et al. [2013]	Female Obese: $n = 11$ age 40 ± 3 years BMI pre 34 ± 2 kg/m^2 BMI post 34 ± 2 kg/m^2 body fat pre 49 ± 1% body fat post 50 ± 2%	Lean: $n = 12$ age 41 ± 2 years BMI pre 23 ± 1 kg/m^2 BMI post 23 ± 1 kg/m^2 body fat pre 32 ± 2% body fat post 32 ± 2%	Leg cycling CWR 60 min at 50% $\dot{V}O_{2peak}$	Obese = Lean RER Lipid oxidation rate (mg·BM^{-1}·min^{-1}, mg·FFM^{-1}·min^{-1})
Ezell et al. [1999]	Female Obese: $n = 5$ age 26.2 ± 2.8 years BMI 30.0 ± 2.7 kg/m^2 body fat 44.4 ± 1.8%	Never obese: $n = 5$ age 25.6 ± 3.5 years BMI 20.6 ± 0.9 kg/m^2 body fat 25.0 ± 2.8%	Leg cycling CWR 60 min at 60-65% $\dot{V}O_{2peak}$	Obese = Never obese RER Total lipid oxidation (g, g·BM^{-1}, g·FFM^{-1})
Goodpaster et al. [2002]	Male Obese: $n = 7$ age 39.3 ± 3.2 years BMI 33.7 ± 1.1 kg/m^2 body fat 30.3 ± 1.3%	Lean: $n = 7$ age 34.3 ± 3.3 years BMI 23.7 ± 0.7 kg/m^2 body fat 20.4 ± 2.3%	Leg cycling CWR 60 min at 50% $\dot{V}O_{2max}$	Obese > = Lean RER Lipid oxidation rate (μmol·FFM^{-1}·min^{-1}) Lipid energy expenditure (MJ, % total)
Grams et al. [2017]	Male/Female Overweight: $n = 5/7$ age 45 ± 11/48 ± 9 years BMI 27.3 ± 1.3/28.4 ± 3.3 kg/m^2 body fat 26.5 ± 1.6/37.9 ± 3.5%	 Normal weight: $n = 10/11$ age 54 ± 6/49 ± 6 years BMI 24.1 ± 1.7/23.4 ± 3.2 kg/m^2 body fat 17.6 ± 2.7/25.3 ± 6.1%	Vacuuming/Floor Walking/Platform Stepping/Leg Cycling CWR (Vacuum) 12 min self paced CWR (Walk) 6 min self paced CWR (Step) 12 min self paced CWR (Cycle) 10 min at 50% $\dot{V}O_{2max}$	Obese < = Lean Lipid oxidation rate (kcal·min^{-1}) Lipid energy expenditure (% total) Peak lipid oxidation rate (kcal·min^{-1}, mg·kgFFM^{-1}·min^{-1}, %$\dot{V}O_{2max}$)
Hickner et al [2001]	Female Obese African American: $n = 11$ age 30.9 ± 2.2 years BMI 38.0 ± 1.8 kg/m^2 body fat 41.2 ± 1.3% Obese Caucasian: $n = 9$ age 34.1 ± 2.5 years BMI 34.8 ± 0.9 kg/m^2 body fat 39.3 ± 2.7%	Lean African American: $n = 7$; age 28.4 ± 2.8 years; BMI 23.1 ± 1.2 kg/m^2; body fat 25.8 ± 2.8% Lean Caucasian: $n = 9$; age 24.7 ± 1.8 years; BMI 23.5 ± 1.0 kg/m^2; body fat 26.4 ± 2.0%	Leg cycling CWR (2 in succession) 10 min at 15 W 10 min at 65% $\dot{V}O_{2peak}$	Obese < = Lean RER Lipid oxidation rate (g·min^{-1}, g·FFM^{-1}·hr^{-1})
Horowitz et al. [2000]	Female Obese: $n = 5$ age premenopausal BMI 37.7 ± 0.8 kg/m^2 body fat 48.6 ± 1.9%	Lean: $n = 5$ age premenopausal BMI 20.9 ± 0.4 kg/m^2 body fat 25.4 ± 1.5%	Leg cycling (recumbent) CWR 90 min at 50% $\dot{V}O_{2peak}$	Obese > Lean Lipid oxidation rate (μmol·FFM^{-1}·min^{-1})
Kanaley et al. [1993]	Female Obese lower body: $n = 11$ age 36 ± 2 years BMI 31.5 ± 0.4 kg/m^2 body fat 50 ± 3% Obese upper body: $n = 13$ age 36 ± 2 years BMI 33.4 ± 0.5 kg/m^2 body fat 48 ± 2%	Non-obese: $n = 8$ age 36 ± 1 years BMI 22.1 ± 0.6 kg/m^2 body fat 30 ± 1%	Leg cycling CWR 150 min at 45% $\dot{V}O_{2peak}$	Obese = Non-obese Lipid oxidation rate (μmol·min^{-1}) Total lipid oxidation (mmol)
Kanaley et al. [2001]	Female Obese lower body: $n = 11$ age 32.0 ± 1.7 years BMI 32.5 ± 0.5 kg/m^2 body fat 38.2 ± 0.6% Obese upper body: $n = 12$ age 32.5 ± 1.7 years BMI 33.5 ± 1.0 kg/m^2 body fat 38.9 ± 0.5%	Non-obese: $n = 8$ age 35.7 ± 1.4 years BMI 21.7 ± 1.7 kg/m^2 body fat 20.8 ± 1.4%	Treadmill CWR 30 min at 70% $\dot{V}O_{2peak}$	Obese > = Non-obese RER Lipid oxidation rate (μmol·FFM^{-1}·min^{-1})
Keim et al. [1996]	Male/Female Fatter: $n = 8/8$ age 34 ± 1/29 ± 2 years body fat 22.1 ± 0.6/36.2 ± 1.8%	Leaner: $n = 8/8$ age 29 ± 1 years/32 ± 1 years body fat 12.4 ± 0.8/20.6 ± 0.9%	Leg cycling Intermittent Incremental 30, 60, 90, 120 W/5-min stage (female) 30, 60, 90, 120, 150 W/5-min stage (male)	Fatter < = Leaner RER Lipid oxidation rate (mg·FFM^{-1}·min^{-1})

Table 3.2

Findings Regarding Exercise Lipid Use for Included Studies and Outcome Measures

Determining Factor	Study	O < NW	O = NW	O > NW	Qualifications
Exercise duration (min)	Kanaley et al. [2001]		15	30	For lipid oxidation rate (LBO and UBO) or RER (UBO)
Exercise intensity (%$\dot{V}O_{2peak/max}$)	Hickner et al. [2001]	65	~40		For lipid oxidation rate for Caucasians; O < NW (RER) for Caucasians at both intensities and O = NW (lipid oxidation rate, RER) for AA at both intensities
	Keim [1996]	40–60	30		For men; O = NW at all intensities for women
	Lanzi et al. [2014]		60–85	20–55	For RER
		85	50–80	20–45	For lipid oxidation rate ($g \cdot min^{-1}$)
		65–85	35–60	20–30	For lipid oxidation rate ($mg \cdot kgFFM^{-1} \cdot min^{-1}$)
	Mohebbi & Azizi (2011)	60–80	20–50		For AM and PM
Exercise mode	Grams et al. [2017]	Platform stepping	Vacuuming Floor Walking Leg cycling		For women; O < NW for men for stepping and cycling (lipid oxidation rate) or all four activities (% lipid energy)
	Larsen et al. [2009]		Leg cycling	Arm cranking	For fatmax % $\dot{V}O_{2max}$; O = NW for Fatmax $g \cdot min^{-1}$, $mg \cdot kgBM^{-1} \cdot min^{-1}$, $mg \cdot kgFFM^{-1} \cdot min^{-1}$
Exercise work rate (W)	Keim et al. [2009]	120	30–90, 150		For men
	Lanzi et al. [2014]		75–150	30–60	For RER
		150	90–135	30–75	For lipid oxidation rate
Outcome measure	Goodpaster et al. [2002]		Lipid energy (MJ) Lipid oxidation rate ($\mu mol \cdot FFM^{-1} \cdot min^{-1}$)	Lipid energy (%) RER	$P = 0.08$
	Grams et al. [2017]	Lipid energy (%)	Lipid oxidation rate ($kcal \cdot min^{-1}$)		For walking (men)
		Lipid oxidation rate ($kcal \cdot min^{-1}$)	Peak lipid oxidation rate ($kcal \cdot min^{-1}$, $mg \cdot kgFFM^{-1} \cdot min^{-1}$, %$\dot{V}O_{2max}$)		For stepping (both sexes) and cycling (men)
		Lipid energy (%)	Peak lipid oxidation rate ($kcal \cdot min^{-1}$, $mg \cdot kgFFM^{-1} \cdot min^{-1}$, %$\dot{V}O_{2max}$)		For stepping (both sexes) and vacuuming, walking, cycling (men)
Hickner et al. [2001]	RER		Lipid oxidation rate ($g \cdot min^{-1}$, $g \cdot FFM^{-1} \cdot hr^{-1}$)		For obese Caucasian and AA v. lean Caucasian
Kanaley et al. [2001]			RER	Lipid oxidation rate ($\mu mol \cdot FFM^{-1} \cdot min^{-1}$)	For LBO at 30 min
Lanzi et al. [2014]	Lipid oxidation rate ($g \cdot min^{-1}$)		RER		At 150 W and 85% $\dot{V}O_{2peak}$
	Lipid oxidation rate ($mg \cdot FFM^{-1} \cdot min^{-1}$)		RER		At 65–85% $\dot{V}O_{2peak}$
	Maximal lipid oxidation rate (%$\dot{V}O_{2peak}$, $\%HR_{max}$, RER) and zone (%$\dot{V}O_{2peak}$)		Maximal lipid oxidation rate ($mg \cdot FFM^{-1} \cdot min^{-1}$)		

Table 3.2 (continued)

	Larsen et al. [2009]		Lipid oxidation rate ($g \cdot min^{-1}$) Maximal lipid oxidation rate ($g \cdot min^{-1}$, $g \cdot BM^{-1} \cdot min^{-1}$, $g \cdot FFM^{-1} \cdot min^{-1}$)	Maximal lipid oxidation rate (%$\dot{V}O_{2max}$)	For arm cranking
	Mohebbi & Azizi (2011)	Lipid oxidation rate ($mg \cdot FFM^{-1} \cdot min^{-1}$)	Maximal lipid oxidation rate ($mg \cdot FFM^{-1} \cdot min^{-1}$, %$\dot{V}O_{2max}$		For lipid oxidation rate at 60–80% $\dot{V}O_{2max}$
		Maximal lipid oxidation rate (%$\dot{V}O_{2max}$) Maximal lipid oxidation rate (%$\dot{V}O_{2max}$)	Lipid oxidation rate ($mg \cdot FFM^{-1} \cdot min^{-1}$) Maximal lipid oxidation rate ($mg \cdot kgFFM^{-1} \cdot min^{-1}$) Minimal lipid oxidation rate (%$\dot{V}O_{2max}$)		For lipid oxidation rate at 20–50% $\dot{V}O_{2max}$
Subject fat deposition	Kanaley et al. [2001]		LB Obese	UB Obese	For RER at 30 min; O < NW for LB and UB Obese for lipid oxidation rate at 30 min
Subject race	Hickner et al [2001]	Caucasian Obese	AA Obese		For cycling at 15 W (RER, lipid oxidation rate) and 65%$\dot{V}O_{2peak}$ (lipid oxidation rate)
Subject sex	Grams et al. [2017]	Male Obese	Female Obese		For cycling (% lipid energy, lipid oxidation rate) and vacuuming, walking (% lipid energy)
	Keim et al. [1996]	Male Obese	Female Obese		For cycling at 40–60% $\dot{V}O_{2max}$; O = NW for men and women at 30% $\dot{V}O_{2max}$

AA for African American subjects, *M* morning assessment, *LB* lower body, FFM fat-free mass, *HR* heart rate, *PM* evening assessment, *RER* respiratory exchange ratio, *UB* upper body obese, $\dot{V}O_{2max}$ maximal rate of oxygen consumption, $\dot{V}O_{2peak}$ peak rate of oxygen consumption

Table 3.3

Quality Assessment of Studies: General Methodological Quality

Study	Aim clearly stated and defined	Eligibility and inclusion criteria explained	Study population clearly specified and defined	Sample size justification provided	Subjects recruited from same or similar population	Independent variable clearly defined, valid and reliable	Dependent variable clearly defined, valid, and reliable	Avg score
Ardèvol et al. [1998]	2.0	2.0	2.0	0.0	0.5	1.5	2.0	1.43
Balci [2012]	2.0	2.0	2.0	0.0	0.5	2.0	2.0	1.50
Chatzinikolaou et al. [2008]	2.0	1.0	2.0	0.0	0.5	1.5	2.0	1.29
Colberg et al. [1996]	2.0	2.0	2.0	0.0	0.5	2.0	2.0	1.50
Devries et al. [2013]	2.0	2.0	2.0	0.0	1.0	2.0	2.0	1.57
Ezell et al. [1999]	2.0	2.0	2.0	0.0	0.5	1.5	2.0	1.43
Goodpaster et al. [2002]	2.0	2.0	2.0	1.0	1.5	2.0	2.0	1.79
Grams et al. [2017]	2.0	2.0	2.0	0.5	1.0	2.0	2.0	1.64
Hickner et al [2001]	2.0	1.5	2.0	0.0	0.5	1.5	2.0	1.36
Horowitz et al. [2000]	2.0	1.5	2.0	2.0	1.0	2.0	2.0	1.79
Kanaley et al. [1993]	2.0	1.5	2.0	0.0	0.0	2.0	2.0	1.36
Kanaley et al. [2001]	2.0	2.0	2.0	0.0	2.0	2.0	2.0	1.71
Kaim et al. [1996]	2.0	2.0	2.0	0.0	0.5	2.0	2.0	1.50
Lanzi et al. [2014]	2.0	2.0	2.0	0.0	1.5	2.0	2.0	1.64
Larsen et al. [2009]	2.0	1.5	2.0	0.0	0.5	2.0	2.0	1.43
Mclanson et al. [2009]	2.0	2.0	2.0	0.0	2.0	2.0	2.0	1.71
Mittendorfer et al. [2003]	2.0	1.0	2.0	0.0	0.5	2.0	2.0	1.36
Mohebbi & Azizi (2011)	2.0	2.0	2.0	0.0	1.0	2.0	2.0	1.57
Pérez-Martin et al. [2001]	2.0	1.0	1.5	0.0	1.0	2.0	2.0	1.36
Santiworakul et al. [2014]	2.0	1.5	1.5	2.0	0.0	1.5	2.0	1.50
Slusher et al. [2015]	2.0	2.0	2.0	0.0	0.0	2.0	2.0	1.43
Steffan et al. [1999]	2.0	1.0	2.0	0.0	1.0	2.0	2.0	1.43
Thyfault et al. [2004]	2.0	2.0	2.0	0.0	0.0	2.0	2.0	1.43
Wong et al. [2006]	2.0	1.5	2.0	0.0	0.5	2.0	2.0	1.43

Table 3.4

Quality Assessment of Studies: Important Variables

Study	Subject Characteristics									Exercise Characteristics			Avg score
	Sex	Race	FFM	CRF	Fat depot	MC	IS	Acute diet	Chronic diet	Mode	Dur	Int	
Ardévol et al. [1998]	2.0	0.0	NA	0.0	0.0	0.0	0.0	2.0	0.0	2.0	1.0	0.0	0.64
Balci [2012]	2.0	0.0	1.0	0.0	0.0	NA	0.0	2.0	0.0	2.0	2.0	1.0	0.91
Chatzinikolaou et al. [2008]	2.0	0.0	NA	0.0	0.0	NA	0.0	1.0	0.0	2.0	2.0	1.0	0.80
Colberg et al. [1996]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.5	2.0	2.0	1.0	0.63
Devries et al. [2013]	2.0	0.0	2.0	2.0	0.0	0.0	0.0	2.0	0.0	2.0	2.0	1.0	1.08
Ezell et al. [1999]	2.0	0.0	2.0	2.0	0.0	2.0	0.0	2.0	2.0	2.0	2.0	1.0	1.42
Goodpaster et al. [2002]	2.0	0.0	2.0	2.0	0.0	NA	2.0	2.0	0.5	2.0	2.0	1.0	1.41
Grams et al. [2017]	2.0	0.0	1.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0	1.0	1.0	0.75
Hickner et al [2001]	2.0	2.0	2.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0	1.0	1.0	1.00
Horowitz et al. [2000]	2.0	0.0	2.0	2.0	2.0	2.0	0.0	2.0	0.0	2.0	2.0	1.0	1.42
Kanaley et al. [1993]	2.0	0.0	0.0	2.0	2.0	2.0	0.0	2.0	0.5	2.0	2.0	1.0	1.29
Kanaley et al. [2001]	2.0	0.0	2.0	0.0	2.0	2.0	0.0	2.0	0.5	2.0	2.0	1.0	1.29
Keim et al. [1996]	2.0	0.0	2.0	2.0	0.0	0.0	0.0	1.0	0.0	2.0	1.0	0.0	0.83
Lanzi et al. [2014]	2.0	0.0	2.0	1.0	0.0	NA	0.0	2.0	2.0	2.0	1.0	1.0	1.08
Larsen et al. [2009]	2.0	0.0	1.0	0.0	0.0	NA	0.0	2.0	0.0	2.0	1.0	1.0	0.82
Melanson et al. [2009]	0.0	0.0	NA	0.0	0.0	0.0	0.0	2.0	2.0	2.0	2.0	1.0	0.90
Mittendorfer et al. [2003]	2.0	0.0	2.0	2.0	0.0	NA	0.0	2.0	0.0	2.0	2.0	1.0	1.08
Mohebbi & Azizi (2011)	2.0	0.0	2.0	0.0	0.0	NA	0.0	1.0	0.0	2.0	1.0	1.0	0.82
Pérez-Martin et al. [2001]	0.0	0.0	2.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0	1.0	1.0	0.73
Santiworakul et al. [2014]	2.0	0.0	1.0	0.0	0.0	NA	0.0	2.0	0.0	0.0	0.0	2.0	0.58
Slusher et al. [2015]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0	2.0	1.0	0.64
Steffan et al. [1999]	2.0	0.0	NA	0.0	0.0	0.0	0.0	1.0	0.0	2.0	1.0	1.0	0.64
Thyfault et al. [2004]	2.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	2.0	2.0	1.0	0.75
Wong et al. [2006]	2.0	0.0	NA	0.0	0.0	NA	0.0	1.0	0.0	2.0	2.0	2.0	0.82

CRF cardiorespiratory fitness ($\dot{V}O_{2max/peak}$), *dur* duration, *Fat depot* fat-deposition pattern, *FFM* fat-free mass, *IS* insulin sensitivity, *int* intensity, *MC* menstrual cycle

3.5 Discussion

3.5.1 Lipid Reliance During Exercise for Overweight/Obese and Normal-weight Individuals

This purpose of this systematic review was to form a consensus opinion regarding the degree to which overweight/obese individuals rely on lipid as fuel during exercise compared to normal-weight counterparts. After reviewing the literature, we found that some or all relevant outcome measures cited in 21 of the 24 included studies indicate that the null hypothesis regarding an influence of obesity on exercise lipid use cannot be rejected (Table 3.1). This means that the preponderance of evidence suggests that obese and non-obese subjects rely on lipid to a similar extent during exercise. However, eight of these 21 studies also include report of a significant difference in exercise lipid use for overweight/obese compared to normal-weight subjects based on other exercise characteristics (e.g., mode, duration, intensity and work rate), subject factors (e.g., sex, race and fat-deposition pattern) and/or outcome measures included in for assessment (Table 3.22). Furthermore, numerous factors should be controlled to validly test this hypothesis and our quality-assessment tool, which was specifically designed to measure how well these variables were accounted for, revealed low ratings for many of the included studies (Table 3.4). Collectively, these findings suggest that more research is required to clarify what is, in all likelihood, a multifactorial complex influence of overweight/obesity on exercise metabolism.

3.5.2 Subject Characteristics that Might Influence Exercise Lipid Use Irrespective of Body Fatness

3.5.2.1 Sex

Six of the 24 included studies assessed both male and female subjects; however, only three controlled for the potential influence of sex by reporting results for male and female subjects separately. All three provide evidence that male obese subjects oxidize lipid less effectively during exercise compared to normal-weight counterparts (Grams et al., 2017; Keim et al., 1996; Perez-Martin et al., 2001), while two of the three studies suggest that this is not the case for female subjects. Specifically, Keim et al. (1996) found an obesity-related reduction in lipid-oxidation rate when cycling at 40, 50, and 60% $\dot{V}O_{2max}$ that was exclusive to male subjects, while Grams et al. (2017) reported that male obese subjects satisfied energy expenditure during physical activities of daily living and leg cycling with a lower contribution from lipid compared to normal-weight counterparts. Conversely, obesity-related decrements were not present for female subjects for any of the activities assessed except stair climbing (Grams et al.,

2017). Collectively, these findings suggest that males might be more susceptible to an obesity-related decline in exercise lipid use, which resonates because sexual dimorphism with regard to exercise metabolism has been confirmed for normal-weight individuals. Specifically, a number of studies indicate that females rely more on lipid during exercise at a given percentage of $\dot{V}O_{2max/peak}$ compared to males (Blatchford, Knowlton, & Schneider, 1985; Carter, Rennie, & Tarnopolsky, 2001; Froberg & Pedersen, 1984; Horton, Pagliassotti, Hobbs, & Hill, 1998; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). The reason(s) for this enhanced capacity is/are unclear, but might relate to increased β- (Horton et al., 1998) and/or decreased α-adrenergic (Hellström, Blaak, & Hagström-Toft, 1996; Mittendorfer, Horowitz, & Klein, 2002) activity resulting in greater FFA circulation/uptake (Blatchford et al., 1985; Mittendorfer et al., 2002), greater uptake of a given level of circulating FFA (Tarnopolsky et al., 1990), and/or greater storage/utilisation of IMTG (Tarnopolsky et al., 1990). Regardless of this distinction, however, a greater capacity for exercise lipid use for females raises the possibility that females possess inherent characteristics that allow them to fend off a decline in the ability to oxidize lipid that accompanies overweight/obesity. In support of this contention, findings from eight of the nine included studies that assessed females exclusively suggest an unchanged (Ardevol et al., 1998; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Steffan et al., 1999; Thyfault et al., 2004) or enhanced (Horowitz & Klein, 2000; Kanaley et al., 2001) capacity for exercise lipid use in the overweight/obese compared to normal-weight group. However, for the nine included studies that assessed males exclusively, only three found evidence that lipid use was reduced with obesity (Chatzinikolaou et al., 2008; Lanzi et al., 2014; Mohebbi & Azizi, 2011), and other subject characteristics could influence exercise fuel use irrespective of body fatness and sex (e.g., race, fat-deposition pattern, IS and, for female subjects, phase of menstrual cycle) that were not controlled in the aforementioned two studies. To clarify ambiguity regarding the influence of sex on exercise fuel use with overweight/obesity, future research should include both male and female subjects with results determined according to sex.

3.5.2.2 Race

Research suggests that African Americans demonstrate a reduced capacity for lipid oxidation at rest (Chitwood, Brown, Lundy, & Dupper, 1996; Weyer, Snitker, Bogardus, & Ravussin, 1999), during exercise (Chitwood et al., 1996) and when challenged with a high-fat diet (Berk, Kovera, Boozer, Pi-

Sunyer, & Albu, 2006). However, only one included study controlled for the potential influence of race when comparing exercise lipid use for overweight/obese compared to normal-weight subjects. Importantly, Hickner et al. (2001) found a higher RER and lower rate of lipid oxidation for obese African American and Caucasian women when cycling at 15 W; however, this was only the case when lean Caucasian women represented the control subjects. Conversely, an ~30% lower rate of lipid oxidation demonstrated by lean African American compared to Caucasian women during the cycling bout was quantitatively similar to that observed in obese subjects of both races (Hickner et al., 2001). The reason(s) for a reduced capacity for lipid oxidation in African American subjects is/are unclear, but might relate to reduced IS and mitochondrial capacity (DeLany et al., 2014) and/or a lower proportion of type I muscle fibers along with greater activity of "anaerobic" metabolic enzymes (Ama et al., 1986). Regardless of this distinction, however, an "obesity-like" decrement in exercise lipid use for normal-weight African American subjects suggests that races should be studied separately in future research investigating the influence of body fatness on exercise metabolism.

3.5.2.3 Fat-free Mass

If a comparison of the capacity for lipid use is made using an absolute measurement (e.g., lipid oxidation rate in $g \cdot min^{-1}$ or $\mu mol \cdot min^{-1}$ and/or total lipid oxidation in grams, μmol or kilocalories), it is important to express the value relative to the subject's FFM because it will be influenced by the amount of metabolically-active tissue they possess (Goodpaster et al., 2002). Conversely, if relative measurements (e.g., RER or lipid use as a percentage of total energy expenditure) are cited, no adjustment is required. In 19 of the 24 included studies, absolute measurements were recognized as some (Balcı, 2012; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Grams et al., 2017; Hickner et al., 2001; Kanaley et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Perez-Martin et al., 2001; Santiworakul et al., 2014; Thyfault et al., 2004) or all (Horowitz & Klein, 2000; Kanaley et al., 1993; Larsen et al., 2009; Mittendorfer et al., 2004; Mohebbi & Azizi, 2011; Slusher et al., 2015) outcome measures and in eight cases, some (Balcı, 2012; Grams et al., 2017; Larsen et al., 2009; Santiworakul et al., 2014) or all (Colberg et al., 1996; Kanaley et al., 1993; Slusher et al., 2015; Thyfault et al., 2004) were stated with no adjustment made for FFM. Interestingly, in seven of these eight studies, when the comparison of overweight/obese and normal-weight subjects was made using the unadjusted absolute

measures, no difference was reported (Balcı, 2012; Colberg et al., 1996; Kanaley et al., 1993; Larsen et al., 2009; Santiworakul et al., 2014; Slusher et al., 2015; Thyfault et al., 2004). Given that overweight/obese subjects typically possess greater FFM, failure to express an absolute measurement of lipid oxidation relative to FFM could cause a type II error by masking an obesity-related decrement that might be present. However, in three of those seven studies, relative measures were also reported that indicated similar exercise lipid use regardless of body fatness (Colberg et al., 1996; Santiworakul et al., 2014; Thyfault et al., 2004) while in two others, support was provided by values of the maximal rate of lipid oxidation, which were normalized to FFM even though lipid oxidation rates for individual stages of the test were not (Balcı, 2012; Larsen et al., 2009). Alternatively, for the 11 studies that included a report of total lipid oxidation or lipid oxidation rate as a function of FFM, only four suggest no difference in the adjusted measurement between groups (Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Mittendorfer et al., 2004). Conversely, for the other seven, one provides conclusive evidence that the FFM-adjusted rate of lipid oxidation is reduced for overweight/obese compared to normal-weight subjects (Perez-Martin et al., 2001), and four others suggest that it can be the case, but only under certain circumstances; for example, in Caucasian, but not African Americans (Hickner et al., 2001); for male, but not females (Keim et al., 1996); and during exercise at higher, but not lower intensities (Lanzi et al., 2014; Mohebbi & Azizi, 2011). Collectively, these observations are consistent with the contention that neglecting the need to normalize an absolute measurement of lipid oxidation can “mask” a deficiency associated with the overweight/obese condition. Finally, the two other studies that included a report of lipid oxidation rate as a function of FFM provide evidence that the adjusted rate is greater for overweight/obese subjects (Horowitz & Klein, 2000; Kanaley et al., 2001) if sufficient duration for the increment to manifest is allowed (e.g., 30, but not 15 minutes; Kanaley et al., 2001). Once again, this ambiguity likely reflects confounding effect(s) of other variables that can influence exercise lipid use irrespective of body fatness that were not controlled in these investigations. Nevertheless, in future research, the authors should recognize the influence of FFM on absolute measurements of lipid use and the corresponding need to control for this factor to assess the influence of overweight/obesity on exercise metabolism.

3.5.2.4 Aerobic Fitness

Endurance-trained individuals demonstrate an enhanced capacity to oxidize lipid during exercise (Costill, Fink, Getchell, Ivy, & Witzmann, 1979; Jansson & Kaijser, 1987) and longitudinal studies confirm a decrease in carbohydrate reliance and increase in lipid use during endurance exercise at the same absolute work rate after chronic endurance training (Martin et al., 1993; Phillips et al., 1996). Consequently, it has been suggested that to properly test the hypothesis that exercise lipid use is altered with overweight/obesity, lean and obese subjects must be matched for "aerobic fitness" (Goodpaster et al., 2002). However, only seven of the 24 included studies made mention of such matching and all used $\dot{V}O_{2peak/max}$ as the proxy measure (Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 1993; Keim et al., 1996; Mittendorfer et al., 2004). This could be problematic because the aspect of aerobic fitness that limits the rate of lipid oxidation is likely different than that which limits the rate of O_2 consumption. Specifically, the capacity to oxidise lipid is dictated by peripheral factors (e.g., mitochondrial mass and oxidative enzyme activity) which allow a particular metabolic rate to be sustained with minimal perturbation of mitochondrial phosphorylation and redox potentials (Bassett Jr & Howley, 2000). Conversely, $\dot{V}O_{2peak/max}$ is generally limited by convective and/or diffusive steps along the O_2-transport cascade (Wagner, 2000). Consequently, it is not surprising that training-related changes in mitochondrial capacity and $\dot{V}O_{2peak/max}$ can be dissociated (Henriksson & Reitman, 1977; Örlander, Kiessling, Karlsson, & Ekblom, 1977. With respect to lipid oxidation, this has resonance because Ara et al. (2011) reported a peak rate of lipid oxidation that occurs at a higher work rate relative to $\dot{V}O_{2peak/max}$ in obese and post-obese compared to control subjects despite similar $\dot{V}O_{2peak/max}$ values. Collectively, these findings suggest that the aspect of aerobic fitness that should be matched to properly assess whether lipid use is altered with overweight/obesity is related to muscle metabolic as opposed to central circulatory capacity and a parameter that reflects this capacity is the highest metabolic rate that can be sustained without perturbation of cellular physophorylation and redox states (i.e., the metabolic rate at the "lactate threshold"; $\dot{V}O_{2LT}$). The influence of differences in $\dot{V}O_{2LT}$ for subjects matched according to $\dot{V}O_{2peak/max}$ can be accounted for when normalizing exercise intensity across subjects (see below).

3.5.2.5 Insulin Sensitivity

Excess adiposity, particularly in the abdominal region (Carey, Jenkins, Campbell, Freund, & Chisholm, 1996), is often accompanied by a loss of IS, which means that the capacity for insulin-mediated glucose uptake by skeletal muscle is blunted. While this IR does not adversely affect contraction-mediated glucose uptake (Goodyear & Kahn, 1998), evidence suggests that overweight/obese IR subjects demonstrate a reduced contribution from muscle glycogen and increased contribution from lipids during exercise compared to BMI-matched subjects without IR (Braun et al., 2004). Interestingly, evolutionary preservation of IR has been suggested to reflect its 'usefulness' for protecting survival in circumstances where glucose sparing takes priority (e.g., acute trauma and prolonged fasting) (Soeters & Soeters, 2012). Collectively, these observations raise the intriguing possibility that exercise lipid reliance might be altered with IR irrespective of body fatness and if this is the case, a comparison of exercise lipid use between overweight/obese and normal-weight subjects could be confounded by the difference in IS that would typically be present. Interestingly, 12 of the 15 included studies that contained a report of IS found a lower value in the overweight/obese group (Ardevol et al., 1998; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Goodpaster et al., 2002; Horowitz & Klein, 2000; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009; Perez-Martin et al., 2001; Slusher et al., 2015; Thyfault et al., 2004); however, no attempt was made to control for IR in any of the studies assessed in this review. Indeed, only one included mention of its potential influence with Goodpaster et al. (2002) reporting that despite relative hyperinsulinemia, fasting insulin levels were not associated with exercise fatty-acid oxidation for the obese individuals they assessed. This implies that IR was not responsible for the higher proportional contribution of lipid to exercise energy expenditure they found for these subjects (Goodpaster et al., 2002). Nevertheless, it seems reasonable to suggest that future research designed to determine how exercise lipid use is influenced by overweight/obesity should include matching of the IS status of the two groups (i.e., by recruiting normal-weight control subjects with a level of IR that matches the overweight/obese group or recruiting overweight/obese subjects without IR).

3.5.2.6 Menstrual Phase

As part of its role as the primary female sex hormone, estrogen improves numerous aspects of energy homeostasis including IS and catecholamine-stimulated lipolysis (Mauvais-Jarvis, Clegg, &

Hevener, 2013). It is, therefore, not surprising that a number of studies confirm increased exercise lipid use during the luteal phase of the menstrual cycle when circulating estrogen level is high (Campbell, Angus, & Febbraio, 2001; Wenz, Berend, Lynch, Chappell, & Hackney, 1997; Zderic, Coggan, & Ruby, 2001). However, only four of the nine included studies that assessed female subjects exclusively involved control for this factor and, interestingly, two of these report greater exercise lipid use for overweight/obese compared to normal-weight subjects (Horowitz & Klein, 2000; Kanaley et al., 2001). Importantly, both of these assessed females during the follicular phase when the cyclical increment in the capacity for exercise lipid use would be absent (Wenz et al., 1997). Conversely, the five investigations that involved the exclusive study of women without controlling for their menstrual phase provide no evidence for an overweight/obesity-related increment (Ardevol et al., 1998; Devries et al., 2013; Hickner et al., 2001; Steffan et al., 1999; Thyfault et al., 2004). One possible explanation is that a propensity for greater exercise lipid oxidation associated with overweight/obesity in women might only be identifiable when the methodology ensures that control subjects are not experiencing their cyclical elevation. Consequently, much like what has been recommended for sex-comparative studies (Devries, Hamadeh, Phillips, & Tarnopolsky, 2006), it stands to reason that comparisons of exercise metabolism between overweight/obese and normal-weight women should be performed in both phases of the menstrual cycle to elucidate the effect of overweight/obesity on exercise lipid use for women with both low and high levels of circulating estrogen.

3.5.2.7 Fat-deposition Pattern

While it is typical to refer to a generic influence of overweight/obesity on myriad aspects of physiology, there is growing appreciation that in many cases, the influence can be markedly different depending upon where excess fat is situated. For example, greater visceral and ectopic deposition and a lower percentage localized subcutaneously in the gluteofemoral region are patterns associated with IR and dyslipidemia (Patel & Abate, 2013; Stefan, Schick, & Häring, 2017). This distinction resonates with respect to exercise fuel use because a key factor believed to underpin the disparity is the degree to which lipolysis is stimulated in these regions by circulating epinephrine (Jansson & Kaijser, 1987). Interestingly, it has been shown that normal-weight women with a lower abdominal-to-lower-body fat-mass ratio possess a higher maximal rate of lipid oxidation during incremental exercise compared to women with a

higher proportion of fat located centrally (Isacco, Thivel, Duclos, Aucouturier, & Boisseau, 2014). Furthermore, compared to normal-weight women with a high abdominal-to-lower-body fat-mass ratio, normal-weight women with a lower ratio mobilize and oxidize more lipid during 45 minutes of CWR cycling at 65% $\dot{V}O_{2max}$ in association with differences in circulating hormones related to lipolysis (e.g., growth hormone, insulin and atrial natriuretic peptide) (Isacco et al., 2013). Considering these findings, fat-deposition pattern is another variable that could confound a comparison of exercise lipid use for overweight/obese compared to normal-weight subjects. However, only two included studies were constructed with overweight/obese subjects classified into two groups according to fat-deposition pattern and results suggest that the decrement in the capacity for exercise lipid use reported for normal-weight women with upper- compared to lower-body adiposity is not present in obese subjects. Specifically, despite demonstrating a lower increase in FFA availability compared to lower-body obese and non-obese subjects (Kanaley et al., 1993), upper-body obese women oxidized a similar amount of lipid during 150 minutes of CWR cycling at 45% $\dot{V}O_{2peak}$ (Kanaley et al., 1993) and demonstrated similar RER and average lipid oxidation rate during 30 minutes of CWR cycling at 70% $\dot{V}O_{2peak}$ (Kanaley et al., 2001). Collectively, these findings suggest that obese subjects with different fat-deposition patterns can be grouped together when comparing exercise fuel use with normal-weight subjects. However, given the aforementioned findings for normal-weight subjects, it still might be necessary to control for this factor to decrease the likelihood of type II error by reducing the variability in criterion measurement of lipid use for subjects in the control group against which overweight/obese subjects are compared.

3.5.2.8 Acute and "Long-term" Dietary Habits

It is well established that a subject's dietary habits influence substrate utilization. For example, Black, Prentice, and Coward (1986) demonstrated that the macronutrient ratio of food intake (i.e., the "food quotient"; FQ) approximates RQ for subjects in energy balance and more recently, Miles-Chan, Dulloo, and Schutz (2015) confirmed that the principal exogenous factor that influences post-absorptive RQ is dietary FQ. Specifically, these authors found a positive relationship between percent dietary carbohydrate intake and post-absorptive RQ under both normo- and under-/overfeeding conditions with an average increase from ~0.80 to ~0.90 in response to an increase in carbohydrate ingestion from 30 to 60% of total energy intake (Miles-Chan et al., 2015). Importantly, research confirms that fluctuating the

carbohydrate (Gregory et al., 2011) or fat (Helge et al., 2001) content of the diet in this manner also influences RQ during exercise, which implies that a controlled feeding period should be imposed prior to assessing exercise lipid use. However, unlike carbohydrate oxidation, which adapts rapidly in response to changes in carbohydrate intake (Abbott et al., 1988; Acheson et al., 1984), lipid oxidation responds slowly when a high-fat diet is implemented (Flatt, 1995; Schrauwen, van Marken Lichtenbelt, Saris, & Westerterp, 1997) with a time course further lengthened by obesity (Thomas et al., 1992) and a family history of T2D (Ukropcova et al., 2007). Being that a balanced controlled diet might reflect a change in lipid intake for some subjects, this implies that dietary control prior to testing exercise metabolism should be more extensive than a simple acute manipulation such as testing in the fasted state or the fasted state following ingestion of a standardized meal. It is also important to recognize that energetic intake should be closely matched to expenditure during any controlled-feeding period because lipid is the predominant substrate used/stored in response to short-term fluctuations in energy balance (Abbott et al., 1988). Finally, following any period of dietary control, to ensure that the metabolic response to exercise will not be affected by the final meal of the feeding regimen, subjects should be tested after a sufficient period of fasting (e.g., >6 hours) (Montain, Hopper, Coggan, & Coyle, 1991).

Given the profound influence of prior feeding habits on substrate oxidation, it is surprising that only seven of the 24 included studies involved anything more than acute dietary control (Colberg et al., 1996; Ezell et al., 1999; Goodpaster et al., 2002; Kanaley et al., 1993); Kanaley et al., 2001; Lanzi et al., 2014; Melanson et al., 2009). Moreover, in four of those seven, a general guideline regarding a minimum amount of carbohydrate intake was all that was provided (Colberg et al., 1996; Goodpaster et al., 2002; Kanaley et al., 1993; Kanaley et al. 2001), while in two others, subjects were not tested in the fasted state after completing their controlled period (Ezell et al., 1999; Melanson et al., 2009). Hence, acute and long-term dietary control with macronutrient standardization in energy balance was only enforced in one of the included studies and, unfortunately, the results from this study are inconclusive because obese subjects oxidized lipid more, less or equally as effectively as normal-weight subjects during exercise depending upon the outcome measure that was considered (Lanzi et al., 2014). Future studies designed to clarify the influence of overweight/obesity on exercise lipid use should include control of both macronutrient

composition and energy balance for an extended period to ensure weight stability with similar prevailing FQ.

3.5.3 Exercise Variables That Might Influence Exercise Lipid Use Irrespective of Body Fatness

3.5.3.1 Mode

The two modes of exercise most often used for exercise testing are treadmill walking/running and leg-cycle ergometry (Beltz et al., 2016). Consequently, it is not surprising that 23 of the 24 included studies involved one of these modes of exercise. However, in one of those 23, arm cranking was performed in addition to leg cycling so that potential differences in substrate use in arm compared to leg muscle could be explored (Larsen et al., 2009). Specifically, citing research indicating that arm muscles retain a better capacity for glucose clearance in older hypertensive individuals (Olsen, Sacchetti, Dela, Ploug, & Saltin, 2005) and patients with T2D (Reynolds IV, Supiano, & Dengel, 2007), Larsen et al. (2009) hypothesized that the influence of T2D on exercise metabolism would be different for the two modes of exercise. However, in addition to lean control subjects against which exercise substrate use by subjects with T2D was compared, these researchers also included a group of healthy obese control subjects; hence, a comparison of the capacity for exercise lipid use for healthy obese compared to normal-weight subjects in arm compared to leg muscle could also be made. Interestingly, these authors found that the maximal rate of lipid oxidation during incremental exercise occurred at a higher percentage of $\dot{V}O_{2max}$ for obese compared to lean subjects during arm cranking, but not leg cycling (Larsen et al., 2009). While lipid oxidation rates at the variety of intensities investigated during the test or the maximal lipid oxidation rate were not different between obese and lean subjects, this finding suggests that testing of the upper-body musculature might allow for an obesity-related increment in exercise lipid use to be identified. The reason(s) for different metabolic activity in arm compared to leg muscle is/are unclear, but might be related to differences in muscle phenotype (Larsen et al., 2009), which resonates because a high proportion of type I fibres provides a greater ‘window’ for the loss of IS that occurs with T2D (Gaster, Staehr, Beck-Nielsen, Schrøder, & Handberg, 2001). Collectively, these findings appear to provide further support for our contention that state of IR is an important factor to control when assessing exercise metabolism for overweight/obese compared to normal-weight subjects (see above). Furthermore, they

indicate that future studies of exercise metabolism with overweight/obesity should include both leg and arm ergometry (in particular, utilizing the CWR exercise model).

3.5.3.2 Duration

During prolonged moderate-intensity exercise, reliance on muscle glycogen decreases while whole-body rate of lipid oxidation increases in association with increased availability and oxidation of plasma FFA (Romijn et al., 1993; van Aggel-Leijssen, Saris, Hul, & van Baak, 2001). If the time course of this up-regulation of lipid use is different for overweight/obese compared to normal-weight subjects, the duration of an exercise bout used to assess exercise metabolism must be selected accordingly. In this regard, Kanalay et al. (2001) observed a similar rate of lipid oxidation for obese and non-obese women after 15 minutes of treadmill walking at 70% $\dot{V}O_{2max;}$ however, for the obese women, lipid-oxidation rate increased from minute 15 to minute 30 whereas for non-obese women, it did not. The end result was that 30 minutes of exercise required the same energy expenditure, but 30% more lipid oxidation for obese women. Unfortunately, these researchers terminated exercise at 30 minutes; hence, it cannot be determined whether non-obese subjects would have eventually "caught up." Nevertheless, these findings imply that a longer duration of exercise is required to reveal an obesity-related increment that might be present and findings by Horowitz and Klein (2000) provide further support. Specifically, these researchers found that whole-body lipid oxidation from 60–90 minutes of cycling at ~54% $\dot{V}O_{2max}$ was ~25% greater for obese compared to lean women (Horowitz & Klein, 2000). Conversely, Goodpaster et al. (2002) reported that the lower RER they observed for obese compared to lean men at 30, 45, and 60 minutes of cycling at 50% $\dot{V}O_{2max}$ was also present at minute 15, which suggests that a longer exercise challenge might not be required to reveal an obesity-related increase. Finally, other than the three studies mentioned above, the other 10 included studies that involved assessment of lipid oxidation for CWR bouts ≥30 minutes in length provided no evidence of a greater capacity for exercise lipid use for overweight/obese subjects (Balcı, 2012; Colberg et al., 1996; Devries et al., 2013; Ezell et al., 1999; Kanaley et al., 1993; Melanson et al., 2009; Mittendorfer et al., 2004; Slusher et al., 2015; Thyfault et al., 2004; Wong & Harber, 2006). Hence, the preponderance of evidence suggests that an obesity-related increase in exercise lipid use was not masked by insufficient duration. Nevertheless, it seems reasonable to suggest that studies designed to investigate the influence of overweight/obesity on exercise lipid use

should involve criterion exercise challenges that are at least 30 minutes in length to allow any duration-dependent increase in lipid mobilisation and subsequent oxidation to occur (Pillard et al., 2007). This is also important to consider in light of the practice of using incremental exercise with relatively short stages to assess substrate use for the work rate maintained during the stage; for example, as was done in eight of the 24 included studies (Ardevol et al., 1998; Balcı, 2012; Keim et al., 1996; Lanzi et al., 2014; Larsen et al., 2009; Mohebbi & Azizi, 2011; Perez-Martin et al., 2001; Steffan et al., 1999). Indeed, Steffan et al. (1999) confirmed that changes in RER after 3 minutes of CWR exercise at both 50 and 75% $\dot{V}O_{2max}$ resulted in significant differences at both minutes 8 and 15 compared to the value measured at minute three and that which was measured during a 3-minute stage of an incremental bout. The authors rightly concluded that "steady-state" substrate use cannot be predicted from graded-exercise tests (Steffan et al., 1999).

3.5.3.3. Intensity

It is well established that intensity of effort influences the proportional use of substrate during physical activity. For example, in support of the 'crossover concept' (Brooks & Mercier, 1994), Venables et al. (2005) assessed 300 healthy men and women during incremental treadmill exercise and found that carbohydrate oxidation continued to increase from low to high work rate while lipid use only did so until an intermediate intensity was encountered. Indeed, once the peak rate of lipid use was reached, lipid reliance decreased progressively until its contribution became negligible at the highest work rates (e.g., ≥~84% $\dot{V}O_{2max}$). The observation that the highest rate of lipid oxidation occurs at an intermediate relative work rate (e.g., ~48% $\dot{V}O_{2max}$) forms the basis for using moderate-intensity exercise to asses lipid-oxidizing capacity during exercise. However, Venables et al. (2005) also observed considerable inter-subject variability with the maximal rate occurring anywhere between 25 and 77% of $\dot{V}O_{2max}$ in the cohort they recruited. Consequently, it stands to reason that exercise intensity should be assigned for such testing in a subject-specific manner. The traditional way to normalize endurance-exercise intensity is by quantifying work rate relative to the subject's $\dot{V}O_{2max}$ based on the assumption that different individuals exercising at the same percentage of $\dot{V}O_{2max}$ will experience similar physiological challenges. However, it is now well established that the metabolic response to an increasing exercise work rate is not linear with characteristics of nonlinear behaviour that are unique for different individuals (DiMenna & Jones, 2009;

Lansley, Dimenna, Bailey, & Jones, 2011). Consequently, exercise performed at the same percentage of $\dot{V}O_{2max}$ can represent a markedly different physiological challenge (including the ability to satisfy the required energy turnover via lipid oxidation) for two $\dot{V}O_{2max}$-matched subjects. Specifically, assigning exercise intensity relative to $\dot{V}O_{2max}$ fails to account for other important parameters of aerobic fitness; for example, the lactate threshold (LT) and the asymptote of the hyperbolic relationship between power production and time to exhaustion (i.e., the "critical power"; CP), which serve as the upper boundaries of the moderate- and heavy-intensity domains, respectively (DiMenna & Jones, 2009). Importantly, Venables et al. (2005) showed that LT, which occurred at ~65% $\dot{V}O_{2max}$ in their group of subjects (range, 45–89%), was aligned with a higher work rate compared to the maximal rate of lipid oxidation. Consequently, in lieu of a direct measurement of the maximal rate of lipid oxidation, it seems reasonable to conclude that tests designed to assess exercise lipid use should be performed at intensities below LT with the specific work rate assigned relative to it.

Fourteen of the 17 included studies that involved CWR exercise and three of the eight that involved incremental exercise were designed with exercise intensity assigned relative to $\dot{V}O_{2max}$. Moreover, in the five other studies that involved incremental testing, stages were performed at the same absolute work rates for all subjects. Conversely, only two studies involving CWR exercise were designed with intensity assigned relative to "VT" (i.e., "ventilatory threshold," which is one of myriad terms used to describe LT (Binder et al., 2008), and in both cases, exercise was performed at VT, which implies that most subjects were operating at an intensity that exceeded that which allowed for their maximal capacity for lipid use (Santiworakul et al., 2014; Wong & Harber, 2006). Furthermore, in one of those studies, the parameter was identified according to the ventilatory equivalent for CO_2, which is the point at which respiratory compensation begins (i.e., the "second ventilatory threshold" or VT_2) (Wong & Harber, 2006). Importantly, VT_2 emerges subsequent to the initial VT (i.e., VT_1) during incremental tests with sufficient sensitivity to reveal the "isocapnic region" within which arterial partial pressure of CO_2 is maintained despite lactic acidosis (Whipp, Davis, & Wasserman, 1989). Conversely, Santiworakul et al. (2014) used the V-slope method for identification, which would be preferred because it reveals VT_1 (and, therefore, LT) regardless of the incremental protocol that is employed. These researchers found no difference in

lipid-oxidizing capacity at LT for obese compared to lean subjects; however, a number of other factors were poorly controlled in this study (Table 3.4) so their finding should be interpreted with caution.

3.5.4 Evidence in Favor of a Limitation in Lipid Oxidation During Exercise for Overweight/Obese Subjects

Despite similar rates of skeletal-muscle fatty-acid uptake compared to normal-weight counterparts, obese individuals demonstrate lower rates of lipid oxidation during fasting when lipid use should predominate (Kelley et al., 1999). With this in mind, it is attractive to speculate a mitochondrial defect underpinning the overweight/obese condition that would also create a limitation during moderate-intensity exercise where lipid should still contribute significantly as fuel (Goodpaster et al., 2002). However, only seven of the 24 studies in this review provide any evidence that obese subjects have a reduced capacity for exercise lipid use compared to non-obese counterparts (Chatzinikolaou et al., 2008; Grams et al., 2017; Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014; Mohebbi et al., 2011; Perez-Martin et al., 2001). Furthermore, in one of those studies, Chatzinikolaou et al. (2008) reported a higher RER for obese compared to lean men during 30 minutes of intermittent circuit resistance training, a physical activity during which lipid would not be expected to contribute to a great extent in healthy individuals. Consequently, the practical relevance of this finding can be questioned. Conversely, five studies indicating an obesity-related decrement involved a comparison during stationary cycling, which likely represents exercise during which such a decrement would be more relevant. Specifically, stationary cycling would typically be recommended as a low-impact activity for overweight/obese individuals to reduce body fat and improve metabolic health. A reduced capacity for lipid oxidation during this type of exercise would, therefore, be important to consider when determining the appropriate training program to manage and/or treat the disease. However, it is important to note that in four of those five studies, the conclusion must be prefaced because the decrement was only present under certain circumstances. For example, Lanzi et al. (2014) found that while obese subjects demonstrated a lower rate of lipid oxidation per FFM at higher exercise intensities (i.e., 65–85% $\dot{V}O_{2peak}$), their lipid use at moderate (35–60% $\dot{V}O_{2peak}$) intensities was not different compared to lean counterparts. Moreover, while obese subjects experienced their maximal rate of lipid oxidation at a lower relative exercise intensity compared to normal-weight individuals (~47 vs. ~57% of peak power output), the rate of lipid oxidation at that apex (~6 $mg \cdot kgFFM^{-1} \cdot min^{-1}$) was not different between groups (Lanzi et al., 2014). Finally, obese subjects

demonstrated a lower RER and higher rates of lipid oxidation at low exercise intensities (20–55% and 20–30% $\dot{V}O_{2peak}$, respectively) in that study (Lanzi et al., 2014). This discrepancy exemplifies why it is important to pay careful attention to the method used to normalize exercise intensity when exercise metabolism is being assessed (see above). Moreover, subject characteristics other than overweight/obesity can play a role. For example, Hickner et al. (2001) reported a higher RER during cycling at the same absolute work rate (15 W) for obese African American and Caucasian women; however, this was only the case when lean Caucasian women represented the control subjects. Conversely, when obese women of both races were compared to lean African American women, no differences were present. This suggests that some genotypes and/or phenotypes might convey a lipid metabolic defect regardless of body fatness. Hickner et al. (2001) also found no difference between exercise RER for obese subjects of both races compared to lean African American women when subjects cycled at the same relative intensity (65% $\dot{V}O_{2peak}$); however, in this case, Caucasian, but not African American obese women, possessed a decrement compared to their lean Caucasian counterparts. In addition to subject characteristics, this again exemplifies how the method used to assign intensity can affect the conclusion. More evidence of the potential confounding influence of intensity assignment and subject characteristics other than overweight/obesity comes from the work of Keim et al. (1996), who had subjects cycle at the same absolute work rates (30, 60, 90, and 120 W for all subjects with an additional bout at 150 W for male subjects) and found that fatter men had a higher RER when cycling at 120 W compared to leaner men. However, no differences were present for men at the four other work rates or for women at any of the work rates they performed. Furthermore, these researchers used each subject's $\dot{V}O_2$/work-rate relationship from the incremental protocol to estimate substrate-oxidation rates at 30%, 40%, 50%, and 60% of their measured $\dot{V}O_{2max}$ and found a different pattern for fatter compared to leaner men (Keim et al., 1996). Specifically, lipid-oxidation rate tended to increase for leaner men as exercise intensity increased whereas for fatter men, it decreased. Consequently, lipid-oxidation rate, which did not differ between groups at 30% $\dot{V}O_{2max}$, was significantly lower for fatter compared to leaner subjects at 40%, 50%, and 60% $\dot{V}O_{2max}$. Conversely, body-fat percentage was not associated with substrate-oxidation rates for female subjects at any of the estimated work rates and regardless of body fatness, like the fatter men, the women's highest rate of lipid oxidation occurred at the lowest exercise intensity

estimated in this study (Keim et al., 1996). These findings with respect to sex cohere with those of Grams et al. (2017) who reported that overweight men use less kilocalories of lipid per minute compared to lean counterparts when cycling for 10 minutes at 50% $\dot{V}O_{2max}$ while overweight women do not. However, it is important to note that this was not the case during 12 minutes of self-paced stepping, which was also performed in this study as both sexes experienced an overweight-related decrement during this activity (Grams et al., 2017). Finally, in the one study that indicated an obesity-related decrement during treadmill exercise, Mohebbi and Azizi (2011) found that obese men demonstrated a lower lipid-oxidation rate during incremental exercise at higher work rates (60–80% $\dot{V}O_{2max}$), but lipid use at lower work rates (20–50 $\dot{V}O_{2max}$) and the maximal rate of lipid oxidation were not different compared to normal-weight controls. Collectively, these findings suggest that if a decrement in exercise lipid use is present for obese compared to non-obese subjects, its identification requires careful consideration of the mode and intensity of the criterion exercise and the race and sex of the subject.

Of the 24 included studies, only one provides unmitigated evidence to support the contention that exercise lipid use is reduced for overweight/obese subjects. Specifically, Perez-Martin et al. (2001) stratified subjects of both sexes into normal and overweight groups and found that overweight subjects demonstrated a higher RER and lower rate of lipid oxidation for all stages of an incremental cycling protocol comprising 6-minute bouts at 30%, 40%, 50%, and 60% of their estimated maximal work rate. Furthermore, the maximal rate of lipid oxidation and the crossover point from predominantly lipid to carbohydrate fuel occurred at lower relative work rates for the overweight subjects (Perez-Martin et al., 2001). In a separate assessment, these researchers confirmed that when similar 6-minute bouts were performed on separate days, $\dot{V}O_2$ and $\dot{V}CO_2$ values were not different, which suggests that substrate oxidation rates were not affected by the sequential nature of the incremental protocol. However, the degree to which 6 minutes of exercise at a given work rate is sufficient for all subjects to achieve substrate-oxidation rates that faithfully reflect the subject's "true" capacity for lipid oxidation at that work rate can be questioned (Kanaley et al., 2001; Steffan et al., 1999).

4.4 The Influence of Overweight/Obesity on the Source of Exercise Lipid Use

In contrast to the aforementioned line of reasoning, there are a number of reasons why obese individuals might demonstrate a greater reliance on lipid during exercise compared to normal-weight

counterparts. Firstly, it is intuitive that greater fat mass in adipose tissue of obese individuals could provide for greater plasma FFA availability in response to a given exercise challenge. However, a review of the literature confirms an obesity-related downregulation of lipolysis per given quantity of fat mass (Karpe, Dickmann, & Frayn, 2011) and reduced lipolytic response to exercise for overweight/obese subjects (Mittendorfer et al., 2004). Alternatively, the "window" for lipid contribution as fuel might be widened by the IR that often accompanies obesity, which has been linked to a decrease in muscle glycogen use during exercise (Braun et al., 2004). Interestingly, obesity is also associated with greater storage of lipid as IMTG (Goodpaster et al., 2002), which could serve to fill the void created by this decreased reliance on muscle glycogen (Braun et al., 2004; Goodpaster et al., 2002). Finally, it is well established that IR is accompanied (and possibly caused by) elevated IMTG (Goodpaster, Thaete, Simoneau, & Kelley, 1997). Although cause/effect characteristics remain to be clarified, these associations, therefore, suggest a series of obesity-related changes involving accumulation of lipid in muscle and blunting of IS that conspire to create a different pattern of exercise fuel use overweight/obese individuals with IR compared to normal-weight individuals without it. The four included studies that involved stable-isotope infusion to determine the source of oxidized lipid in addition to the whole-body measurement derived from indirect calorimetry provide important insight in this regard. Specifically, in three of those studies (Goodpaster et al., 2002; Horowitz & Klein, 2000; Mittendorfer et al., 2004), the contribution of non-plasma lipid (presumably predominantly IMTG) was increased with obesity while in the other (Thyfault et al., 2004), an ~15% increase that did not reach statistical significance was observed. However, this enhanced ability to utilize IMTG for obese subjects only allowed for greater whole-body lipid oxidation in two of these studies (Goodpaster et al., 2002; Horowitz & Klein, 2000). Conversely, in the other two, obese subjects demonstrated a concomitant reduction in the ability to oxidize plasma FFA such that a similar whole-body rate of lipid oxidation was observed compared to normal-weight subjects (Mittendorfer et al., 2004; Thyfault et al., 2004). Consequently, mixed conclusions regarding the degree to which exercise lipid use is increased, decreased or unaffected for overweight/obese individuals might be attributable at least in part to a difference in the net effect of changes in the non-plasma and plasma contributions with some overweight/obese subjects able to maintain the capacity for plasma lipid along with an increased use of IMTG while others cannot. The reason(s) for such a disparity is/are unclear but

might have to do with the degree to which lipolytic activity is reduced in association with obesity. For example, in addition to an increased reliance on nonsystemic fatty acids and decreased reliance on systemic fatty acids, Mittendorfer et al. (2004) reported an inverse relationship between adiposity and the lipolytic response to exercise that they linked to reductions in peak plasma epinephrine concentration and adipose-tissue catecholamine sensitivity. The end result was that unlike lean controls, it was FFA availability, not uptake, that rate limited plasma lipid oxidation in their overweight/obese male subjects (Mittendorfer et al., 2004). However, other studies provide no evidence of such a limitation. For example, Lanzi et al. (2014) found reduced lipolytic activity at all work rates encountered during incremental exercise for obese males; however, the increased quantity of adipose tissue compensated for the deficit thereby allowing for greater FFA availability during exercise and, by extension, greater and unaffected lipid-oxidation rates at low and moderate intensities, respectively. Indeed, these researchers only observed lower lipid-oxidation rates for obese subjects at higher intensities, which is consistent with the existence of a “downstream” limitation once these work rates were encountered. Moreover, Horowitz and Klein (2000) found similar whole-body lipolytic activity for women with abdominal obesity compared to lean controls, while Goodpaster et al. (2002) reported an ~50% greater rate of uptake of FFA during exercise for obese males despite similar reliance on these sources.

Collectively, the contrasting reports detailed above suggest a complex interplay with regard to the capacity to maintain plasma FFA availability/uptake/oxidation alongside an obesity-related increase in IMTG availability/oxidation with this interplay ultimately dictating the net effect on whole-body lipid oxidation. Specific factors that might influence this interplay are subject sex, fat-deposition pattern, and/or degree of obesity; however, the degree to which IR is present and has progressed might be the most important one to consider. Blaak et al. (2000) found that total lipid oxidation was similar for patients with T2D compared to healthy subjects matched for body composition during 60 minutes of moderate-intensity cycling although the patients demonstrated an increased reliance on triglyceride-derived lipids to compensate for a lower exercise-induced rate of appearance/oxidation of plasma FFA (Blaak, van Aggel-Leijssen, Wagenmakers, Saris, & van Baak, 2000). This contrasts findings by Braun et al. (2004), who reported similar FFA concentrations along with increased lipid contribution to energy expenditure during 50 minutes of moderate-intensity cycling for overweight/obese normoglycemic women with IR compared

to women without IR matched for body composition. Although the source of oxidized lipid was not reported in this study, it seems reasonable to conclude that an increased reliance on IMTG to fill the void created by deceased muscle glycogen use in association with the initial stages of insulin desensitization had not yet been offset by the decrease in plasma lipid use that ultimately accompanies its progression to T2D (Blaak et al., 2000). A sequence of alterations that might explain the relationship between overweight/obesity and the progression from IR to T2D and how that relationship dictates whether exercise lipid use is increased, decreased or unchanged by overweight/obesity is depicted in Figure 3.2.

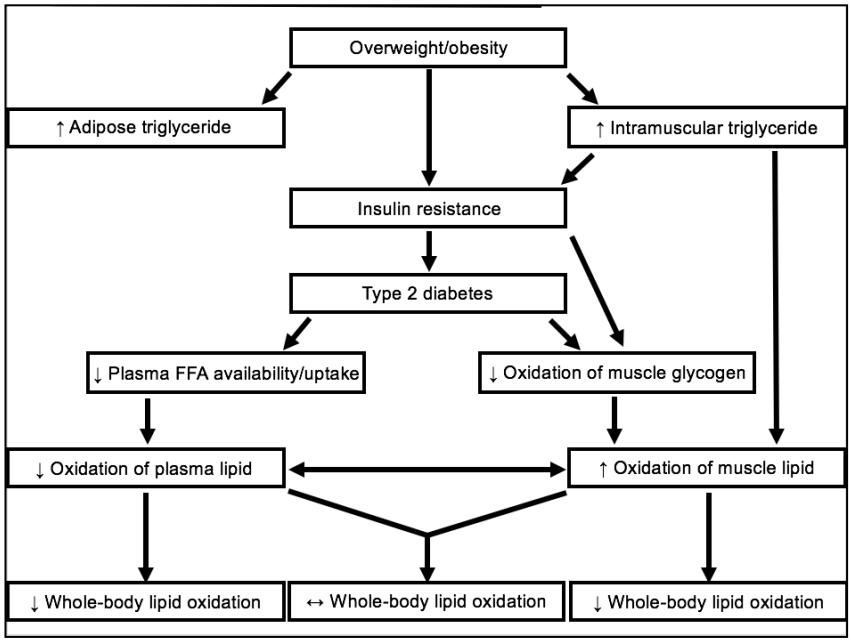

Figure 3.2. Relationship between overweight/obesity and the progression from IR to T2D

3.6 Conclusion

The contention that there is a lipid-oxidizing decrement associated with overweight/obesity comes predominantly from research indicating that obese individuals demonstrate lower rates of lipid oxidation during fasting when lipid use should predominate. With respect to moderate-intensity exercise where lipid should still contribute significantly, it is difficult to reconcile how a “normal” capacity for lipid oxidation could be maintained if such a deficiency is present. Nevertheless, we found that the preponderance of evidence suggests that obese and non-obese subjects rely on lipid to a similar extent during exercise as

normal-weight counterparts. Moreover, it is possible that exercise lipid use might be enhanced for overweight/obese subjects due to an increased presence of and contribution from muscle lipid stores. Regardless of this distinction, however, if the capacity for exercise lipid use is, indeed, preserved in overweight/obese subjects, it refutes the contention that a lipid-oxidizing defect contributes to the overweight/obese condition and supports the importance of exercise as an adjunct to diet for both loss of body fat and maintenance/restoration of metabolic health.

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Chapter 4 – Controlled Clinical Trial

4.1 Assessing Defects in Functional Substrate Selectivity in Individuals at Risk for Type 2 Diabetes

4.1.1 Research Question

Do individuals with the overweight/obese condition rely on lipid oxidation to a greater, lesser, or the same extent during a physiologically-equivalent exercise challenge compared to age-, gender-, and fitness-matched normal-weight subjects?

4.1.2 Hypothesis #1

Rates of lipid oxidation during exercise, adjusted for fat-free mass, will be lower in individuals with the overweight/obese conditions.

4.1.3 Hypothesis #2

The lower rates of lipid oxidation will be related to changes associated with the loss of metabolic health (e.g., insulin resistance) rather than the overweight/obese state per se.

4.2 Methods

4.2.1 Setting

The research was conducted at the Mt. Sinai St. Luke's Hospital, Division of Endocrinology, Diabetes, and Metabolism, Clinical Research Unit. The protocol included informed consent, screening, and all tests and procedures involved in this application. Participants were recruited via printed flyers, online advertisement, and presentations at schools and community centers in the area. The research was approved by the Institutional Review Board (IRB) of ICAHN School of Medicine at Mount Sinai, Mount Sinai Hospital, New York, New York.

4.2.2 Study Design

4.2.2.1 Participants and Recruitment Methods

Both men and women (age 18-45 years) of all races were invited to participate in this cross-sectional study. To maximize recruitment potential, we used multiple mediums and vehicles, including contacting community neighborhood leaders, reaching out to student and staff at the nearby Columbia University, advertising online (e.g. Craigslist, Inc.), and posting flyers in the neighborhood. Below is our IRB-approved flyer.

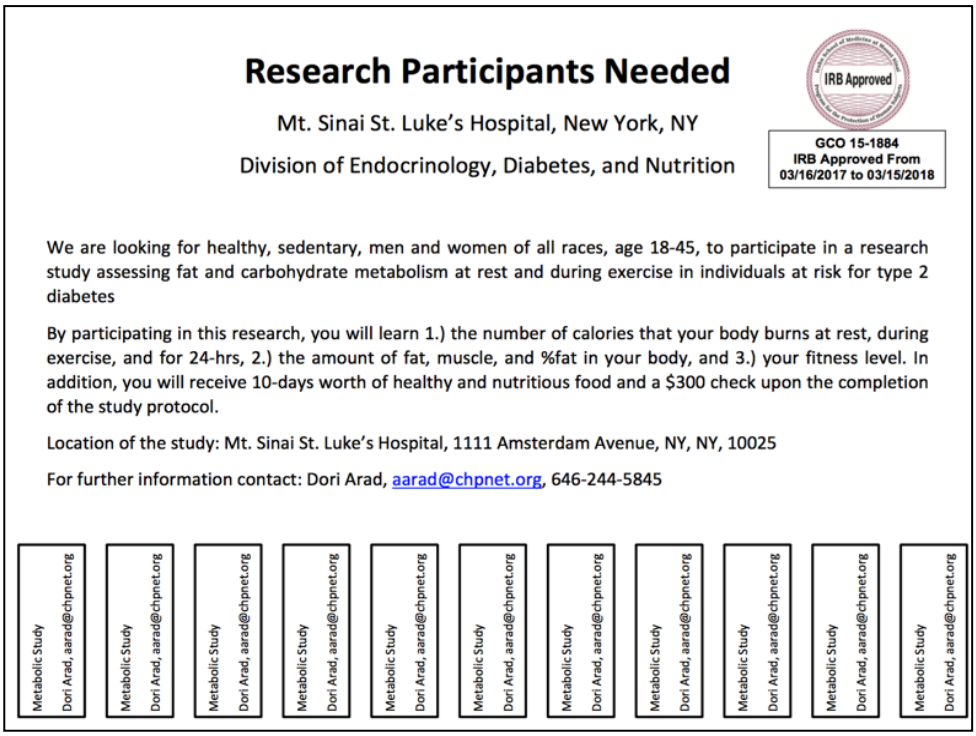

Figure 4.1. IRB-approved flyer

4.2.3 Inclusion and Exclusion Criteria

4.2.3.1 Inclusion Criteria

Inclusion criteria were participants should be non-diabetic, sedentary (≤2 x 30 min of exercise per week) men and women of all races aged 18-45. Eligible participants were recruited and stratified into four groups matched for age, fat-free mass, and cardiorespiratory fitness level (see Table 4.1).

Table 4.1

Participant Groupings

Group	BMI	Family History of Diabetes	Metabolic Syndrome Risk Factors
Metabolically unhealthy overweight/obese	≥26 kg·m^{-2}, <35 kg·m^{-2}	FH+	≥2
Metabolically healthy but overweight/obese	≥26 kg·m^{-2}, <35 kg·m^{-2}	FH-	≤1
Metabolically unhealthy normal weight	≥19 kg·m^{-2}, ≤24 kg·m^{-2}	FH+	≥2
Metabolically healthy but normal weight	≥19 kg·m^{-2}, ≤24 kg·m^{-2}	FH-	≤1

- **Family History of DM (FH+)** was defined as a first-degree relative (parents or siblings) with type 2 diabetes
- **Metabolic-syndrome risk factors** were based on the definition set by the American Heart Association, World Health Organization, and the National Heart, Lung, and Blood Institute. These included:
    - waist circumference ≥35 inches for females and ≥40 inches for men or waist-to-hip ratio >0.85 for females and <0.90 for men;
    - triglycerides ≥150 $mg \cdot dl^{-1}$ or receiving drug therapy for hyperlipidemia;
    - HDL ≤50 $mg \cdot dl^{-1}$ for females and ≤40 $mg \cdot dl^{-1}$ for males;
    - blood pressure >130/85 mmHg or receiving drug therapy for hypertension; and
    - fasting blood glucose ≥100 $mg \cdot dl^{-1}$ or receiving drug therapy for hyperglycemia.

In order to participate, FH+, metabolically *unhealthy* normal-weight and overweight/obese participants had to possess two or more of the metabolic syndrome risk factors. Conversely, metabolically *healthy* normal-weight and overweight/obese participants had to be free from FH+ and possess one or less of the metabolic-syndrome risk factors.

4.2.3.2 Exclusion Criteria

The exclusion criteria were as follows:

- having experienced weight change ≥±3 kg within the past three months;
- taking any medications that might affect insulin or fat metabolism;
- smoking within the past 6 months;
- consuming >2 oz ethanol per day;
- having irregular menstrual cycle (females);
- being pregnant;
- possessing any indication of having any known cardiovascular disease (e.g., coronary artery disease, previous myocardial infarction, angina, ischemic heart disease, coronary syndrome, congestive heart failure, valvular heart disease, cardio myopathy), pulmonary disease (e.g., COPD/asthma, emphysema), or metabolic disease (e.g., diabetes), or any other chronic

illness as per the discretion of the principal investigator that might interfere with the capacity to exercise;

- possessing fasting blood sugar >126 $mg \cdot dl^{-1}$ and/or 2-hour post oral glucose tolerance test >200 $mg \cdot dl^{-1}$;
- demonstrating an age- and gender-appropriate $\dot{V}O_{2peak}$ better than "average" as defined by the American College of Sports Medicine.

4.2.4 Study Timelines

After screening and the determination of eligibility, the duration of an individual subject's participation in this study was 10 days. The duration anticipated to enroll all study subjects was 24 months. The anticipated date for the investigators to complete this study was 30 months from the approved start date.

4.2.5 Procedures

4.2.5.1 Screening

The screening process included three phases: (a) initial phone screening to collect basic demographic, clinical, and physical activity information; (b) comprehensive on-site medical evaluation, which included physical examination, diabetes screening, blood work, and resting ECG; and (c) maximal exercise testing ("Ramp Exercise Test") to evaluate cardiorespiratory fitness.

4.2.5.2 Initial Phone Screening

Initial phone screening was conducted for any individual who contacted our center and expressed interest in participating in the study. We developed and used a screening script to keep the phone screening consistent and efficient. The script had four parts: (a) a brief overview of the study, including purpose, protocol, significances, commitment, and incentives; (b) basic demographic information such as name, date of birth, sex, race, weight, height, physical address, email, and phone number; (c) clinical information such as weight history and weight change in the past 3 months, smoking habits, alcohol consumption, exercise and physical activity behavior, current medical conditions, medications, supplements, over the counters, family history of diabetes (siblings and parents), and (for females) menstruation, pregnancy, oral-contraceptive medicine, and date of last ; (d) comprehensive physical activity questionnaires (Paffenbarger and IPAQ) to evaluate exercise and physical activity behavior. Only

participants who satisfied the inclusion/exclusion criteria outlined above were invited to the second screening phase, the on-site comprehensive medical examination.

4.2.5.3 Comprehensive On-site Medical Evaluation

Participants underwent a comprehensive medical examination that included blood work, physical examination, and ECG, and a standard test to screen for diabetes to determine if they were eligible to participate in this study. Screening required approximately 4 hours during one morning after an overnight fast (12 hours for which participants did not eat or drink anything except water and did not chew anything containing calories, such as regular gum with sugar). The total amount of blood which was taken during screening was 7 cc (6 cc for blood-lipid analysis and 1 cc for blood-glucose analysis), which is about 1.5 teaspoons (teaspoon equals ~5 cc). This provided enough blood for general blood chemistry and diabetes screening.

4.2.5.3.1 Diabetes screening

To ensure that a participant did not have diabetes, he or she underwent an Oral Glucose Tolerance Test (OGTT). The test was performed at approximately 9:00 am after a 12-hour overnight fast. A small portion of blood (0.5 cc) was taken from a vein in the arm for the microfuge tube/glucose analyzer for blood glucose reading, and participants then consumed a sweet drink containing 75 grams of glucose. For the next two hours, participants remained quietly reading or resting in the lab, after which another 0.5 cc of blood was taken for the same purpose to conclude the test.

4.2.5.3.2 Medical examination

A physician or other certified medical professionals performed a comprehensive physical and medical history to ensure that participants were eligible for this study. A participant's height, weight, waist circumference, blood pressure, and heart rate were obtained and recorded. Approximately 6 cc of blood was taken to measure blood lipids (for example, cholesterol) and also to perform a complete blood count and regular blood chemistries (basic metabolism and liver panel). The blood was drawn from the same site where the blood was taken during the Oral Glucose Tolerance Test (see above). Participants also underwent a resting EKG to ensure they could safely participate in an exercise test. During this visit, they completed a dietary-preference questionnaire, which included food likes, food dislikes, food allergies, food intolerance, and general and specific food restrictions related to clinical, ethnical, or religious

preferences/restrictions. We also asked each participant about their living situation and access to kitchen and refrigerator so that the food given during the protocol was not only based on their liking and needs, but also on their resources, environment, and lifestyle. Upon physician approval, only participants who satisfied the inclusion/exclusion criteria outlined above were invited to the third screening phase, the maximal exercise test (Ramp Cycle Test).

4.2.5.4 Ramp Cycle Test

This procedure was performed to measure the participants' cardiorespiratory fitness. They performed this test one time, immediately after the RMR test (described below) while they were still fasting. The night before this test, participants followed the directions given to them by the investigators; for example, they should not engage in moderate or strenuous physical activity and they should abstain from ingesting caffeine or alcohol for 48 hours prior to initiation of the testing protocol. The test was performed on a cycle ergometer. During this test, the subject was connected to a Care Fusion Encore Vmax System in order to collect gas-exchange data. Participants breathed through a mouthpiece that was connected through tubing attached to the machine. During the test, their blood pressure, heart rate, and heart activity were continually monitored for safety. As the test progressed, they were required to gradually work harder until they reached their individual level of tolerance. Once they reached that point, the test was ceased and a cool-down cycling (e.g., at 25 W) was performed for 5–10 minutes. To assess whether the $\dot{V}O_{2peak}$ from this test represented the true $\dot{V}O_{2max}$, subjects then performed a verification bout, which involved a square-wave transition in work rate to that which was achieved upon completion of the ramp test, after which subjects pedaled until they reached a limit of tolerance once again (typically 2–3 minutes). A licensed physician was present for each of these tests. If a subject felt ill at any time during the test, he or she was instructed to stop exercising immediately and inform the tester to receive proper medical treatment if needed. Upon the successful completion of the test, those demonstrating an age and gender-appropriate $\dot{V}O_{2peak}$ of "average" or lower, as defined by the American College of Sports Medicine, were invited to participate in the study, whereas those demonstrating a value better than "average" were excluded.

4.2.6 Study Protocol

4.2.6.1 Testing Protocol and Timeline

Table 4.2

Substrate Selectivity Testing Protocol

Substrate Selectivity Testing Protocol									
	9-day Weight Maintenance Controlled Diet Period (15% protein, 50% carbohydrate, 35% fat)								
Pre	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
RMR + RAMP/ VER			CWRE 70%GET			BC + CWRE GET			RMR + OGTT

Participation in this study extended over a period of 9 days. During this time, each participant reported to the lab three times to conduct five different tests. The 9-day substrate-selectivity testing protocol consisted of two distinct exercise tests (CWRE; Day 4 and Day 7); a body composition assessment (BC; Day 7); one resting metabolic rate test (RMR; Day 9); and a test to measure insulin resistance (OGTT: Day 9). All tests and procedures are explained in detailed below. Participants were required to follow a 9-day weight maintenance controlled dietary protocol designed to keep their weight from changing and continue their present level of physical activity over the course of the study. They were provided with food and dietary guidelines that were specifically designed to make sure they did not gain or lose weight during the course of the study. Eating plan and caloric load were individualized for each participant based on the comprehensive dietary questionnaire they completed during screening and the resting metabolic rate they demonstrated prior to protocol initiation.

4.2.6.2 Diet Intervention and Follow-up

To ensure weight stability and similar macronutrient composition between subjects during the entire testing period, subjects adhered to a 9-day weight-maintenance controlled diet period. The caloric load was calculated based on the subjects' resting metabolic rate (150% RMR), which has been shown to ensure weight stability at this activity level. The diet was comprised of 15% protein, 50% carbohydrate, and 35% fat. This macronutrient proportionality was in accordance with the USDA guidelines for a healthy eating pattern for adults. During the screening process, participants completed a dietary preference questionnaire inquiring about their specific liking and restrictions as well resources and lifestyle. The diet

was designed by a clinical dietitian and individually tailored to include a wide choice of foods based on subject preference and any restrictions they might have. To determine a diet that was appropriate, the clinical dietitian met with the subject to derive a fixed menu for each day and the subject only ate what was written on this menu. The food was provided in the form of frozen and fresh foods. If a subject had to purchase any items, he or she was reimbursed according to hand-in receipts. Subjects received specific instructions about the meal plan and portion sizing to ensure that they ate the correct amount. During this time, they continued their previous level of physical activity and also weighed themselves daily so that they could report their weight to the study coordinator. The diet was adjusted if necessary to make sure a subject's weight did not change. Moreover, each participant was required to fill out a diet adherence sheet that outlined in detail what he or she ate and how much of the food provided was consumed.

4.2.6.3 Resting Metabolic Rate Test (RMR)

Each participant performed this procedure two times: (a) prior to protocol initiation to measure metabolic rate and total energetic requirement for the 9-day weight-maintenance diet; and (b) at protocol conclusion (Day 9) to measure resting substrate selectivity (i.e., rates of lipid and carbohydrate oxidation) after 9 days of energy and weight stability. The night before this test, participants followed the directions given to them by the investigators; for example, they did not engage in moderate or strenuous physical activity and they abstained from ingesting caffeine or alcohol. The RMR test was done in the morning after the subjects had not consumed any calorie-containing foods or beverages for 12 hours. The duration of the test was approximately 1 hour, during which the subject lay quietly in bed with a large, comfortable plastic hood placed over his or her head. This hood was connected to a machine that analyzed gas exchange.

4.2.6.4 Constant Work-Rate Exercise Test (CWRE)

On Day 3 and Day 6, participants underwent a constant-work-rate exercise test to assess the energy and fuel they used during moderate-intensity exercise. Unlike the ramp test described above, this test did not require maximal exertion. The test was performed in the morning after 12 hours of overnight fasting. The night before this test, subjects followed the directions given to them by the investigators (see above). During this test, they were again connected to the Vmax system to collect gas-exchange data (see above). The subjects' heart rate and blood pressure were also monitored for safety. For the first 5

minutes of the test, participants cycled freely with the work rate set at 0 W. After that, the work rate was increased in a square-wave manner to a predetermined level and remained fixed for the next 55 minutes. Participants were allowed to listen to music of their choice during the cycling bout. A licensed physician was in proximity and available to address any emergency that may occur. Appropriate emergency equipment was present in the room. If a participant felt ill at any time during the tests, he or she was instructed to stop exercising immediately and tell the tester in order to receive proper medical treatment if needed.

4.2.6.5 Body Composition Measurements (Bod Pod)

On Day 6, body composition was assessed using a Bod Pod. The BOD POD Gold Standard Body Composition Tracking System, manufactured and distributed exclusively by COSMED USA, is an air displacement plethysmograph that uses whole-body densitometry to determine body composition (fat and lean tissue) in adults and children. In this research application, to control for any differences that may have been present between subjects/groups in the amount of fat-free mass, lipid and carbohydrate oxidation were expressed relative to fat-free mass obtained from this measurement. For this test, participants were asked to sit calmly with their hands on their lap in the chamber with a window while wearing a swim cap and tight-fitting underwear.

4.2.6.6 Oral Glucose Tolerance Test (OGTT)

On Day 9, participants underwent an OGTT to assess insulin sensitivity. The test was performed immediately following the RMR test while the participants were still fasting. After the IV was inserted, two 4-cc tubes of blood were collected for plasma insulin and glucose analysis. After that, the participants drank a 75g sugary drink, after which an additional two 4-cc tubes of blood were collected at 30 minutes, 60 minutes, 90 minutes, and 120 minutes following ingestion. Blood samples obtained were processed and plasma and serum stored immediately in a -70 degree Fahrenheit freezer located in the facility in proximity to the testing room until processing.

4.3 Results

Prior to conducting each analysis, the requisite assumptions of normality and homogeneity of variance were investigated using visual inspection and statistical tests. Regarding the former assumption, normal distribution of variables was examined using Q-Q plots and Shapiro-Wilks tests for each variable

of interest. Results indicated that the observations for each of the resting and exercise conditions met requirements of normality. Figure 1 below presents the Q-Q plots for fat oxidation rate in mg·min^{-1}, adjusted for kg fat-free mass, for the 70% (A) and 100% (B) GET constant work-rate exercise tests. As is evident from the plots below, the assumption of normality was upheld in the present data. Levene's tests of homogeneity of variance suggested that no violations to this assumption were evident for the aforementioned variables.

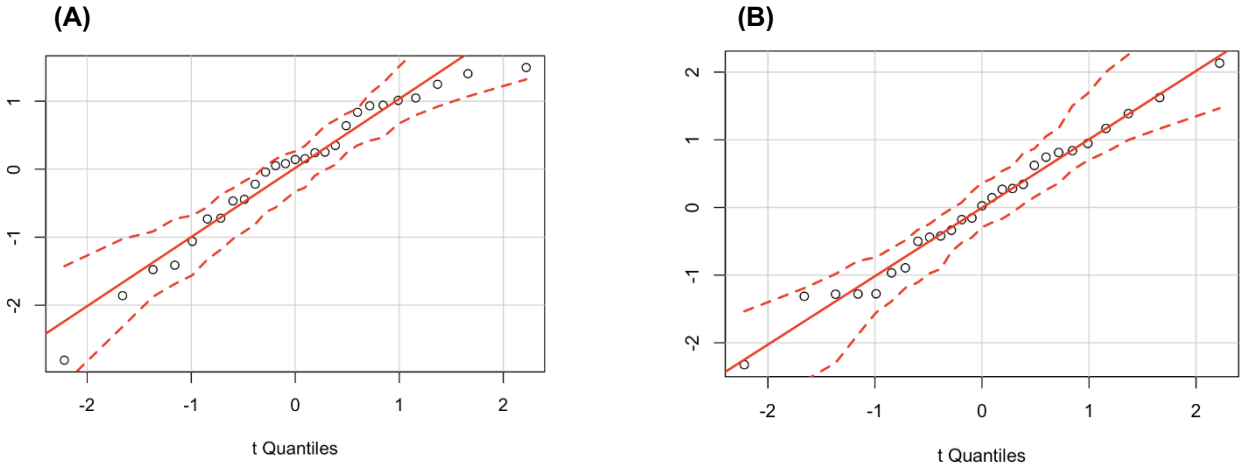

Figure 4.2. Q-Q plots for fat oxidation rate in mg min^{-1}

4.3.1 Subject Characteristics

The physical characteristics of the groups (i.e., metabolically healthy normal weight, MHNW; metabolically unhealthy normal weight, MUNW; metabolically healthy obese, MHO; and metabolically unhealthy obese, MUNW) are presented in Table 4.3 below. Based on the results of ANOVA and planned contrasts investigations conducted for each variable, individuals in the MHO and MUO groups had, on average, higher body mass, percent body fat, BMI, and waist circumference compared to individuals in the MHNW and MUNW groups ($p < .05$). One-way ANOVA also revealed a significant difference in mean age between the groups ($F(3,25) = 3.669$, $p < .05$). However, a follow-up analysis with planned contrasts and Tukey's post-hoc indicated that the different in mean age only existed between the MHNW and the MUO groups ($p < .05$). Fat-free mass and blood glucose during fasting and 2-hr post 75g glucose drink were not significantly different between the four groups in the one-way ANOVA and planned contrasts analyses.

Table 4.3

Subject Characteristics

Variable	MHNW	MUNW	MHO	MUO	p-Value
N	8 (4M, 4F)	7 (4M, 3F)	8 (4M, 4F)	7 (5M, 2F)	
Race	White (2) Asian (2) Black (1) Hispanic (1)	White (4) Asian (3)	White (3) Black (4) Hispanic (1)	White (5) Asian (2)	
Age, yr	25.8 ± 2.9	31.4 ± 4.9	31.6 ± 7.8	35.3 ± 5.0	p < .05
Body Mass, kg	63.0 ± 9.6	67.5 ± 7.5	84.1 ±6.2	96.0 ±13.2	p < .05
FFM, kg	49.3 ± 11.6	47.3 ± 6.6	54.7 ± 9.5	58.9 ± 12.4	NSD
Body Fat, %	21.6 ± 9.0	29.6 ± 3.3	33.6 ± 10.6	38.8 ± 9.0	p < .05
BMI, $kg \cdot m^2$	23.0 ± 1.3	23.4 ± 1.2	30.2 ± 3.3	31.9 ±3.3	p < .05
WC, in	28.3 ± 4.3	33.3 ± 2.4	35.9 ± 2.7	40.4 ± 5.8	p < .05
WHR	0.76 ± 0.07	0.86 ± 0.05	0.81 ± 0.08	0.90 ± 0.10	p < .05
FBG, $mg \cdot dl^{-1}$	93.7 ± 4.4	99.9 ± 9.0	92.3 ± 10.2	99.5 ± 8.4	NSD
2-hrs, $mg \cdot dl^{-1}$	100.2 ± 17.7	119.4 ± 38.6	107.6 ± 12.3	124.3 ± 35.9	NSD

Values are mean±SD. M, male; F, female; BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; FBG, fasting blood glucose; 2-hrs, blood glucose post 2-hrs 75g glucose drink; HDL, high-density lipoprotein; FFM, fat-free mass; MHNW, metabolically healthy normal weight ; MUNW, metabolically unhealthy normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NSD, no significant difference.

4.3.2 Incremental Test Parameters

Maximal exercise testing was performed to determine GET and cardiorespiratory fitness level ($\dot{V}O_{2max}$) (Table 4.4). One-way ANOVA and follow-up planned contrasts for specific group differences indicated no statistical differences between the groups in any of the Peak or GET values below ($p > .05$).

Table 4.4

Incremental Test Parameters

Variable	MHNW	MUNW	MHO	MUO	P-Value
N	8 (4 M, 4F)	7 (4M, 3F)	8 (4M, 4F)	7 (5M, 2F)	
WR_{peak}, W	175 ± 42	150 ± 21	183 ± 46	184 ± 37	NSD
$\dot{V}O_{2max}$, $ml \cdot min^{-1}$	2213 ± 629	1880 ± 320	2275 ± 518	2427 ± 601	NSD
$\dot{V}O_{2max}$, $ml \cdot min^{-1} \cdot kgFFM^{-1}$	44.4 ± 5.0	40.1 ± 6.8	41.7 ± 6.0	41.1 ± 4.3	NSD
70% GET, W	46 ± 13	39 ± 9	52 ± 19	49 ± 14	NSD
70% GET, % WR_{peak}	26 ± 5	27 ± 5	28 ± 5	26 ± 4	NSD
100% GET, W	66 ± 19	56 ± 13	75 ± 27	70 ± 20	NSD
100% GET, % WR_{peak}	53 ± 7	51 ± 5	54 ± 7	55 ± 5	NSD
100% GET, $ml \cdot min^{-1}$	1146 ± 262	981 ± 189	1217 ± 279	1332 ± 391	NSD

Values are mean±SD. M, male; F, female; WR_{peak}, peak work rate; $\dot{V}O_{2peak}$, peak rate of oxygen consumption; GET, gas exchange threshold; MHNW, metabolically healthy normal weight ; MUNW, metabolically unhealthy normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NSD, no significant difference.

4.3.3 Weight and Energy Stability

Participants were required to follow a 9-day controlled dietary regiment with a specific number of calories and macronutrient composition to ensure weight and energy stability within and between subjects during the assessment period. Tables 4.5 and 4.6 below present data related to mean weight (kg), weight change, and 95% CI, by group, recorded on the first and last day of the assessment period, and diet adherence. Weight change was not significantly different between the group ($F(3,26) = 1.009$, $p = 0.4$). Diet adherence was similar between the groups and significantly high overall (90%). Each participant received, on average, 20 food items to eat each day. On average, 1.4 food items were excluded each day, whereas 0.6 were added.

Table 4.5

Weight and Energy Stability

Group	Day 1	Day 9	Weight Change	95% CI
MHNW	63.1 ± 9.8	62.8 ± 9.3	0.3 ± 0.9	[-0.4, 1.1]
MUNW	68.0 ± 7.2	67.3 ± 7.3	0.6 ± 0.9	[-0.1, 1.4]
MHO	83.4 ± 5.7	82.5 ± 5.9	0.9 ± 0.8	[0.2, 1.5]
MUO	97.2 ± 13.5	96.2 ± 13.0	1.0 ± 0.7	[0.3, 1.7]

Values are mean±SD for body weight in kg.

Table 4.6

Average Dietary Adherence, %, by Group

Group	MHNW	MUNW	MHO	MUO	Overall
Adherence, %	93 ± 4	91 ± 8	84 ± 16	89 ± 5	90 ± 9
Exclusion, #	1.3 ± 0.7	1.6 ± 1.4	2.9 ± 2.9	1.9 ± 1.0	1.9 ± 1.0
Inclusion, #	0.3 ± 0.2	0.3 ± 0.3	1.0 1.2	0.8 ± 0.8	0.6 ± 0.7

Values are presented as percent from total, and actual numbers. % Adherence indicates the total # of items prescribed vs. eaten. Exclusion indicates the total number of items that were prescribed, but not eaten ("omitted"). Inclusion includes the total number of items that were eaten, but not prescribed ("added").

For the present investigation, a series of ANOVAs were conducted to evaluate the differences between the four groups of interest: MHNW, MUNW, MHO, MUNW. Specifically, the analyses identified differences between the four groups in fat oxidation, expressed as both RER and as a rate ($mg \cdot min^{-1}$) adjusted for FFM, during the basal condition and two constant-work-rate exercise bouts (70% and 100%

GET). In addition, planned contrasts were examined to evaluate any differences between MHNW and MUO (Research Question #1) and between MUNW and MHO (Research Question #2) with *alpha* levels adjusted according to the step-down procedure outlined by Holm. Effect sizes were also investigated based on the omega-squared statistic, and correlation coefficients were used to examine the planned contrast analyses. Finally, Tukey's post-hoc analyses were used to explore remaining comparisons between groups.

To reduce within-group error variance and more accurately assess the effect of the experimental manipulation, a series of ANCOVAs were also conducted using GET (ml/*min, adjusted for kg body weight) as a covariate, following the same process outlined above for the ANOVAs, planned contrasts, and Sidak's post-hoc analyses. Partial Eta Squared, instead of Omega Square, was used to assess effect size for the ANCOVA.

4.3.4 Lipid Oxidation During Basal Condition After Eight Days of a Weight-Stable and Energy-Balanced Dietary Protocol

A one-way ANOVA and follow-up planned contrasts analysis indicated no significant differences between the groups for lipid oxidation, expressed as RER or as $mg \cdot min^{-1}$ adjusted for FFM (Table 4.7). Figure 2 below presents a graphical depiction of lipid oxidation as (A) RER and as (B) $mg \cdot min^{-1}$ adjusted for FFM for each of the four groups. Resting energy expenditure, expressed as $kcal \cdot min^{-1}$, adjusted for kg FFM, was also not statistically different between the groups in the ANOVA of planned contrasts comparisons.

Table 4.7

Lipid Oxidation During Basal Condition After Eight Days of a Weight-Stable and Energy-Balanced Dietary Protocol

Variable	MHNW	MUNW	MHO	MUO	p-Value
N	8 (4 M, 4F)	7 (4M, 3F)	8 (4M, 4F)	7 (5M, 2F)	
REE, $kcal \cdot min^{-1} \cdot kgFFM^{-1}$	1164 ± 508	1448 ± 161	1406 ± 233	1724 ± 315	NSD
RER	0.82 ± 0.05	0.84 ± 0.05	0.81 ± 0.05	0.85 ± 0.05	NSD
Fat Oxidation, $mg \cdot min^{-1} \cdot kgFFM^{-1}$	1.16 ±0.50	1.12 ± 0.46	1.16 ± 0.48	1.04 ± 0.35	NSD

Values are mean±SD. M, male; F, female; REE, resting energy expenditure; $\dot{V}O_2$, rate of oxygen consumption; RER, respiratory exchange ratio; MHNW, metabolically healthy normal weight ; MUNW, metabolically unhealthy normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NSD, no significant difference.

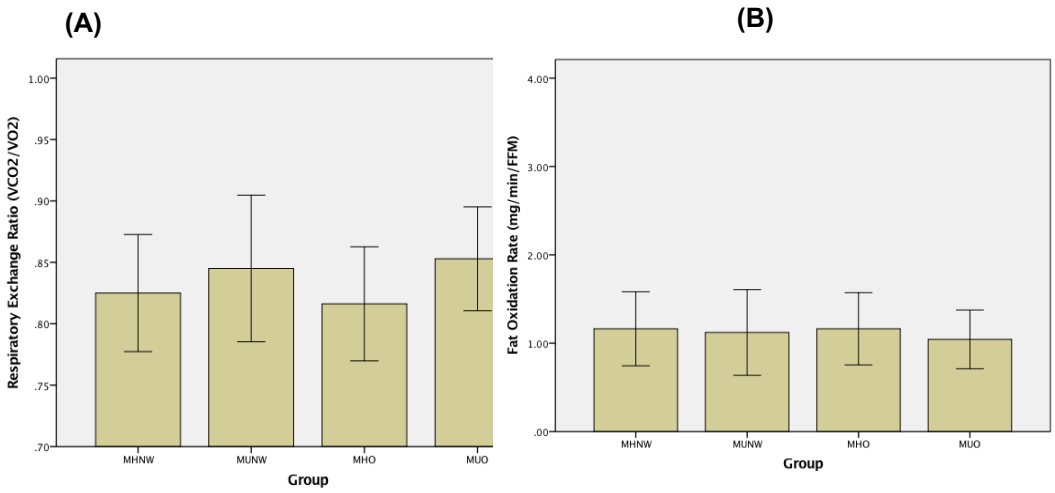

Figure 4.3. Lipid oxidation during basal condition after eight days of a weight-stable and energy-balanced dietary protocol as RER (A) and as mg·min^{-1} adjusted for kg fat-free mass (B).

4.3.5 Lipid Oxidation During the 70% GET Constant Work-Rate Exercise

In the one-way ANOVA (Table 4.8), lipid oxidation, expressed in mg·min^{-1} adjusted for kg fat-free mass, was significantly different ($F(3,26) = 4.058$, $p < .05$) between the groups, with a large effect size ($\omega^2 = .25$). Total energy burn, expressed in kcal·min^{-1} adjusted for fat-free mass, and lipid oxidation, expressed as RER, were similar between the groups ($p > .05$). Planned contrasts were examined to assess group differences in order to answer each of the research questions outlined above. These results revealed a statistically significant difference between MHNW and MUO (mean $\pm$ SD: 3.86 $\pm$ 1.10 vs. 2.73 $\pm$ 0.93; t (26) = 2.282, $p < .05$) with a medium effect size ($r = 0.41$), and between MUNW and MHO (mean $\pm$ SD: 2.35 $\pm$ 0.41 vs. 3.65 $\pm$ 1.15; t (26) = -2.685, $p < .025$) with a medium effect size ($r = 0.47$). In addition, Tukey post-hoc analyses also revealed a significant difference between MHNW and MUNW groups ($p < .05$).

When ANCOVA was applied to evaluate group differences in lipid oxidation while accounting for variability attributed to differences in GET (ml/min/kg BW), both RER ($p = .005$, $\eta^2 = 0.40$) and lipid oxidation, expressed as mg*min*kg FFM, were significantly different between the groups. Planned contrasts and Sidak post-hoc analyses were also examined and revealed a statistically significant difference in lipid oxidation, expressed as mg·min^{-1}·kgFFM^{-1}, between MHNW vs. MUO ($p = .007$), MUNW

vs. MHO (p = .009), and MHNW vs. MUNW (p < .05). In addition to lipid oxidation as mg*min*kg FFM, RER was also significantly different between MHNW vs. MUO (p = .001).

Table 4.8

Lipid Oxidation During the 70% GET Constant Work-Rate Exercise (one-way ANOVA)

Variable	MHNW	MUNW	MHO	MUO	P-Value	Omega Squared
N	8 (4 M, 4F)	7 (4M, 3F)	8 (4M, 4F)	7 (5M, 2F)		
Total Energy, $kcal \cdot min^{-1} \cdot kgFFM^{-1}$	0.08 ± 0.02	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	NSD	---
RER	0.86 ± 0.04	0.90 ± 0.03	0.87 ± 0.04	0.91 ± 0.04	NSD	---
Lipid Oxidation Rate, $mg \cdot min^{-1} \cdot kgFFM^{-1}$	3.9 ± 1.1	2.4 ± 0.4	3.7 ± 1.2	2.7 ± 0.9	0.012	0.25

Values are mean±SD. M, male; F, female; RER, respiratory exchange ratio; MHNW, metabolically healthy normal weight; MUNW, metabolically unhealthy normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NSD, no significant difference.

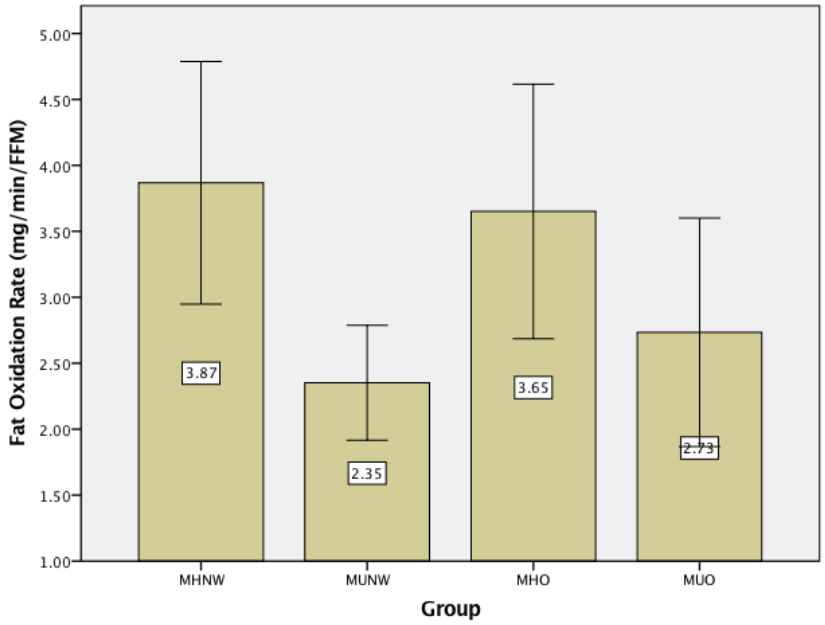

Figure 4.4. Group results for fat oxidation in $mg \cdot min^{-1}$ adjusted for FFM, for each of the four groups

4.3.6 Lipid Oxidation During the 100% GET Constant Work-Rate Exercise

In the one-way ANOVA (Table 4.9), RER and total energy in kcal·min^{-1} adjusted for fat-free mass were similar between the groups ($p > .05$). However, lipid oxidation rate in mg·min^{-1} adjusted for kg fat-free mass was approaching statistical significance ($F(3,26) = 2.674$, $p = .068$), with a large effect size ($\omega^2 = 0.15$). Similar trends were observed when planned contrasts analyses were conducted to assess differences between the designated groups ($p > .05$). Accounting for variability attributed to differences in GET (ml*min*kg BW) with the ANCOVA resulted in a similar, albeit smaller p-value ($p = .061$), and a similar, albeit bigger effect size ($\eta^2 = 0.46$).

Table 7

Lipid Oxidation During the 100% GET Constant Work-Rate Exercise

Variable	MHNW	MUNW	MHO	MUO	P-Value	Omega Squared
N	8 (4 M, 4F)	7 (4M, 3F)	8 (4M, 4F)	7 (5M, 2F)		
Total Energy, kcal·min^{-1}·kgFFM^{-1}	0.12 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.12 ± 0.02	NSD	---
RER	0.90 ± 0.03	0.93 ± 0.04	0.93 ± 0.03	0.93 ± 0.04	NSD	---
Lipid Oxidation Rate, mg·min^{-1}·kgFFM^{-1}	3.9 ± 1.7	2.1 ± 0.7	2.9 ± 1.1	2.6 ± 1.3	.068	0.15

Values are mean±SD. M, male; F, female; RER, respiratory exchange ratio; MHNW, metabolically healthy normal weight; MUNW, metabolically unhealthy normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NSD, no significant difference.

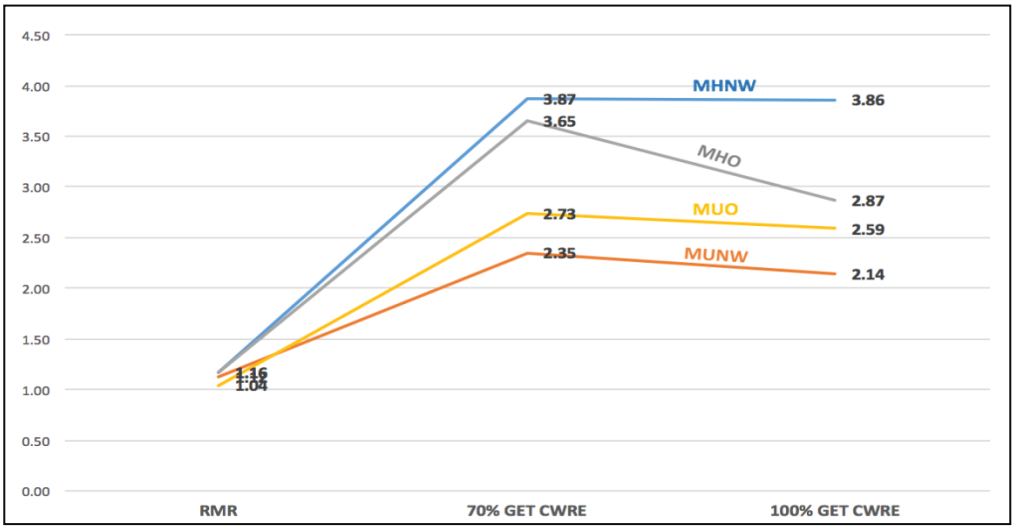

Figure 4.5 (for illustration purposes only). Group means for lipid oxidation rate in mg·min^{-1} adjusted for kg FFM in each of the three conditions: RMR, 70% GET CWRE, and 100% GET CWRE

4.3.7 Lipid Oxidation During the 70% GET Constant Work-Rate Exercise (Independent t-test)

An independent t-test was conducted to assess differences in RER and lipid oxidation as mg·min^{-1} adjusted for kg fat-free mass, between normal weight (MHNW + MUNW; n = 14) and overweight/obese (MHO + MUO; n = 15) individuals (Table 4.10). Effect sizes were also investigated based on correlation coefficients. Lipid oxidation was similar between the groups.

Table 4.10

Lipid Oxidation During the 70% GET Constant Work-Rate Exercise (Independent t-test)

Variable	Normal Weight	Overweight·Obese	P-Value	Correlation Coefficient
N	15 (8 M, 7 F)	15 (9 M, 6 F)		
RER	0.89 ± 0.04	0.89 ±0.04	NSD	---
Fat Oxidation Rate, mg·min^{-1}·kgFFM^{-1}	3.1 ± 1.1	3.2 ± 1.1	NSD	---

Values are mean±SD. M, male; F, female; RER, respiratory exchange ratio; NSD, no significant difference

4.4 Discussion

The main original finding from this investigation is that a reduced rate of lipid oxidation during exercise in association with the overweight/obese condition was only observed when the "metabolic health" of the overweight/obese subject was also compromised. Moreover, normal-weight individuals with a similar derangement in metabolic profile also demonstrated a lower lipid oxidation rate during exercise, while "metabolically-healthy" overweight/obese subjects possessed a capacity that was not different compared to their metabolically-healthy normal-weight counterparts. Collectively, these findings supported our experimental hypotheses and suggest that the lower rate of lipid use that has been reported for overweight/obese subjects in some previous investigations reflects the loss of metabolic health often associated with excessive fat deposition, as opposed to the overweight/obese condition per se.

In the present study, we assessed the capacity for exercise lipid use during stationary cycling at work rates where lipid should still contribute significantly to energetic demand. Specifically, subjects cycled at work rates assigned relative to the "gas-exchange threshold" (GET), which approximates the lactate threshold (LT) that defines the upper-region of the moderate-intensity domain. Importantly, the

work rate at LT has been shown to be correlated with the one that allows for the maximal capacity for lipid use (Achten & Jeukendrup, 2004); hence, assigning work rate relative to GET should allow for a consistent comparison across subjects. Consequently, the ~40% and ~25% reductions in the rate of lipid use at 70% GET that we observed in association with metabolic compromise in normal-weight and overweight/obese subjects, respectively, suggest that the metabolic health of an individual is a key determinant that influences his or her capacity to use lipid as fuel effectively. This coheres with findings from Ghanassia et al. (2006) who observed a lower maximal rate of lipid oxidation in sedentary subjects with type 2 diabetes compared to age-, sex- and BMI-matched sedentary controls with normal glucose tolerance. Indeed, patients with type 2 diabetes demonstrated a lower lipid oxidation rate at five different exercise work rates that were assessed in that study. Importantly, our metabolically-unhealthy subjects did not yet possess type 2 diabetes; hence, our findings implicate an impaired capacity for lipid oxidation during exercise that manifests early during the progression of insulin desensitization. A similar conclusion can be drawn from the findings of Robinson, Hattersley, Frost, Chambers, and Wallis (2015) who reported that the maximal lipid oxidation rate was positively correlated with insulin sensitivity in healthy recreationally-active men.

The present findings might help to clarify previous ambiguity regarding the influence of overweight/obesity on the capacity for exercise lipid use. Specifically, while the preponderance of evidence suggests that overweight/obese individuals oxidized lipid during exercise similarly compared to their normal-weight counterparts, seven of 24 studies that made this comparison did include evidence of an overweight/obesity-related reduction during exercise (see Chapter 3 for review). However, one of these studies involved intermittent bouts of resistance training (i.e., an activity for which lipid use would not be expected to contribute to a great extent), while others involved an incremental exercise challenge where the time to achieve and work at “steady state” was limited. This is important to consider because in a study that included assessment of substrate use during both incremental (Bruce protocol; 3-minute stages) and constant-work-rate (15 minutes) treadmill bouts, Steffan et al. (1999) found that RER values measured at 8 and 15 minutes of the constant work-rate bout were significantly less than and not correlated with the values measured during the 3-minute stage of the incremental test at the same relative work rate ($\dot{V}O_{2max}$). As for the other two, both involved constant-work-rate cycling like that which

was assessed in the present study; however, duration was less than 30 minutes for each of these bouts. This has resonance because Kanaley et al. (2001) found that a lipid-use increment in exercise lipid use was identifiable after 30, but not 15 minutes of exercise for obese compared to normal-weight subjects. Hence, longer constant-work-rate exercise bouts appear to be required to adequately test lipid-oxidizing capacity across different types of subjects. Finally, the obesity-related decrement reported by Hickner et al. (2001) during the 10-minute constant-work-rate cycling bouts they employed was only present when obese subjects were compared to Caucasian normal-weight controls. Conversely, when compared with normal-weight African American controls, no decrement was observed. Considering what was discovered from the present research in concert with the fact that African Americans typically demonstrate lower insulin sensitivity compared to Caucasians, it is possible that Hickner et al.'s findings represented the deleterious effect of metabolic health on exercise lipid use as opposed to overweight/obesity per se.

The link between a reduced capacity to oxidize lipid as fuel and loss of insulin sensitivity that we observed could be rooted in an increased accumulation of lipid in skeletal muscle, which has the potential to create "lipotoxic" repercussion (Moro et al., 2008). Indeed, it is attractive to speculate an inherent defect in mitochondrial lipid oxidation that results in lipid accumulation exceeding use, which could also predispose the overweight/obese condition (Kelley et al., 1999). However, the preponderance of evidence in support of this notion has been derived from studies that have assessed lipid use during basal conditions when lipid use should predominate (Kelley et al., 1999) and the degree to which a mitochondrial defect during a condition with such a low metabolic demand could mediate insulin sensitivity has been questioned (Holloszy, 2008). It is also important to note that a reduced capacity for lipid use during the basal condition is not a universal finding. Although not the primary purpose of the present investigation, we also assessed lipid use during fasting for our four groups of subjects and found no deficit associated with either metabolic compromise or the overweight/obese state. Importantly, prior to this measurement, we imposed an 8-day controlled-feeding period with fixed macronutrient proportions and weight stability. In this regard, Galgani et al. (2008) emphasize the importance of this dietary control when assessing fuel proportionality because of the profound effect of acute energy balance and dietary macronutrient consumption on substrate selectivity.

With respect to determining the influence of overweight/obesity on exercise lipid use, a number of methodological aspects of the present study deserve mention. In the preponderance of previous research, exercise intensity was assigned relative to the peak work rate achieved on a maximal incremental test (i.e., "$\dot{V}O_{2max}$"). This method of normalizing exercise work rate would not be expected to provide for a consistent comparison across subjects because Venables et al. (2005) observed that the maximal rate of lipid use occurred anywhere between 21 and 77% of $\dot{V}O_{2max}$ in their cohort of healthy individuals. Conversely, as previously mentioned, the maximal rate of lipid oxidation is correlated with LT; hence, assigning work rate relative to that parameter appears to be a superior alternative. We also modeled the $\dot{V}O_2$ response to the exercise transition to estimate the point at which a "steady state" level of energy expenditure occurred because prior to this point, gas-exchange values do not faithfully indicate metabolism and fuel proportionality. Consequently, regardless of the rapidity with which they made the transition to the higher work rate (i.e., $\dot{V}O_2$ kinetics), subjects were compared for the same duration of exercise (i.e., the initial 45 minutes after attainment of steady state). Finally, we assessed fuel use for 45 minutes of exercise to ensure that the greater temporal increase in lipid use during exercise that has been reported for overweight/obese subjects by Kanaley et al. (see above) would be accounted for in our comparison.

A few limitations to our study need to be discussed. As previously mentioned, a potential interpretation of our findings is that the condition we defined for our two groups of metabolically-unhealthy subjects could be rooted in a mitochondrial defect that restricts oxidation of available lipids. Indeed, independent of adiposity, individuals with type 2 diabetes, a disease of insulin resistance, have been shown to demonstrate a decrease in mitochondrial biogenesis, reduce mitochondrial content, and lower concentration of oxidative enzymes compared with individuals without the disease (DeLany et al., 2014). However, a potential problem with this interpretation is that in our study, we did not assess mitochondrial activity and/or function directly, but instead, lipid oxidation was used as a proxy. Nevertheless, it has been proposed that a reduced mitochondrial oxidative capacity can lead to impaired lipid oxidation (Morino, Petersen, & Shulman, 2006) and therefore, the ability, or lack thereof, to increase lipid oxidation in response to an increase in energy needs and/or lipid flux might represent an early biomarker of metabolic abnormalities that lead to type 2 diabetes.

Our participants underwent body-composition analysis using the Bod Pod to quantify fat and fat-free components of their body. This is important because a greater fat-free mass might translate to a greater metabolic and fat-burning capacity, independent of fat mass (Goodpaster, Wolfe, & Kelley, 2002). Consequently, measurements of lipid oxidation expressed in absolute terms (e.g., as mg·min) should be normalized to fat-free mass to control for this factor. Given that individuals with excess lipid storage typically possess greater fat-free mass compared with normal-weight counterparts, failure to express an absolute measurement of lipid oxidation relative to fat-free mass could lead to a type II error by masking an obesity- and/or insulin-resistance-related decrement that might be present. Indeed, most studies that have failed to normalize exercise lipid oxidation to fat-free mass have found no difference in exercise lipid oxidation between normal-weight and overweight/obese individuals (Balcı, 2012; Colberg et al., 1996; Kanaley et al., 1993; Larsen et al., 2009; Santiworakul et al., 2014, Slusher et al., 2015; Thyfault et al., 2004). Normalizing lipid oxidation rate for fat-free mass might explain the reason for the discrepancy, and our findings are consistent with previous studies that have measured and normalized rates of lipid oxidation to fat-free mass (Hickner et al., 2001; Keim et al., 1996; Lanzi et al., 2014, Mohebbi & Azizi, 2011; Perez-Martin et al., 2001). However, there is a growing appreciation that in many cases, fat deposition pattern (e.g., subcutaneous vs. muscle) can also influence the insulin-resistant state and/or patterns of fuel selection and metabolism independent of body weight (Fried, Lee, & Karastergiou, 2015; Patel & Abate, 2013; Stefan, Schick, & Häring, 2017). For example, lower rates of lipid oxidation have been observed in normal-weight women with a higher abdominal-to-lower-body fat mass ratio compared with women with a lower abdominal-to-body fat mass ratio during 45 minutes of steady-state exercise at 65% $\dot{V}O_{2max}$. Based on this observation, patterns of fat-deposition might be an important factor to consider when a comparison of exercise lipid use is made between overweight/obese and normal-weight individuals. Interestingly, in our study, the normal-weight and overweight/obese unhealthy subjects demonstrated a higher waist-to-hip ratio, which may, in part, explain our observation. Furthermore, the normal-weight unhealthy subjects in our study had, on average, a higher body-fat percentage compared with their normal-weight healthy counterparts (e.g. ~28 vs. 21%; $p > .05$). However, we did not assess patterns of fuel storage and/or fat deposition in discrete compartments (e.g., abdominal, gluteal, muscle, etc.) and, therefore, cannot exclude the possibility that fat-deposition pattern influenced our results.

Previous research suggests that a racial difference might exist in the capacity for, and oxidation of, lipids during basal (DeLany et al., 2014; Chitwood, Brown, Lundy, & Dupper, 1996; Weyer, Snitker, Bogardus, & Ravussin, 1999) and exercise (Chitwood et al., 1996) conditions. For example, Chitwood et al. (1996) reported higher metabolic reliance on carbohydrate during rest and exercise in normal-weight Black women compared with their White counterparts. In this study, Blacks exhibited higher fasting plasma insulin levels that may, in part, explain their findings. As previously mentioned, in concert with these observation, our findings help to inform those of Hickner et al. (2001) by implying that differences in lipid-oxidizing capacity that were observed might simply reflect the metabolic-health differences of the races as oppose to race per say. In our study, we did not control for race, but we did control for factors that might predispose and/or be strongly associated with poor metabolic health. Overall, we observed significantly lower rates of lipid oxidation in the metabolically-unhealthy compared with metabolically-healthy groups with all of our participants who self-identified as Black (n = 5) falling in the latter category (normal-weight, n = 1; overweight/obese, n = 4). Based on this observation, it is attractive to speculate that racial differences observed in previous studies have little to do with race per se, but much to do with related metabolic-health differences. With that said, our study was not designed to assess racial differences and future studies should evaluate whole-body exercise lipid oxidation in various races with factors that might influence the comparisons rigidly controlled (e.g., metabolically-unhealthy and healthy Black and White classifications).

Another potential limitation of the present research is the observed differences in age between the metabolically-healthy normal-weight (~26 yrs) and metabolically-unhealthy overweight/obese (~35 yrs) groups. When adjusted for metabolic rate, exercise lipid oxidation expressed as RER and as a rate relative to FFM (i.e., $mg \cdot min^{-1} \cdot kg^{-1}FFM$), was significantly lower in the metabolically-unhealthy obese compared with the metabolically-healthy normal-weight group. Consequently, one might speculate that advancing age (or possibly the associated change in muscle phenotype brought on by passage of years with insufficient exercise) influenced our results. However, in a study of 32 women aged 18–73 yrs, Calles-Escandon, Arciero, Gardner, Bauman, and Poehlman (1995) observed no association between age and lipid oxidation when the effect of fat-free mass was statistically removed. Furthermore, other studies that have found differences in lipid oxidation between two or more groups stratified by age have

used age gaps that were three (Solomon et al., 2008), four (Levadoux et al., 2001) and five (Melanson et al., 1997) times the one that was present in our study. It is also important to note that lipid oxidation was also significantly lower in the metabolically-unhealthy normal-weight group compared with both the metabolically healthy normal-weight and obese groups despite no statistically significant difference in age across these groups. Finally, while mean age was lower in the metabolically-unhealthy normal-weight compared with metabolically-unhealthy obese group, RER and rates of lipid oxidation were similar, which support the contentions that decrements in the capacity to rely on lipid during exercise found for the former group in our study are related to the influence of metabolic health as opposed to that of aging per se. In closing, with regard to these limitations, future research should be designed to confirm our contentions regarding the relative contribution of age and these other variables to exercise lipid use being that answering these questions was outside the scope of the present research.

In summary, we have confirmed that when important pre-test diet and exercise aspects of control are present, overweight/obese subjects do not demonstrate a reduced capacity for exercise lipid use compared to their normal-weight counterparts. However, when metabolic health is compromised, an exercise lipid-use decrement is, indeed, present and of similar magnitude regardless of whether overweight/obesity accompanies the metabolic demise. Although the cross-sectional nature of this study precludes determination of a cause/effect relationship, these findings are consistent with the contention that a sluggish capacity for lipid use is extant early in the progression to insulin desensitization regardless of whether overweight/obesity is present.

Chapter 5 – Discussion

The overarching objective of this dissertational work was to evaluate a 9-day protocol designed to assess metabolic function in individuals at risk for developing type 2 diabetes by measuring whole-body substrate selectivity during exercise with various factors that can affect exercise lipid use rigidly controlled. Specifically, in Chapter 4, we demonstrated how our assessment protocol was effective for clarifying lingering ambiguity in the research regarding the influence of overweight/obesity on the capacity for lipid oxidation during exercise that we documented in Chapter 3. We, therefore, contend that this protocol can be advanced as a standardized one designed to assess exercise lipid use as a proxy for metabolic health to establish prognosis and potential exercise-related treatment options early in the progression of insulin desensitization in a variety of at-risk subjects.

The etiology of type 2 diabetes is complex and the causes have been debated. One theory that has gained traction in recent years suggests that type 2 diabetes, and the insulin-resistant state that predates it, are rooted in dysfunctional lipid metabolism (i.e., a reduced capacity to use lipid for energy production in circumstances where lipid would be preferred; e.g., the fasting state). Within this schema, intramyocellular lipids that would serve to supply a readily available source of energy is instead accumulate in muscle resulting in weight gain, insulin resistance and, eventually, type 2 diabetes (McGarry, 1992). Indeed, reports from biochemical analysis demonstrated a six-fold increase in intramyocellular lipid content in subjects with type 2 diabetes compared with sex- and age-matched non-diabetic controls independent of body weight (Jansson & Kaijser, 1987; Perseghin et al., 2002). Moreover, an inverse relationship between insulin sensitivity and intramyocellular lipid content was reported in unaffected normal-weight adults (Krssak et al., 1999), and a similar relationship was also found in obese (Kelley et al., 1999; Weyer et al., 2001) and normal-weight offspring of subjects with type 2 diabetes (Jacob et al., 1999). Collectively, these observations suggest that dysfunctional lipid metabolism might be responsible, in part, for the metabolic decline associated with the progression to type 2 diabetes.

Elevated intramyocellular lipid content can be the result of excess free-fatty acid uptake, inadequate lipid use, or both. However, evidence suggests that free-fatty acid uptake by skeletal muscle is similar between normal-weight healthy and obese individuals with insulin resistance (Horowitz & Klein,

2000; Kanaley et al., 2001; Kelley et al., 1999). This implies that pathological lipid accumulation in muscle is related to an inability to use it. Early evidence in the support of this notion comes from studies that assessed lipid oxidation during fasting conditions when lipid should predominate (Filozof et al., 2000; Kelley et al., 1999; Kim et al., 2000; Ukropcova et al., 2007). However, reduced lipid use in the fasting state for individuals with or at risk for type 2 diabetes is not a universal finding. Indeed, evidence exists to show that the capacity to use lipid in the basal state; e.g., after an overnight fast of 12 hours, is not limited in individuals with insulin-resistance and/or obesity (Colberg et al., 1996; Ekelund et al., 2007; Galgani, Moro, & Ravussin, 2008; Goodpaster et al., 2002; Kaneley et al., 2001; Perez-Martin et al., 2001; Steffan et al., 1999; Weyer et al., 2001). Moreover, in some studies, fasting lipid oxidation was enhanced for these types of subjects (Blaak et al., 2006; Felber et al., 1987; Schutz et al., 1992). The reason(s) for this ambiguity is/are not clear but might be related to a number of factors that were not adequate controlled when fasting lipid oxidation was measured. For example, weight stability and energy balance in the days prior to measurement have been shown to be important factors influencing post-absorptive substrate selectivity and oxidation (Goris & Westerterp, 2000). Moreover, day-to-day variations in energy balance and macronutrient intake can profoundly affect the type of fuel used by skeletal muscle (Schutz et al., 1992; Weyer et al., 2001). For example, Schutz et al. (1992) demonstrated that one-day increases in energy intake followed by one-day decreases produce significantly different RQ values. Collectively, these findings mean that energy balance (and, by extension, body weight) and macronutrient composition of the diet should be tightly controlled in the days prior to an assessment of substrate selectivity. Interestingly, these factors were not adequately controlled in many of the studies mentioned above and, therefore, the interpretation of the results might be limited. Conversely, our protocol requires that subject follow a 9-day controlled dietary protocol specifically designed to keep their weight, energy intake and macronutrient proportions constant. Moreover, each participant receives specific instructions about the meal plan and portion sizing and was asked to complete a diet adherence sheet that indicates how closely they follow the diet. Using this protocol, we found that body weight did, indeed, remain constant while fasting lipid oxidation was similar between metabolically-healthy and metabolically-unhealthy individuals. This suggests that dysfunctional lipid metabolism cannot be identified during the basal state

for these types of subjects. We also used our assessment protocol to show that that overall adherence to the type of diet we employed is very high (~90%).

The degree of consistency we observed for our basal measurements supports our contention that our assessment protocol can be effective for assessing lipid use during circumstances where a greater metabolic challenge to lipid use (and, therefore, greater potential to unveil a possible early defect) is present. One such condition is moderate-intensity exercise because metabolic demand is higher yet lipid can still contribute significantly as fuel (Galgani, Heilbronn, et al., 2008). For example, in the clinical trial we used to apply our assessment protocol in the exercise setting, we observed an ~6-fold increase in energy use along with an ~3-fold increase in lipid oxidation during the moderate-intensity exercise test we had subjects perform. Consequent to these characteristics of energetic demand, we did, indeed, observed differences consistent with defective lipid oxidation in some types of subjects. Specifically, lipid oxidation was significantly lower in metabolically-unhealthy normal-weight and overweight/obese subjects compared with metabolically-healthy subjects regardless of body composition. Consequently, our assessment protocol provided data that allow us to conclude that an impairment in lipid use for metabolically-unhealthy subject that was not apparent during basal condition does, indeed, become evident when a comparison is made using the exercise condition.

It stands to reason that the exercise condition would provide a particularly appropriate one during which to assess the capacity for lipid use. Skeletal muscle is the major site for fuel metabolism and energy production and the amount of skeletal muscle one has influences the capacity to oxidize lipid (Storlien et al., 2004; Venables et al., 2005), especially during exercise. Furthermore, both cross-sectional and longitudinal analyses confirm that endurance-trained individuals demonstrate a greater capacity for lipid use during exercise. Specifically, endurance-training-related adaptations include increased muscle size and content of type I muscle fibers, increased capillary density and blood flow to the working muscles, increased hemoglobin and myoglobin concentrations, increased mitochondrial content and function and increased content of oxidative enzymes (Cox, 1991; Hellsten & Nyberg, 2015; Jones & Carter, 2000; Lundby & Jacobs, 2016; Neufer, 1989). Each of these factors can account for a propensity for greater lipid oxidation during exercise (Costill et al., 1979; Jansson & Kaijser, 1987). However, there is large interpersonal variability with regard to endurance-training-induced skeletal muscle adaptation

(Timmons, 2010). Therefore, when assessing impairments in lipid use in association with factors that might predispose and/or lead to type 2 diabetes, it is vital to control for physical activity level and aerobic fitness (Goodpaster et al., 2002). When testing our assessment protocol, we accounted for this factor by only recruiting sedentary subjects who self-reported exercising two or fewer times per week. Furthermore, each subject underwent a maximal incremental exercise test to determine their cardiorespiratory fitness (i.e., $\dot{V}O_{2max}$) prior to enrollment and those demonstrating an age and gender-specific classification as "better than average" according to American College of Sports Medicine criteria were excluded.

Another key factor to consider when a comparison of exercise lipid oxidation is made between groups is the intensity of the exercise bout. The capacity to rely on lipid during exercise decreases when exercise work rate surpasses that which is aligned with the lactate threshold (Venables et al., 2005) and the point at which that occurs is vastly different based on fitness level and other factors (e.g., muscle phenotype) (Achten & Jeukendrup, 2004). Previous research found that in sedentary individuals, the highest rate of lipid oxidation during exercise occurs at approximately ~50% $\dot{V}O_{2max}$, which formed the basis for using the highest work rate achieved on an incremental test as a point of reference to prescribe exercise at the same relative intensity for different subjects or the same subject under different circumstances. However, Venables et al. (2005) reported large intersubject variability with the maximal rate of lipid oxidation occurring anywhere between 25 and 77% of $\dot{V}O_{2max}$ in the cohort they recruited. Using GET as a non-invasive proxy for lactate threshold in our study, we found that as a percentage of $\dot{V}O_{2max}$, GET occurred on average at 53% with values ranging from 45 to 67%. Being that we only included subjects who were sedentary and unfit, this relatively large interpersonal variability supports our contention that normalizing exercise intensity relative to $\dot{V}O_{2max}$ is not sufficient for ensuring that all subjects are exercising at the same relative challenge.

As discussed above, one of the main objectives of this work was to resolve the ambiguity regarding the capacity for lipid oxidation in individuals with the overweight/obese state, a condition strongly associated with diabetes and other metabolic disorders. This has clinical and practical significance because the degree to which the overweight/obese condition is associated with a dysfunctional lipid metabolism is an important factor to consider when designing an exercise intervention to "treat" this disease. For example, if lipid use is impaired in individuals with the overweight/obese

condition, it supports the contention that a mitochondrial defect exists; hence, exercise that is specific for improving mitochondrial capacity (e.g., high-intensity interval training) would be warranted. Conversely, if the capacity for lipid use is similar, or enhanced, with the overweight/obese condition, mitochondrial function would, apparently, be sufficient; hence, a training work rate situated closer to the one at which lipid use is maximized might be preferred as a way to perturb the intramyocellular lipid pool and reduce its pathogenic influence. Our findings supports our experimental hypotheses and suggest that the lower rate of lipid use that has been reported for the overweight/obese subjects in some previous studies reflect the loss of metabolic health often associated with excessive fat deposition as opposed to the overweight/obese conditions. Therefore, individuals with markers of metabolic derangements, such as those associated with the metabolic syndrome, may benefit more from an exercise program that focuses specifically on improving mitochondrial function. Conversely, in the absence of metabolic derangements, e.g. metabolically-healthy obese, mitochondrial function and the capacity for lipid oxidation is likely preserved and therefore, an exercise intensity situated closer to the one at which lipid use is maximized (e.g. endurance training) might be preferred as a way to reduce muscle fat. It is important to keep in mind however that a large intra-subject variability exists with regard to the work-rate at which maximal fat oxidation occurs (Venables, Achten, & Jeukendrup, 2005) and therefore, the tradition way to normalize endurance-exercsie intensity by quantifying work-rate relative to the individual $\dot{V}O_{2max}$ may not be sufficient. Instead, normalizing endurance-exercise intensity relative to lactate threshold, or GET (non-invasive proxy for lactic threshold), as a way to ensure that work-rate is carried at, or close to, the individuals maximal fat oxidation capacity, might be preferred for clinical and practical applications.

In our study, lipid use was not different between metabolically-healthy and -unhealthy individuals despite a statistical and clinical difference between the groups observed in the exercise condition. This suggests that dysfunctional lipid metabolism may not be reliably identified during the basal state for these types of subjects and future studies should consider assessing dysfunctional lipid metabolism in one or more at-risk phenotypes using the exercise condition (e.g. white vs. blacks, lower-body obese vs. upper-body obese, men vs. women). In addition, our novel and robust protocol can be advanced as a standardized one designed to assess exercise lipid use as a proxy for metabolic health to establish prognosis and potential exercise-related treatment options early in the progression of insulin

desensitization in a variety of at-risk subjects. For example, lingering question with regard to metabolic risk associated with patterns of fat storage (e.g. lower-body obese vs. upper-body obese, muscle vs. abdominal vs. subcutaneous), gender (e.g. female vs. male), race/ethnicity (e.g. white vs. black), genetics (nature vs. nurture), and more.

In closing, with respect to the exercise challenge we employed, control of numerous factors that are present with our assessment protocol allowed us to confidently clarify ambiguity regarding the influence of overweight/obesity on exercise lipid use. Specifically, in previous studies that have addressed this topic, exercise intensity was prescribed relative to $\dot{V}O_2$peak (Ara et al., 2011; Colberg et al., 1996; Croci et al., 2014; Devries et al., 2013; Ezell et al., 1999; Geerling et al., 1994; Goodpaster et al., 2002; Guesbeck et al., 2001; Helge et al., 1999; Horowitz & Klein, 2000; Kanaley et al., 1993; Lanzi et al., 2014; Larsen et al., 2009; Mittendorfer et al., 2004; Perez-Martin et al., 2001; Steffan et al., 1999; Zunquin et al., 2009; Thyfault et al., 2004), which means that subjects were likely working at different intensities relative to their maximal capacity for lipid oxidation (Meyer, Gabriel, & Kindermann, 1999; Weltman et al., 1990). Furthermore, in some studies, fitness level was not measured or controlled. In association with the aspects of control we applied for pre-test feeding (see above), we believe our exercise assessment protocol can be useful as a standardized method for assessing whole-body substrate selectivity and, by extension, metabolic function in individuals at risk for developing type 2 diabetes.

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