Changes in Fat Oxidation with Endurance Activity in Adults with and without Type 2 Diabetes

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Impaired metabolism of fatty acids is associated with obesity and type 2 diabetes (T2D). Based on evidence in lean adults, the expected response of skeletal muscle to aerobic training is an increase in the oxidation of fatty acids. However, considerably less is known about the response of fat oxidation to aerobic training in those with obesity and/or T2D. **PURPOSE:** 1) To determine if sedentary overweight adults with and without type 2 diabetes exhibit significant improvements in fatty acid metabolism at rest and during physical activity due to endurance training. 2) To compare changes in the oxidation of intramuscular triacylglycerols (IMTG) during sub-maximal exercise between those with and without T2D. **METHODS:** 13 (10 without T2D, 3 with T2D) overweight (BMI: 28-40 kg/m^2) men and women aged 28-55 completed an 8-week aerobic exercise intervention. Pre and post intervention, all subjects underwent a DEXA, maximal graded exercise test, and indirect calorimetry with non-radioactive labeled isotopes palmitate and acetate to determine energy expenditure, fat oxidation, and source of fatty acids for oxidation at rest and during exercise. **RESULTS:** VO_2max improved by an average of 14% (40.8$\pm$1.6 to 46.5$\pm$1.7ml/kg LBM/min) in the OW group ($p<0.01$) and 13.4% (34.8+4.5 to 38.0+1.7 ml/kg/LBM/min) in the T2D group ($p=0.10$). A non-significant increase in whole body fat oxidation during exercise was measured in both the OW (6.2%) and T2D (5.1%) group. There were no changes in whole body fat oxidation at rest in either group. Before and after intervention, IMTG oxidation during exercise was 4.13 $\pm$ 1.7 and 5.5 $\pm$ 2.3 uMol/kg

LBM/min in OW and 3.42 $\pm$ 1.9 and 2.41 $\pm$ 2.8 uMol/kg LBM/min in T2D. These changes were not significant due to the intervention (p=0.62). **CONCLUSIONS:** Eight weeks of moderate intensity aerobic exercise results in increased cardiorespiratory fitness but not a significant increase in whole body fatty acid oxidation during rest and exercise in overweight adults with or without type 2 diabetes. Moreover, oxidation of fatty acids from IMTG was not enhanced by the 8-week intervention.

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PREFACE

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1.0 INTRODUCTION

The most plentiful source of energy within the body is fat. Found as adipose triglycerides, within lipoproteins, as plasma free fatty acids, and within muscle cells themselves, fat provides the primary source of energy for skeletal muscle during fasting and resting states and during low to moderate intensity exercise. A defect or decreased tendency of skeletal muscle to oxidize fatty acids in favor of the oxidation of carbohydrate is a characteristic and perhaps a contributor to obesity and insulin resistance (Kelley, D. E. et al. 1999). As a lean, healthy individual becomes more physically fit, the capacity of skeletal muscle to oxidize fatty acids during exercise increases. An unknown is if this characteristic of lower fatty acid oxidation with obesity and insulin resistance can be preferentially altered with an increase in physical activity. Therefore, the purpose of this investigation was to examine the effects of physical activity training on fatty acid oxidation in obese adults with and without type 2 diabetes (T2D). This study was a sub-investigation of a larger clinical trial in the Division of Endocrinology, Department of Medicine, at the University of Pittsburgh.

The recent dramatic rise in obesity and T2D is a global health pandemic. Recent data from the Centers for Disease Control approximates that over 65% of Americans are either overweight or obese, and greater than 13 million Americans are living with T2D (CDC et al. 2004). The cornerstones of T2D and obesity treatment are lifestyle modifications, such as increasing physical activity and reducing caloric intake. While caloric restriction is most

effective in reducing body weight, exercise is essential for maintaining weight loss (Kayman, S. et al. 1990; Wing, R. R. and Hill, J. O. 2001). The differing effects of reducing caloric intake versus increasing physical activity on fat oxidation may explain this divergent effect, yet the physiological basis underpinning this important effect of treatment is not known.

Weight loss alone may *decrease* absolute rates of fat oxidation along with resting metabolic rate (Kelley, D. E. et al. 1999; Christiansen, M. P. et al. 2000). Alternatively, when non-obese adults perform endurance physical activity, skeletal muscle *increases* its ability to use fat as a substrate for oxidation (Romijn, J. A. et al. 1993; Martin, W. H., 3rd 1997; Christiansen, M. P. et al. 2000). This increase in skeletal muscle fat oxidation may positively impact metabolic pathways, increasing the success of weight loss and weight maintenance efforts. Whether this adaptation occurs at the same rate in obese individuals, and particularly individuals with T2D, is not yet recognized. A reduced-calorie diet and regular physical activity are essential for combating obesity and its related diseases; therefore, understanding the mechanisms that make individuals most successful is vital. This sub-investigation was undertaken to answer unresolved questions regarding the metabolism of fatty acids during endurance activity in people who are obese and overweight with T2D. Furthermore, this investigation sought to determine whether any potential up-regulation of fatty acid oxidation was primarily attributable to plasma or non-plasma sources.

1.1 RATIONALE

Obesity is a prime risk factor for hepatic and skeletal muscle insulin resistance as well as for T2D. There is accumulating evidence that obesity's role in contributing to the development of

insulin resistance and T2D is, in part, due to the accumulation of lipid within and around muscle cells (Kelley, D. E. et al. 2002). Previous research also points to a decrement or disturbance in skeletal muscle's ability to oxidize fatty acids both at rest and during physical activity (Kelley, D. E. et al. 2002). Because fatty acids are a significant substrate for skeletal muscle during fasting, resting, and moderate intensity physical activity, a disturbance in fatty acid oxidation within skeletal muscle could have a profound effect on total body homeostasis. To date, research has not shown whether these disturbances are caused by obesity or whether they perhaps predispose some individuals to becoming overweight, but the known negative health consequences are evident. For this reason, it is imperative to investigate fatty acid metabolism differences and discover whether lifestyle modifications, such as an increase in physical activity, can reverse any possible defects.

Zurlo's (Zurlo, F. et al. 1990) observational study of Pima Indians, a population with a known significantly increased incidence of obesity and T2D, found an association of reduced fatty acid metabolism and obesity. This three year follow up study concluded that a decreased ratio of fat to carbohydrate oxidation was associated with weight gain, independent of physical inactivity and may contribute to the familial aggregation of obesity seen in this population. Zurlo's observations came nearly 30 years after Randle proposed the theory of a lipid-induced impairment of glucose metabolism that could cause insulin resistance (Randle, P. J. et al. 1964). This "glucose-fatty acid cycle" and whether impairments in fatty acid or glucose metabolism are the primary culprit of insulin resistance continue to be debated today (Unger, R. H. 2008).

Physical activity is the only known, non-pharmacological, method of increasing fatty acid oxidation within muscle cells. The expected response of muscle tissue to an increase in regular aerobic activity is an increased reliance on fatty acids as the primary fuel substrate, thus sparing

glycogen stores. Current literature is not decisive as to whether muscle substrate oxidation in overweight individuals or those with T2D responds to physical activity training in this expected manner. This lack of any conclusive pattern in these populations is likely due to differences in length and intensity of the exercise intervention, testing conditions, possible gender differences, as well as concurrent alterations in caloric and macronutrient intake that may alter substrate oxidation. Because an increase in physical activity theoretically *may* be an excellent treatment for the lipotoxicity associated with obesity and T2D, answering this question is imperative.

A second, much more clearly documented benefit of regular physical activity is a *non*-insulin dependent uptake of plasma glucose by myocytes in individuals with type 2 diabetes (Goodyear, L. J. et al. 1992). To treat damaging hyperglycemia, most pharmaceuticals either increase beta cell production and the release of insulin or target insulin dependent receptors of myocyte and hepatocyte cells, allowing a greater influx of glucose into the mitochondria for oxidation. If indeed the primary cause of many cases of insulin resistance and T2D seen with obesity is due to a defect in fatty acid delivery, uptake, or oxidation, and not glucose metabolism, the best approach to treating these conditions would indeed be methods that target fatty acid oxidation directly, such as physical activity.

In addition to conflicting reports regarding fatty acid utilization in people with overweight and T2D, there are further gaps in the literature. At the commencement of this project there were limited reports on fatty acid utilization after endurance training in overweight subjects and no reports in subjects with T2D. Furthermore, it is not known if any seen changes are attributable to an increased uptake and oxidation of plasma free fatty acids or whether non-plasma fatty acids, i.e. fatty acids stored within myocytes as intramuscular triacylglycerols (IMTGs) are mobilized for this purpose. The goal of this study was to address the unanswered

question of the effect of endurance training on substrate oxidation in subjects with T2D and to offer clarity to conflicting data in obesity. Results of this study may lead to a better understanding of the abnormal metabolism of lipids attributed to obesity and T2D and whether physical training can positively alter any defects. Such information may provide a better insight into the disease processes, identifying alternate targets for prevention and treatment options for overweight, obesity, and T2D.

1.2 PURPOSE

In this prospective intervention study, we examined the extent to which an 8-week endurance physical activity program increases the capacity of skeletal muscle to oxidize fatty acids during exercise in overweight adults with and without T2D. By studying overweight subjects with and without T2D, we intended to examine whether having T2D further limits one's ability to oxidize fatty acids more than obesity may alone. We specifically tested whether these improvements came predominantly from non-plasma fatty acids sources, i.e. increases in IMTG oxidation or simply from a greater uptake and oxidation of plasma fatty acids.

1.2.1 Specific Aims

The primary aims of this project were to:

1. Determine if sedentary overweight adults with and without T2D exhibit significant improvements in fatty acid oxidation at rest and during physical activity due to endurance training.

2. Compare changes in non-plasma fatty acid oxidation during sub-maximal exercise between previously sedentary overweight adults with and without T2D after endurance training.

The secondary aims of this project were to:

1. Compare changes in fitness due to endurance training in previously sedentary overweight adults with and without T2D.
2. Compare changes in body weight and composition due to endurance training in previously sedentary overweight adults with and without T2D.

1.2.2 Research Hypotheses

Primary Hypotheses:

1. Overweight adults with T2D will have improvements in fatty acid oxidation, both at rest and during exercise with endurance training but not to the same extent as overweight adults without T2D.

2. Overweight adults with T2D will have improvements in non-plasma fatty acid oxidation with endurance training but not to the same extent as overweight adults without T2D.

Secondary Hypotheses:

1. Overweight adults with and without T2D will have significant improvements in fitness due to endurance training.

2. Overweight adults with and without T2D not have significant changes in body weight or body composition due endurance training.

1.3 SIGNIFICANCE

A recent study estimates that T2D is now the 5th leading cause of mortality worldwide with over 2.9 million deaths caused by diabetes in the year 2000 (Roglic, G. et al. 2005). In the US, the prevalence of T2D has more than doubled over the last 20 years (Flegal, K. M. et al. 2002). Obesity, defined as a body mass index (BMI) of greater than or equal to 30 kg/m^2, has increased at a similar rate. While there are many risk factors of T2D in addition to obesity (age, family history of T2D, ethnicity, impaired glucose tolerance, increased waist to hip ratio, and physical inactivity) the rapid acceleration of T2D and obesity are highly correlated. It is well accepted that increasing rates of obesity have contributed significantly to the escalating incidence of T2D. Research by Mokdad et al shows a positive relationship between increasing BMI and increased risk of T2D (Mokdad, A. H. et al. 2003). This relationship is expressed as an increasing odds ratio in which adults who are normal weight (BMI <25 kg/m$^{2)}$, overweight (BMI 25.0-29.9 kg/m^2), obese class 2 (BMI 30-39.9 kg/m^2) and obese class 3 (BMI> 40 kg/m^2) have odds ratios for developing T2D of 1.00, 1.59, 3.44, and 7.37 respectively.

Weight loss via a reduced calorie diet is an accepted safe and potent tool for managing glucose in adults with T2D. The positive effects of decreasing weight by as little as 5-10% are well documented and benefit all genders and ethnicities. Specifically, a reduced calorie weight

loss diet improves glucose control and reduces the risks associated with diabetic cardiovascular disease, including unsafe blood lipids, blood pressure and central adiposity (Schaumberg, D. A. et al. 2005). The benefits of increased physical activity *exclusive* of weight loss are also well evidenced with improvements in glycemic control, body composition, blood lipids, blood pressure, cardio-respiratory fitness and fibrinolytic functioning (Boule, N. G. et al. 2001; Di Loreto, C. et al. 2005). Furthermore, the National Weight Control Registry has shown that long-term maintenance of weight loss is strongly related to the amount of physical activity sustained over time (Wing, R. R. and Phelan, S. 2005).

While total energy balance is the ultimate determinant of weight maintenance, obesity, a family history of obesity and prior-obesity alter the control of metabolic pathways that regulate energy storage and expenditure (Ranneries, C. et al. 1998; Blaak, E. E. et al. 2000; Kanaley, J. A. et al. 2001). Increasing skeletal muscle fat oxidation via physical activity may positively impact these pathways to improve and maintain weight loss. The effect of endurance activity on rates of fat oxidation in obese adults with and without T2D has not been elucidated. This sub-investigation was undertaken to answer this previously unknown question.

In the fasted state, lean individuals oxidize a higher proportion of fat than obese individuals (Lean, M. E. and James, W. P. 1988; Zurlo, F. et al. 1990), and skeletal muscle increases its reliance on fat as a substrate versus glucose during sub-maximal exercise (Carter, S. L. et al. 2001). The effect of a single bout of exercise on the rate of fat oxidation in overweight adults is unclear, with reports of increased rates, no change, and decreased rates (Ranneries, C. et al. 1998; Ezell, D. M. et al. 1999; Mensink, M. et al. 2005). Few studies have tested the effects of endurance training on fat oxidation in the obese. One such project reported greater reliance on fat oxidation during fasting conditions after a 16 week weight loss and endurance exercise

intervention (Goodpaster, B. H. et al. 2003). This project did not measure fat oxidation *during* exercise bouts nor did it include individuals with T2D. A review of the literature finds no studies that examine the effect of endurance training on fat oxidation during exercise in individuals with T2D.

Current literature suggests that adults with T2D may not have the same metabolic adaptations as their non-diabetic counterparts, in part due to mitochondrial defects associated with T2D (Schrauwen, P. et al. 2002; Menshikova, E. V. et al. 2005). In 2002, Kelley et al demonstrated that mitochondria of insulin resistant T2D subjects were smaller and had reduced oxidative capacity when compared to insulin-sensitive subjects without T2D (Kelley, D. E. et al. 2002). Weight loss without an increase in physical activity does not appear to improve this impaired capacity to oxidize fats. This finding may therefore be an indication as to why weight maintenance following weight loss in the absence of regular physical activity is so difficult to achieve. Thus, the contribution of this 8-week exercise intervention is to provide a further understanding of how physical activity affects muscle's ability to oxidize fatty acids in overweight adults with and without T2D. This information may contribute to our understanding of the potentially powerful role of exercise in the prevention and treatment of obesity and T2D.

2.0 REVIEW OF THE LITERATURE

The intention of this study was to determine whether 8 weeks of physical endurance training increases the capacity of skeletal muscle to oxidize fatty acids in overweight adults with and without T2D. We then compared the two groups to determine if the presence of T2D further inhibits fatty acid oxidation compared to obesity alone. This study also sought to determine if any improvements seen in fatty acid oxidation were from an increased oxidation of IMTGs or simply from a greater uptake and oxidation of plasma fatty acids. Such information may contribute to a better understanding of the role of physical activity in the prevention and treatment of obesity and T2D as well as provide insight as to why physical activity may be so vital in preventing the regain of weight after a significant weight loss. The following review of the literature provides support for the importance of these research questions.

2.1 OBESITY AND TYPE 2 DIABETES

The prevalence of overweight and obesity in both adults and children in the United States is a leading public health concern (NTF 2000; Flegal, K. M. et al. 2005). For both clinical and research purposes, overweight and obesity are most often defined in terms of body mass index or BMI. A person's BMI is his weight in kilograms divided by the square of his height in meters.

A BMI $\geq$ 25 kg/m^2 is defined as overweight and a BMI $\geq$ 30 kg/m^2 is defined as obese. The importance of addressing weight and its health effects on a national level was evidenced by the 2001 release of the Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity. The US government has targeted efforts to treat and prevent overweight and obesity in many different forums including in public schools, in the work place, directly to families and communities, through the media, and via health care providers. One such initiative was the 2003 US Preventative Services Task Force recommendation that clinicians screen all adult patients for obesity and offer intensive counseling and behavioral intervention to maintain a significant weight loss (USPSTF 2003).

This national emphasis on treating and preventing overweight and obesity in our population has unfortunately not yet reversed the prevalence rate among the majority of the US population. Despite these public health efforts, the most recent data published from the National Health and Nutrition Examination Survey (NHANES) in April, 2006, concludes that the prevalence of overweight among children and adolescents has actually continued to rise as has the prevalence of obesity among men (Ogden, C. L. et al. 2006). The percent of children and adolescents who are at risk of becoming overweight or are overweight was 28.2% in 1999-2000 and increased to 33.6% in 2003-2004. Similarly, the prevalence of obesity among men has increased to 31.1% in 2003-2004, up from 27.5% in 1999-2000. This most recent data estimates that 66.3% of all US adults are either overweight or obese with 32.2% falling into the obese category. The prevalence of overweight and obesity in women remained stable during this six year time period.

Similarly, the prevalence of T2D has more than doubled in the United States over the last 20 years. From 1980 through 2002, the number of Americans with diabetes increased from 5.8

million to 14.7 million (Centers for Disease Control. 14 May, 2004). In 1998, King et al, made projections on the worldwide prevalence of diabetes if the current rapid rate of increase continues. By the year 2025, the authors estimate that the worldwide prevalence of diabetes will topple 300 million (King, H. 1998). The treatment of diabetes and its complications has created an enormous burden on our medical and economic systems. The total annual economic cost of diabetes in 2002 was approximately $132 billion, or one out of every 10 health care dollars spent in the United States (American Diabetes Association, June 2005). Heart disease and stroke are the most life-threatening consequences of diabetes and occur at twice the rate in people with diabetes compared to those without. In fact, more than 65 percent of deaths in patients with diabetes are attributed to heart and vascular disease (Centers for Disease Control, 2000). Discovering and understanding the most effective methods to prevent and treat T2D and obesity is vital to controlling these costs and improving the health of our population.

2.1.1 Health Risks of Overweight, Obesity, and Type 2 Diabetes

Scientists and clinicians have attempted to quantify the health risk of overweight and obesity in a variety of ways from economic costs, to years of life lost, to specific rates of morbidity and mortality. Regarding morbidity and mortality, it is evidenced that as BMI increases over 20 kg/m^2, so does the risk of morbidity from many health conditions as does the risk of mortality (NIH 1998). Some health conditions that are strongly correlated with increasing BMI include hypertension, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, and some types of cancers (NTF 2000). Additionally, several studies show that having a BMI of 30 kg/m^2 or greater increases the risk of death from all causes by 50-

100% compared with adults with a BMI between 20-25 kg/m^2 (Troiano, R. P. et al. 1996; NIH 1998). In 1991, over 280,000 deaths were attributable to obesity among US adults (Allison, D. B. et al. 1999). In 2004, an analysis of the two major risk factors for obesity, poor nutritional intake and physical inactivity, accounted for over 400,000 annual deaths in the United States (Mokdad, A. H. et al. 2004).

Data from the National Health Interview Survey demonstrated that from 1997 to 2003, the incidence of diagnosed diabetes increased from 4.9 to 6.9 per 1000 people, an increase of 41% (Geiss, L. S. et al. 2006). After adjusting for education and income level, smoking status, gender, and physical inactivity, the authors concluded that age, race/ethnicity, and BMI were all major predictors of new incidence of type 2 diabetes. Similarly, a study by Daousi C et al conducted in the United Kingdom concluded that of patients in a community care setting, those with a BMI >30 kg/m^2, had significantly poorer glycemic control, higher blood pressures, and worse lipid profiles. They were also on more medications than those with a BMI< 30 kg/m^2 (Daousi, C. et al. 2006). These finding are consistent with previous landmark studies that demonstrated that a change in lifestyle behaviors (i.e. decreased caloric intake and increased physical activity) that results in decreased body weight can significantly decrease the incidence of type 2 diabetes (Tuomilehto, J. et al. 2001) (Knowler, W. C. et al. 2002). The Diabetes Prevention Program, a multi-center, randomized clinical trial with over 3,000 adults of both genders and multiple racial and ethnic groups, demonstrated that improved lifestyle behaviors can reduce the incidence of T2D by 58% (Knowler, W. C. et al. 2002). Both decreased caloric intake and increased physical activity were emphasized in the diabetes prevention program, and research concluded that both behaviors played a role in preventing the onset of T2D. The current

study will add to the understanding of the role of physical activity and specifically the oxidation of fatty acids in treating obesity and T2D.

2.1.2 Management of Type 2 Diabetes with Lifestyle Changes

The benefits of tightly controlling glucose levels in individuals with T2D have been confirmed many times and were most clearly documented in the landmark UK Prospective Diabetes Study (1998). Compared with conventional care, long-term intensive treatment and control of hemoglobin A1c (HbA1c) resulted in a 25% risk reduction in all microvascular endpoints. Significant reductions in macrovascular disease risk were not seen in this project; however, and it should be noted that weight management was not a goal of this clinical trial. Recently, a follow up to the Diabetes Control and Complications Trial established that intensive glucose management in patients with type 1 diabetes does reduce many of the risk factors associated with cardiovascular disease, such as LDL cholesterol, triglycerides, and several inflammatory markers (Nathan, D. M. et al. 2005).

A reduced calorie diet is a safe and potent tool for managing glucose in adults with T2D. Many sources have documented the benefits of decreasing body weight by as little as 5-10 percent in both genders and all ethnicities. Specifically, a reduced calorie diet results in improvements in glucose control as measured by fasting glucose, HbA1c, and insulin sensitivity. Weight loss also improves many of the risk factors associated with diabetic cardiovascular disease including blood lipids and blood pressure.

The benefits of increased physical activity *exclusive* of weight loss in the treatment of T2D have also been well evidenced. A meta analysis reviewing the effect of exercise on

glycemic control in adults with T2D demonstrated an overall mean difference in HbA1c of 0.66% between exercise and control groups, a reduction that if sustained over time would result in significant reductions in diabetic complications (Boule, N. G. et al. 2001). Physical activity has additional cardio protective benefits including favorable changes in body composition, blood lipids, blood pressure, cardio respiratory fitness, and fibrinolytic functioning (Ruderman, 1995). DiLoreto et al (2005) most recently punctuated this in a post hoc analysis of a physician based, randomized intervention in which increased amounts of moderate walking resulted in decreases in all markers of glycemic control and cardiovascular risk factors as well as significant decreases in money spent on managing diabetes and other health conditions. In addition to having favorable effects on specific disease risk factors, physical activity is directly related to the long-term maintenance of a weight loss. The relationship of regular physical activity and weight loss maintenance, as evidenced by the National Weight Control Registry and other long-term clinical trials is discussed below. All of these data indicate that a lifestyle approach of incorporating both a reduced caloric intake and an increase in physical activity is a safe and powerful tool in the management of T2D.

2.1.3 The Role of Physical Activity in Weight Loss Maintenance

Significant weight loss via a reduced calorie diet is possible for people who are overweight, obese, and/or diagnosed with T2D. The *maintenance* of a significant weight loss over a long period of time however remains a clinical and public health challenge. Even well controlled weight loss studies in which subjects are given long term support show a significant amount of weight regain over time (Vogels, N. et al. 2005) (Leser, M. S. et al. 2002).

The National Weight Control Registry (NWCR), established in 1994, is the largest prospective study of long-term successful weight loss maintenance. Because maintaining weight loss seems so elusive, the NWCR was developed to identify and investigate the characteristics of people who have succeeded at long-term weight loss. The NWCR is currently tracking over 5,000 adults who have lost significant (30+lbs) amounts of weight and kept it off for long periods of time (1 year+). The importance of physical activity in the maintenance of weight loss is evident in the NWCR population. Successful weight maintainers in the NWCR report expending an average of 2,800 kcal/week in physical activity, with more than 90% of successful NWCR participants reporting this high level of physical activity. One report analyzing weight loss maintenance after 1 year of enrolling in the NWCR concluded that time spent in physical activity is a significant predictor of weight maintenance and that alternatively low physical activity is a predictor of weight regain (McGuire, M. T. et al. 1999).

In addition to observational data supporting the importance of exercise in weight maintenance, other studies have also found a strong correlation between physical activity and weight maintenance (Donnelly, J. E. et al. 2003). A meta-analysis of 6 randomized controlled trials compared the effects of a reduced calorie diet alone versus a reduced calorie diet with an exercise program on weight loss and subsequent weight maintenance after one year (Wing, R. R. 1999). Study participants in the reduced calorie diet and exercise arm fared better at maintaining their weight than the reduced calorie diet only group in all 6 studies, though only two of the 6 studies reached a statistically significant difference between the groups. A larger meta-analysis that included both observational, as well as randomized controlled trials also concluded that although statistical significance could not be reached, subjects who included a regular physical activity program in their lifestyle tended to maintain a greater weight loss, and the level of

activity was proportional to the amount of weight loss maintained (Fogelholm, M. and Kukkonen-Harjula, K. 2000). Although high levels of physical activity are associated with long-term success of weight maintenance (Jakicic, J. M. and Otto, A. D. 2005; Jakicic, J. M. and Otto, A. D. 2005), the exact physiologic mechanism beyond its contribution to a caloric deficit is not known. Because physical activity in the only known safe method for increasing total fat oxidation, perhaps a significant increase in physical activity can compensate for any impairment in fat oxidation that exists in obese or post-obese individuals.

2.2 FATTY ACID OXIDATION

Because maintaining a significant weight loss seems to be so elusive for many patients, scientists have begun to investigate whether metabolic differences exist between people that make weight loss maintenance more difficult for some. One such consequence of weight loss reported by several researchers is a reduced capacity for fat oxidation after weight loss (Bryson, J. M. et al. 1996) (Kelley, D. E. et al. 1999). Such a reduction could at least in part explain why maintaining weight loss is so difficult and why physical activity, besides creating a caloric deficit, appears to be so crucial for weight loss maintenance. Prior to reviewing literature regarding the oxidation of fatty acids in specific states such as during exercise, in overweight versus non-overweight individuals and in the presence of diagnosed T2D, it is important to review the metabolic process of fatty acid oxidation.

2.2.1 Overview of Fatty Acid Oxidation

Endogenous triacylglycerols provide the largest amount of stored fuel in the body. While triacylglycerols are stored primarily in adipose tissue, they are also present in significant amounts in skeletal muscle and plasma. In a lean adult male, endogenous triacylglycerols represent an enormous amount of energy, approximately 133,840 kilocalories. This large fuel source is utilized at its highest rate during periods of fasting, such as overnight, and during prolonged bouts of moderate intensity physical activity.

For fatty acids to be used as energy by the body's muscles, triacylglycerols must be hydrolyzed from adipose tissue, muscle or plasma. These fatty acids must then be transported to skeletal muscle and taken up by mitochondria for oxidation. The majority of free fatty acids (FFA) released from adipose tissue are transported across the muscle membrane via transport proteins while a much smaller amount diffuse across the membrane. Once in the cytosol, FFAs from adipose tissue or from within skeletal muscle itself must join with fatty acid binding proteins (FABP) for transport to the surface of the outer mitochondrial membrane. FFAs are then converted to a fatty acyl carnitine compound by binding with coenzyme A (CoA). Finally, fatty acyl carnitine is moved across the outer mitochondrial membrane via carnitine palmitoyltransferase I (CPT-I) and then across the inner mitochondrial membrane via carnitine palmitoyltransferase II (CPT-II). Once inside the mitochondria, the carnitine is removed, the CoA is rebound, and the fatty acyl-CoA molecules are free to enter the beta-oxidation pathway. Beta-oxidation of fatty acids is a cyclic set of 4 reactions in which two carbons are removed in each cycle. For each two carbons cleaved from the chain, 5 adenosinetriphosphate (ATP) molecules are produced. These ATPs are a result of the formed reducing equivalents $FADH_2$ and NADH entering the electron transport chain. Each cycle of reactions also produces an

acetyl-CoA molecule that produces an additional 12 ATP molecules via the tricarboxylic (TCA) cycle. (Refer to Figure 1.)

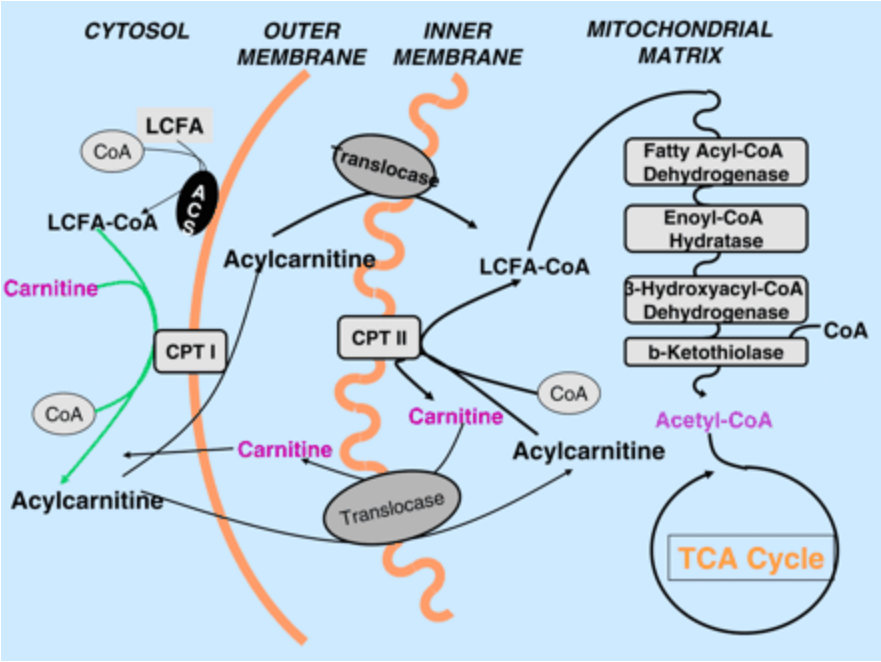


Figure 1: Fatty acid transport into the mitochondria and subsequent beta-oxidation

Keins B Physiol Rev 2006;86:205-243.

During periods of fasting, such as overnight, adipose tissue provides the majority of the body's energy needs by releasing fatty acids into the blood stream for oxidation (Klein, S. et al. 1986). Hormones that stimulate and inhibit hormone sensitive lipase (HSL) regulate this process of lipolysis. During such periods, the amount of fatty acids released from adipose tissue is significantly greater than the amount of oxidized fatty acids. The liver re-esterifies those fatty acids not oxidized back into triacylglycerols.

During exercise, the control of skeletal muscle fat metabolism appears to be regulated at 4 possible sites (Kiens, B. 2006). The first site of control is the lipolysis of fatty acids from

adipose tissue and its subsequent delivery to muscle. The second site is the movement of FFAs across the muscle membrane into the cytosol. The third site of control is the regulation of HSL activity and the fourth is the regulation of FFAs across the mitochondrial membrane by carnitine palmitoyltransferase 1 (CPT-1).

2.2.2 Fat Oxidation During Aerobic Activity in Non-Overweight Individuals

During low and moderate intensity exercise in healthy, lean individuals, skeletal muscle metabolism gradually increases its reliance on fat oxidation (Jeukendrup, A. E. et al. 1998) (Horowitz, J. F. and Klein, S. 2000). This increase during prolonged bouts of moderate physical activity is estimated to be 5-10 times greater than the rate of fatty acid oxidation at rest (Krogh A, L. J. 1920). The increased demand for fatty acids for energy is supplied by both an estimated 2-3 fold increase in the lipolysis of fatty acids from adipose tissue (Wolfe, R. R. et al. 1990) (Klein, S. et al. 1994) as well as a decrease in the amount of released fatty acids that are reesterified by the liver (Wolfe, R. R. et al. 1990) as blood flow is largely redirected to exercising muscles for delivery of oxygen as well as released fatty acids. This metabolic shift during an acute bout of exercise has been well described in healthy individuals (Schaumberg, D. A. et al. 2005) (Boule, N. G. et al. 2001) as well as in obesity and T2D (Colberg, S. R. et al. 1996) (Mensink, M. et al. 2001) (Kang, J. et al. 1999) (Borghouts, L. B. et al. 2002).

As exercise intensity increases from rest, the absolute contribution of fat to total energy production increases. The absolute contribution of fat to total energy production peaks between 50-65% of maximal oxygen uptake (VO_2max) and decreases as exercise intensity nears approximately 85% of VO_2max. Aerobic training significantly enhances the body's ability to

use fat as a fuel source during moderate intensity activity even further. This adaptive response is possible for several reasons. First, there is an increase in capillary density from training that allows a greater blood flow to reach exercising muscle, thus increasing the delivery of fatty acids. Aerobic training also increases the density of mitochondria in skeletal muscle, providing a greater capacity to oxidize fat (Holloszy, J. O. 1967). There is also an increase in the enzyme CPT-1, the enzyme that transports fatty acids across the outer mitchondria membrane (Mole, P. A. et al. 1971) as well as an increase in fatty acid binding proteins that regulate myocyte fatty acid transport (Turcotte, L. P. et al. 1999). This adaptive response to training has been shown to a significant degree in athletes (Jansson, E. and Kaijser, L. 1987) as well as in sedentary non-obese adults (Kiens, B. et al. 1993), and the elderly (Sial, S. et al. 1998). A few studies have found a positive response in obese adults (van Aggel-Leijssen, D. P. et al. 2001; van Aggel-Leijssen, D. P. et al. 2002) and perhaps even in adults with impaired glucose tolerance (Mensink, M. et al. 2005). (This study will be discussed in more detail later.) Whether endurance training improves the ability of people with T2D to utilize fatty acids for energy during physical activity has not yet been reported.

2.2.2.1 Non-plasma, Intramuscular Triacylglycerol Oxidation

In addition to fatty acids from plasma and adipose tissue, intramuscular triacylglycerols (IMTGs) may also provide an additional source of fatty acids for oxidation to exercising muscles (Klein, S. et al. 1994). Using stable isotope technology, several researchers have noted the possible contribution of this additional fuel source. The initial proof of this additional active fuel source during prolonged activity was evidenced by reports from several investigators showing the rate of fatty acid disposal (uptake from plasma) is actually lower than the rate of fatty acid

oxidation during the first 120 minutes of moderate intensity exercise (Kanaley, J. A. et al. 1993; Martin, W. H., 3rd et al. 1993; Romijn, J. A. et al. 1993). This negative balance suggests that fatty acids are coming from another energy source, presumably IMTGs. Coined the "athelete's paradox," it has been shown that the amount of IMTG stored in the muscles of endurance athletes is similar to that stored as IMTG in obese individuals (Goodpaster, B. H. et al. 2001) (van Loon, L. J. and Goodpaster, B. H. 2006). However, the cause for the existence of such a large amount of IMTGs in sedentary obese adults has not been determined. This large fat reserve may be the result of increased delivery or uptake of plasma FFAs to muscle tissue in obese individuals. However, it is also important to examine the capacity of skeletal muscle to oxidize FFAs. The "health" of the muscle may determine if excess FFAs will be disposed and oxidized or directed towards intracellular storage. Therefore, it is conceivable that one of the reasons for excess fat accumulation in skeletal muscle in T2D is the possibility of deficient oxidative capacity in this tissue, specifically in regards to utilization of IMTGs. (Refer to Figure 2.)

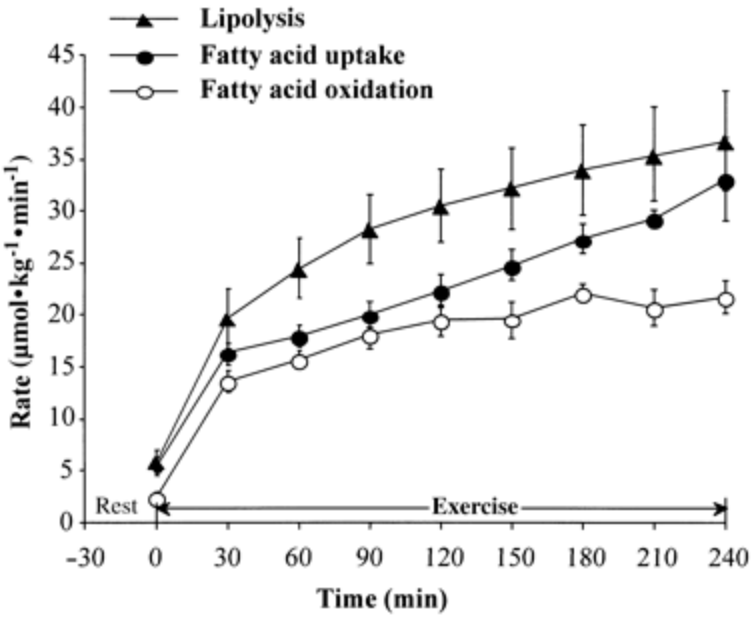


Figure 2: Rates of lipolysis, fatty acid uptake, and fatty acid oxidation at rest and during 4 h of treadmill exercise. Source: Horowitz JF and Klein S Am J Clin Nutr 2000;72:558S-563 Rates of lipolysis (3 x the glycerol rate of appearance in plasma), fatty acid uptake, and fatty acid oxidation at rest and during 4 h of treadmill exercise performed at 45% of maximal oxygen uptake in untrained subjects.

The use of labeled isotope fatty acid tracers have permitted estimates of the portion of fatty acids used for energy from sources other than plasma. These non-plasma fatty acids from triglycerides can be estimated as the difference between total fat oxidation, as measured by indirect calorimetry, and plasma FFA oxidation as measured by a labeled carbon fatty acid infusion. Early research suggests that during the first two hours of exercise, IMTGs provide as much as 50% of the fatty acids used for oxidation by exercising muscle in young, healthy lean subjects (Martin, W. H., 3rd et al. 1993; Stellingwerff, T. et al. 2007). Interestingly, Boon found a much lesser contribution of IMTGs to total fatty acid oxidation and no differences between

long-term exercising endurance trained males and matched sedentary subjects when studying older (58±2 years), but not elderly, adults (Boon, H. et al. 2007).

2.2.3 Obesity and T2D are Associated with a Decline in Fat Oxidation

Previous research strongly indicates that there is a decline in FFA oxidation with obesity and T2D (Kelley, D. et al. 1994; Kelley, D. E. et al. 1999). Several examples depicting reduced rates of fat oxidation post weight loss were described earlier. Because these two conditions of obesity and T2D frequently occur together, it is possible that both share disturbances in fat metabolism. Some scientists have theorized that a decline in FFA oxidation leads to increased fat storage within adipose tissue and skeletal muscle, thereby promoting obesity and insulin resistance, a hallmark characteristic of T2D (Blaak, E. E. 2004). While weight loss does improve insulin resistance in obese adults with and without T2D, it does not seem to improve this impaired capacity to utilize fat for energy. This has been demonstrated in obese subjects *after weight loss* during beta adrenergic stimulation (Blaak, E. E. et al. 1994) as well as at rest (Kelley, D. E. et al. 1999) and in adults with obesity and T2D during exercise (Blaak, E. E. et al. 2001). This may be an indication as to why exercise helps to maintain weight loss and may even suggest that impairments in fat oxidation are a primary cause of obesity rather than a consequence (Blaak, E. E. and Saris, W. H. 2002). Also supporting this theory is a prospective study of Pima Indians that found that a decreased reliance on fat as a fuel source, as measured by 24 hour RQ, is a risk factor for weight gain (Zurlo, F. et al. 1990). Thus, a thorough understanding of how physical activity affects muscle's ability to oxidize fat in people with

obesity and T2D will provide important data concerning the role of exercise in the prevention and treatment of these diseases.

2.2.3.1 Imtramuscular Triacylglycerol Utilization During Exercise in Obesity and Type 2 Diabetes

Two separate cross sectional studies revealed no differences in total fat oxidation during a single bout of moderately intense exercise but rather a difference in the *source* of fatty acids used for oxidation when comparing obese adults with lean adults matched for age and aerobic capacity (Horowitz, J. F. and Klein, S. 2000) (Goodpaster, B. H. et al. 2002). The population of the first study was women with abdominal obesity while the second study evaluated obese men. Both projects reported significantly higher non-plasma, i.e. IMTG, fatty acid oxidation during exercise in obese adults, indicating a variance from the "normal" pattern of substrate oxidation during exercise in lean adults. The additive effect of having T2D was compared with non-diabetic obese matched controls in a similar study of fat oxidation during a single bout of moderate intensity exercise (Blaak, E. E. et al. 2000). Again, total rates of fat oxidation were similar between the two groups, but those with T2D had significantly lower rates of FFA appearance and disappearance compared with control subjects at rest and during exercise. Similarly, the oxidation of plasma fatty acids was significantly lower in T2D than in controls at rest and during exercise while rates of non-plasma fatty acids was greater in T2D in both conditions. This was the first study to show an additional impairment in the oxidation of plasma fatty acids with T2D over obesity. The authors hypothesize several reasons for this impairment. First, the lower rate of appearance of FFA may be explained by an increased re-esterification within adipose tissue. Second, people with T2D may have an increase in muscle lipolysis, which may flood the muscle with FFA, thus decreasing the blood tissue concentration gradient,

resulting in a lower plasma fatty acid uptake and oxidation. Lastly, T2D may cause a disturbance in plasma fatty acid transport across the cytoplasm. This last hypothesis is supported by Blaak's evidence of a reduced concentration of fatty acid binding proteins in the skeletal muscle of adults with T2D (Blaak, E. E. et al. 2000) as well as data showing increased lipid droplet concentration in myocytes of individuals with T2D. These droplets located very near the mitochondria are not reliant on cytosolic transport (Hoppeler, H. 1999).

2.2.4 Effects of Weight Loss on Fatty Acid Oxidation

Larson et al. compared resting metabolic rate (RMR) and respiratory quotient (RQ) in post obese individuals with a weight stable control group matched for age and body weight. After adjusting for fat-free mass, fat mass, age, and gender, energy expenditure (i.e. RMR), was not significantly different between the groups. Post obese subjects, however, did have significantly higher RQs over a 24-hour period compared with control subjects. This suggests that post-obese individuals have lower rates of fat oxidation, predisposing a greater rate of fat storage, which may contribute to the tendency to regain lost weight (Larson, D. E. et al. 1995). Postprandial fat oxidation has also been shown to be reduced in post obese adults measured by RQ compared with subjects matched for age and weight (Raben, A. et al. 1994). Astrup et al also found a significant difference in fat oxidation between post-obese and matched control subjects after each group consumed a high fat diet (Astrup, A. et al. 1994). In this study, control subjects appropriately increased their ratio of fat to carbohydrate oxidation after following a high fat diet for three days while post-obese subjects did not. This low capacity for fat oxidation could contribute to a positive fat balance and weight regain, especially when eating a fat-rich

diet. Faraj et al conducted a more detailed study in which post-obese women that were 2-3 years post-gastric banding were age and weight matched with control subjects (Faraj, M. et al. 2001). This study evaluated fat metabolism using a labeled fatty acid (oleate) isotope tracer. The post-obese subjects did not increase their enrichment of non-esterified fatty acids (NEFA) in circulation after 8 hours, while NEFAs were markedly increased in control subjects. This suggests a tendency towards peripheral fat storage in the post-obese subjects. Post-obese subjects also had lower postprandial insulin, leptin, and acylation-stimulating protein levels.

Individuals with T2D can achieve the same amount of weight loss as those without T2D; however, a study looking at weight maintenance after a behavioral weight loss intervention suggests that those with T2D may have a more difficult time maintaining that weight loss (Guare, J. C. et al. 1995). In this particular study, women with T2D lost an average of 7.4 k g in a 16-week behavioral weight loss program. Age and weight matched controls lost an average of 6.4 kg . At the year one follow-up, those with T2D had regained 5.4 kg compared with 1.0 kg in the control group. While this only suggests that having T2D may predispose an individual to weight regain, other research suggests this may not be due solely to differences in behaviors. One such study which examined obese adults with and without T2D 5 years after a weight loss program concluded that both groups had significant weight regain and reduced resting fat oxidation as well as reduced metabolic flexibility during a hyperinsulinemic clamp after weight regain. Those with T2D, however, had a significantly lower RQ during the hyperinsulinemic clamp study than those without T2D (Poynten, A. M. et al. 2003). Because a regular exercise program appears to be crucial for successfully maintaining weight loss long term, it is important to investigate whether some people with obesity and/or T2D have impairments in fatty acid oxidation that may predispose them to regain lost weight. In doing so, it is also of importance to

examine the effects of an endurance exercise-training program on fat oxidation during exercise as well as at rest.

2.2.5 Exercise Training Effects on Fat Oxidation in the Presence of Obesity and Type 2 Diabetes

Little is known about substrate oxidation *after* endurance training in obesity or T2D and much of the data is conflicting. Following are a few of the key studies. Please note that there is a lack of available research regarding endurance training without a concurrent weight loss intervention, which prevents the separate analysis of the two interventions on metabolic changes. At least two separate studies have shown that endurance training can blunt the decline in resting fat oxidation that normally occurs with weight loss (Nicklas, B. J. et al. 1997) and (van Aggel-Leijssen, D. P. et al. 2001). More recently, a study by Mensink et al concluded that a combined lifestyle intervention of weight loss and aerobic activity prevented the *further deterioration* of impaired fat oxidation during exercise in adults with impaired glucose tolerance (Mensink, M. et al. 2005). In this intervention, 9 adults with impaired glucose tolerance and 7 matched control subjects participated in a 12 month program that included regular individual meetings with a dietitian to reduce caloric intake and increase physical activity to at lest 30 minutes of walking/5 days/week. While control subjects saw a decline in their capacity to oxidize fatty acids over time, as measured by indirect calorimetry, those in the intervention group, although they did not significantly improve, did maintain their baseline ability to oxidize fatty acids even with a small amount of weight loss.

Current literature provides conflicting results of the effect of endurance training on fat metabolism *during* exercise. An eight-week exercise training intervention at 45% of VO_2max in obese men resulted in an increased total fat oxidation without a change in lipolysis or FFA availability (Kempen, K. P. et al. 1995) as did a similar study at 40% VO_2max that further showed not only an increase in total fat oxidation in obese men, but that the increase was largely due to an increase in non-plasma fatty acid oxidation (van Aggel-Leijssen, D. P. et al. 2002). These two studies are contrasted with at least one other published work in which fat oxidation during exercise in obese women *after* exercise training was not increased, but rather only carbohydrate oxidation was increased (Kanaley, J. A. et al. 2001).

To date, there are no published data investigating the effects of endurance training on fat oxidation during physical activity in individuals with T2D. Nor have there been many published studies on the effects of endurance training on fat oxidation in individuals with obesity without the confounding factor of weight loss. The results of this proposed study more clearly define the metabolic benefits that obese adults with and without T2D may expect from exercise training.

2.3 SUMMARY

Significant weight loss via a reduced calorie diet is possible for obese people with and without T2D. However, regardless of the presence of diabetes, maintaining a significant weight loss remains a clinical and public health challenge. Even well controlled long-term weight loss studies (in which subjects are given long term support) report a significant amount of weight regain over time.

This observation has led scientists to investigate whether metabolic differences exist that make weight loss maintenance more difficult for some people. One such consequence of weight loss reported by several researchers is a reduced capacity for fat oxidation after weight loss. Such a reduction could in part explain why maintaining weight loss is so difficult and why physical activity, besides creating a caloric deficit, appears to be so crucial for weight loss maintenance. As physical activity is the only known proven safe method for increasing the body's ability to utilize stored fat for energy, this study may also provide metabolic insights into alterations in fatty acid metabolism associated with obesity and T2D.

During a session of prolonged moderate physical activity, skeletal muscle gradually increases its reliance on fat oxidation. In healthy individuals aerobic training enhances this metabolic adaptation; however, whether individuals with T2D respond to exercise training with similar metabolic adaptations remains to be determined. To date, there are no published prospective studies comparing rates of fat oxidation during physical activity before and after endurance training among individuals with obesity and T2D. This project tested the hypothesis that adults with T2D will not see the expected increase in total or plasma fatty acid oxidation, measured by indirect calorimetry and a labeled isotope tracer, compared with people without T2D. Knowing whether physical training can positively alter the abnormal metabolism of lipids associated with obesity and T2D will contribute to a more clear understanding of the diseases and provide further insight into the possible benefits of increased physical activity.

3.0 METHODS

3.1 INTRODUCTION

Whether obese individuals with and without T2D can improve their ability to oxidize fatty acids with endurance physical training remains to be shown. Therefore, the purpose of this study was to examine changes in fatty acid oxidation *during* exercise *after* obese adults with and without T2D complete an 8-week endurance physical activity program. This study also examined whether any seen changes in total fatty acid oxidation were derived from plasma or non-plasma fatty acid sources.

3.2 SUBJECTS

Sedentary overweight men and women were recruited to participate in this sub-investigation of a larger clinical trial. No distinction was made based on race or ethnicity. To be eligible for the trial subjects were between the ages of 28 to 55, overweight or obese, and with or without T2D. This specific age range was chosen because the parent investigation included methods for comparing mitochondria within skeletal muscle between the groups. An age too young or too old could confound the effect of aerobic training on mitochondrial enzyme activity. Younger people were excluded because common mitochondrial abnormalities are unlikely to be

manifested yet, and older adults may bear too many mitochondrial mutations as a result of aging (Pesce V, e. a. 2001; Short K, e. a. 2005). Another requirement was that subjects had to be previously sedentary as defined by self-reported physical activity of less than 3 sessions of no more than 20 minutes per week for the previous six months.

The BMI of eligible subjects was 28.0-39.9 kg/m^2, with a maximum weight of 350 lbs. due to testing equipment maximum specifications. The lower BMI range (28.0 kg/m^2) was chosen to eliminate subjects that may have undiagnosed impaired glucose tolerance. Including such subjects may mask a clear difference between those with and without T2D. In addition this helped to further differentiate these two subject groups from the lean control group in the parent project, which had an upper limit BMI of 25 kg/m^2. To determine eligibility for subjects with T2D, a diagnosis of T2D was determined from either two separate fasting glucose measurements of $\geq$ 126 mg/dl, confirmation of diagnosis from the subject's physician if treated by diet and exercise alone, or proof of prescription medication for the treatment of T2D.

We recruited subjects from the local area using a variety of methods. Our primary method of recruitment was via several telephone audix announcements to University of Pittsburgh and University of Pittsburgh Medical Center employees. We also posted Institutional Review Board approved flyers throughout the Oakland area and utilized the Obesity and Nutrition Research Center's subject database.

The following criteria determined eligibility of individuals in the main trial. The same criteria were thus applied to individuals in this sub-investigation.

3.2.1 Exclusion Criteria

1. Participating in regular physical activity of at least 20 minutes on three separate days per week during the previous six months prior to screening.
2. Weight change > 3kg in the preceding month.
3. Planned or established pregnancy.
4. Presence of severe cardiorespiratory disease or any other condition that precludes exercise.
5. Presence of any systemic or organ disease that might significantly affect insulin resistance or cause muscular disease.
6. Symptomatic diabetic neuropathy.
7. Resting systolic blood pressure $\geq$ 150 mmHg or a resting diastolic blood pressure $\geq$ 95 mmHg either with or without medication.
8. $HbA_1c \geq 10\%$.
9. Treatment with any of the following medications: beta blockers (due to blunting effect of heart rate response to exercise), insulin, oral glucocorticoids, fibrates, niacin, and any weight-loss drug.
10. Exclusion laboratory criteria: hematocrit <34%; platelets count <70,000/uL, serum creatinine >1.5 mg/dl; HbA1c >10%; serum TSH <0.1 or >8 mU/ml; ALT > 2.5 x times the upper limit of the laboratory's normal range; alkaline phosphatase >150 IU, fasting triglycerides >450 mg/dl, cholesterol >300mg/dL, fasting blood glucose >200, presence of proteinuria grade 2+ or more on urine sediment analysis.
11. Any other physical complication that would prevent optimal participation in the moderate-intensity walking component of the study.

3.3 EXPERIMENTAL DESIGN

This prospective experimental study examined whether an 8-week physical activity program increased the capacity of skeletal muscle to oxidize fatty acids *during* exercise in overweight and obese adults with and without T2D. This research project was conducted as a sub-investigation of a larger research project entitled, "Mitochondrial Dysfunction in Type 2 Diabetes Mellitus and Capacity for Fat Oxidation During Exercise" (Principal Investigator: Frederico Toledo, MD.) Both projects utilized the same subjects while analyzing different physiological markers. For this reason, the inclusion and exclusion criteria for this dissertation project reflect criteria of the larger parent project. Specific reasons are explained within this chapter as each criterion is discussed. Subjects in both groups defined below underwent a series of baseline assessments (described in section 3.4) prior to entering the 8-week aerobic exercise intervention. All assessments were repeated after the completion of the 8-week program.

Overweight Group (OW): Subjects *without* T2D, BMI between 28-40 kg/m^2.

T2D Group: Subjects *with* T2D, BMI between 28-40 kg/m^2.

The University of Pittsburgh Institutional Review Board approved the larger clinical trial as well as the assessments for this sub-investigation. Prior to participating in screening assessments for this study, each participant signed an informed consent. Prior to enrollment, subjects successfully completed a physical examination with a physician, a maximal graded exercise test, which was reviewed by a cardiologist, and preliminary blood work to ensure safety in beginning a physical activity program. For safety reasons, subjects taking medications with

the potential to cause hypoglycemia were required to monitor their blood glucose levels using a glucose meter.

The assessments for this trial were performed at baseline and completion of the exercise intervention and included measures of resting and exercising total fatty acid oxidation, as well as the utilization of stable isotopes to determine the contributing source of the fatty acids. In addition to these measures, participants underwent evaluations of body weight, body composition and aerobic fitness at baseline and post-exercise training. These assessments are described in detail below (see section 3.4.)

Please see Appendix A: Study Flow Sheet for detailed schedule of testing visits.

3.3.1 Exercise Intervention

The basic exercise intervention prescription was the same for all subjects. Each subject's goal was to progress to a minimum of 4-5 aerobic exercise sessions of 45 minutes duration per week by the eighth and final week. The subjects began with 20-30 minute sessions 4-5 days per week and increased gradually to 45 minutes as able. This progression was monitored and supported by an exercise physiologist (EP). During the 8-week aerobic training program, participants came at least twice a week to the Obesity and Nutrition Research Center (ONRC) Exercise Room for supervised exercise sessions with an EP. A minimum of two required visits per week was chosen to facilitate adherence to the program as well as respect participants' schedules. Sessions were conducted either one on one or with two subjects and one EP.

The recommended primary mode of activity for all subjects was walking, because walking is a natural, easy, affordable, and low injury risk form of exercise. Moreover, moderate intensity walking is known to increase mitochondrial activity in obesity and T2D (Toledo, F. G.

et al. 2007). Continuous walking was encouraged. However, given daily time constraints and research demonstrating the benefits of short bouts of activity (Jakicic, J. M. et al. 1995) participants were allowed to count 10 minute "bouts" of walking throughout the day to reach their 45-minute goal. Participation in other forms of aerobic exercise, such as biking and swimming that were maintained for a period of at least 10 minutes was acceptable. We encouraged all subjects to use a stationary bicycle for a portion of each session at the ONRC exercise room as all exercise-testing sessions were completed on a cycle ergometer.

Participants learned how to use the heart rate monitor at their first session and encouraged to exercise at a level that maintained their heart rate in the target range of 50-70% of their predetermined maximum heart rate. We calculated individual target ranges from the maximum heart rate attained during the initial maximal graded exercise test. This range coincides with the rating of perceived exertion (RPE) of "somewhat hard" (Dunbar, C. C. et al. 1992). Introduced to the Borg 15 Point Rating of Perceived Exertion Scale at the time of their maximal exercise test during screening, participants were taught to "anchor" their exertion level of "somewhat hard." We also encouraged them to reevaluate what "somewhat hard" meant to them each session. Together, these two tools allowed participants to maintain the appropriate heart rate during physical activity training to produce maximum metabolic improvements. This was especially important given the relatively short length of the intervention (8 weeks). Additionally, learning to use the RPE scale may have helped participants maintain appropriate intensity of activity after the study ended. We instructed subjects not to participate in "hard" or vigorous physical activity for safety reasons as well as and to maintain the integrity of the study.

All subjects had an exercise diary to record time spent in physical activity, mode of activity, intensity, and blood glucose if appropriate for those with T2D. This information was

collected each week. Only sessions that included all required data (mean heart rate and duration) were included in caloric expenditure calculations.

3.4 ASSESSMENT COMPONENTS

Subjects completed a set of exercise tests at baseline to assess fitness and rates of fatty acid oxidation at rest and during sub-maximal exercise. They repeated this battery of tests after the 8-week training phase to assess changes in fitness and the body's ability to oxidize fatty acids due to the intervention. These tests included a maximal graded exercise test, a 60-minute sub-maximal exercise test with the fatty acid palmitate, a 60-minute sub-maximal exercise test with acetate, and a dual energy x-ray absorptiometry (DEXA) scan to determine fat-free mass.

3.4.1 Maximal Graded Exercise Test (VO_2max)

Subjects completed a graded exercise test (GXT) to assess cardio-respiratory fitness (VO_2max) at baseline and completion of the intervention. All exercise tests were conducted at the ONRC Exercise Physiology Lab at UPMC-Montefiore Hospital under the direction of a physician and an EP. The test was an incremental modified Bruce protocol on an electronically braked cycle ergometer (Bosch ERG 601, Germany). This 6-12 minute GXT protocol is well suited for overweight adults. We recorded heart rate, blood pressure, and electrical activity of the heart prior to, during and immediately following the test. Prior to beginning the maximal exercise test, the Borg RPE Scale was presented to participants as described earlier. Resistance began at 25 watts for women and 50 watts for men, and was increased by 25 watts every 2

minutes until subjects reached volitional fatigue. Subjects breathed through a mouthpiece connected to a two-way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected via open-circuit spirometry into a 5-liter mixing chamber (Rayfield RMC-1, Waitsfield, VT) containing a bi-directional turbine to measure expiratory flow. We used a customized computer program to analyze and integrate signals for the determination of oxygen consumption (VO_2) every 30 seconds. During screening, the test was stopped and subjects excluded from further participation if there was >2mm ST-segment depression on the electrocardiogram or evidence of cardiovascular instability, such as hypotension, dangerous arrhythmias or angina. A collaborating cardiologist reviewed the electrocardiogram for study eligibility.

3.4.2 Sub-maximal Exercise Tests

To assess the body's ability to oxidize fat during exercise, subjects returned to the Exercise Laboratory after completing the VO_2max test for two additional baseline exercise tests. There were no less than 48 hours between any of the three exercise tests. These two sub-maximal exercise tests, repeated after the training intervention, were 60-minute cycle ergometer exercise sessions completed after an overnight fast, one with a ^{13}C-palmitate and one with a ^{13}C-acetate infusion. At 50% of their pre-determined VO_2max, these sessions quantified rates of total fat oxidation during sub-maximal exercise using gas exchange indirect calorimetry and differentiated plasma versus non-plasma sources of fatty acids using a palmitate isotope tracer infusion. The second 60-minute cycle test with ^{13}C-acetate was used to correct for any labeled substrate that was "trapped" in the TCA cycle and thus unaccounted for with the ^{13}C-palmitate infusion.

Because meal composition can affect rates of fat oxidation, we admitted the subjects to the General Clinical Research Center (GCRC) the evening prior to each sub-maximal test. They ate a standard dinner (10 kcal/kg; 50% carbohydrate, 30% fat, 20% protein) and fasted until completion of the study the next morning. Providing a standard meal ensures a consistent dietary fat intake for all subjects and also ensures adequate physical rest in the hours prior to each exercise test. We instructed subjects to avoid strenuous physical activity for 48 hours prior to each exercise test and to eat at least 200 g of carbohydrate for the 48 hours preceding each study to ensure adequate glycogen stores for the exercise test. We collected a 12-hour urine collection in this overnight period for estimation of protein oxidation.

3.4.2.1 Tracer Methods

To determine rates of non-plasma free fatty acid oxidation, we administered a ^{13}C-labelled palmitate tracer infusion during the first of the two sub-maximal exercise tests. ^{13}C-labeled palmitate is a non-radioactive isotope of the fatty acid palmitate, the basic molecular building block of fats. Because ^{13}C-labeled palmitate converts into carbon dioxide (CO_2) by cells, this enables the tracing of its metabolic fate after oxidation using mass spectroscopy chemical analysis. The carbon-13 isotope (^{13}C) is an atomic-weight variant of the element carbon, which is *not* radioactive and is naturally present in organic compounds in nature. Approximately 16% of naturally occurring palmitate contains at least one ^{13}C atom. In contrast to other stable isotopes, such as deuterium, ^{13}C has no known side effects because it is metabolically indistinguishable from natural carbon-12.

Prior to beginning the exercise test, two peripheral veins were cannulated, one for the infusion of the palmitate tracer, the second for blood sampling. The arm to be used for blood sampling was kept warm with a hearting pad to help maintain the flow of blood to the vein

throughout the exercise test. Before beginning the tracer infusion resting blood and breath samples were collected for biochemical determinations and background $^{13}CO_2$ and ^{13}C-palmitate enrichment.

A priming dose of approximately 18 µmol/kg $NaH^{13}CO_3$ was infused to shorten the time required to achieve equilibration of recovered expired $^{13}CO_2$. The dosage of ^{13}C-palmitate (in a human albumin solution) was adjusted to patient weight, yielding approximately 0.15 µmol/kg/min. This infusion ran for a minimum of 30 minutes prior to exercise initiation to achieve the equilibrium of the isotope in plasma. We measured resting energy expenditure and substrate utilization prior to beginning the exercise test via indirect calorimetry. We also collected baseline blood and breath samples prior to exercise to measure resting rates of FFA oxidation. Seated on the cycle ergometer, each subject cycled for approximately 60 minutes at a work rate corresponding to 50±10% of his or her predetermined VO_2max. This was determined using a work rate-VO_2 regression equation obtained during the incremental VO_2max test. Since VO_2 during exercise was precisely determined after the first 15 minutes of exercise, the work rate was monitored and either increased or decreased to precisely maintain 50% of VO_2max. This work rate was then held constant throughout the remainder of the 60 minutes.

During the 60-minute exercise test, we collected data at 4 intervals: 15, 30, 45 and 60 minutes. At each time point, blood samples were collected to measure plasma free fatty acid (FFA) and ^{13}C-palmitate enrichment. Substrate oxidation was measured via indirect calorimetry. For each time point determination, subjects breathed through a mouthpiece connected to the two-way breathing valve for 5 minutes. In order to allow for sufficient gas exchange equilibrium with the dead-space in the tubing, only the average of the last 2 minutes of VO_2 and carbon dioxide production (VCO_2) data were used. At the completion of each indirect calorimetry

measurement, a separate breath sample collected determined breath $^{13}C/^{12}C$ enrichment. We measured VO_2 and VCO_2 production from whole body gas exchange indirect calorimetry and then calculated rates of total lipid and carbohydrate oxidation. The gas analyzer was calibrated prior to each test. Subjects were given water and cooled by a fan throughout the tests.

The measurement of palmitate oxidation using tracer methodology is an estimate; a significant portion of the labeled isotope can be lost or "trapped" in the Krebs cycle. Measuring acetate recovery during a separate, but similar exercise test can correct for this loss (Wolfe, R. R. et al. 1990; Sidossis, L. S. et al. 1995). Many scientists use an estimated acetate correction factor. To provide the most accurate measurement of palmitate oxidation, particularly since our sample size is modest, we chose to conduct a second sub-maximal exercise test using ^{13}C-labeled acetate for each subject. Further, the acetate recovery factor may be reduced in subjects with T2D compared to those without diabetes (Blaak, E. E. et al. 2000) as well as high inter-subject variability (Schrauwen, P. et al. 1998).

This second sub-maximal exercise test followed the same protocol as the palmitate test; however, the infused labeled tracer used was ^{13}C-labeled acetate. The acetate test was performed no sooner than 48 hours and no more than a week after the palmitate test and was completed at baseline and post-intervention by all subjects. We measured the recovery of acetate with a continuous infusion of non-radioactive ^{13}C-labeled acetate (0.08 μmol/kg) and subsequent breath samples at the same 4 time intervals. To eliminate a possible training effect from the palmitate to the acetate test, the order of the isotope infusions between subjects was alternated. For example, subject 1 received palmitate/acetate pre and post intervention and subject 2 received acetate/palmitate pre and post intervention.

3.4.2.2 Indirect Calorimetry and Fatty Acid Oxidation Calculation

Systemic net carbohydrate and fat oxidation rates were calculated from the indirect calorimetry data with the equations of Frayn et al. (Frayn, K. N. 1983). To estimate protein oxidation, urea nitrogen was measured in overnight 10-12h urine collections. We calculated free fatty acid oxidation from total fat oxidation, assuming 860 g/mol as the average molecular weight of triglyceride.

To determine whether predicted increases in fat oxidation came from an increase in plasma or non-plasma FFAs, we utilized the stable-isotope tracer methodology described above. These samples were then analyzed by mass spectroscopy to assess the $^{13}C/^{12}C$ isotopic enrichment of expired CO_2 by the ONRC Stable Isotope Lab, which routinely performs these assays. We used the following equation to calculate the percent of the infused tracer (palmitate) that was oxidized:

$$\% \text{ Infused tracer oxidized} = V^{13}CO_2 = (ECO_2 - E_{bkg}) \times VCO_2 \times 100 / (16 \times F \times c)$$

Where, $V^{13}CO_2$ is the expired CO_2 production (ml/min; 1 mmol CO_2=22.4 mL); ECO_2 is the breath $^{13}C/^{12}C$ ratio at a given time and E_{bkg} is the background $^{13}C/^{12}C$ ratio at rest. F is the palmitate infusion rate, and c is the acetate recovery factor.

The acetate correction factor was calculated as follows:

$$c = (ECO_2 - E_{bkg}) \times VCO_2 / (2 \times \text{acetate infusion rate})$$

Next, plasma FFA oxidation was calculated by:

Plasma FFA oxidation = FFA Rd * % infused palmitate oxidized

Where FFA Rd equals the rate of palmitate disposal, which was calculated with Steele's equations, based on the relative enrichment of plasma labeled/non-labeled palmitate and the known rate of labeled palmitate infusion (Wolfe, R. R. 2005). Triglyceride fatty acid oxidation was estimated as the difference between total fat oxidation from indirect calorimetry minus plasma FFA oxidation from labeled palmitate.

3.4.2.3 Body Weight and Body Composition

Each participant completed a DEXA scan prior to beginning and upon conclusion of the training phase to determine lean body mass. Because DEXA technology quantifies lean body mass, this information allowed us to normalize fat oxidation rates for each subject based on his or her total lean body mass. The DEXA scan, which takes about 15 minutes to complete, was operated by the ONRC and was performed on the 8th floor of UPMC-Montefiore Hospital. ONRC staff calibrated the DEXA scanner each day for quality assurance using a calibration block. Additionally, the DEXA was calibrated monthly using an aluminum spine phantom. All females of childbearing potential had a urine pregnancy test that was verified as negative prior to this procedure.

Body weight was also assessed via the DEXA scan as part of the body composition analysis. Subjects were encouraged to maintain their regular dietary patterns and weight throughout the exercise intervention. Body weight was measured bi-weekly throughout the

intervention to ensure participants were not altering their dietary intake. Weight as assessed on the day of the pre and post-DEXA scan was used in data analyses as evidence of weight stability.

3.5 STATISTICAL ANALYSIS

Statistical analyses were performed using JMP (Cary, NC) statistical software, version 7.0.1, with a defined statistical significance of $p \leq 0.05$. Data presented to describe the characteristics of study participants in both groups (age, BMI, fasting glucose, etc) were analyzed using independent t-tests. T-tests were also used to assess any differences between the OW and T2D groups in resting and exercising RQ and rates of total fatty acid and glucose oxidation prior to beginning the intervention. Analyses were conducted to determine if the data were normally distributed prior to conducting additional analyses.

Two-way repeated measures analysis of variance (ANOVA) (group x time) was used to assess changes in the outcomes variables (total and source of fatty acid oxidation) after subjects completed the intervention. Linear regression analysis was used to examine the relationship between exercise adherence and changes in substrate utilization.

3.5.1 Power Analysis

The power analysis for this study assumed a power level of 0.80 and a P value <0.05 for significance. To detect an improvement of at least 3.0 mg/kg/min in the rate of fat oxidation with the exercise intervention using paired t-test, and estimating a standard deviation of change to be 3.0, it was anticipated that 13 subjects per group would be required to detect differences in

fat oxidation rates with training. A similar study with 13 participants found a 24% increase ($p<0.05$) in fat oxidation during exercise after 12 weeks of endurance training (Pruchnic, R. et al. 2004). It was further estimated that data may be lost from no more than 20% of subjects due to failure to complete the testing and an additional 10% data loss from poor muscle biopsy samples in the parent project. Thus, it was estimated for this project that 18 subjects must be recruited per group to achieve approximately 13 data-complete subjects within each group.

3.5.2 Retention

In combination with the parent project, "Mitochondrial Dysfunction in Type 2 Diabetes and Capacity for Exercise," study participants were compensated $300 after completing all baseline assessments. Subjects were compensated an additional $300 after successfully participating in the intervention and completing the post-intervention assessments. These funds were approved and provided by the primary funding source, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, and the University of Pittsburgh Institutional Review Board.

4.0 RESULTS

Sixteen previously sedentary adults participated in this experimental study that included an 8-week aerobic physical activity program. Participants underwent the assessments described above both prior to and on completion of the program. The independent variables in this trial were the presence or absence of T2D and exercise training. The primary dependent variable was change in whole body fat oxidation, with change in plasma fatty acid and IMTG oxidation as secondary variables. The results of this study are presented in 4 main sections. First, a description of the study participants at baseline followed by an analysis of adherence to the exercise intervention, i.e. changes in fitness, and its effects on body weight and composition. Next, a comparison of substrate utilization *at rest* before and after the 8-week exercise intervention. The final section addresses the primary hypothesis that T2D further inhibits the oxidation of fatty acids by skeletal muscle *during* moderate intensity exercise beyond overweight or obesity alone. Changes in the source of fatty acids; i.e. plasma free fatty acids vs. non-plasma free fatty acids, are discussed within this section as well.

4.1 SUBJECTS: CHARACTERISTICS AND RETENTION

The subjects in this trial were 16 adults (11 females and 5 males) with and without T2D participating in a larger clinical trial at the University of Pittsburgh, School of Medicine,

Division of Endocrinology. Subjects were between 39 and 55 years of age with a BMI of 28-39 kg/m^2. This included 10 subjects in the overweight (OW) group and 6 subjects in the T2D group.

While 16 subjects successfully passed the screening exams and were enrolled into this research study, three subjects were lost to follow up prior to completing the post-intervention assessments. All analyses on the outcome variables include only subjects that completed the intervention and assessments. Thus, baseline data are presented on 16 subjects and analysis of all other measures includes 13 subjects. This consists of 10 subjects in the OW group and 3 subjects in the overweight with T2D group. One participant in the T2D group was unable to complete the post-VO_2 max test due to illness; therefore the number of participants in this group is 2 for this pre-post comparison of VO_2max. This study remains ongoing and additional subjects with T2D will be recruited to increase statistical power.

At baseline, there were no significant differences comparing these two groups for participant age, body weight, BMI, waist circumference, or fasting glucose. There were significant differences between the groups in VO_2max and hemoglobin A1c (HbA1c) measurements. All subjects were otherwise healthy and had successfully completed all screening requirements. Detailed baseline subject characteristics are shown in Table 1.

Table 1. Baseline Characteristics

	All Subjects N=16	Overweight N=10	Overweight with T2D	
			Enrolled N=6	Completers N=3
Age (years)	48.4±1.6	48.9±5.7	47.7±2.5 (p=0.69)	48.67±3.8 (p=0.95)
Weight (kg)	90.9±5.1	88.2±5.9	95.5±7.8 (p=0.46)	101.1±9.7 (p=0.31)
BMI (kg/m^2)	32.9±1.2	31.8±1.3	34.7±1.3 (p=0.17)	36.6±1.4 (p=0.08)
VO_2max (ml/kgLBM/min)	38.5±1.6	40.8±1.5	34.6±2.1* (p=0.03)	34.8±4.5 (p=0.12)
Waist (cm)	105.7	103.9±4.7	108.6±6.0 (p=0.55)	113.9±7.3 (p=0.35)
Fasting glucose (ml/dL)	101.8±3.0	93.6±3.9	115.5±14.3 (p=0.09)	98.7±0.7 (p=0.51)
HbA1c (%)	5.8±0.13	5.5±0.12	6.3±0.2* (p=0.004)	6.1±0.3* (p=0.05)
Gender	11 female/5 male	7 female/3 male (70% female)	4 female/2 male (67% female)	2 female/1male 67% female

4.2 FITNESS TRAINING, BODY WEIGHT AND COMPOSITION

We hypothesized that aerobic training increases total fatty acid oxidation in both groups, but not to the same extent. It is thus imperative to verify all subjects did indeed participate in the prescribed exercise program and become more physically fit during the 8-week exercise intervention by VO_2max criteria. Data presented regarding activity during the 8 weeks includes

twice weekly-supervised exercise sessions at the ONRC exercise facility as well as self-reported data from participants. If participants were unable to provide the details of an unsupervised exercise session, such as heart rate or length of session, the session was omitted from the analysis. Data are presented first regarding adherence to the prescribed plan and caloric expenditure during exercise. As a separate measure of fitness, participants completed a VO_2max test on the cycle ergometer following the same protocol used at baseline. These results are presented below.

4.2.1 Participation in the Exercise Intervention

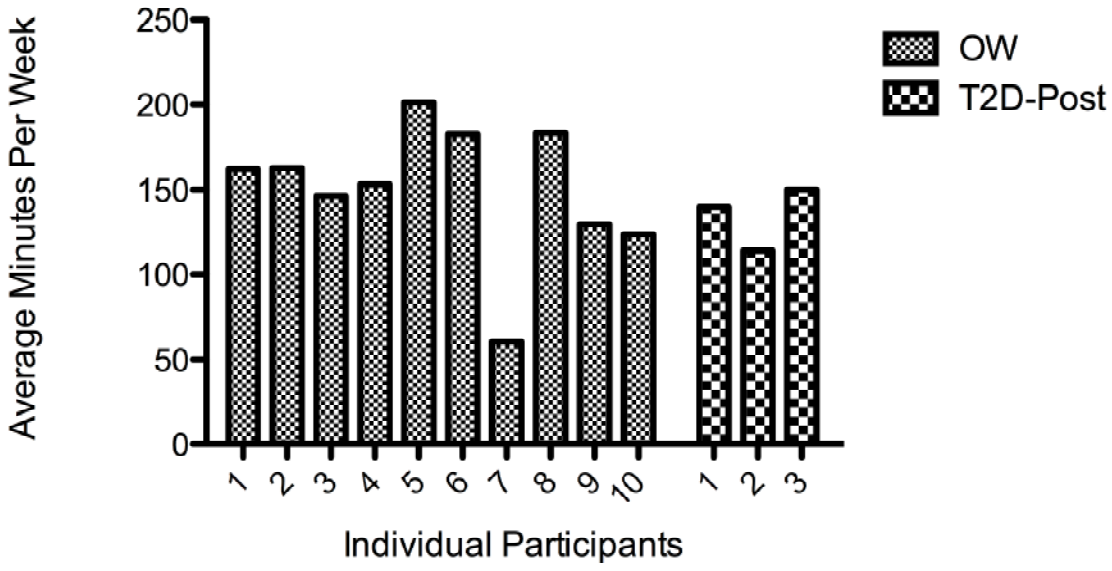
Adherence to the supervised exercise sessions was excellent with only 5 total missed supervised sessions among the 13 participants that completed the trial. While on average, the participants fell slightly short of the recommended 4-5 total sessions per week and minimum of 180 minutes per week; the adherence was similar between the two groups. An overview of their participation in the exercise program and energy expended during exercise is given below in Table 2. Adherence of individual subjects to the recommended minutes of exercise per week is shown graphically in Figure 3.

Table 2: Adherence to the Prescribed Exercise Intervention

Values are means + SEM; HR_{max} = Maximal Heart Rate

	Sessions/Week		Minutes/Week	Kcal/Week	% HR_{max}
OW (N=10)	3.9±0.3		155.6±11.8	937.4±54.9	80.1±2.0
	LAB	SELF			
Weeks 1-4	2.7±0.4	1.3±0.3	159.2±15	949.5±20.2	80.0±2.3
Weeks 5-8	2.6±0.1	1.1±0.2	152.0±12.2	925.2±60.3	80.2±2.4
T2D (N=3)	3.6±0.5		142.9±11.6	971±313.9	79.3±5.5
	LAB	SELF			
Weeks 1-4	2±0.6	1.4±0.8	138.7±20.2	917.5±320.9	79.1±6.6
Weeks 5-8	2±0.9	1.8±1.1	147.1±15.3	1024.5±325.2	79.5±5.7
p-value	0.62		0.45	0.86	0.87

Figure 3: Average Weekly Minutes of Exercise by Individual Participants During the 8-Week Intervention

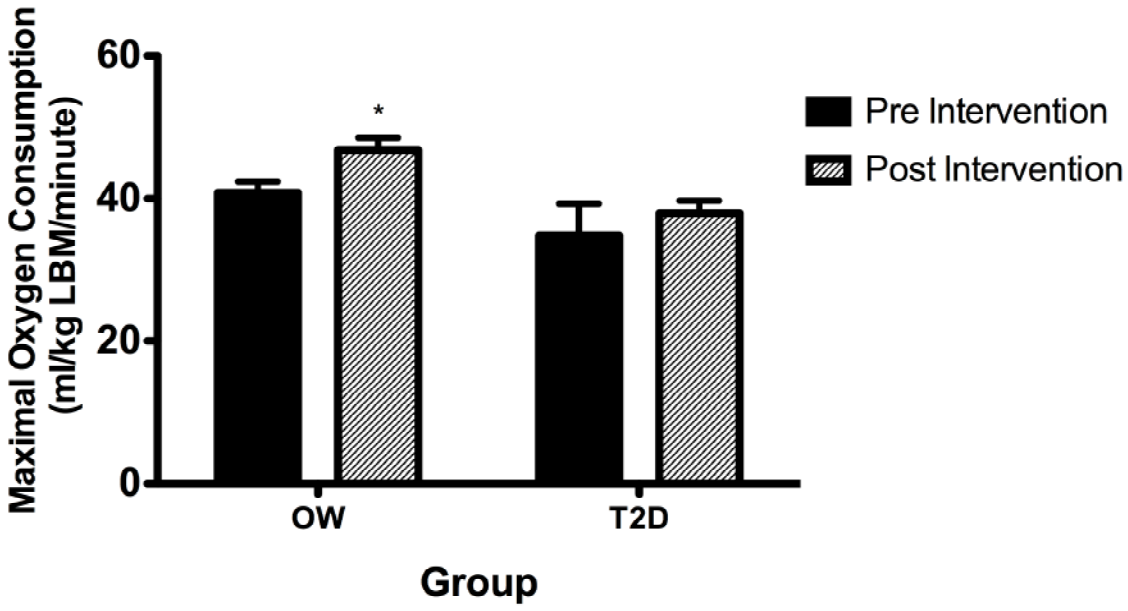


4.2.2 Fitness Response to the Exercise Intervention

Participants in both groups improved their maximal oxygen consumption (VO_2 max) as assessed from pre and post maximal effort graded exercise tests (Figure 4). Prior to entering the exercise intervention, the OW group had an average VO_2max of 40.8$\pm$1.6 ml/kg LBM/min (N=10), while the T2D group measured 34.8$\pm$4.5 ml/kg LBM/min (N=3). After the exercise intervention, average VO_2max for the OW and T2D groups were 46.8$\pm$1.7 ml/kg LBM/min (N=10) and 38.0$\pm$1.7 ml/kg LBM/min (N=2) respectively. Expressed as a percent change, the OW and T2D groups increased their VO_2max by 14% and 13.4% respectively. This increase in maximal oxygen consumption was significant ($p<0.05$) but did not reach significance in the T2D group ($p<0.15$). This is also evidenced by an increased time to exhaustion during the post-intervention GXT by both groups. The OW group increased by 15.9% (142 seconds, N=10) and the T2D group improved by 7.5% (108 seconds, N=2). This change was significant ($p<0.01$) in the OW group, but not in the T2D group ($p=0.10$). Maximal heart rate and RPE achieved during the graded exercise tests were unchanged from pre to post in both groups indicating that subjects gave maximal and equivalent effort on each test.

Figure 4: Change in Maximal Oxygen Consumption

Values are means ± SEM; LBM = lean body mass



4.2.3 Body Weight and Composition Changes

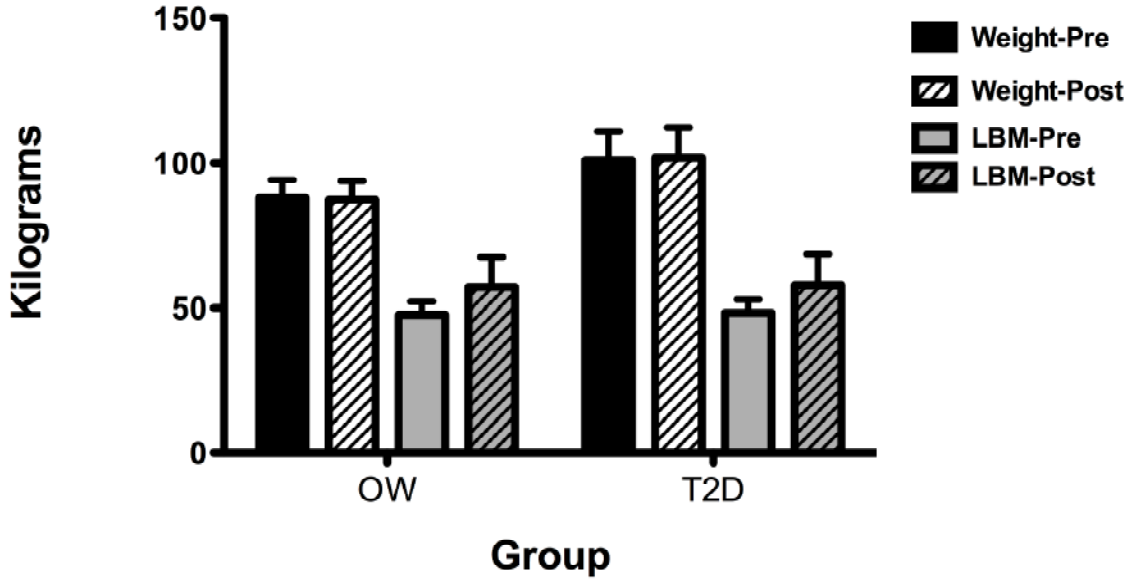
A change in body weight due to a change in caloric intake may cause a change in the amount of total triglyceride or glucose required during rest or exercise as well as the ratio of the substrates. For this reason, we asked participants not to change their dietary intake during the exercise intervention to best see the effect of the exercise intervention alone on substrate utilization. Body weight and lean tissue were assessed in the fasting state prior to and after the 8-week intervention by DEXA scan. Both are reported below in Figure 5.

After the 8-week intervention, body weight change in the OW and T2D groups was -0.67 ± 0.95 kg and +0.8 ± 1.16 kg respectively. Average change in lean body mass in the OW and

T2D groups was +0.71 ± 0.42 kg and +0.66 ± 0.43 kg respectively. Neither change in body weight (p=0.94) or amount of lean tissue (p=0.12) after the intervention was significant.

Figure 5: Change in Weight and Lean Body Mass (LBM)

Values are means ± SEM



4.3 ASSESSMENTS MEASURED DURING REST

Prior to beginning each 60-minute sub-maximal exercise test on the cycle ergometer, participants rested quietly in a reclined chair for at least 30 minutes. Measurements of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were taken via indirect calorimetry approximately 10 minutes prior to beginning the exercise session and just prior to beginning the exercise

session. Measurements from both time points were averaged and this average was used in the following analyses.

Resting metabolic rate (RMR) did not change significantly in either of the groups after the 8-week intervention. RMR in the OW group went from 1586±104 to 1621±106 kcal/24 hours, and the T2D group went from 1746±232 to 1867±157 kcal/24 hours. This increase was not significant ($p=0.34$) nor was there an interaction effect of group and intervention ($p=0.60$). Percent of total calories derived from fat during the resting period varied widely between subjects. RMR was measured using the same mouthpiece as during exercise, not a separate canopy system. On average, the T2D group obtained a greater portion of their energy from fat sources than the OW group both pre and post. There was a slight decrease in RQ in the OW group and an increase in RQ in the T2D group after the intervention. These changes were not significant and are shown in Table 3.

Prior to intervention, there were no significant differences between the OW and T2D groups in resting RQ ($p=0.28$), resting total triglyceride (TG) oxidation ($p=0.18$), or resting total glucose oxidation ($p=0.27$). A test of the main effect of the intervention (time) on these resting measurements revealed no significant change in RQ ($p=0.59$), total TG oxidation ($p=0.62$), or total glucose oxidation ($p=0.60$) during the resting period *after* participants completed the 8-week intervention. Additionally, there was no interaction effect of group and intervention for any of the three measurements; RQ ($p=0.38$), total TG ($p=0.44$), and total glucose ($p=0.43$). Results of these measurements are given in Table 3.

Table 3: Resting Measurements Before and After Intervention

Values are means ± SEM. TG: triglyceride.

	OW N=10		T2D N=3		
	Pre	**Post**	**Pre**	**Post**	**p-value**
RQ	0.82±0.03	0.81±0.02	0.75±0.00	0.79±0.02	0.59
TG (mg/min)	54.28±14.4	57.99±5.3	94.52±12.6	78.17±16.2	0.62
Glucose (mg/min)	111.24±41.6	101.61±29.5	19.81±0.7	66.72±16.9	0.60
Energy from Fat (%)	45.8±2.0	49.5±6.2	70.2±2.1	53.7±9.2	0.52

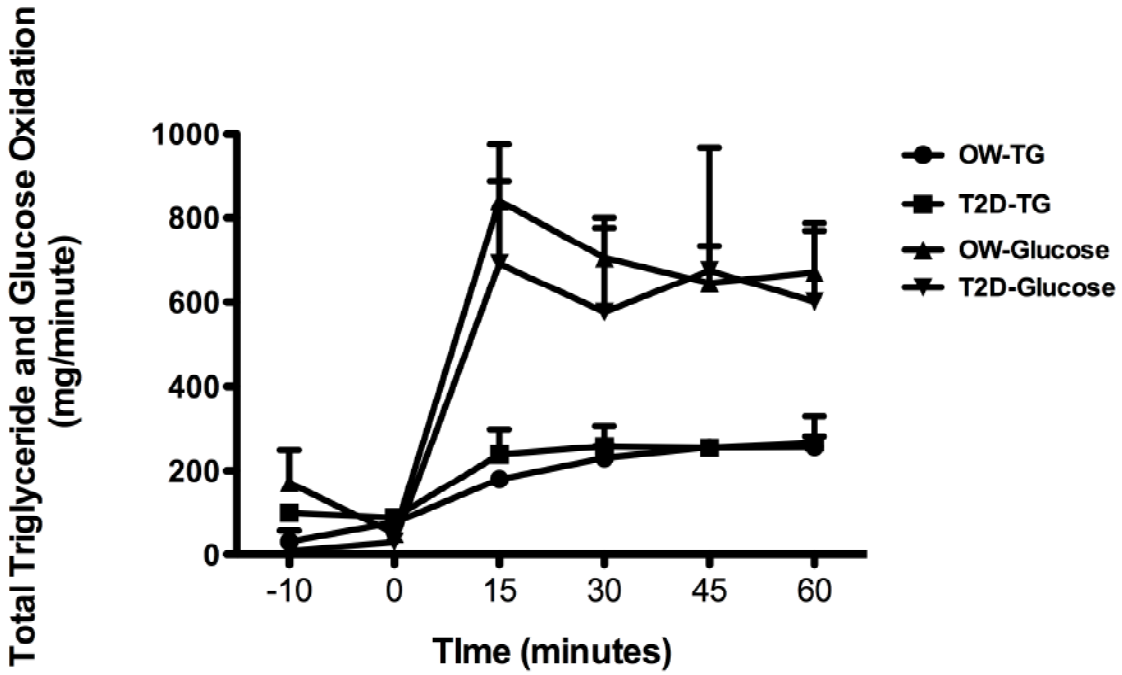
4.4 ASSESSMENTS MEASURED DURING SUB-MAXIMAL EXERCISE

To assess any change in fatty acid utilization during exercise it was necessary that subjects attained a steady state of substrate oxidation. Measurements of total TG and glucose oxidation during 10 minutes of rest prior to the exercise test through the 60-minute sub-maximal session in which participants received the palmitate infusion are shown in Figure 6. This clearly displays the expected rise in TG oxidation over the first 30-minutes of the exercise period and then a leveling off as subjects reach a steady state of fatty acid oxidation. This level is held over the 30-60-minute time period in both groups. All analyses regarding the effect of the intervention on

substrate utilization during exercise are calculated from the 30, 45, and 60-minute time points in which this steady state was obtained.

Figure 6: Total triglyceride and glucose oxidation at rest and during sub-maximal exercise.

Values are means + SEM; TG = triglyceride



4.4.1 Substrate Utilization During Exercise *Prior* to Intervention

To compare exercising substrate utilization between the two groups *prior* to the 8-week intervention, average values for RQ, total TG, and total glucose utilization were calculated from the 30, 45, and 60-minute time points. An independent t-test found no significant differences between the two groups in these measures. Average RQ for the OW group was 0.85 $\pm$ 0.01 and 0.83 $\pm$ 0.01 for the T2D group (p=0.29). Average total TG oxidation in the OW and T2D groups

was 247.17 ± 28.5 mg/min and 278.92±36.8 mg/min respectively (p=0.51). Similarly, no differences were found between the groups in total glucose oxidation (p=0.52), with the OW and T2D groups averaging 673.9 ± 98.9 mg/dl and 565.94 ± 127.7 mg/min respectively.

4.4.2 Proportion of Total Energy Derived from Fat and RQ

The trend in proportion of energy derived from fat during the 30 to 60 minute time period *during* the sub-maximal exercise tests was consistent yet not significant in both study groups (p=0.47). Prior to intervention, the OW group derived 45.3±3.5 % of total energy expenditure from fat and the T2D group derived 48.4±5.5%. After intervention, the OW group was utilizing fat for 48.2±3.6% and the T2D group 50.8±7.0% of total energy expenditure. This is a 6.2% increase in the OW group and 5.1% increase in the T2D group. This change in fat oxidation was not significant (p=0.47) nor was it different between the two groups (p=0.96). This increase in proportion of energy derived from fat is depicted in Figure 7.

Change in RQ during the 60-minute sub-maximal exercise test is shown in Figure 8. No significant main effect of the intervention (time) on change in RQ was detected at the 30 (p=0.75), 45 (p=0.81) or 60-minute (p=0.07) time points. Additionally, there was not a significant interaction effect of group and intervention at the 30 (p=0.69), 45 (p=0.86) or 60-minute (p=0.53) time points. Average values for each time point, pre and post, are given in Appendix A.

Figure 7: Change in Percent Energy Derived from Fat During Exercise

Values are means ± SEM

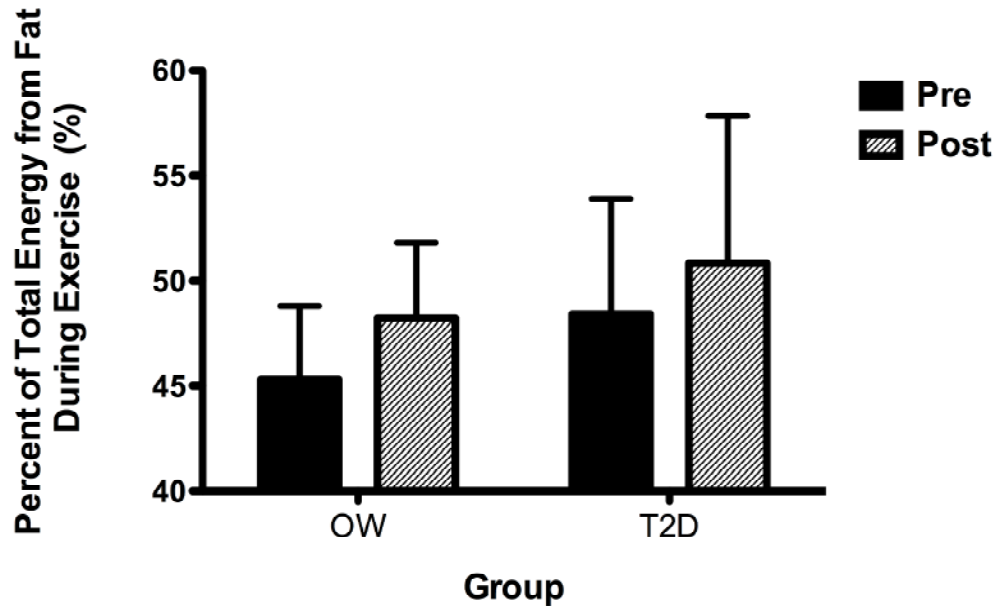
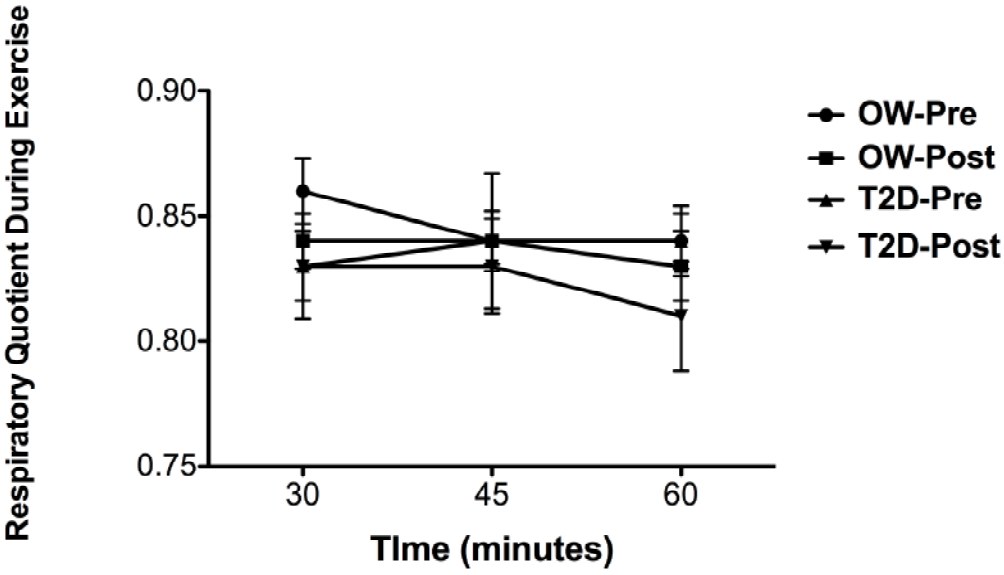


Figure 8: Respiratory Quotient During Exercise

Values are means ± SEM

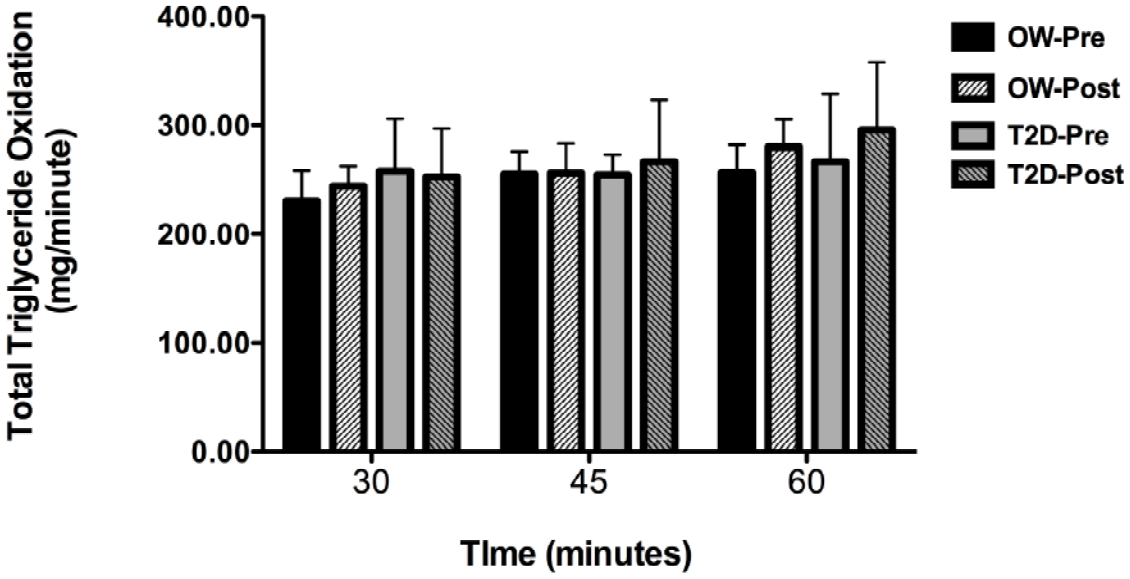


4.4.3 Change in Total Triglyceride Oxidation

Change in total TG oxidation is shown in Figure 9. Total TG oxidation was calculated for each data collection point during the pre and post 60-minute sub-maximal exercise tests in which the participants received the stable isotope palmitate. Repeated measures ANOVA showed no significant change after 8-weeks of exercise training in either the OW or the T2D group at 30 (p=0.88), 45 (p=0.77), or 60–minute time points (p=0.09) due to the intervention. Additionally, there was not an interaction effect of group and intervention at 30 (p=0.72), 45 (p=0.80) or 60- minutes (p=0.86). Average values for each time point, pre and post, are given in Appendix A.

Figure 9: Change in Total Triglyceride Oxidation During Exercise

Values are means ± SEM



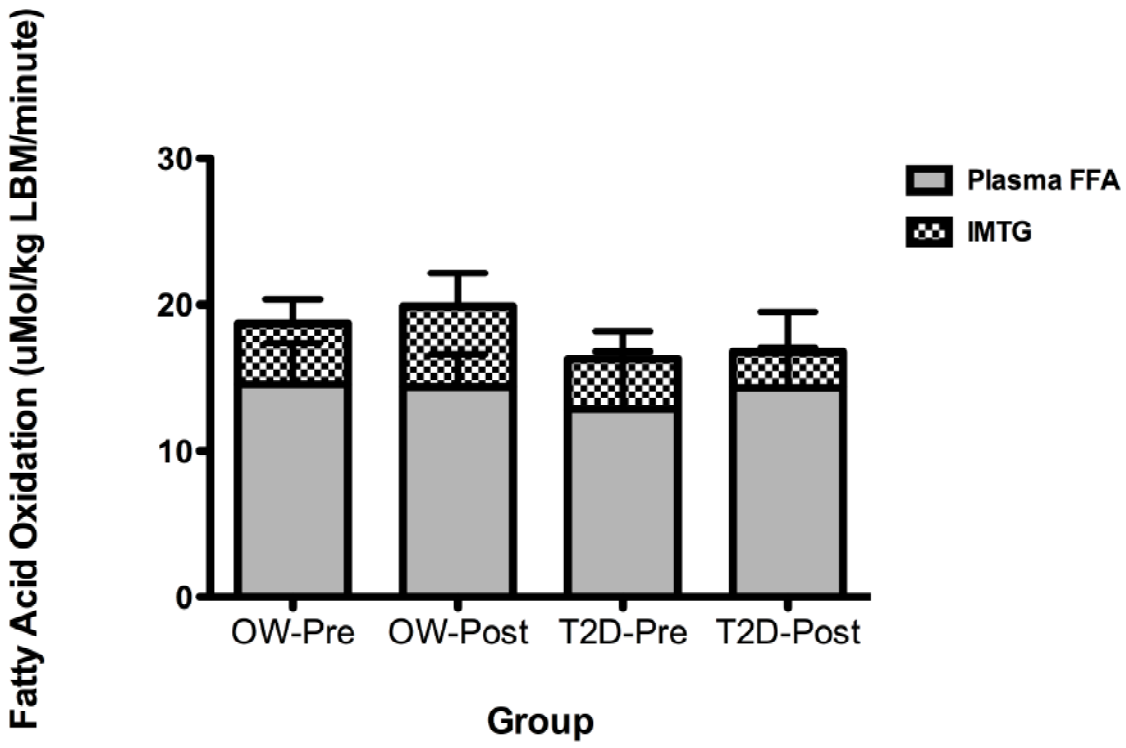
4.4.4 Non-Plasma Fatty Acid Oxidation

Subjects in the OW group derived 4.13 ± 1.7 uMol/kg LMB/min of total fatty acids from non-plasma or IMTG stores during exercise at baseline. Following the intervention this went to 5.5 ± 2.3 uMol/kg LBM/min. Prior to intervention the T2D group oxidized 3.42 ± 1.9 uMol/kg LBM/min of IMTG during exercise. After intervention, IMTG oxidation was measured at 2.41 ± 2.8 uMol/kg LBM/min in the T2D group. This change was not significant ($p=0.62$) nor was there an interaction effect of group and intervention ($p=0.53$).

Utilization of plasma FFAs by the OW group was 14.6 ± 2.8 uMol/kg LBM/min prior to intervention and 14.4 ± 2.2 uMol/kg LBM/minute during exercise after the intervention. Oxidation of plasma FFAs by the T2D group went from 12.86 ± 3.9 to 14.33 ± 2.7 uMol/kg LBM/min after participating in the exercise intervention. These changes due to intervention were not significant ($p=0.92$) nor were there an interaction effect of group and intervention ($p=0.51$). Contribution of oxidized fatty acids from IMTG and plasma sources are shown in Figure 10.

Figure 10: Proportion of IMTG and Plasma Fatty Acids Oxidized During Exercise

Values are means + SEM



4.4.5 Change in Total Glucose Oxidation

No significant differences were detected in total glucose utilization after exercise training at the 30 (p=0.80), 45 (p=0.49), or 60-minute (p=0.07) time points. While the T2D group tended to oxidize less total glucose than the overweight group throughout the 30-minute steady state period, an interaction effect of group and intervention was not evident at any of the three time points (p=0.63,p=0.48, p=69). Average values for all pre and post-intervention time points are shown in Appendix A.

5.0 DISCUSSION

5.1 INTRODUCTION

Prior research concerning the response of fatty acid oxidation to chronic exercise training has been largely limited to healthy, non-obese individuals. Trials with overweight and obese subjects have found equivocal results that may be due to variations in study methods, such as length and intensity of the intervention and testing conditions. Peer reviewed data on this topic with the added condition of T2D is extremely limited. Because impaired fatty acid oxidation is associated with both diseases, obesity and T2D, discovering whether any impairment can be positively affected by aerobic activity is of key significance. This study therefore was undertaken to extend the current body of knowledge to those with T2D. We also sought to determine if having T2D contributes an additional impairment to fatty acid oxidation beyond that of overweight or obesity alone. Using stable isotope tracer methods we further investigated the contribution of plasma vs. non-plasma fatty acids to overall fat oxidation.

Two groups of overweight, previously sedentary adults were compared in this project before and after participating in an 8-week moderate intensity exercise intervention: those with T2D and those without. It was hypothesized that both groups would have an increase in total and non-plasma fatty acid oxidation, but that those with the co-morbidity of T2D would have a blunted increase compared to those without T2D.

5.2 SUBJECT RECRUITMENT AND RETENTION

To provide adequate power for statistical analysis this study was originally slated to recruit 18 subjects in each group allowing for normal participant attrition. Recruitment and subsequent time to complete the study was lengthier than expected, leading to a smaller enrollment than planned. For this reason, recruitment remains ongoing and all attempts to achieve adequate power based on subject number will be made.

As part of the larger clinical trial, subjects were asked to attend five separate visits prior to beginning the exercise intervention and four separate visits after completing the intervention. This included a screening visit, a maximal graded exercise test, a muscle biopsy and DEXA scan, and two separate overnight stays with the sub-maximal exercise tests with isotope infusion the following morning. These visits were then repeated post-intervention without the screening visit. This rigorous schedule was a significant participant burden that may have affected recruitment and retention efforts. This is reflected by the finding that 35 volunteers attended screening visits to achieve 16 subjects successfully enrolled in the study. Thus, future investigations will need to plan for additional resources and strategies to recruit subjects into such a time intensive study in this population group.

Ten adults were enrolled into the OW group and six into the T2D group. Regrettably, 3 subjects from the T2D group were lost to follow up during the intervention, which is 50 percent of the subjects in this group. This dropout rate is significantly greater than the 10% to 20% dropout rates typically observed in intervention studies with a similar duration of intervention. These participants withdrew from the study due to unwillingness to complete supervised exercise sessions and/or inability to schedule the post-intervention measurements according to protocol,

which again may reflect the concern when conducting research that requires a significant time commitment from participants related to supervised sessions and lengthy assessment periods.

A smaller than planned sample size leaves the study underpowered for some of the intended statistical analyses. For the purpose of this dissertation project, analyses were carried out and results reported as intended. These questions will be reanalyzed after more subjects have completed the trial.

5.3 AEROBIC EXERCISE INTERVENTION

Prior to beginning the intervention, the two groups were not significantly different in age, weight, or waist circumference and genders were represented equally in both groups. Subjects in the T2D group were, however, significantly less physically fit than their OW counterparts as measured by VO_2max during screening procedures. There was not a correlation between VO_2max and total TG or IMTG oxidation. In spite of this initial difference in fitness, after intervention both groups exhibited a similar increase in VO_2max with the OW group improving maximal oxygen consumption by 14% and the T2D group by 13.4%. This increase in VO_2max is similar to other studies of previously sedentary individuals tested on cycle ergometers after an intervention of similar length (Hoppeler, H. et al. 1985) and exhibits that the exercise intervention was successful in eliciting an improvement in fitness.

Subjects were asked to not change their caloric or macronutrient intake during the testing procedures and throughout the intervention. This eliminated the possible confounder of a change in body weight or substrate availability due to dietary intake. Both groups remained weight

stable and exhibited only small changes in body composition. This is expected with a relatively short intervention period of 8 weeks.

5.4 RESTING MEASUREMENTS

Small changes in resting energy expenditure (REE) can have profound effects on body weight over the course of a year or more. Long-term (25 weeks) aerobic exercise has been shown to increase in REE in the absence of weight loss in overweight subjects (Hunter, G. R. et al. 2006). Both groups, but not all subjects, were found to have a non-significant increase in REE after participating in 8 weeks of activity. This increase was not directly correlated with time spent in physical activity or caloric expenditure during exercise. Resting measurements were taken using the same methods and equipment for indirect calorimetry as during the exercise intervention; a separate canopy was not used in these measurements.

Percent of energy derived from fat varied greatly between subjects and this variation may have been exacerbated by our choice of equipment for resting measurements. While changes were not significant in either group, the T2D group tended to increase carbohydrate and decrease fat oxidation at rest. Conversely, the OW group responded with a moderate increase in percent of calories derived from fat and thus a slight decrease in RQ. Differences were not found between the two groups in these measurements, however. These findings are similar to those of van Aggel-Leijssen et al which found no change in total fat oxidation at rest following 12 weeks of aerobic activity (van Aggel-Leijssen, D. P. et al. 2002).

Women typically derive a significantly greater portion of total energy from fat compared to men. However because genders were represented equally with approximately 70% females in

each group, this was not an issue. Statistical tests conducted also confirmed that gender was not a cofounder. Though current literature shows that phases of the menstrual cycle do not affect substrate oxidation (Jacobs, K. A. et al. 2005), attempts were made to measure all pre-menopausal women (n=2) in the follicular phase to reduce intra-subject variability.

5.5 SUBSTRATE OXIDATION DURING EXERCISE

Neither group exhibited a significant change in total fatty acid oxidation due to the 8-week intervention. Expressed as percent change, the OW and T2D groups oxidized 6.2% and 5.1% respectively more energy from fat during exercise after participating in the intervention. This failure to support the main hypothesis was *not* due to a failure of the participants to adhere to the prescribed exercise program as evidenced by a significant increase in VO_2max in the OW group and a similar yet non-significant change in the T2D group. The lack of significance in T2D is likely due to the small sample size (n=3) of participants for analysis. Improvements in fitness are further validated by similar HR_{max} and RPE values at the termination of the pre and post maximal graded exercise tests in both groups.

Previous research regarding changes in exercising fat metabolism in obesity and T2D after endurance training varies in their findings. A recent study by Wolf et al utilized a similar exercise protocol in overweight women and reported 12% increase in total fat oxidation (Wolf, D. L. 2006). The intervention however was 16 weeks in length and only included women. Our results are consistent with that of Kanaley et al who reported no significant changes in total fat oxidation after 16 weeks of moderate intensity aerobic exercise (70% VO_2max) in obese women

(Kanaley, J. A. et al. 2001). This study did report a significant increase in carbohydrate oxidation.

While the intensity of the interventions in the studies described above were very similar and found conflicting results, other research suggests that training intensity may be an important factor in governing fatty acid oxidation. When obese men were randomized to either 40% or 70% VO_2max training regime for 12 weeks only the lower intensity group displayed an increase in exercising fat oxidation (van Aggel-Leijssen, D. P. et al. 2002). Most recently, a study in which the exercise intervention was tailored to target maximal fat oxidation ($LIPOX_{max}$) for each individual found a significant increase in fat oxidation in overweight adults with T2D after 10 weeks of $LIPOX_{max}$ exercise training (Bordenave, S. et al. 2008). The authors report average intensity in which subjects achieved $LIPOX_{max}$ was 37% VO_2max. A critical methodological issue may be the pairing of the appropriate training *and* testing intensity to maximize the response of fat oxidation.

Analysis of our study did not find a relationship between training intensity and change in TG oxidation. However, participants trained at a high intensity of approximately 80% of maximal heart rate with no participants exercising at an average training HR below 69% of their predetermined HR_{max}. Whole body and non-plasma fatty acid oxidation were measured at 50% of $VO2_{max}$, however the more vigorous intervention in this study may not have specifically affected fat oxidation. Additionally since training intensity varied little among participants this limits the ability to correlate intensity with other measurements. Because Kanaley et al found a relationship between a similarly high training intensity and glucose oxidation this was also analyzed but found to be not statistically significant in either group or when groups were combined.

Length of intervention may also be critical for inducing metabolic flexibility during exercise. This study was 8 weeks in length while other exercise interventions tend to be 10-24 weeks in duration. Eight weeks was chosen in this project in part to reduce participant burden and drop out and because it is known that 8 weeks is sufficient time to elicit mitochondrial changes, the primary aim in the parent trial. Bordenave's study in men with T2D found an increase in whole body fat oxidation with 10 weeks of training (Bordenave, S. et al. 2008). The training intensity was individualized to target the intensity in which maximal fat oxidation was measured for each subject in a sub-maximal test prior to intervention. Taken together with Wolf et al's (Wolf, D. L. 2006) positive response of fat oxidation when overweight women trained at a high intensity for 16 weeks and with Van Aggel-Leijssen's (van Aggel-Leijssen, D. P. et al. 2002) comparison of training at 40% and 70% VO_2max for 12 weeks, it is plausible that exercise must be sustained for a longer intervention period at high intensities to achieve a measurable change in fat oxidation.

Lastly, our participants completed the baseline and post-intervention sub-maximal exercise tests at the same absolute workload. Because this workload was not adjusted to reflect improvements in fitness, i.e. higher maximal oxygen consumption, subjects were likely tested at a significantly lower intensity post-intervention. This lower intensity would elicit lower total energy and thus lower total triglyceride needs than at baseline. This may have led to an underestimation of change in total triglyceride oxidation.

5.5.1 Contribution of Non-Plasma Fatty Acids to Total Fat Oxidation

IMTG oxidation accounted for approximately 22% and 20% of fatty acids oxidized during physical activity in the OW and T2D groups respectively. This proportion did not change

significantly after intervention. Because no significant change in whole body fat oxidation was seen after 8 weeks of aerobic exercise it is reasonable that the source of fatty acids was not altered as well.

Whether moderate exercise training can increase IMTG breakdown and oxidation remains controversial. The "athlete's paradox" contrasts obese subjects with highly trained endurance athletes and finds both with similar IMTG stores (Goodpaster, B. H. et al. 2001). It is presumed that the athlete readily utilizes IMTG as a substrate during exercise while this same depot contributes to insulin resistance in obesity. A published review of the literature suggests that those with T2D have a reduced capacity to mobilize and/or oxidize IMTG (van Loon, L. J. C. 1997). A more recent study demonstrates an increase in IMTG content in previously sedentary insulin-resistant overweight adults after 16 weeks of exercise training (Dube, J. J. et al. 2008). This is preceded by the examination of an acute bout of exercise comparing men with long-term diagnosis of T2D with healthy matched controls (Boon, H. et al. 2007). In this study, Boon et al. did not find evidence of an impaired mobilization or oxidation of IMTG in T2D subjects.

Disagreement among findings of impairment or conversely the ability to mobilize IMTG in obese and T2D subjects likely are due to methodological differences among published reports. These variances are likely not only due to exercise intervention variances such as intensity and duration as discussed in regards to whole body fat oxidation, but also to laboratory techniques for measuring and quantifying IMTG content and utilization.

5.6 LIMITATIONS

The primary limitation of this study is the small number of subjects, particularly in the T2D group. The time and physically intense pre and post-testing regimen were likely a significant participant burden that limited recruitment and retention of subjects. Efforts remain underway to recruit further subjects and increase the statistical power of these analyses. A second limitation of this study is the method used to measure resting energy expenditure. Because of the length and nature of the sub-maximal exercise tests with isotope tracers and other required procedures in the larger clinical trial, we did not feel it realistic to require a separate RMR measurement using the more accurate canopy. Other limitations may be the length of the exercise intervention at 8 weeks as well as the moderately high intensity of the intervention. A longer intervention period may elicit greater mobilization of IMTG and an overall greater shift towards preferentially oxidizing fatty acids. Likewise, it may be that maximal fat oxidation is occurring at lower intensity than this intervention was conducted. Therefore a more controlled intervention at an intensity that specifically targeted maximal fatty acid oxidation may have elicited a stronger response.

5.7 CONCLUSIONS

In conclusion, 8 weeks of aerobic exercise did not significantly increase the reliance on fatty acids as an energy source at rest or during physical activity in adults who are overweight with or without T2D. Likewise, the intervention did not alter the contribution of non-plasma fatty acids to the total fatty acid pool in either group as quantified using stable isotope technology. A

correlation was not found between fitness, time spent in exercise, or caloric expenditure during exercise and change in these parameters.

5.8 RECOMMENDATIONS FOR FUTURE RESEARCH

The primary goal for further research is to first increase the sample size of both the OW and T2D groups to adequately answer the proposed questions. Prior to enrolling more participants however, a few directions should be considered to answer the proposed questions in a timely manner. Because there are limited publications regarding the response of total fat oxidation to endurance training in the presence of obesity and T2D, it may be reasonable to enroll additional participants to complete the sub-maximal tests *without* using isotope technology, only indirect calorimetry. While this would only measure total fat oxidation and not the source of fatty acids being oxidized, this may be a time and cost effective solution. This would reduce not only the time to complete the study by eliminating two overnight stays per participant as well as the need for intravenous access during the tests, but also drastically reduce the cost, participant risk, and staff needed to complete these tests. This may be prudent given that if no change is seen in total fat oxidation it is unlikely that a significant change in the source of fatty acids would be detected.

There are other considerations to ensure the integrity of the study as we move forward. These include the progression of participants in their weekly minutes spent in physical activity toward the minimum goal of 180 minutes per week by the end of the 8-week intervention. It is also suggested that all participants be required to progress through the 8-week intervention in a

more uniform manner so that during week 1, all participants complete 20 minutes of exercise each session, 25 minutes in week 2, 30 minutes in week 3 and so on.

As there was a *tendency* for both groups to increase whole body fat oxidation and in light of the recently published report by Bordenave et al., further research to define the most appropriate exercise prescription to enhance the oxidation of fatty acids is warranted. This would include not only a further evaluation of exercise intensity, but also exercise mode and duration of exercise sessions. Given the plausible varying effects of low vs. moderate vs. high intensity exercise on substrate utilization and accompanying improvements in insulin resistance, weight loss maintenance, and adherence, a subsequent evaluation of the long term benefits of each is warranted.

At the time this project was proposed and at its completion to this point, an obvious gap in the literature is a detailed time course of changes in substrate utilization due to aerobic exercise in lean, overweight and T2D subjects. This lack of knowledge explains in part why researchers have employed such different methodology in length and intensity of the intervention and thus why these important questions remain unresolved. A project that utilized indirect calorimetry at regular intervals throughout the aerobic exercise intervention would provide insight into when and if subjects become more metabolically flexible over time with endurance training. A comparison should be made between previously sedentary adults who are lean, overweight, and overweight with T2D to best understand any differences between each condition.

APPENDIX A

Average values of RQ, total TG, and total glucose during sub-maximal exercise before and after an 8-week aerobic exercise intervention.

			OW N=10	**T2D** N=3
30 Minute Time point	**RQ**	Pre	0.86	0.83
		Post	0.84	0.83
	Total TG (mg/min)	Pre	230.1	258.1
		Post	243.8	252.5
	Total Glucose (mg/min)	Pre	705.4	576.9
		Post	654.8	591.9
45 Minute Time point	**RQ**	Pre	0.84	0.84
		Post	0.84	0.83
	Total TG (mg/min)	Pre	255.0	254.6
		Post	255.9	266.9
	Total Glucose	Pre	645.5	675.2

	(mg/min)	Post	646.5	581.2
60 Minute Time point	**RQ**	Pre	0.84	0.84
		Post	0.83	0.81
	Total TG (mg/min)	Pre	256.4	267.1
		Post	279.9	295.6
	Total Glucose (mg/min)	Pre	670.7	601.2
		Post	590.8	479.9

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