THE COMBINED EFFECTS OF ACUTE AEROBIC EXERCISE AND OMEGA-3 SUPPLEMENTATION ON GLUCOSE METABOLISM IN HEALTHY, NORMOGLYCEMIC MEN

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To the Dean of the Graduate School:

I am submitting herewith a thesis writing by Ronique Pleasant entitled "The Combined Effects of Acute Aerobic Exercise and Omega-3 Supplementation on Glucose Metabolism in Healthy, Normoglycemic Men". I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters of Science with a major in Exercise Physiology.

Kyle Biggerstaff, Major Professor

We have read this thesis and recommend its acceptance.

Department Chair

Accepted:

Dean of Graduate School

ABSTRACT

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THE COMBINED EFFECTS OF ACUTE AEROBIC EXERCISE AND OMEGA-3 SUPPLEMENTATION ON GLUCOSE METABOLISM IN HEALTHY, NORMOGLYCEMIC MEN

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This study evaluated the effects of acute aerobic exercise and n-3 supplementation on glucose, insulin, and c-peptide concentrations in normoglycemic men. Eleven sedentary, healthy men completed four interventions: rest without n-3 (CON), exercise without n- 3 (EX), rest with n-3 (SUP), and exercise with n-3 (EX-SUP). Significant decreases from the CON group was seen in the incremental area under the curve during an oral glucose tolerance test for insulin in the EX (3371.0 ± 69.1 μU/ml; $p = .01$) and EX-SUP (3350.9 ± 1124.6 μU/ml; $p = .04$) groups, and in c-peptide in the EX (241.9 ± 52.8 ng/ml; $p = .03$) and EX-SUP (231.9 ± 76.6 ng/ml; $p = .02$) groups. These data suggest that aerobic exercise effected only insulin and c-peptide concentrations, n-3 alone did not effect any variable, and the combination of aerobic exercise and n-3 effected only insulin and c-peptide concentrations in healthy, normoglycemic men.

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CHAPTER I

INTRODUCTION

In 2007, the Centers for Disease Control and Prevention (CDC) reported that 23.6 million people (7.8% of population) had type 1 or 2 diabetes; 17.9 million being diagnosed with diabetes and 5.7 million undiagnosed. In 2006, diabetes was the seventh leading cause of death in the United States, and overall people with diabetes have twice the risk for death than people without diabetes that are similar in age (CDC, 2007). Diabetes also increases the risk for heart disease and stroke (American Diabetes Association [ADA], 2009). In many cases diabetes can be prevented.

Diabetes is a group of metabolic diseases characterized by hyperglycemia (high blood glucose concentration). Hyperglycemia is a result of defects in insulin secretion and/or action (ADA, 2009). There are several risk factors for diabetes that include age, obesity, family history, physical inactivity, and ethnicity. There are two classifications of diabetes, type 1 (insulin dependent) and type 2 (non-insulin dependent). Basic treatments for type 1 diabetes consist of healthy eating, regular physical activity, and insulin therapy. Treatment for type 2 diabetes also consists of healthy eating and physical activity, along with blood glucose testing, medications to control glucose concentrations, and insulin therapy (CDC, 2007; National Diabetes Information Clearinghouse [NDIC], 2006). Insulin is a hormone that helps regulate blood glucose concentrations, and is secreted in response to high a concentration of blood glucose. Skeletal muscle is the most responsive to insulin, and clears most of the glucose from the blood (Aronoff, Berkowitz, Shreiner, & Want, 2004; Jue et al., 1989; Turcotte & Fisher, 2008). An impaired

response to insulin results in an inability of maintaining normal blood glucose concentrations (Turcotte & Fisher, 2008).

Acute exercise and exercise training has become a primary prevention method for type 2 diabetes due to the effects it has on regulating glucose metabolism and increasing insulin action (Horowitz, 2007; Pan et al., 1997). Other benefits of exercise for diabetics are improvements in hypertension, body composition, and lipid profiles (ADA, 2002; Pan et al., 1997; Wallberg-Henriksson, Rincon, & Zierath, 1998). Regular physical activity results in metabolic adaptations of sustained improvements in whole-body and muscle insulin sensitivity (Hawley & Lessard, 2008). Although, participating in regular physical activity has several health benefits, improved insulin action and insulin sensitivity seem to be short lived. This is supported by past studies by Heath el al. (1983) and King et al. (1995) that reviewed the acute effects of exercise on insulin action, and found that improvements in insulin sensitivity can occur for 24-72 hr after the last session of exercise. King et al. (1995) exercised a group of moderately trained middle-aged men for 5 days at 75% VO_2peak for 45 min, and then gave them an oral glucose tolerance test (75 g) ~30 min and 1, 3, 5, and 7 days post exercise. The results of this study revealed that insulin action increased significantly from days 3 to 5 ($p < .05$), but overall insulin response on days 5 and 7 did not differ. Insulin sensitivity also increased immediately after exercise (1,162 ± 373 μU/ml; $p < .05$) compared to day 1 and 3, significantly increased for day 1 (362 ± 205μU/ml)) and day 3 (378 ± 179 μU/ml) compared to day 5 (843 ± 441 μU/ml) and day 7 (971 ± 510 μU/ml), and significant improvements were only seen up to day 3. Heath et al. (1983) examined the effects of 10 days of detraining on a group of eight healthy, trained men and women that had been exercising vigorously, either by running or cycling 5-7 d/wk for 45 min a day for the past 6 months. These trained persons were given a glucose tolerance test (100 g) on the first day of

physical inactivity, which was also the day after their last exercise session, and the morning of the 11th day after the 10 days of physical inactivity. On the 12th day of the study these participants performed one session of their normal exercise routine, followed by a third oral glucose tolerance test the next day. Results revealed that after 10 days of detraining insulin response during an oral glucose tolerance test were 55-120% greater ($p < .05$) than in the trained state (117 ± 9 μU/ml), and after detraining a single session of exercise insulin response was significantly (147 ± 15 μU/ml; $p < .05$) blunted. These finding suggest that a decrease in insulin response after an oral glucose tolerance test are an effect of the last exercise session, and regular physical activity has an even greater effect. Therefore, regular exercise is necessary to maintain improvements of insulin response.

Several clinical trials have demonstrated that lifestyle interventions through diet, along with an increase in physical activity, decrease the risk for diabetes in persons with impaired glucose tolerance (Gill & Cooper, 2008). Impaired glucose tolerance is when glucose concentrations are between 140-199 mg/dL after an oral glucose tolerance test (OGTT), and is associated with insulin resistance and indicates prediabetes (NDIC, 2006; Trevisan, Vedovato, & Tiengo, 1998). Pan et al. (1997) conducted a 6-year clinical trial that took 530 men and women, over 25 years of age, with impaired glucose tolerance, and randomly assigned them to a diet only, exercise only, or diet and exercise intervention. The purpose of this study was to determine which intervention delayed the incidence of type 2 diabetes. The diet only intervention encouraged participants to eat more vegetables, less simple sugars, control alcohol intake, and reduce caloric intake. The exercise only group encouraged participants to increase their leisure physical activity by 1 hr/day, and 2 hr/day for those less than 50 years old. The diet and exercise group were given the same instructions as the other two interventions. All interventions

displayed a decrease of incidence of diabetes; 33% in diet only, 47% in exercise only, and 38% in diet and exercise.

Another clinical study by Tuomilehto et al. (2001) assigned 522 middle-aged, overweight men and women with impaired glucose tolerance to a 3-year lifestyle intervention, and separated them into an intervention group or control group. This study also evaluated the effects of dietary adjustment and regular physical activity on the incidence of type 2 diabetes, with the overall goals being 1) a 5% or more reduction in weight, 2) less than 30% total intake of fat for energy consumed, 3) less than 10% intake of saturated fat for energy consumed, 4) a 15 g increase of fiber intake per 1000 kcal, and 5) participate in moderate exercise for at least 30 min/day. Participants were encouraged to frequently ingest whole grain products, vegetables, fruits, low fat milk and meat products, soft margarines, and monosaturated fatty acid vegetable oils. There was a 58% reduction in the incidence of diabetes for this study. Other similar clinical studies, such as Knowler et al. (2002) and Ramachandran et al. (2006) also concluded that healthy lifestyle changes decrease the risk for type 2 diabetes.

Evidence shows that diet modifications decrease the risk for type 2 diabetes, but dietary supplementation could be another preventative method to control type 2 diabetes. The consumption of additional amounts of omega-3 fatty acids (n-3) has been found to improve risk factors for cardiovascular disease, and also possibly decreases risk for developing type 2 diabetes (Kesavulu, Kameswararao, Apparao, Kumar, & Harinarayan, 2002; Nettleton & Katz, 2005). Fatty acids are classified into saturated fatty acids and unsaturated fatty acids, with unsaturated fatty acids being monounsaturated or polyunsaturated. Polyunsaturated fatty acids consists of n-3, which is an essential fatty acid found in oily fish (Martin de Santa Olalla, Sanchez Muniz, &

Vaquero, 2009). Studies that solely use n-3 and/or diet modifications to improve glucose metabolism have been inconsistent.

For example, the KANWU study (Vessby et al., 2001), found that substituting a monosaturated fatty acid diet for a saturated fatty acid diet slightly improved insulin sensitivity in healthy persons. The study was a 90 day experiment using 162 healthy men and women aged 30-65 years, which were assigned to a high saturated fatty acid or monosaturated fatty acid diet. Within the groups was a second random assignment to n-3 supplements (3.6 g/day) or placebo. The participants on the saturated fatty acid diet had a decrease of 10% ($p = .03$) in insulin sensitivity, and a 2% ($p = .51$) in the monosaturated fatty acid diet; insulin secretion was not affected in either group. The addition of n-3 supplements had no effect on insulin sensitivity or secretion, and had no interaction between the treatment effects. Studies that used overweight individuals, such as Mori et al., (1999) and Browning et al., (2007) found improvements in insulin sensitivity with n-3 supplementation/diet and weight loss, but results from Mori et al. (1999) revealed that the n-3 treatment alone had no effect on glucose-insulin metabolism. Another study using 162 healthy men and women, in a similar research design to the KANWU study, also used 3.6 g/day supplementation of fish oil as a second randomized assignment along with monosaturated and saturated fat diets and found no effect on insulin sensitivity or glucose tolerance (Giacco, et al., 2007). A study completed by Mostad, Bjerve, Bjorgaas, Lydersen, and Gill (2006) only used fish oil supplementation (1.8 g/d, 3.0 g/d, and 5.9 g/d) for a 1 week and 9 week intervention, with 26 type 2 diabetic participants. This study concluded that a decrease in insulin sensitivity, as well as a moderate increase in blood glucose concentrations occurred in the groups that consumed higher amounts of fish oil.

Several studies have examined the combined effects of n-3 fatty acids and aerobic exercise on body composition and/or cardiovascular risk factors (Warner, Ullrich, Albrink, & Yeater, 1989; Brilla & Landerholm, 1990; Westerveld, et al., 1993; Axelrod, et al., 1994; Hill, Buckley, Murphy, & Howe, 2007), but very little research has been conducted to evaluate the effects on glucose metabolism. A study by Dunstan et al. (1997) examined the effects of dietary fish supplementation and aerobic exercise on serum lipids and glycemic control in dyslipidemic type 2 diabetics. The study design was an 8 wk intervention using 55 sedentary, dyslipidemic, type 2 diabetics, and assigned them to a low-fat diet with or without a daily fish meal (3.6 g/d) and a moderate (55-65% VO_2max) or light (heart rate $\leq$100 bpm) exercise group. The addition of fish to the low-fat diet resulted in a decrease in triglycerides (0.08 ± 1.3 mmol/l, p = .03), a decrease in HDL_3 cholesterol (0.05 ± 0.07 mmol/l, p = .02), an increase in HDL_2 cholesterol (0.06 ± 0.07 mmol/l, p = .1), and an increase in LDL cholesterol (0.22 ± 0.42 mmol/l, p = .01) and glycated hemoglobin (0.33 ± 0.17%, p = .06). The fish meal and light exercise program demonstrated a significant increase in glycated hemoglobin (0.50 ± 0.24%, p = .05), which was attenuated in the fish meal and moderate exercise program (0.19 ± 0.25%, p = .44) and a 21.71 ± 10.7 pmol/l (p = .05) decrease in fasting serum insulin (78.2 ± 47.2 pmol/l) compared to control group. This result of this study suggested that a low-fat diet consisting of one fish meal a day can result in substantial improvements in triglycerides and increased HDL_2 cholesterol in dyslipidemic type 2 diabetics, and the addition of moderate exercise training can assist in maintaining normal glycemic control. Other studies such as, Delarue, Labarthe, and Cohen (2003), Delaure et al. (1996), and Bortolotti, Tappy, and Schneiter (2007) evaluated the effects of n-3 supplementation during exercise on the rate of glucose disappearance, hepatic glucose production, and lipid and carbohydrate oxidation with 6 g/d or 7.2 g/d of n-3 supplementation. These studies revealed that 6 g/d of fish oil for 20 days decreased hepatic glucose production (-

21%; $p < .05$) and the rate of glucose disappearance (-26%; $p < .05$) during exercise (Delarue, Labarthe, & Cohen, 2003), but neither 6 g/d nor 7.2 g/d resulted in any significant difference in energy metabolism or lipid oxidation.

Problem Statement

Regular physical activity has been encouraged as a method to regulate glucose metabolism and increase insulin sensitivity. The use of n-3 supplementation can improve lipid profiles, but how it effects glucose metabolism is still not clear. Mechanisms for improving insulin sensitivity with acute exercise are different than that of the dietary supplementation of n-3 (Anderson & Ma, 2009; Cartee et al., 1989; Das, 2005; Holloszy, 2005; Horowitz, 2007; Nugent, et. al., 2001; Sugii, et al., 2009; Turotte & Fisher, 2008). Improvements in insulin sensitivity from a single session of acute exercise are short-lived (48–72 hr), unless regular exercise is maintained (Turcotte & Fisher, 2008; Mikines, Sonne, Farrell, Tronier, & Galbo, 1988). Improvement of insulin response from dietary supplements takes days to weeks. Studies that have examined the effects of n-3 supplementation on insulin action in participants that are diabetic or are at risk for diabetes have had inconsistent results. If the regular consumption of n-3 has positive effects on insulin action, then a two-fold intervention plan (exercise plus n-3 supplementation) could be beneficial in the regulation of glucose metabolism, as well as used a potential prevention method of type 2 diabetes. The purpose of this study will be to determine effects of acute aerobic exercise and n-3 supplement, on glucose, insulin, and c-peptide (amino acid used to gauge insulin secretion) responses in healthy, normoglycemic men.

Null Hypothesis

Insulin, glucose, and c-peptide responses will be compared across 4 conditions: 1) rest without n-3 supplementation (CON), 2) exercise without n-3 supplementation (EX), 3) rest with n-3 supplementation (SUP), and 4) exercise with n-3 supplementation (EX-SUP). The null hypotheses are:

1. There will be no effect of exercise on glucose, insulin, or c-peptide concentrations.
2. There will be no effect of n-3 fatty acid supplementation on glucose, insulin, or c-peptide concentrations.
3. There will be no interaction of exercise and n-3 fatty acid supplementation on glucose, insulin, or c-peptide concentrations.

Definitions

1. Acute exercise is a single bout of exercise.
2. Aerobic exercise is a type of exercise that is rhythmic in movement and involves large muscle groups (American College of Sports Medicine [ACSM], 2006)
3. Impaired glucose tolerance is defined as blood glucose concentrations between 140-199 mg/dl during a 2 hr oral glucose tolerance test (ADA, 2009).
4. Impaired fasting glucose is defined as blood glucose concentrations between 100-125 mg/dL after an 8-hr fast (ADA, 2009).

5. Insulin resistance is commonly seen in obesity and during the development of type 2 diabetes; defined as a reduction in the body's ability to clear glucose from the circulation in response to insulin (Turcotte & Fisher, 2008).

6. Insulin sensitivity is defined as an efficient submaximal insulin response to stimulate glucose transport (Turcotte & Fisher, 2008).

7. Moderate intensity exercise is exercise that elicits a noticeable increase in heart rate and breathing in the range of 64-76% of maximal heart rate or 40-59% of heart rate reserve (ACSM, 2006).

8. Normoglycemic is defined as fasting plasma glucose less than 100 mg/dL (ADA, 2007).

9. Oral glucose tolerance test is a test that measures blood glucose concentrations after a fast and during the 2 hours after drinking a drink rich in glucose; blood glucose levels are measured at 0, 15, 30, 60, 90, and 120 minutes post-ingestion of 75 g of glucose solution.

10. Type 2 diabetes is a condition when the body does not produce enough insulin, or when cells do not respond to insulin (ADA, 2009).

11. Vigorous intensity exercise is exercise that elicits a substantial increase in heart rate and breathing in the range of 77-93% of maximum heart rate or 60-84% heart rate reserve (ACSM, 2006).

Assumptions

This study was conducted the following assumptions:

1. Each participant's dietary recall will be as accurate as possible.

2. Each participant will fully comply with the consumption of n-3 supplement.

Limitations

This study was conducted with the following limitations:

1. Results of this study may not be generalized to a population outside of healthy, sedentary men.

2. The limited sample size may not provide practical implications.

3. Participant's prior diet and exercise history may effect results.

Significance of Study

Diabetes has become more prevalent and is expected to continually increase in the years to come. Diabetes is also one of the leading causes of death in the United States, making the prevention of diabetes very important (CDC, 2007). The typical treatment of diabetes is with a healthy diet and regular physical activity, but methods for preventing diabetes are not clear (CDC, 2007; NDIC, 2006). Findings have been inconsistent on the effects of solely consuming n-3 fatty acids to improve glucose metabolism, but it is thought to be possible method for preventing type 2 diabetes (Ebbesson, Risisca, Ebbesson, Kennish, & Tejero, 2005; Kesavulu et al., 2002). Therefore, by recognizing the combined and independent role of n-3 fatty acids and exercise on glucose metabolism, additional knowledge on how to prevent diabetes will be provided.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this study is to evaluate the effects of acute aerobic exercise and n-3 supplementation on glucose, insulin, and c-peptide responses in healthy, normoglycemic men. To better understand the effects that acute exercise and n-3 fatty acids have on glucose metabolism, the following topics were reviewed: (a) normal glucose metabolism, (b) the physiological effects of diabetes, (c) glucose metabolism during exercise in normoglycemic individuals, (d) the relationship between regular and acute physical activity on glucose metabolism, (d) effects of n-3 fatty acid on glucose and insulin responses, and (e) effects of n-3 fatty acids and exercise on glucose and insulin responses.

Normal Glucose Metabolism

Glucose is a simple six-carbon sugar used as a source of energy for the body, derived from the digestion of carbohydrates and the breakdown of glycogen in the liver. The concentration of glucose in blood plasma is precisely regulated by glycogenolysis, gluconeogensis, and intestinal absorption during the fed state (Aronoff et al, 2004). Glucose is absorbed by the small intestine and released into the blood stream where it either (1) becomes an energy source for cell metabolism, (2) is stored as glycogen in the liver and muscles, or (3) is converted to fat in the form of triacylglycerol. Normal blood glucose concentration is approximately 100 $mg\,dl^{-1}$, which is required for normal function of the central nervous system and other organs and cells that require glucose (ADA, 2007; Brooks, Fahey, & Baldwin, 2005). Glucose molecules are broken down through a metabolic pathway called glycolysis. Glucose

molecules that do not undergo glycolysis are linked together to form glycogen, which is then broken down by a process called glycogenolysis.

The uptake and use of glucose by cells is dependent on several factors that include the type of tissue, the amount of glucose available in the blood and tissue, the physiological status of the tissue, and the presence of the endocrine hormones of the pancreas, insulin and glucagon (Brooks et al., 2005). Insulin is a protein made of two polypeptide chains that consist of 51 amino acids, and is secreted from beta cells of the pancreas. This hormone is secreted when blood glucose concentrations are higher than normal, making it responsible for stimulating the transport of glucose from the blood stream and into tissues. The most responsive tissue to insulin is skeletal muscle. Most glucose that is cleared from the blood in response to insulin is stored as glycogen in the skeletal muscle (Jue et al., 1989; Turcotte & Fisher, 2008). Skeletal muscle is an important factor in the regulation of normal blood glucose concentrations; therefore, if insulin-stimulated glucose transport is altered or diminished, it results in an inability to keep blood glucose concentrations at normal ranges (Turcotte & Fisher, 2008). Glucagon is a catabolic hormone consisting of 29 amino acids, and is secreted by alpha cells of the pancreas (Aronoff et al., 2004). Glucagon is secreted when blood glucose concentrations fall below normal levels, and is responsible for stimulating hepatic glucose production to increase blood glucose concentration. This hormone effects hepatic metabolism by enhancing glycogenolysis and increasing gluconeogenesis (Cyrer, Davis, & Shamoon, 2003).

Diabetes and the Physiological Effects

Diagnosis of Type 2 Diabetes

Diabetes is a disease that inhibits the body from producing or properly utilizing insulin. In type 2 diabetes the body does not produce insulin, due to the insulin producing beta cells in the pancreas being destroyed. Type 2 diabetes derives from the body's progressive impaired ability to secrete sufficient amounts of insulin in response to a rise in glucose concentrations, eventually causing the body to become insulin resistant (ADA, 2007; Gannon & Nuttall, 2006). By using either a fasting plasma glucose test (FPG) or an oral glucose tolerance test (OGTT), it can be determined if a person is prediabetic or diabetic. A FPG is commonly used for diagnosing diabetes in nonpregnant adults and children, and is most accurate when performed in the morning after an 8 hr fast (NDIC, 2006). If blood glucose concentration is < 100 mg/dl after a FPG, this is considered to be normal; between 100-125 mg/dl is considered to be impaired glucose tolerance; and ≥ 126 mg/dl is diagnosed as diabetes and a follow up test should be performed to confirm the diagnosis (ADA, 2009). When performing an OGTT, fasting blood glucose concentration is measured before they are given a glucose-rich drink that consists of 75 g of glucose dissolved in water. Two hours after consuming the drink, blood glucose concentration is measured again (NDIC, 2006). If the blood glucose concentration is < 140 mg/dl, this is considered to be normal; if between 140-199 mg/dl, this is considered to be impaired glucose tolerance; and ≥ 200 mg/dl, the diagnosis is diabetes and should also be confirmed with another test on a separate day (ADA, 2009). A diagnosis of impaired glucose tolerance or impaired fasting glucose is considered to be prediabetes, and increases the risk for type 2 diabetes, as well as stroke and heart disease.

Complications of Diabetes

Diabetes causes acute and chronic complications consisting of hyperglycemia (high blood glucose concentrations) and hypoglycemia (low blood glucose concentrations). Hyperglycemia develops from frequent high blood glucose concentrations and/or poor glycemic control. Symptoms of hyperglycemia include frequent urination, excessive thirst, weight loss, fatigue, and/or blurred vision; acute consequences being ketoacidosis (diabetic coma) or hyperosmolar nonketotic syndrome, also known as prolonged hyperglycemia (Kisiel & Marsons, 2009). Chronic effects of hyperglycemia can lead to the damage, dysfunction, and failure of the eyes, kidneys, heat, blood vessels, and nerves of the body (Kisiel & Marsons, 2009). Hypoglycemia occurs when blood glucose concentrations decrease below normal ranges and can be the effect of diabetic treatment (too much insulin or antidiabetic oral agents administered), not consuming enough carbohydrates, missed meals, and/or excessive exercise (Cefalu, C. A. & Cefalu, 2005; McAulay, Deary, & Frier, 2001;). The symptoms of hypoglycemia include sweating, hunger, anxiety, trumulousness, dizziness, light-headedness, and several other symptoms (Cefalu, C. A. & Cefalu, 2005; Cryer et al., 2003; McAulay, Deary, & Frier, 2001). Moderate hypoglycemia can result in impaired motor function and or confusion, while severe hypoglycemia can lead to a coma, seizure, brain damage, and/or death (Cryer et al., 2003; Davis & Alonso, 2004). Other chronic complications of diabetes are adult-onset blindness, nontraumatic lower-limb amputation, and end-stage renal failure, as well as a diabetics risk for heart disease and stroke being increased 2 to 4 times more (Cefalu, C. A. & Cefalu, 2005; McAulay, Deary, & Frier, 2001).

Glucose Metabolism During Exercise

Blood Glucose Homeostasis During Exercise

Blood glucose homeostasis at rest and during exercise is regulated by several physiological systems, including the endocrine system and sympathetic nervous system (Suh, Paik, & Jacobs, 2007). Important tissues involved in energy metabolism, such as the liver, pancreas, adrenal medulla, adipose tissue, and skeletal muscle are innervated and physiological functions are regulated by the autonomic nervous system (Nonogaki, 2000). Glucose metabolism is effected by the subdivision of the autonomic nervous system, being the sympathetic and parasympathetic responses (Carnethon & Craft, 2008). An increase in plasma glucose concentrations, after a meal for example, will signal for the parasympathetic neurons to stimulate the pancreas to produce insulin, while the liver increase insulin sensitivity and ceases the production of glucose to accommodate for the increasing in glucose uptake (Carnethon & Craft, 2008). This increase in parasympathetic activity is also referred to as anabolic (synthesis) effect of glucose and is a method for storing energy when physical demands are low (Carnethon & Craft, 2008; Nonogaki, 2000). A catabolic (break down) effect of glucose occurs with an increase in sympathetic neural activity (Nonogaki, 2000). An increase in sympathetic activity blunts pancreatic insulin secretion, the stimulation of hepatic glucogensis, and decreases the uptake of glucose. This response ensures the availability of glucose as an energy source during times of high psychological or physical demands (Carnethon & Craft, 2008).

Muscle glycogen is the primary source of energy for muscle contractions during the early stages of exercise, and circulating glucose and nonesterified fatty acids are more essential as exercise duration increases (Suh et al., 2007). Hepatic glucose production is regulated by the

stimulation/inhibition of hepatic portal venous levels of glucagon and insulin, epinephrine and norephrine, and the synthesis of glucose by glucocorticoids (Stanley & Connett, 1991). The release of epinephrine from the adrenal medulla and the sympathetic response of glucagon release from the pancreas also contribute to hepatic glucose production (Nonogaki & Iguchi, 1997; Shimazu, 1996). Changes in these hepatic glucose controllers occur early during exercise. Generally during exercise there is no decrease in blood glucose concentrations until after approximately 1 hr of moderate to vigorous exercise (Stanley & Connett, 1991; Wolfe, Nadel, Shaw, Stephenson, & Wolfe, 1986).

During moderate intensity exercise the liver maintains glucose homeostasis by matching the increase of glucose utilization in the muscle to glucose production, as long as there is a sufficient supply of glycogen in the liver. During prolonged exercise energy requirements are increased, and stored liver glycogen decreases. In order to maintain glucose homeostasis during prolonged exercise, the liver must conserve by channeling carbon-based compounds (lactate, glycerol, amino acids) into gluconeogenic pathways (Suh et al., 2007). Insulin concentrations also decrease to minimize glucose uptake by nonactive muscles in attempts to maintain homeostasis (Brook et al., 2005; Vranic, Kawamori, Pek, Kovacevic, & Wrenshall, 1976).

Skeletal Muscle Glucose Uptake During Exercise

A single aerobic exercise session and exercise training have the ability to regulate glucose metabolism and increase the action of insulin for several hours as a result of reduced muscle glycogen concentrations (Cartee et al., 1989; Horowitz, 2007; Holloszy, 2005; Turotte & Fisher, 2008). Exercise increases skeletal muscle glucose uptake, but the magnitude of this increase is dependent on the intensity and duration of the exercise (Suh et al., 2007). During

exercise there is a substantial increase in blood flow to working muscles, allowing glucose to become more available to the muscles (DeFronzo, Ferrannini, Sato, Felig, & Wahren, 1981). This is possible because exercise stimulates muscle membrane glucose transport, and insulin and muscle contractions stimulate the translocation of GLUT4 (Richter, Derave, & Wojtaszewski, 2001; Suh et al., 2007). The glucose transporter protein GLUT4, facilitates movement of glucose across the plasma membrane of a cell, and is stimulated by insulin. There are multiple factors in the working skeletal muscle that may be responsible for the response of GLUT4 (Ryder, Chibalin, & Zierath, 2001; Turcotte & Fisher, 2008). It is possible that increases in the cytosolic concentrations of calcium ions and nitric oxide during exercise are enough to stimulate glucose transport in muscle (Turcotte & Fisher, 2008). Another possible factor could be the increase in the ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) (Turcotte & Fisher, 2008). The hydrolysis of ATP occurs in the working muscle, and the increase of adenosine diphosphate (ADP) concentrations provides a substrate for a reaction to occur to replenish ATP and produce AMP. The increase in the AMP/ATP ratio seems to activate the protein AMP-activated protein kinase (AMPK), which also stimulates glucose transport (Fisher, Gao, Han, Holloszy, & Nolte, 2002; Fujii et al., 2005; Fujii, Jessen, & Goodyear, 2006; Hayashi et al., 2000; Mu, Brozinick, Valladares, Bucan, & Birnbaum, 2001;).

Physical Activity and Glucose Metabolism

Effects of Regular and Acute Physical Activity

For the promotion and maintenance of health, all healthy adults 18-65 years of age need to participate in moderate-intensity aerobic physical activity for at least 30 min 5 d/wk, or vigorous-intensity aerobic activity for at least 20 min 3 d/wk. The combination of moderate and

vigorous-intensity activity can also be performed (Haskell et al., 2007). Regular physical activity has numerous health benefits, with many of these benefits being related to reduced insulin needs and improved glucose tolerance. Participation in regular physical activity produces metabolic adaptations of sustained improvements in whole-body and muscle insulin sensitivity (Hawley & Lessard, 2008). As mentioned previously, insulin action improves for several hours following a single session of aerobic exercise as a result of reduced muscle glycogen concentrations (Cartee et al., 1989; Holloszy, 2005; Horowitz, 2007; Turotte & Fisher, 2008). The reduction of muscle glycogen after exercise seems to be result of an increase in activity of glycogen synthase, which is a rate-limiting enzyme involved in the formation of glycogen once glucose has entered the cell (Turcotte & Fisher, 2008). The increase of glycogen synthase after exercise is considered to be attributable to a decrease in glycogen content in the exercised muscle, and inverse relationship found between glycogen concentrations in skeletal muscle and glycogen synthase activity after exercise (Bogardus, et al., 1983; Turcotte & Fisher, 2008). Insulin response is increased in skeletal muscle due to the decrease in glycogen concentration in order to replenishing muscle glycogen, resulting in enhanced insulin sensitivity (Richter et al., 2001) and an increase in whole-body glucose disposal after exercise (Brun, Guintrand-Hugret, Boegner, Bouix, & Orsetti, 1995; Hayashi et al., 2005; Wojtaszewski et al., 2000). The enhancements of these metabolic responses are also short lived if another bout of exercise is not performed after approximately 48-72 hr (Mikines et al., 1988; Turcotte & Fisher, 2008).

The increase of insulin sensitivity and glucose uptake in the skeletal muscle after exercise has been supported by animal and human studies (Houmard, Shaw, Hickey, & Tanner, 1999; Khayat, Patel, & Klip, 2002; Richter, Mikines, Galbo, & Kiens, 1989; Richter, Ploug, & Galbo, 1985; Wallberg-Henrikkson, Constable, Young, & Holloszy, 1988). In healthy men a single exercise session has been found to increase insulin stimulated whole-body glucose uptake up to

16 hrs after exercise (Bogardus et al., 1983; Mikines et al., 1988), as well as decreases in the insulin response to OGGT (Young, Enslin, & Kuca, 1989); another indication of increased insulin sensitivity.

A study Brestoff et al. (2009) performed a study that evaluated whether an acute session of endurance exercise or sprint interval exercises using a cycle ergometer enhanced insulin sen itivity. This study consisted of 13 healthy and recreationally (< 1.5 d/wk) active men and women (age 20.7 ± 0.4 yrs) that were divided into an acute session of endurance exercise (45 min at ~75% VO_2peak) group or a sprint interval exercise (five 30 sec sprints at ~125% VO_2peak) group. Participants were given an OGTT at baseline with no exercise performed, and then the morning following each exercise session after a 12 hr fast. Variables measured were insulin and glucose concentrations area under the curve and during the OGTT, and insulin sensitivity was measured using the composite whole-body insulin sensitivity index for OGTT equation. No significant changes in plasma glucose concentrations were found for either exercise group, but the OGTT revealed lower concentrations of insulin (p = .012) after acute endurance exercise. The area under the curve for insulin concentrations after endurance exercise was 25.6% (p = .019) lower than baseline and 18.6% (p = .045) lower than sprint interval exercise. Relative to baseline the acute endurance exercise increased insulin sensitivity by ~70-100% and ~40-80% relative to sprint interval exercise; insulin sensitivity was not enhance in the sprint interval exercise group. The findings of this study demonstrated that acute endurance exercise enhances insulin sensitivity in healthy men and women.

Another study by Hayashi et al. (2005) also demonstrated the effects of acute exercise on insulin sensitivity by evaluating the effects of a single bout of exercise at different intensities on glucose effectiveness, glucose disappearance, and insulin sensitivity. This study used six healthy, active men (age 28.5 ± 2.0 yr) and exercised them on a cycle ergometer at 50% and 70% VO_2max

for 30 min. Immediately (30 min) after exercise the participants were given a stable-labeled frequently sampled intravenous glucose tolerance test and blood samples were taken up to 180 min to measure insulin and glucose concentrations. Insulin sensitivity and glucose effectiveness were measured with a two-compartment minimal model, and glucose disappearance was calculated as the slope of the least squares regression line related to the natural logarithm of glucose concentration to the time when blood samples were drawn. The results of this study revealed significant improvements only at 70% VO_2max; glucose effectiveness (1.02 ± .11 dl/kg/min), glucose disappearance (3.48 ± .30 %/min) insulin sensitivity (18.34 ± 3.20 dl/kg/min/(μU/ml)) being higher ($p < .05$) than the control (no exercise) group (.53 ± .12), and glucose effectiveness being higher ($p < .05$) than 50% VO_2max measurements (.72 ± .08).

A study by Poehlman, Dvorak, and DeNino (2000) compared the effects of resistance training and aerobic training on insulin sensitivity, measured by a hyperinsulinemic-euglycemic clamp technique, in 51 young, untrained, nonobese women (18-57 yr of age) in a 6 month randomized program. Possible mechanisms for altering insulin sensitivity, such as body composition, regional adiposity, and skeletal muscle characteristics, were also measured. Participants were divided into an endurance training group, resistance training group, or a control group and trained 3 d/wk nonconsecutively for 6 months. The endurance training consisted of a base training phase and interval recovery phase for a total of 16 weeks. The first four weeks of training were 25 min of slow jogging with time increasing time by 5 min and intensity by 5% heart rate max per 4 wk phase, progressing to 40 min at 90% of heart rate max. Interval training was designed to increase exercise intensity and duration to successfully complete 60 min at 85% of heart rate max. Resistance training was performed at approximately 80% of 1-RM on the following weight machines: leg press, bench press, leg extensions, arm curls, and leg curls. The resistance training intervention resulted in an increase ($p < .05$) of body weight ($p < .05$), body

mass index ($p < .05$), and free fat mass (2 kg; $p < .001$). There were no changes of free fat mass in the endurance trained or control groups, and no changes in fat mass in any of the interventions. Insulin sensitivity increased in both endurance (16%, $p < .05$; respectively) and resistance (9%, $p < .06$; respectively), with improvements in glucose disposal with endurance training (15%, $p < .05$; respectively). No significant changes were found in regional adiposity or skeletal muscle characteristics. This study concluded that enhancements in glucose uptake after physical training can occur in this group of young women, regardless of changes in free fat mass or body composition, along with improvements in insulin sensitivity with resistance training.

Another study by Short et al. (2003) examined the effects of a 4 month moderate intensity aerobic exercise program on insulin sensitivity in 102 healthy, sedentary men and women (21-87 yr of age). One main goal of this study was to evaluate if this aerobic training program would lead to similar improvements in insulin sensitivity across this age span. A stationary bicycle was used for exercise, with initial training 3 d/wk for 20 minutes at 70% maximal heart rate and progressing to 4 d/wk for 40 minutes at 80% maximal heart rate. Participants were instructed to maintain their body weights during the study. Insulin sensitivity was determined by using an intravenous glucose tolerance test. There were no significant changes in fasting glucose or insulin concentrations, but insulin sensitivity was altered. An increase in insulin sensitivity of 72% ($p < .001$) was found in the younger group (20-39 yrs), 20% ($p < .11$) in the middle age group (40-59 yrs), and 5% ($p < .42$) in the older group ($\geq$ 60 yrs). These changes were not related to any changes of VO_2 peak, body composition, muscle metabolic parameters, or any other variables other than age. The conclusion of this study suggested that moderate aerobic exercise training can improve insulin sensitivity in young men and women, but not older persons and that specific exercise programs may be needed to prevent the onset of diabetes in older persons.

Specific exercise prescriptions for improving insulin action are still in question. Some studies suggest that vigorous intensity exercise of ≥ 70% VO_2 peak (Hayshi et al., 2005; Kang et al., 1996; Seals, Hagberg, Hurley, Ehsani, & Holloszy, 1984) is enough to improve insulin sensitivity, while other suggest mild to moderate intensity exercise is sufficient (Mayer-Davis et al., 1998; Oshida, Yamanouchi, Hayamizu, & Sato, 1989). Houmard et al. (2004) evaluated different exercise training interventions of different intensities and training volumes in attempt to find an effective exercise prescription that improves insulin action. The study consisted of 154 sedentary, overweight or obese men and women (age 50-55 yrs) that were assigned to either a control or exercise group for six months. Exercise was performed on cycle egometers, treadmills, and/or elliptical machines; groups were a low volume/moderate intensity (~12 miles/wk at 40-55% VO_2peak), low volume/high intensity (~12 miles/wk at 65-80% VO_2peak), and high volume/high intensity (~20 miles/wk at 65-85% VO_2peak). A 3-hr intravenous glucose tolerance test was performed at baseline and 24 hr after the last exercise session. Fasting insulin concentrations decreased (p = .001) in the low volume/moderate intensity and high volume/high intensity exercise groups, but there were no differences in fasting glucose concentration or glucose effectiveness any exercise group. All exercise groups had an increase in insulin sensitivity (p = .001), with the low volume/moderate intensity and high volume/high intensity groups having ~85% increase and the low volume/high intensity group having ~40% increase. The results of this study concluded that a low volume/moderate intensity and high volume/high intensity exercise training program is an effective exercise prescription to improve insulin action. The actual time per week for the low volume/moderate intensity and high volume/high intensity was ~170 min, and the low volume/high intensity exercise was ~115 min. These findings also suggest that duration of exercise should be considered for improving insulin action.

Regular Physical Activity and Prevention of Diabetes

The role of exercise has become a primary prevention or delay of type 2 diabetes (Gill & Cooper, 2008). Regular, long-term exercise is proven to be most beneficial in improving diabetic's glucose control and insulin resistance, hypertension, body composition, weight loss, and lipid profiles (ADA, 2002; Pan et al., 1997; Wallberg-Henriksson et al., 1998). Exercise training studies have found improvements in glucose tolerance with seven consecutive days of training in individuals with early type 2 diabetes (Holloszy, Schultz, Kusnierkiewicz, Hagberg, & Ehsani, 1986; Lampman & Schteingart, 1991; Trovati, et al., 1984). Evidence has been provided that exercise training improves insulin-mediated glucose disposal, insulin sensitivity in skeletal muscle and adipose tissue with or without body composition changes (Koivisto, Yki-Jarvinen, & Defronzo, 1986; Mayer-Davis et al., 1998), blood pressure, and lipids in type 2 diabetics (Krotkiewski, et al., 1985; Schneider, Khachadurian, Amorosa, Clemow, & Ruderman, 1992). Since the benefits of improved blood glucose and insulin action from exercise are short lived if exercise is not performed again after 48-72 hr, there is an important emphasis for consistent exercise (Mikines, et al., 1988; Schneider, Amorosa, Khachadurian, & Ruderman, 1984; Turcotte & Fisher, 2008).

Longitudinal cohort studies reported by Gill and Cooper (2008) have provided consistent evidence that physical activity has a protective effect for the development of type 2 diabetes. A study by Manson et al. (1991) performed an 8 year follow-up on 87,253 women free of cardiovascular disease and diagnosed diabetes, and aged 34-59 years. These women were divided into a nonexercise and exercise group, with the exercise group participating in vigorous exercise at least once per week. The results revealed that the women that participated in vigorous exercise at least once a week had approximately a 33% lower risk for diabetes than those that did

not exercise weekly. Risks for the participants were determined by the rate of occurrence of diabetes in the physical activity categories then divided by the incidence rate in the nonexercise group, after proper adjustments for age and body mass index. A cohort study of Manson et al. (1991) examined the relationship between moderate intensity exercise (walking) and the incidence of type 2 diabetes, compared to the relationship found with vigorous intensity exercise in the Manson et al. (1991) study (Hu, et al. 1999). The Hu et al. (1999) study also compared the amount of time spent performing moderate activities to the occurrence rate of type 2 diabetes. The study concluded that increasing physical activity levels (≥ 2.1 MET-hr/week) and walking lowers the risks for type 2 diabetes; greater physical activity levels (≥ 10 MET-hr/week) having approximately a 45% lower risk. Risk rates for participants were determined using the same methods as the Manson et al. (1991) study. The results of these two studies are evidence that the risk for diabetes can be reduced with participation in vigorous and moderate intensity activities. In men there is approximately a 40-50% reduction in the risk for diabetes with participation in vigorous physical activity (Gill & Cooper, 2008). Helmrich, Ragland, Leung, and Paffenbarger (1991), took 5,990 men aged 39-55 years and recorded weekly energy expenditure in walking, stair climbing, and sports for 14 years. Energy expenditure of ≥ 3500 kcal/week was associated with a 50% reduction in risk for diabetes. Manson et al. (1992) recorded weekly vigorous activity in 21, 271 men aged 40-84 years for 5 years and reported that vigorous activity at least once a week was also associated with a decrease in risk for diabetes.

Effects of Omega-3 Fatty Acids on Glucose Metabolism

Free fatty acids lipids are composed of hydrocarbon linear long chains with an even number of carbon atoms and a carboxylic edge. These fatty acids are classified into two groups, saturated fatty acids and unsaturated fatty acids, with unsaturated fatty acids being

monounsaturated or polyunsaturated (Martin de Santa Olalla et al., 2009). Polyunsaturated fatty acids have three families, n-9, n-6, and n-3. The distinction between these polyunsaturated fatty acids is based upon the location of the first double bond on the fatty acid molecule. Fatty acids n-3 and n-6 are essential fatty acids that humans and other mammals cannot synthesize; therefore they must be consumed from the diet (Martin de Santa Olalla et al., 2009; Simopoulos, 1991). Omega-6 fatty acids are represented by linoleic acid (LA), which can be found in the seeds of most plants, with the exception of cocoa, palm, and coconut. Omega-3 fatty acids are represented by alpha linolenic acid (ALA), which is found in the chloroplast of green leafy vegetables (Simopoulos, 1991). Both essential fatty acids are metabolized into long-chain polyunsaturated fatty acids (LCPUFA) of 20 and 22 carbon atoms; LA is metabolized to arachidonic (AA) and LNA to eicosapentaenoic acid (EPA) and docasahexaenoic acid (Simopoulos, 1991). Omega-6 LCPUFA occurs mainly in the diet and can be found in sunflower oil, corn, rapeseed, safflower, oils, nuts, grains, and seeds (Martin de Santa Olalla et al., 2009; Simopoulos, 1991; Whelan & Rust, 2006). Major sources for n-3 LCPUFA are oily fish from marine or farm origin (salmon, sardines, mackerel, and tuna) and vegetable oils (Kris-Etherton, et al. 2000; Martin de Santa Olalla et al., 2009). Omega-3 fatty acids are also essential to the grow, development, and conception of humans, as well as play an important role in the treatment and prevention of cardiovascular diseases, diabetes, arthritis, and other autoimmune disorders (Connor, 2000; Simopoulos, 2000; Simopoulos, 1991).

Consuming more n-3 LC-PUFAs has been found to improve several cardiovascular risk factors in persons with diabetes, as well as possibly decreasing the risk of conversion from impaired glucose tolerance to type 2 diabetes (Kesavulu et al, 2002; Nettleton & Katz, 2005). There were previous concerns that an increased consumption of n-3 LC-PUFAs would have

negative effects on glucose control, insulin activity, and low-density lipoprotein cholesterol concentrations in persons with type 2 diabetes, but further research has suggested there are more benefits than risk (ADA, 2002; Kris-Etheron, Harris, & Appel, 2002; Nettleton & Katz, 2005). Studies that used low doses of n-3 LC PUFAs (1 to 2 g/day) showed no deterioration in glucose control (Axelrod, et al., 1994; Luo, et al., 1998; Sirtori, et al., 1998; Westerveld, et al., 1993), but studies that used much larger amounts (10 g/day or more) did show adverse effects in glucose control and insulin activity (Borkman et al. 1989; Luo et al., 1998).

It is well known that obesity increases the risk of developing diabetes, but the effect of total dietary fat and its composition on developing obesity and insulin resistance is still not clear. Low fat, high protein, and high complex carbohydrate diets help to maintain a healthy weight in normal-weight individuals, and promotes weight loss and improved glucose tolerance in overweight individuals (Nettleton & Katz, 2005; Swinburn, Metcalf, & Ley, 2001). A study by Ebbesson et al. (2005) provided a positive correlation between consuming high amounts (4-8 g/day) of n-3 fatty acids and glucose tolerance and insulin sensitivity after a 2 hr OGTT. This study measured plasma n-3 fatty acid concentration in 447 Norton Sound Eskimos, as well as evaluated their traditional diet of fish and marine mammals. The results of this study suggest that the participants high consumption of fish and marine mammals, which provided a higher consumption of n-3 fatty acids, actually contributed to decreased prevalence of insulin resistance and type 2 diabetes. Another study by Abete, Parra, Crujeiras, Goyenechea, and Martines (2008) found that adding three serving a week (7.4 ± 1.7 g/d) of fatty fish to an energy restricted diet significantly reduced insulin resistance, as well as revealed a positive correlation between leptin (hormone associated with fat metabolism) and insulin concentrations ($r = .502$; $p = .004$). This study randomly assigned 18 men and 14 women (BMI = 31.6 ± 3.5 kg/m^2; aged 36 ± 7 yrs) to

control (without fatty fish) or fish based diet for 8 wks. The decrease in insulin was determined using the homeostatic model assessment index (HOMA-IR = insulin x glucose/22.5). A statistical difference in the decrease in leptin levels was observed between the groups, with the control group having a 3.3 ng/ml^{-1} decrease and the fish diet group having a 14.0 ng/ml^{-1} decrease. The conclusions for this study suggest that incorporating (7.4 ± 1.7 g/d) of fatty fish 3 d/wk improves insulin sensitivity and leptin concentrations in an obese population, providing a nutritional intervention to improve glucose metabolism and body weight regulation.

It has been established that n-3 fatty acids do have beneficial health effects, but not all these benefits are clear. Plasma lipid concentrations have been lowered with consumption of n-3 PUFA in patients with high cholesterol plasma concentrations, but the effects seem to be lower in healthy patients, suggesting that n-3 fatty acids effects are health-status sensitive (Lichtenstein & Schwab, 2000; Martin de Santa Olalla, et al., 2009). Studies have found that total cholesterol is decreased, high density lipoproteins (HDL) are increased, and the changes in low density lipoproteins (LDL) are controversial. The effects of n-3 fatty acids on serum lipids also seem to be dependent on the amount of fatty acids in the diet and if this amount is kept constant (Martin de Santa Olalla, et al., 2009; Simopoulos, 1991; Vessby et al., 2001). The consumption of n-3 fatty acids has also been associated with improving insulin action in humans, but findings are inconsistent (Bloedon et al., 2008; Giacco et al., 2007; Itoh et al., 2007; Tsitoouras, Gucciardo, Slabe, Heward, & Harman, 2008). Epidemiological data suggest that diets high in saturated and trans fatty acids and high sucrose diets make an individual more susceptible to developing insulin resistance (Fedor & Kelley, 2009; Martin de Santa Olalla, et al., 2009). In most animals studies this has proven to be true, but type 2 diabetics results are inconsistent and confounded by differences in each participant's body weight (Martin de Santa Olalla, et al., 2009; Mayer-Davis

et al., 1997; Nagy, Levy, & Grunberger, 1990; Pascoe, Jenkins, Kusunoki, & Storlien, 1992; Sevak, McKeigue, & Marmot, 1994; Salomaa et al., 1990; Vessby et al., 1994).

If n-3 can improve glucose metabolism the improvements of insulin sensitivity may be a result of a few other biological effects. Improvements could be due to the preferentiality of EPA and docasahexaenoic acid (DHA) into cell membranes, which would increase membrane fluidity, the number of insulin receptors, and the receptors affinity for insulin (Anderson & Ma, 2009; Das, 2005). Another reason could be due to PPARγ (peroxisome proliferator-activated receptor gamma), a nuclear receptor in the insulin-responsive tissue of skeletal muscle and the liver, increasing the expression and translocation of the glucose transporters GLUT-1 and GLUT-4 and improving glucose uptake (Anderson & Ma, 2009; Nugent, et. al., 2001; Sugii, et al., 2009;). The inconsistent findings in human studies suggest that there may be other factors to address when using n-3 fatty acids to effect glucose metabolism. None the less, it is recommended to consume at least two meals per week rich in n-3 LC-PUFA to reduce risks for sudden death and cardiovascular disease (Kris-Etherton et al., 2002; Lichtenstein et al., 2006).

Effects of Omega-3 Fatty Acids and Exercise on Glucose Metabolism

Thus far, there is very little research examining the combined effects of n-3 fatty acids and exercise on glucose metabolism. The majority of studies have evaluated the combined effects of n-3 fatty acids and aerobic exercise on body composition and/or cardiovascular risk factors (Axelrod, et al., 1994; Brilla & Landerholm, 1990; Hill, Buckley, Murphy, & Howe, 2007; Warner, Ullrich, Albrink, & Yeater, 1989; Westerveld, et al., 1993). One study by Dunstan et al. (1997) did examine glycemic control along with serum lipids (triglycerides, total cholesterol, LDL, and HDL) in dyslipidemic type 2 diabetics, after dietary fish supplementation and aerobic

exercise training. Dunstan et al. (1997) set out to address the concerns of using dietary fish supplementation to improve lipid profiles in type 2 diabetics, due to large amounts (5.5 g) of n-3 fatty acids previously being found to increase plasma glucose, glycated hemoglobin, plasma total cholesterol, LDL cholesterol, and apolipoprotein B (Glauber, Wallace, Griver, & Brechtel, 1988; Schectman, Kaul, & Kissebah, 1988). This study was an 8 wk intervention that used 55 sedentary, type 2 diabetics, with fasting serum triglycerides > 1.8 mmol/l and/or HDL cholesterol < 1.0 mmol/l, and BMI < 36 kg/m^2. The participants were randomly assigned to a low-fat diet with or without one fish meal daily (3.6 g/d), and to moderate (55-65% VO_2max) or light (heart rate ≤100 bpm) exercise programs. The aerobic exercise interventions were incorporated to evaluate if exercise could prevent any glycemic deterioration. An OGTT, fasting serum glucose, insulin, lipids, and glycated hemoglobin were measured before and after the interventions. The addition of fish to the low-fat diet resulted in a reduction in triglycerides (0.08 ± 1.3 mmol/l, p = .03), a reduction in HDL_3 cholesterol (0.05 ± 0.07 mmol/l, p = .02), an increase in HDL_2 cholesterol (0.06 ± 0.07 mmol/l, p = .01), and an insignificant increase in LDL cholesterol (0.22 ± 0.42 mmol/l) and glycated hemoglobin (0.33 ± 0.17%, p = .06). The fish meal and light exercise program demonstrated a significant increase in glycated hemoglobin (0.50 ± 0.24%, p = .05), which was attenuated in the fish meal and moderate exercise program (0.19 ± 0.25%, p = .44). A decrease in fasting serum insulin (21.71 ± 10.7 pmol/l, p = .05) was also found with the fish meal and light exercise program. This study concluded that a low-fat diet consisting of one fish meal a day results in substantial improvements in triglycerides and increased HDL_2 cholesterol in dyslipidemic type 2 diabetics, as well as moderate exercise training preventing the deterioration of glycemic control.

Other studies have evaluated the effects of n-3 supplementation during exercise. For example, a study by Delarue, Labarthe, and Cohen (2003) examined the effects of n-3 fatty acids (6 g/d) for 3 wks on the rate of glucose disappearance, hepatic glucose production, and lipid and carbohydrate oxidation during exercise in six healthy, untrained males. A previous study by Delaure et al. (1996) found that n-3 fatty acid supplementation decreased the stimulation of carbohydrate oxidation by 35% and increased the fat oxidation by 35% during an oral glucose load in resting participants; therefore Delarue et al. (2003) set out to determine if n-3 fatty acids alters fuel selection during exercise as it did at rest. The Delarue et al. (2003) study lasted for 40 days and was divided into two consecutive interventions of 20 days to ingest 6 g/d of olive oil and then 6g/d of fish oil. Within the two 20 day interventions were two consecutive phases; a 15 day period when participants were asked to maintain their normal diets, and a 5 day period when diets were controlled to consist of 50% carbohydrates, 30% fat, and 20% protein divided between three meals. During each phase participants were asked to maintain their usual physical activities. Cycle exercise at 65% VO_2max was maintained for 90 min before and after the n-3 fatty acid supplement intervention. Blood samples were obtained every 15 min starting at the 30 min time point of exercise; samples analyzed for plasma glucose, non-esterified fatty acids, beta-hydroxybutyrate, lactate, insulin and glucagon concentrations, and plasma isotopic enrichment in ^2H of glucose and glycerol. The major findings of this study were that 6 g/d of fish oil for 20 days decreased hepatic glucose production (-21%; $p < .05$) and rate of glucose disappearance (-26%; $p < .05$) during exercise, reduced glucose metabolic clearance rate ($p < .001$), but no significant difference of whole-body substrate oxidation. This study concluded that it is possible that the reduction in the rate of glucose disappearance was a result of facilitating fat oxidation, and the reduction in hepatic glucose production was a result of a feedback mechanism from the decrease in glucose disappearance.

Another study by Bortolotti, Tappy, and Schneiter (2007) also examined the effects of fish oil on energy metabolism during exercise and at rest. Eight healthy men were used and assigned to a n-3 fatty acid supplement (7.2 g/d) group or no n-3 fatty acid supplement group in a cross over study design for 15 days. Cycling exercise was also used at 50% of VO_2max for 30 min for each intervention. Blood samples were collected at baseline (0 min), 15 min, and 30 min, and were analyzed for plasma non-esterified fatty acids, glucose, and insulin concentrations in post-prandial and postabsorptive conditions. Rates of carbohydrate, fat and protein oxidation and energy expenditure were determined using VO_2 and VCO_2 from collected respiratory gases and urinary urea nitrogen excretion. The results of this study revealed no significant changes in any of the measured variables. It was concluded that n-3 fatty acid supplementation failed to produce significant alterations in energy metabolism and lipid oxidation, but due to the small sample size there could have been some metabolic effects of n-3 fatty acid that were not detected.

Summary

The regulation of glucose metabolism is very important at rest and during exercise. There are several mechanisms that must function efficiently in order to maintain glucose homeostasis. Research has established that regular physical activity is a useful method in improving and maintaining insulin sensitivity and other metabolic disorders, but most studies have concluded that these effects are short lived if regular physical activity is not maintained. When adding n-3 fatty acids to a diabetic's diet it has been shown to have several cardiovascular benefits, as well as improving impaired glucose tolerance. Although, there are established health benefits from consuming more n-3 fatty acids, the result still vary. Research has found that there may be benefits in the combination of participating in regular physical activity and consumption of n-3 fatty acids, but results vary as well. The findings of this study will contribute to a more

definite answer of whether exercise and/or dietary fatty acids are beneficial to improving and maintaining normal glucose metabolism.

CHAPTER III

METHODS

Participants

Eleven healthy men between the ages of 18-45 years were recruited to participate in this study. The following criteria were met for each participant: nonsmoker, nondiabetic, nonhypertensive, have not participated in any regular (>2 times/week) physical training (aerobic activity or weight training) in the previous 3 months, were not taken any medications that would interfere with lipid and glucose metabolism, and were not taking n-3 supplementation in the last 6 months. There were no exclusion criteria for body mass index (BMI) or body composition. Data for each participant was obtained by using procedures approved by the TWU's Institutional Review Board (Appendix A) from the previous study *The Effect of Omega-3 Fatty Acids and Exercise on Insulin Response to an Oral glucose Tolerance Test.*

Research Design

This study was a randomized, crossover study comparing the effects of exercise and n-3 supplementation, where each participant completed four different interventions. The interventions were as follows: (1) rest without n-3 supplementation (CON), (2) exercise without n-3 supplementation (EX), (3) rest with n-3 supplementation (SUP), (4) and exercise with n-3 supplementation (EX-SUP). The order for EX and CON interventions were randomly assigned to each participant to occur during either the 1st or 2nd week of the study. The n-3 supplementation was taken for 42 days, starting on the 2nd day after the CON intervention. Following the 42 days of taking the n-3 supplementation the SUP and EX-SUP interventions were randomly assigned to

each participant first while continuing to take the n-3 supplementation. The remaining rest and exercise interventions, with and without n-3 supplementation were performed 1 week later after the initial interventions.

Procedures

Prequalifying Physiological Assessments

A questionnaire regarding medical and physical history was completed by each participant. Participant's eligibility was determined by using the American College of Sports Medicine contraindications for exercise, and a finger prick blood sample to measure fasting blood glucose with the 2300 Stat Plus Analyzer. Fasting blood glucose concentrations had to be less than 110 mg/dl, indicating that the participant did not have impaired fasting glucose and less risk for diabetes.

Maximal Oxygen Consumption (VO_2max) Test

Each participant performed a modified maximum graded exercise test (GXT) on a treadmill (Quinton ST 65 Treadmill, Quinton Instruments Company, Bothell, WA.) The protocol required participants to walk on the treadmill at a constant speed of 3.5 mph with a grade increase of 5.0% every 2 min until the participant could no longer keep pace with the treadmill. Respiratory gases were analyzed using the ParvoMedics Truemax 2400 metabolic cart (Consentius Technologies, Sandy, UT), after oxygen and carbon dioxide gas and air flow were calibrated using known gases and a 3-liter syringe. VO_2 max, the maximum amount of oxygen consumed during the test, was then interpreted. Heart rate and rhythm were monitored with a 12-lead EKG. Rating of perceived exertion (RPE) was also monitored using a scale ranging from 6 to 20; 6 meaning no exertion at all and 20 meaning maximal exertion. The results were

considered to be maximal if exercise heart rate was within 10 beats of age predicted maximal heart rate (220-age) and if respiratory exchange ratio (RER) exceeded 1.1, according to the measurement of RER from the metabolic cart.

Body Composition

Prior to and at the completion of the study, body mass and height measurements were obtained. Body mass was measured with participants wearing clothing, no shoes using the Tanita BWB-800S Digital Scale (Tanita Corporation of America, Inc., Arlington Heights, IL). A portable stadiometer was used to measure height, with participant's head facing forward and no shoes worn. Body mass index (BMI) was calculated from these measurements using the following equation:

$$BMI = Weight\ (kg)/Height\ (m^2)$$

Exercise and Rest Interventions

Participants were randomly assigned to CON and EX interventions first at the beginning of the study. The two exercise interventions (EX-SUP and EX) both consisted of three consecutive days of exercise. Exercise consisted of walking on the treadmill at 3.5 mph at an incline appropriate for the participant to meet 65% of VO_2 max for 60 min. Exercise sessions were performed 24 hr apart and between the times of 2:00 and 6:00 PM. The speed and grade used for the first exercise session were also used for the remaining exercise session. Participants were then instructed to consume n-3 supplementation for 42 days, and then were randomly assigned to EX-SUP and SUP. Participants were asked to not participate in moderate to vigorous exercise throughout the study, other than what would usually be performed during activities of daily living.

N-3 Supplementation

Participants were asked to consume seven packets (4.55 g; EPA 350 mg, DHA 230 mg) of n-3 supplement per day in addition to a normal diet (Appendix B) for 42 days. Participants were instructed to consume two to three packets with each meal during the day until all seven packets had been ingested each day. Compliance to these instructions was regulated via personal contact when the participants reported to the laboratory to replenish their n-3 supplements.

Diet

For all four interventions the participants were required to record all food and beverage consumption for 3 days (total of 12 days) before each blood draw and oral glucose tolerance test (OGTT) for each intervention. Participants were asked to refrain from any alcohol consumption during all interventions, and to maintain their normal diets, but to eat similar meals during the experimental sessions. In addition, participants were provided a similar submarine sandwich from a local sandwich shop that was limited in red meat, for their evening meal prior to each blood draw and OGTT. A nutrition software program (Nutrionist V, First Databank, San Bruno, CA) was used to analyze total caloric intake and macronutrient contents for all food records.

Blood Sampling and Oral Glucose Tolerance Tests (OGTT)

After the week of rest and 14-16 hr after the third exercise session and all interventions, the participant lay in a supine position as blood was extracted from the antecubital vein into K_2-EDTA and serum-separator vacutainer tubes by a trained phlebotomist. Participants were required to fasted for at least 10 hr prior to all blood draws, and blood collection was performed after the participant was seated for at least 20 min to control for any effect of postural changes in plasma volume. Plasma and serum were separated from the blood by a low-speed centrifugation

(1,500 g, 15 min, 10 ˚C) and transferred in to aliquots, then stored at -70˚C until analysis. The participants were then given an OGTT via a commercially prepared solution (Tru-Glu) that consisted of 75 g of glucose. Additional blood samples were then obtained in the same manner at 15, 30, 60, 90, and 120 min postingestion of glucose solution. Blood samples were mixed and centrifuged at 4˚C at 2500 rpm for 8 min. Plasma was then removed from the samples and stored at -70˚C for further analysis.

Blood Analysis

The plasma samples were previously analyzed for glucose, insulin, and C-peptide levels using a Yellow Springs Instruments Model 2300 Stat Plus Analyzer. Plasma insulin (DSL, Inc.) and C-peptide (DSL, Inc.) were measured with double antibody ^{125}I radioimmunoassays and a 10-detector gamma counter (RIASTAR 5410, Packard Instrument Co.). C-peptide was measured due to it being a good indicator of insulin secretion. All samples for the same individual were analyzed using the same kits with the same lot numbers.

Statistical Analysis

A 2 (rest or exercise) x 2 (with or without supplementation) ANOVA with repeated measures was used to compare incremental area under the curve (IAUC) means of each dependent variable (glucose, insulin, and c-peptide) within each of the four interventions. The IAUC was determined by computer analysis with a trapezoidal model using the following equation:

$$IAUC = [(1/2((\Delta X1)(y15 - y0)) + (\Delta X1)(y30\text{-}y15)) + (\Delta X2)(y60 - y30)) + (\Delta X2)(y90 - y60)) + (\Delta X2)(y120 - y90))) + ((\Delta X1))(y15 - y0)) + ((\Delta X2)(y30 - y0)) + ((\Delta X2)(y90 - y0)) + ((\Delta X2)(y120 - y0)))))) \quad \Delta X1 = 15 \quad \Delta X2 = 30$$

where y = concentration above baseline for either glucose, insulin, or c-peptide at each time point. Since multiple tests were performed a Bonferroni adjustment was conducted to adjust for the alpha level. The chosen level of significance was a p-value less than .05. SPSS®VERSION 15.0 (SPSS, Inc., Chicago, IL) was used to analyze all data.

CHAPTER IV

RESULTS

Description of Participants

Eleven healthy, sedentary men were recruited to participate in this study. All 11 participants completed all four interventions, but only 10 participants were included in the data analysis. One participant was excluded due to an improper compliance to the diet protocol. Descriptive characteristics of the participants are presented below in Table 1 and metabolic and cardiorespiratory data for the VO_2max test can be found in Appendix C.

Table 1. Descriptive Baseline Characteristics of Participants

Age (years)	31.0 ± 10.1
Height (cm)	180.1 ± 4.3
Weight (kg)	85.8 ± 11.7
BMI (kg/m^2)	26.5 ± 4.0
Relative VO_2max (ml/kg/min)	39.4 ± 5.6
Absolute VO_2max (L/min)	3.4 ± 0.4
HR_{max} (bpm)	183 ± 21
$\%HR_{max}$	98 ± 10.0
RER_{max}	1.2 ± 0.2
FPG (mmol/l)	5.0 ± 0.5

Note. N = 10; all values expresses as mean ± standard deviation; BMI = body mass index; VO_2max = maximal oxygen consumption; HR_{max} = maximal heart rate; RER = respiratory exchange ratio; FPG = fasting plasma glucose.

Submaximal Exercise Responses

During the exercise trial the average VO_2 was 28.3 ± 3.7 ml/kg/min, which was 71.0 ± 5.9% of VO_2max. Each participant completed all the exercise sessions with no major complications. Individual mean cardiorespiratory and metabolic data can be found in Appendix D.

Diet History Results

Based on the verbal comments of each participant, there was a 100% adherence to the n-3 supplement of the 10 participants included in the data analysis. A nutrient profile for the n-3 supplement is presented in Appendix B, and a detailed description of each participant's food intake can be found in Appendix E. The average energy consumption for each intervention is presented below in Table 2.

Table 2. Mean Values of Selected Nutrient Variables for all Interventions

	Intervention Group			
	CON	SUP	EX	EX-SUP
Calories (kcal)	2256.5 ± 817.1	2291.5 ± 613.8	2501.9 ± 735.1	2689.9 ± 785.5
Carbohydrates				
% of diet	53.2 ± 9.9	51.2 ± 7.7	52.2 ± 9.2	47.9 ± 6.2
grams	299.8 ± 112.9	295.2 ± 94.9	321.2 ± 91.3	318.8 ± 87.4
Protein				
% of diet	15.7 ± 4.1	16.9 ± 7.8	16.6 ± 5.8	15.8 ± 3.7
grams	88.2 ± 37.7	97.3 ± 63.0	103.9 ± 42.6	104.8 ± 35.0
Total Dietary Fat				
% of diet	31.8 ± 6.9	34.9 ± 4.7	30.8 ± 4.0	36.7 ± 3.9
grams	80.5 ± 35.3	89.5 ± 30.6	85.8 ± 28.9	111.5 ± 41.0
Saturated Fat				
% of diet	10.0 ± 25.4	9.2 ± 2.5	10.4 ± 2.4	10.5 ± 2.3
grams	25.3 ± 11.1	24.6 ± 11.5	29.2 ± 10.6	32.8 ± 14.9
EPA + DHA				
% of diet	0	1.6	0	1.3
grams	0	4.2 ± 0.4	0	4.2 ± 0.4

Note : Values expressed as mean + standard deviation; N-3 supplement included; CON = rest and no n-3, SUP = rest and n-3, EX = exercise and no n-3, EX-SUP = exercise and n-3.

Main Effects of Exercise and Supplementation

All ANOVA assumptions were checked and met. The Test of Within-Subject Effects was used to interpret the main results of ANOVA, with insulin and c-peptide variables having significantly different group means. A description of the main results of ANOVA can be found below in Table 3.

Table 3. Main Results of ANOVA for Each Variable

Variable	df	Mean Squares	F	Sig.	Observed Power
Insulin IAUC	3	1863432.36	5.18	0.01*	0.88
Glucose IAUC	3	968182.53	1.04	0.39	0.25
C-Peptide IAUC	3	4542.25	5.69	0.01*	0.91

Note: df = degrees of freedom; F = MSbetween/MSwithin - evaluates the overall difference between groups; $\eta 2$ = partial eta squared - a measure of relationship; < .05 significance level; * indicates significance.

No significant main effects were seen for n-3 supplementation on any measured variable, exercise had a significant effect on insulin and c-peptide concentrations, and exercise plus n-3 supplementation had a significant effect on insulin concentration. A full description of the main effects of n-3 supplement and exercise is presented below in Table 4.

Table 4. Main Effects of N-3 Supplement and Exercise on Measured Variables

Variable		df	Mean Squares	F	Sig.	η^2	Observed Power
Insulin							
	Effect of Exercise	1	7588814.15	20.56	0.00*	0.70	0.98
	Effect of Supplement	1	2683633.69	2.23	0.17	0.20	0.27
	Supplement x Exercise	1	2895526.86	6.53	0.03*	0.42	0.63
Glucose							
	Effect of Exercise	1	183347.85	0.14	0.72	0.02	0.06
	Effect of Supplement	1	4933938.56	0.82	0.20	0.18	0.24
	Supplement x Exercise	1	858279.05	0.82	0.39	0.08	0.13
C-Peptide							
	Effect of Exercise	1	14719.50	13.91	0.01*	0.61	0.91
	Effect of Supplement	1	4.30	0.00	0.97	0.00	0.05
	Supplement x Exercise	1	1140.84	1.41	0.27	0.14	0.19

Note: df = degrees of freedom; F = MSbetween/MSwithin - evaluates the overall difference between groups; η2 = partial eta squared - a measure of relationship; < .05 significance level; * indicates significance.

Pairwise comparisons for each intervention revealed significant differences for insulin and c-peptide EX and EX-SUP group means being significantly lower than the CON group mean (Appendix F). The IAUC mean values for each variable can be reviewed in graphical depictions presented in Figures 1, 2, and 3.

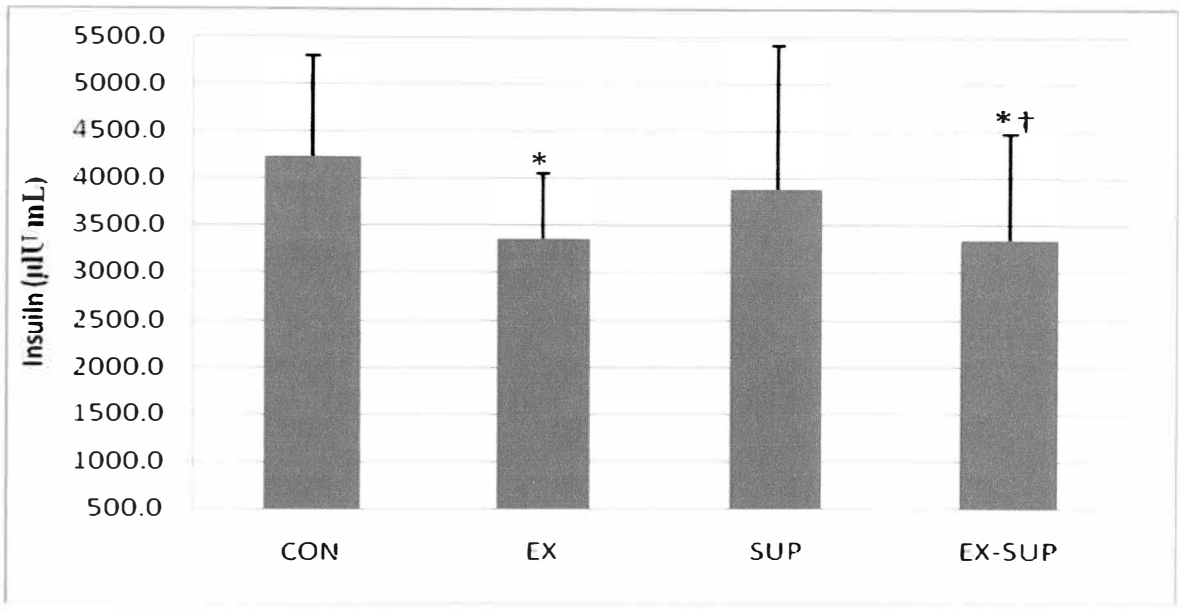

Figure 1. Mean IAUC values for insulin concentrations at each intervention. N = 10; 4242.1 ± 1068.1 (CON), 3371.0 ± 691.1 (EX), 3889.0 ± 1529.8 (SUP), 3350.9 ± 1124.6 (EX-SUP); values expressed as mean ± standard deviation; α = .05; * indicates significantly different from CON; † indicates significantly different than SUP.

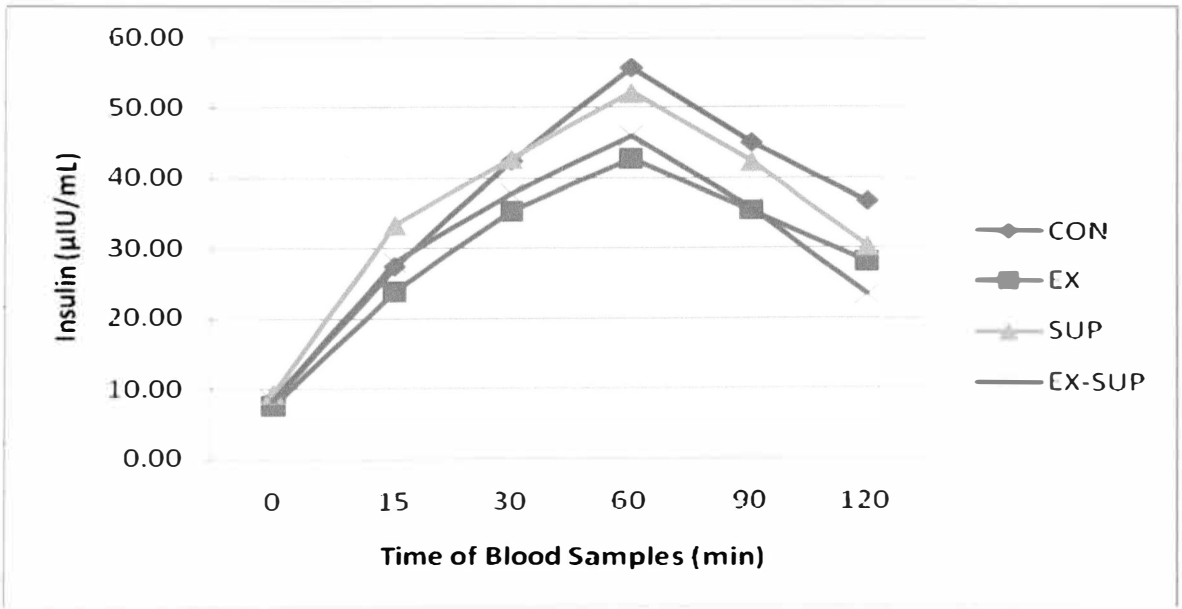

Figure 2. Mean values for insulin concentration at each time point of blood samples for each intervention. IAUC significant difference in means for EX and EX-SUP group; α = .05.

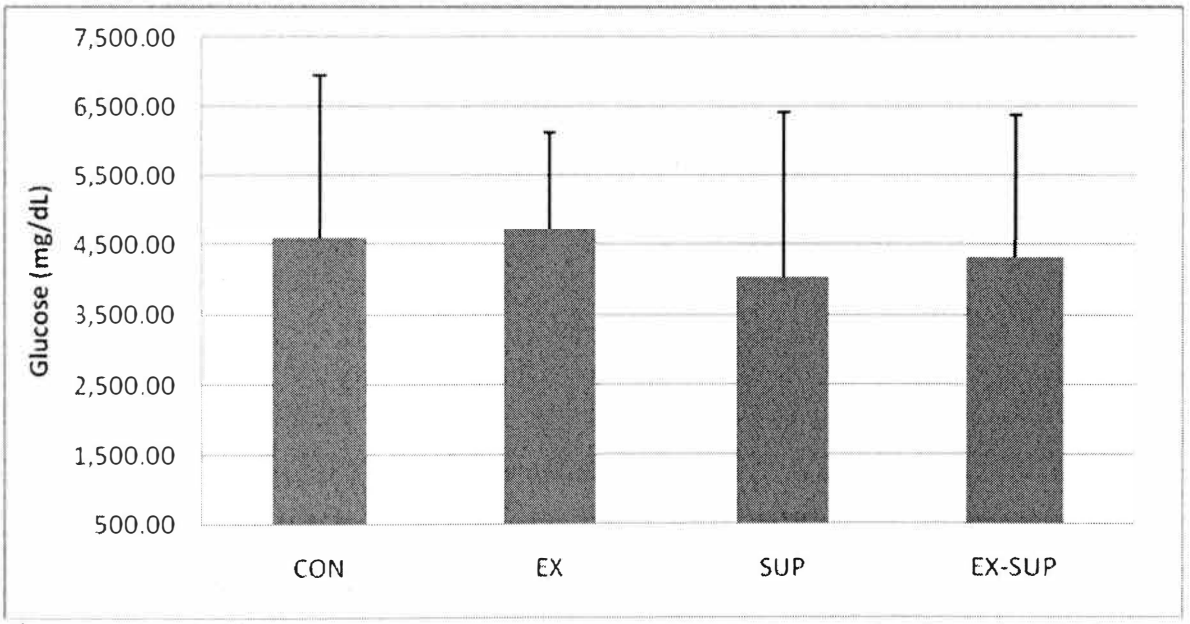

Figure 3. Mean IAUC values for glucose concentrations at each intervention. N = 10; 4611.2 ± 2329.8 (CON), 4746.6 ± 1380.3 (EX), 4044.2 ± 2367.9 (SUP), 4337.1 ± 2053.2 (EX-SUP); values expressed as mean ± standard deviation; $\alpha = .05$; no significant differences existed.

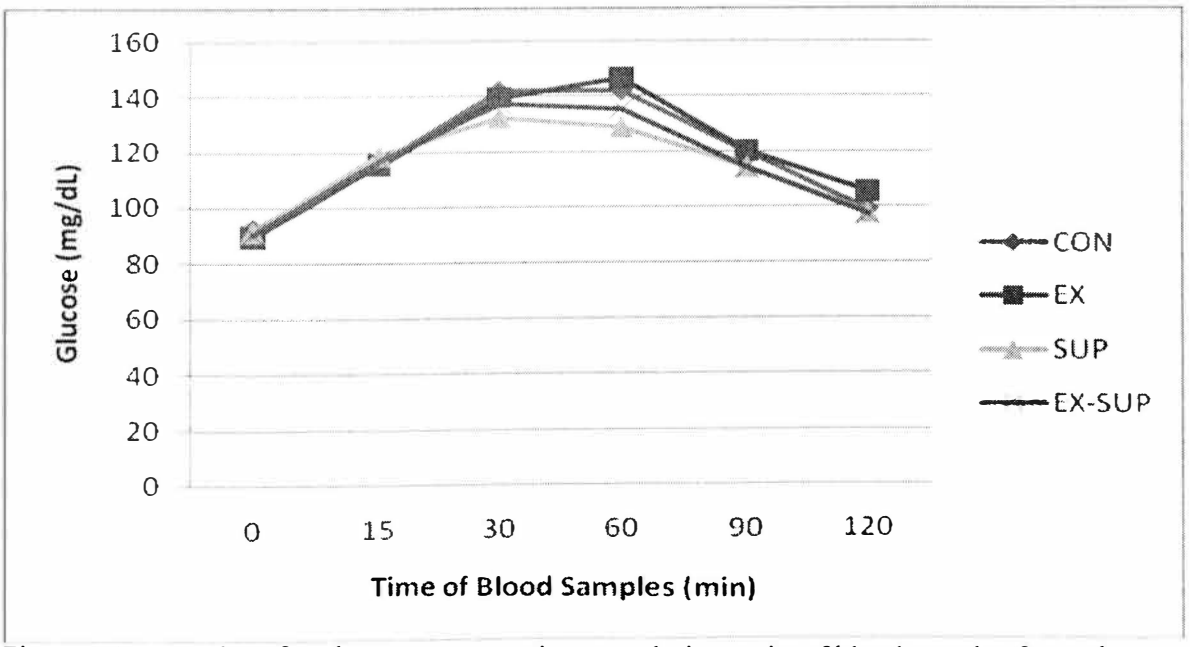

Figure 4. Mean values for glucose concentration at each time point of blood samples for each intervention.

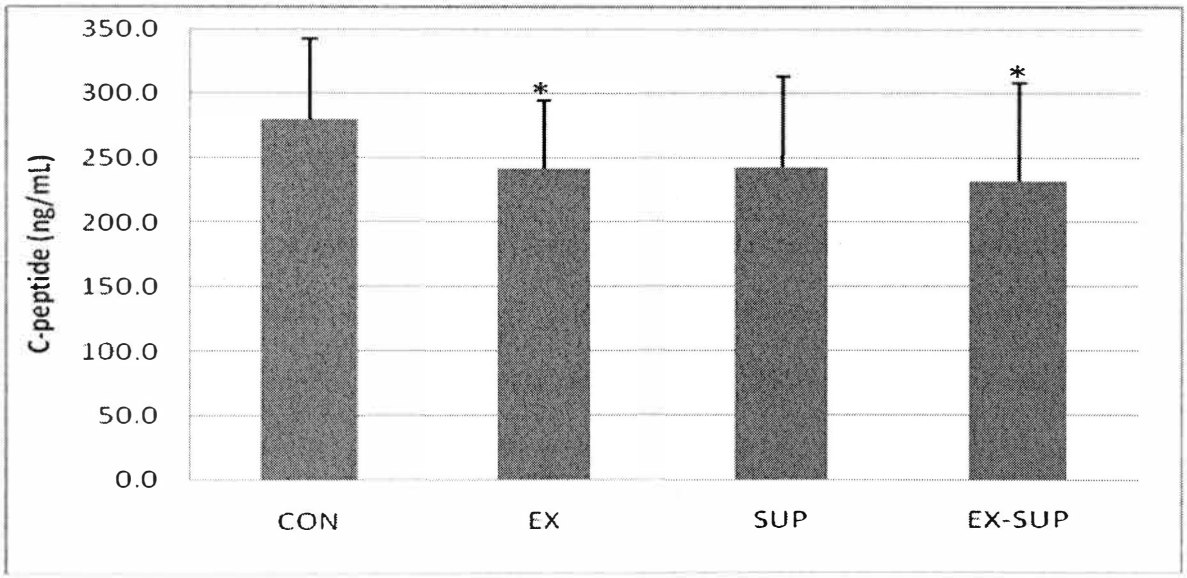

Figure 5. Mean IAUC values for c-peptide concentrations at each intervention. N = 10; 280.2 ± 62.5 (CON), 241.9 ± 52.8 (EX), 242.5 ± 70.2 (SUP), 231.9 ± 76.6 (EX-SUP); values expressed as mean ± standard deviation; α =. 05; * indicates significantly different from CON.

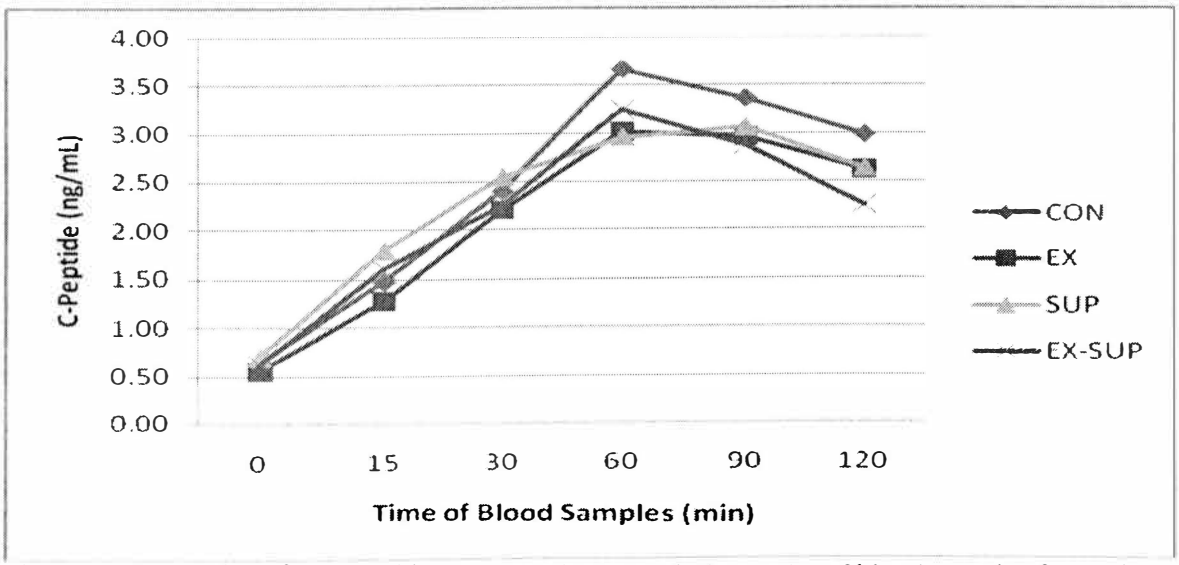

Figure 6. Mean values for c-peptide concentration at each time point of blood samples for each intervention. IAUC significant difference in means for EX and EX-SUP; α = .05.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The intentions of this study were to examine the effects of acute aerobic exercise and n-3 fatty acid supplementation on glucose, insulin, and c-peptide responses in healthy, nonsmoking, nondiabetic, nonhypertensive, normoglycemic, sedentary men. To date few studies have evaluated the combined effects of n-3 fatty acid supplementation and exercise on glucose metabolism (Bortolotti, Tappy, &Schneiter, 2007; Delarue, Labarthe, & Cohen, 2003; Dunstan et al., 1997), with the majority of studies examining the effects of n-3 fatty acid supplementation and aerobic exercise on body composition and/or cardiovascular risk factors (Axelrod, et al., 1994; Hill, Buckley, Murphy, & Howe, 2007; Brilla & Landerholm, 1990; Westerveld, et al., 1993). Therefore, the present study was conducted to better understand the acute effects of aerobic exercise and n-3 fatty acid supplementation on glucose, insulin, and c-peptide responses in healthy individuals.

Summary of Findings

The analysis of variance performed on the collected data revealed that three consecutive days of aerobic exercise at ~71% VO_2max significantly lowered insulin and c-peptide IAUC concentrations after an OGTT. In comparison to the CON group, insulin levels in the EX group were 20.5% (p = .01) lower, and c-peptide levels in the EX group decreased 13.7% (p = .03). The addition of n-3 supplementation to each participant's normal diet resulted in no significant effects for any variable. Exercise plus n-3 supplementation revealed a significant main effect on insulin concentration. The IAUC insulin levels for the EX-SUP group decreased 21.0% (p = .04) from the CON group, and 13.8% (p = .03) from the SUP group. These results indicate that for this

sample aerobic exercise effectively decreased insulin and c-peptide concentrations, and n-3 fatty acid supplementation had no effect on glucose, insulin, or c-peptide concentrations. Although, the combination of n-3 fatty acid supplementation and exercise did decrease insulin concentration. Lastly, these finding also suggest that glucose concentrations are not significantly altered by aerobic exercise or n-3 supplementation independently or combined in this sample. The following hypotheses were accepted after analysis:

1. There will be no effect of n-3 fatty acid supplementation on glucose, insulin, or c-peptide concentrations.

The following hypotheses were rejected after the analysis:

1. There will be no effect of exercise on glucose, insulin, or c-peptide concentrations.
2. There will be no interaction of exercise and n-3 fatty acid supplementation on glucose, insulin, or c-peptide concentrations.

Insulin, C-peptide, and Glucose Responses to Exercise

The present study did not reveal any changes in glucose concentrations during an OGGT after 3 days of aerobic exercise, which was consistent with past studies that examined the effects of aerobic exercise on glucose concentrations in healthy, nondiabetic men and women (Brestoff et al., 2009; Houmard, Shaw, Hickey, & Tanner, 1999; Houmard et al., 2004; Ostergard et al., 2006). Brestoff et al. (2009) reported no significant changes in glucose concentrations during a OGTT after acute aerobic exercise for 45 min at ~75% VO_2peak, and Houmard et al. (2004) reported no significant changes during an intravenous glucose tolerance test after 6 months of aerobic training at 65-80% VO_2peak for ~50 min. Insulin responses during an OGTT were

decreased in the present study, which did agree with past studies that reported changes in insulin concentrations after a single session of aerobic exercise and/or aerobic exercise training for durations of 30-50 min at 40-75%VO_2peak or VO_2max (Ben-Ezra, Jankowski, Kendrick, & Nichols, 1995; Brambrink, Fluckey, Hickey, & Craig, 1997; Brestoff et al., 2009; Houmard et al., 2004). The present study also found a significant decrease in c-peptide concentration during an OGTT, which disagrees with other studies that examined the acute (60% VO_2max) and training (70-80% VO_2max) effect of aerobic exercise in healthy men and women (Brambrink, Fluckey, Hickey, & Craig, 1997; Ostergard et al., 2007; Radikova et al., 2007). Brambrink, Fluckey, Hickey, and Craig (1997) reported that 18-hr after cycle exercise for 30 min at 60% VO_2max, insulin concentration significantly decreased ($p < .05$) and c-peptide concentration remained unchanged during an OGTT. The reduced post-exercise (18 hr) insulin response and unchanged c-peptide response in the Brambrink, Fluckey, Hickey, and Craig (1997) study also suggests an increase in insulin clearance.

Insulin and glucose response are effected by exercise prescription and mode of exercise. Effective exercise prescriptions for improving insulin action are still in question, with some studies suggesting that vigorous intensity exercise of $\geq$ 70% VO_2 peak (Hayshi et al., 2005; Kang et al., 1996; Seals, Hagberg, Hurley, Ehsani, & Holloszy, 1984) is enough to improve insulin sensitivity, while others suggest that mild to moderate intensity exercise is sufficient (Mayer-Davis et al., 1998; Oshida, Yamanouchi, Hayamizu, & Sato, 1989). A significant change in insulin sensitivity and glucose effectiveness has been seen after a single exercise session for 20 min at 70% of VO_2max (Hayashit et al., 2005), but not after 1 hr of a single exercise session at 75% of VO_2max (Venables, Shaw, Jeukendrup, & Wagenmakers, 2007), which suggests that duration of exercise may be a factor in an exercise prescription for improving insulin action. The

chosen exercise prescription of the present study was enough to decrease insulin and c-peptide concentrations, but not glucose concentration during an OGTT. Mode of exercise may be another factor in any inconsistent results in the present study. The amount of muscle utilized during exercise can positively influence glucose and insulin responses (Brambrink, Fluckey, Hickey, & Craig, 1997; Dela, Larsen, Mikines, & Galbo, 1995; Hespel, Vergauwen, Vandenberghe, & Richter, 1995). Treadmill exercise, which utilizes upper and lower body muscle mass, was used in the present study, while cycle ergometers were used in others (Brestoff et al., 2009; Houmard et al., 2004; Ostergard et al., 2007).

Diet and muscle glycogen concentration after acute exercise are also factors that can effect insulin action. Insulin response improves after a single exercise session as a result of reduced muscle glycogen concentration (Caree et al., 1989; Horowitz, 2007), and is decreased following exercise and OGGT (Ben-Ezra, et al, 1995; Young, Enslin, & Kuca, 1989) indicating increased insulin sensitivity and whole-body glucose disposal (Hayashit e al., 2005). Insulin sensitivity can be increased by consuming more carbohydrates after and during recovery of exercise, and can result in glycogen concentration being replenished above basal level (Cartee et al., 1989). Ivy et al. (2002) exercised 7 male cyclists (age 19-26 yr) for 2.5 hr at 65-75% VO_2max, then gave them 32 fluid ounces of a high carbohydrate supplement (216 g carbohydrate + 12 g fat; 756 kcal) over a recovery period of 4 hr. Basal muscle glycogen of these cyclists in this treatment was 150.8 ± 9.5 mmol/l and 41.9 mmol/l after exercise. After the high carbohydrate treatment, 31.1% ($p < .05$) of the muscle glycogen used during exercise had been replenished, and insulin and glucose concentration began to decline after 3 hr. In the present study the EX group carbohydrate consumption consisted of 52% (1284 ± 360.9 kcal) of their diet, and insulin concentration decreased 20.5% ($p = .01$) from the CON group 14-16 hr after exercise

but was not accompanied by a decrease in glucose concentration. It is possible that insulin sensitivity after exercise may have been increased in the present study, but this variable was not measured.

Insulin, C-Peptide, and Glucose Responses to N-3 Supplementation

Improvements in insulin action from consuming of n-3 fatty acids have been found in animal studies (D'Alessandro, Lombardo, & Chicco, 2002; Ikemoto et al., 1996; Luo et al., 1996; Mori et al., 1999; Mori et al., 1997; Nobukata, Ishikawa, Obata, & Shibutani, 2000; Storlien et al., 2000; Storlien et al., 1996). Many studies have evaluated the effects of n-3 fatty acid supplementation on insulin action in diabetic patients and have also found improvements, but results are equivocal (Martin de Santa Olalla, et al., 2009; Mayer-Davis et al., 1997; Sevak, McKeigue, & Marmot, 1994; Salomaa et al., 1990; Vessby et al., 1994); while studies that evaluated the effects in healthy participants have mostly found no improvements (Anderson, Nalsen, Tengblad, & Vessby, 2002; Giacco et al., 2007; Toft, Bonaa, Ingebretsen, Nordoy, & Jenssen, 1995; Vessby et al., 2001).

Studies by Giacco et al. (2007) and Vessby et al. (2001) assessed the effects of diets rich in monosaturated and saturated fats, along with a random assignment of n-3 fatty acid supplementation (3.6 g/d) for 3 months on healthy, nondiabetic men and women. These studies found no changes in insulin sensitivity, insulin secretion, or glucose tolerance during an intravenous glucose tolerance test. In contrast Ramel et al. (2008) did find improvements in fasting insulin (16.4% decrease; $p = .025$) and insulin resistance (17.2% decrease; $p = .022$) after consuming 1.3 g/dn-3 supplement during energy restriction (70% of normal diet) for 8 wk in

nondiabetic, overweight and obese (BMI 27.5-32.5 kg/m^2) men and women (age 20-40 yr) without weight loss.

The consumption of n-3 fatty acid supplementation (4.55 g/d) for 42 days in the present study had no effect on insulin, c-peptide, or glucose concentrations during an OGTT in healthy, nondiabetic men. Although, Giacoo et al. (2007) and Vessby et al. (2001) used lower dosages of n-3fatty acid supplementation for a longer duration of time, the present studies results are similar. The lack of specific diet restrictions in the present study compared to other studies may have contributed to no significant changes in this intervention. It is possible that fish oil may be more active with a diet rich in saturated fat, which could revert the negative effect of fatty acids on insulin action as seen in animals (Storlien et al., 1991). The n-6:n-3 fatty acid ratio may be another factor in determining insulin action through different mechanisms, such as enzyme and/or transcriptional factors or the production of eicosanoids at varying anit-inflammatory potency and membrane fluidity (Accinni et al., 2006; Pischon et al., 2003; Warensjo, Ohrvall, & Vessby, 2006). Insulin sensitivity may also be improved more in people with initial low concentrations of n-3 or n-6:n-3 fatty acid ratio (Vessby et al., 2001), while in healthy persons improvements may be dependent upon status of glucose tolerance (Fasching et al., 1991). The participants in the present study did not have impaired glucose tolerance, but n-3 and n-6:n-3 fatty acid ratio concentrations at the beginning of the study were not measured.

Insulin, C-Peptide, and Glucose Responses to Exercise plus N-3 Supplementation

The majority of studies that have examined the combined effects of aerobic exercise and n-3 fatty acid supplementation have focused on body composition, cardiovascular risk factors, and/or the effects during exercise in healthy and diabetic persons (Axelrod, et al., 1994; Hill,

Buckley, Murphy, & Howe, 2007; Bortolotti, Tappy, & Schneiter, 2007; Brilla & Landerholm, 1990; Delarue, Labarthe, & Cohen, 2003; Dunstan et al., 1997; Warner, Ullrich, Albrink, & Yeater, 1989; Westerveld, et al., 1993). Of these studies that did measure glucose and insulin concentrations the following was found: daily fish meals (3.6 g/d) along with moderate exercise training (55-65% VO_2max) for 8 wk resulted in improvements in triglycerides, HDL_2-C, and no deterioration in glycemic control in dyslipidemic type 2 diabetics (Dunstan et al., 1997); 6 g/d of fish oil for 3 wks during acute aerobic exercise (65% VO_2max) in healthy, untrained men resulted in reductions in hepatic glucose production, reduced rate of glucose disappearance, and reduced glucose metabolic clearance rate (Delarue, Labarthe, & Cohen, 2003); and 7.2 g/d of fish supplementation for 15 days during aerobic exercise (50% VO_2max) in healthy men revealed no significant alterations in glucose, insulin, or plasma non-esterified-fatty acid concentrations (Bortolotti, Tappy, & Schneiter, 2007).

Due to the lack of human studies that have that evaluated the combined effects of n-3 fatty acid supplementation and exercise on glucose metabolism after exercise in healthy persons, no comparisons could be made with the present study. In the present study, if significant alterations in glucose, insulin, and c-peptide concentrations were found from n-3 fatty acid supplementation and exercise alone, then it was assumed that the combination of n-3 fatty acids and exercise would have an equal or greater effect on the measured variables. The present study revealed a significant difference in IAUC mean values between the CON and EX-SUP groups for insulin and c-peptide concentrations, but no significant differences for the SUP group. Although not statistically significant the IAUC insulin concentration for the SUP intervention was lower (3889.0 ± 1529.8 μIU/ml; $p = .999$) than the CON (4242.1 ± 1068.1) intervention and higher ($p = .19$) than the EX-SUP (3350.9 ± 1124.6) intervention. The c-peptide concentration in the SUP

group was also lower (242.5 ± 70.2 ng/ml; $p = .16$) than the CON group (280.2 ± 62.5 ng/ml) and higher ($p = .999$) than the EX-SUP group (231.9 ± 76.6 ng/ml) in the present study.

The measurement of c-peptide concentration is considered to be a good indicator of insulin secretion because of its equimolar secretion with insulin from pancreatic beta cells, assuming that the mean clearance rate of c-peptide are constant with the range of c-peptide concentrations at normal physiological conditions (Bratusch-Marrain, Waldhaust, Gasic, & Hofer, 1984; Gumbiner et al., 1990; Licinio-Paixao et al., 1986; Palmer et al, 2004; Polonsky et al., 1984). The kidney extracts c-peptide and the constant peripheral clearance of c-peptide occurs at varying plasma concentrations and plasma glucose concentrations (Gumbiner et al., 1990; Licinio-Paixao et al., 1986; Palmer et al., 2004). In the present study if n-3 supplementation altered insulin or glucose action during the OGTT, then this could possibly explain the significant difference of means for insulin and c-peptide concentrations between the CON and EX-SUP interventions. Due to no changes occurring in the SUP intervention, it is likely that the significant difference in group means in insulin and c-peptide concentration seen in EX-SUP is a result of exercise alone. As discussed previously and displayed in the present study, a single session of exercise can enhance insulin sensitivity, which would reduce the amount of insulin needed for the clearance of glucose during an OGTT; accompanied by a decline in c-peptide concentration as it is an indicator of insulin secretion.

Conclusions and Recommendations

In conclusion, the results of the present study demonstrate that in healthy men three consecutive days of aerobic exercise at ~71% VO_{2max} had a significant effect on insulin and c-peptide, while consuming 4.55 g/d of n-3 fatty acids for 42 days had no effect on glucose, insulin,

or c-peptide concentration during an OGTT. In addition, the combination of aerobic exercise and n-3 fatty acids only effected insulin concentrations. These findings suggest that n-3 fatty acid supplementation alone does not effect glucose metabolism, but exercise does, and the combination of the interventions provides a greater effect on insulin concentration than n-3 supplementation alone in healthy, normglycemic men.

The following are recommendations for future studies:

1. More acute exercise studies that examine the combined effects of exercise and n-3 fatty acid supplementation on glucose metabolism are needed.

2. More studies that examine the combined and independent effects of aerobic exercise and n-3 fatty acid supplementation on glucose metabolism, without long term diet restrictions of saturated or unsaturated fatty acids.

3. More studies that perform additional combined and independent interventions of acute exercise and n-3 supplementations at different duration, intensities, modes, and dosages.

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APPENDIX A

Institutional Review Board Approval

TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940 898 3378 Fax 940 898 3416
e-mail: IRB@twu.edu

November 3, 2009

Dear Ms. Pleasant:

Re: *The Combined Effects of Acute Aerbolc Exercise and Omega - 3 Supplementation on Glucose Metabolism in Healthy, Normoglycermic Healthy, Normoglycemic Men*

The above referenced study has been reviewed by the TWU Institutional Review Board (IRB) and was determined to be exempt from further review.

If applicable, agency approval letters must be submitted to the IRB upon receipt PRIOR to any data collection at that agency. Because a signed consent form is not required for exempt studies, the filing of signatures of participants with the TWU IRB is not necessary.

Another review by the IRB is required if your project changes in any way, and the IRB must be notified immediately regarding any adverse events. If you have any questions, feel free to call the TWU Institutional Review Board.

Sincerely,

Kathy DeOrnellas, Ph.D.

Dr. Kathy DeOrnellas, Chair
Institutional Review Board - Denton

cc: Dr. Charlotte Sanborn, Department of Kinesiology
Dr. Kyle biggerstaff, Department of Kinesiology
Graduate School

APPENDIX B

Omega-3 Supplement Nutrition Profile

Omega-3 Supplement Nutrition Profile (4.55g)

Energy and Nutrition	Per Packet*
Calories	20.0
Total Fat (g)	2.0
Saturated Fat (g)	0.5
Cholesterol (mg)	8.0
Vitamin C (mg)	12.0
Vitamin E (IU)	3.0
Total Long Chain n-3 Polyunsaturates (mg)	650
EPA (mg)	350
DHA (mg)	230

Information provided by Coromega TM (European Reference Botanical Laboratories, Carlsbad, CA).

APPENDIX C

Metabolic and Cardiorespiratory Data at VO_{2max}

Cardiorspiratory and Metabolic Data at VO_{2max} for All Participants (N = 10)

Participants	VO_{2max} (l/min)	VO_{2max} (ml/kg/min)	HR (bpm)	%HRmax
1	3.0	46.6	155	85
2	2.9	36.3	178	90
3	3.2	33.4	222	116
4	3.6	39.5	191	95
5	4.1	47.2	199	103
6	3.6	37.2	173	100
7	2.9	29.3	185	103
8	3.8	40.4	151	83
9	3.3	41.2	199	103
10	3.5	42.9	203	105
$\mu \pm SD$	3.4 ± 0.4	39.4 ± 5.6	186 ± 22	98 ± 10.1

Participants	RER	SBP (mmHg)	DBP (mmHg)	V_e (l/min)	VCO_2 (l/min)
1	1.4	168	64	112.9	4.1
2	1.0	168	70	67.1	2.7
3	1.2	170	80	104.2	4.0
4	1.4	174	80	147.0	4.9
5	1.3	168	80	174.2	5.2
6	1.3	168	78	120.1	4.6
7	1.3	168	60	127.3	3.8
8	1.3	176	50	101.1	4.9
9	1.4	123	70	145.6	4.7
10	1.3	158	82	104.9	4.5
$\mu \pm SD$	1.3 ± 0.1	164 ± 15	71 ± 11	120.4 ± 29.9	4.3 ± 0.7

APPENDIX D

Metabolic and Cardiorespiratory Data During Exercise Trails

Cardiorspiratory and Metabolic Data During Exercise Trial

Participants N = 10	VO_2 (l/min)	VO_2 (ml/kg/min)	$\%VO_2max$	HR (bpm)
1	2.1	31.5	72	117
2	2.8	35.6	71	152
3	2.5	27.7	81	153
4	2.3	25.1	63	144
5	2.7	30.6	68	158
6	2.6	26.3	71	135
7	2.2	22.2	76	139
8	2.7	28.9	71	124
9	2.2	27.2	63	157
10	2.0	28.3	66	131
$\mu \pm SD$	2.4 ± 0.3	28.3 ± 3.7	71.0 ± 5.9	141 ± 14

Participants N = 10	%HRmax	RER	V_e (l/min)	VCO_2 (l/min)
1	60	0.96	40.6	1.9
2	77	0.95	53.9	2.7
3	80	0.98	65.8	2.5
4	72	0.91	44.3	4.2
5	82	0.95	64.4	2.6
6	78	0.95	50.7	2.5
7	77	1.02	55.4	2.4
8	68	0.91	50.7	2.4
9	81	0.98	46.3	2.1
10	75	0.93	46.0	1.8
$\mu \pm SD$	75 ± 7.0	$.95 \pm .03$	51.8 ± 8.3	2.5 ± 0.7

APPENDIX E

Diet History Summary

Total Energy Intake for Each Participant for Each Intervention N= 10

	Intervention			
Participant	CON	SUP	EX	EX-SUP
	(kcal)	(kcal)	(kcal)	(kcal)
1	3405.6	2579.7	2153.0	2794.3
2	2339.3	2540.8	2552.6	3618.2
3	1672.0	2240.3	2669.9	2212.8
4	3415.7	3010.8	4131.2	4295.1
5	1275.9	1793.7	2007.8	2212.8
6	3120.9	2228.4	2750.5	2119.3
7	2359.9	2968.4	3024.5	2737.1
8	1293.0	1289.5	2331.0	2246.6
9	1884.0	1438.6	1417.5	1673.3
10	1798.5	2825.0	1980.6	2989.8
$\mu \pm$ SD	2256.5 $\pm$ 817.1	2291.5 $\pm$ 613.9	2501.85 $\pm$ 735.1	2689.9 $\pm$ 785.5

Total Carbohydrate Intake for Each Participant for Each Intervention N= 10

	Intervention			
Participant	CON		SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	445.1	1780.4	400.2	1600.8
2	334.5	1338.0	307.9	1231.6
3	193.8	775.2	242.4	969.6
4	365.7	1462.8	302.8	1211.2
5	193.9	775.6	252.5	1010.0
6	462.9	1851.6	301.4	1205.6
7	312.3	1249.2	425.9	1703.6
8	105.5	422.0	129.8	519.2
9	307.1	1228.4	193.0	772.0
10	277.1	1108.4	396.3	1585.2
$\mu \pm SD$	299.8 ± 112.9	1199.2 ± 450.6	295.2 ± 94.9	1180.9 ± 379.5

Participant	EX		EX-SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	265.5	1062.0	366.3	1465.2
2	412.2	1648.8	448.9	1795.6
3	235.6	942.4	192.5	770.0
4	454.5	1817.6	436.9	1747.6
5	260.2	1040.8	265.5	1062.0
6	381.8	1527.2	252.5	1010.0
7	435.7	1742.8	344.1	1376.4
8	246.4	985.6	267.6	1069.6
9	219.8	879.2	239.9	959.6
10	300.4	1201.6	373.6	1494.4
$\mu \pm SD$	321.2 ± 90.3	1284.8 ± 360.9	318.8 ± 87.4	1275.0 ± 349.8

Total Protein Intake for Each Participant for Each Intervention N= 10

	Intervention			
Participant	CON		SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	116.8	467.2	78.7	314.8
2	65.2	260.8	97.5	390.0
3	79.8	319.2	109.4	437.6
4	133.9	535.6	268.5	1074.0
5	42.8	171.2	47.3	189.2
6	154.9	619.6	75.9	303.6
7	99.7	398.8	96.1	384.4
8	70.1	280.4	74.6	298.4
9	76.9	307.6	57.0	228.0
10	41.3	165.2	68.4	273.6
$\mu \pm SD$	88.2 ± 37.7	352.6 ± 150.9	97.3 ± 63.0	389.4 ± 252.1

Participant	EX		EX-SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	91.9	367.6	95.6	382.4
2	65.1	260.4	129.5	518.0
3	166.3	665.2	126.3	505.2
4	103.7	414.8	182.0	728.0
5	79.9	319.6	91.0	364.0
6	122.7	490.8	85.0	340.0
7	134.3	537.2	89.8	359.2
8	165.8	663.2	115.4	461.6
9	43.9	175.6	59.2	236.8
10	65.2	260.8	74.3	297.2
$\mu \pm SD$	103.9 ± 42.6	415.5 ± 170.4	104.8 ± 35.0	419.2 ± 140.1

Total Saturated Fat Intake for Each Participant for Each Intervention N= 10

	Intervention			
Participant	CON		SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	34.7	312.3	29.6	266.4
2	34.1	306.9	38.1	342.9
3	21.4	192.6	23.6	212.4
4	46.9	422.1	41.8	376.2
5	12.3	110.7	10.7	96.3
6	29.4	264.6	17.8	160.2
7	23.4	210.6	32.5	292.5
8	18.9	170.1	12.1	108.9
9	11.3	101.7	10.5	94.5
10	20.2	181.8	28.8	259.2
$\mu \pm SD$	25.3 ± 11.1	227.3 ± 99.5	25.6 ± 11.5	221.0 + 103.0
Participant	EX		EX-SUP	
	(grams)	(kcal)	(grams)	(kcal)
1	30.4	273.6	0.11	0.99
2	24.8	223.2	0.13	1.17
3	38.6	347.4	0.11	0.99
4	48.6	437.4	0.13	1.17
5	24.1	216.9	0.12	1.08
6	27.8	250.2	0.10	0.9
7	35.6	320.4	0.10	0.9
8	25.9	233.1	0.08	0.72
9	8.7	78.3	0.05	0.45
10	27.0	243.0	0.11	0.99
$\mu \pm SD$	29.2 ± 10.5	262.4 ± 94.4	32.8 ± 14.9	0.94 ± 0.22

Total EPH and DHA Intake for Each Participant for Each Intervention N= 10

	Intervention			
Participant	CON (grams)	SUP (grams)	EX (grams)	EX-SUP (grams)
1	0	4.06	0	4.06
2	0.001	5.31	0	4.06
3	0	4.77	0	5.37
4	0	4.06	0	4.06
5	0	4.06	0	4.06
6	0.026	4.06	0	4.06
7	0.023	4.06	0	4.06
8	0	4.06	0	4.06
9	0	4.06	0	4.06
10	0	4.06	0	4.06
$\mu \pm SD$	0.01 ± 0.01	$4.24 \pm .410$	0.0 ± 0.0	4.23 ± 0.40

APPENDIX F

Pairwise Comparison Summary

Pairwise Comparisons for Trials (N = 10)

Measure	Trials (I)	Trials (J)	Mean Difference	St. Error	Sig.
Insulin	1	2	871.14	192.14	0.01*
		3	353.10	344.69	1.00
		4	891.20	259.87	0.04*
	2	1	-871.14	192.14	0.01*
		3	-518.04	346.60	1.00
		4	20.06	208.71	1.00
	3	1	-353.10	344.69	1.00
		2	518.04	346.60	1.00
		4	538.10	210.64	0.19
	4	1	-891.20	259.87	0.04*
		2	-20.06	208.71	1.00
		3	-538.10	210.64	0.19
Glucose	1	2	-135.41	361.71	1.00
		3	567.01	481.35	1.00
		4	274.05	494.35	1.00
	2	1	135.41	361.71	1.00
		3	702.42	503.84	1.00
		4	409.46	392.09	1.00
	3	1	-567.01	481.35	1.00
		2	-702.42	503.84	1.00
		4	-292.96	323.18	1.00
	4	1	-274.05	494.35	1.00
		2	-409.46	392.09	1.00
		3	292.96	323.18	1.00
C-Peptide	1	2	38.37	10.29	0.03*
		3	37.71	14.27	0.16
		4	48.39	12.56	0.02*
	2	1	-38.37	10.29	0.03*
		3	-0.66	14.87	1.00
		4	10.03	13.72	1.00
	3	1	-37.71	14.27	0.16
		2	0.66	14.87	1.00
		4	10.68	9.00	1.00
	4	1	-48.39	12.56	0.02*
		2	-10.03	13.72	1.00
		3	-10.68	9.00	1.00

Note: Mean difference = (I-J); .05 significance level; *indicates significant difference;
Trials 1 = CON, 2 = EX, 3 = SUP, 4 = EX-SUP.

APPENDIX G

IAUC Means for Each Intervention

IAUC Means for Each Participant for Each Intervention N=10

Insulin (μIU/ml)

Participants	CON	EX	SUP	EX-SUP
1	3723.90	2932.20	3431.40	2721.45
2	5356.50	4282.58	7455.75	5588.33
3	3379.80	3610.05	3943.05	3843.30
4	6019.50	4406.03	5119.50	3814.58
5	4288.65	3208.88	4092.98	3658.20
6	5138.10	3346.50	3426.15	3387.90
7	2632.43	2002.28	2539.50	1549.20
8	3059.10	2928.98	1897.95	1996.88
9	4380.98	3397.73	3850.50	3872.33
10	4441.95	3594.30	3133.13	3076.73

C-Peptide (ng/ml)

Participants	CON	EX	SUP	EX-SUP
1	380.73	308.03	333.30	337.78
2	306.63	277.94	354.14	325.05
3	283.65	251.86	240.08	238.50
4	294.38	291.28	296.14	304.10
5	154.80	142.01	170.46	119.19
6	310.93	296.50	227.08	212.18
7	199.79	202.90	121.44	131.15
8	270.80	203.45	229.78	171.47
9	305.75	232.50	223.94	257.18
10	294.98	212.31	228.98	221.93

	Glucose (mg/dl)			
Participants	CON	EX	SUP	EX-SUP
1	7122.60	6151.28	6185.33	4633.20
2	8692.65	6999.08	10037.93	9466.20
3	3408.75	4622.40	2772.45	4482.00
4	3470.85	3917.70	2840.40	3893.40
5	1563.30	3460.05	3009.15	3591.00
6	3661.20	3369.60	2303.10	1617.30
7	3704.40	3665.25	2789.10	3090.15
8	2177.55	3399.30	3446.55	3284.55
9	7002.45	6011.55	3929.85	5159.70
10	5308.20	5869.80	3127.95	4153.95