EFFECT OF 7 DAYS AEROBIC EXERCISE ON INSULIN SENSITIVITY, OXIDATIVE STRESS, TLR2/TLR4 CELL SURFACE EXPRESSION AND CYTOKINE SECRETION IN SEDENTARY OBESE ADULTS

A dissertation submitted to the Kent State University College and Graduate School of Education, Health and Human Services in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

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August 2011
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Aerobic exercise training (AEX) has been well documented to improve glucose tolerance and insulin action in patients who are obese (Goodpaster & Kelley, 2003), insulin resistant (Rodgers, M.A., 1988) and in patients with T2DM (Bruce, 2004; Kirwan et al., 1990; Kirwan, Solomon, Wojta, Staten, Holloszy, 2009; Kirwan, Kohrt et al., 2002; Kirwan, et al., 1990) in the absence of weight loss. Mononuclear cells (MNC) are immune mediators of inflammation that are known to be elevated in diseases such as obesity and insulin resistance. Toll like receptors 2 and 4 (TLR2, TLR4) are predominantly found on the plasma membrane of MNCs and play a key role in the innate immune response. However, it is not known whether improvements in glucose tolerance resulting from short term AEX is due to reductions in proinflammatory mediators.

Thirteen sedentary obese (34.38 ± 1.13 kg/m²) adults, age 58.71 ± 3.21 yr, underwent Oral Glucose Tolerance Tests (OGTT’s), before and after 7 days of supervised AEX at ~70% VO₂max. Insulin sensitivity was assessed via the OGTT, TLR2/TLR4 cell surface expression by flow cytometry, ROS production by chemiluminescence, and MNC derived cytokine (TNF-α, IL-6) secretion by ELISA.

Seven days of AEX resulted in improvements in Matsuda insulin sensitivity (p=0.04) in the absence of weight loss and increased VO₂max, (p = 0.004). Metabolic
improvements were also noted in \( \text{CHO}\text{OX} \) and \( \text{Fat}\text{OX} \) (\( p = 0.02, 0.03 \)), \( \text{Gluc}_{120} \) and \( \text{Ins}_{120} \) responses (\( p = 0.002, 0.007 \)) and \( \text{tAUC} \) for \( \text{Gluc}_{120} \) and \( \text{Ins}_{120} \) (\( p = 0.04, 0.01 \)). \( \text{ROS} \) (\( p = 0.05 \)) and \( \text{IL-6} \) production at \( \text{Gluc}_{120} \) (\( p = 0.05 \)) were also significantly reduced. No changes were observed in either CD14 or CD14 CD16 TLR2 or TLR4 response for any time points.

Seven days of aerobic exercise training is sufficient to produce changes in insulin sensitivity in obese adults and these changes are associated with reductions in ROS and improvements in insulin sensitivity of peripheral tissues. However, because there were no changes in innate response, it is likely that a longer training duration greater than seven days may be required to reduce chronic inflammation in obese sedentary humans.
DEDICATION

This work is dedicated to my family for their unwavering
support during my educational pursuits.
ACKNOWLEDGEMENTS

I would like to extend my profound gratitude to my committee members: Dr. Michael I. Kalinski, Ph.D., my Dissertation Director, Dr. John P. Kirwan, Ph.D., Dr. Jacob Barkley, Dr. Robert Stadulis, and Dr. Jennifer Marcinkiewicz. I am extremely grateful for their professional expertise, encouragement, and guidance through all aspects of this project.

To Dr. Kalinski, who believed in me through all of my personal and family struggles. Thank you for your continued support, understanding and encouragement throughout this project.

To Dr. Kirwan thank you for your role as principal investigator of this project and the generous funding and vast support you provided me in so many aspects; for without your contributions, this project would not have been possible. Everything that I was able to participate on, experience, have access to, study, learn and assimilate has come from being in your lab. I cannot thank you enough and will always consider myself extremely fortunate for this opportunity.

I would like to express my sincere appreciation to the Clinical Research Unit at the Cleveland Clinic. For all the nurses who worked tirelessly to help us collect the data, the lab technicians who provided lab support for sample processing, secretaries that assisted with subject scheduling, and Kay Stelmach, who personally assisted when problems inevitably came up, without their contributions, a project of this magnitude would not have been possible. This project was definitely a team effort and a huge undertaking. I am grateful for the many talented people that I was blessed to work with.
I especially wish to thank Julianne Filion, our clinical nurse coordinator for this project and various other ongoing projects. Julianne assisted in all aspects of the study to include numerous IRB revisions, amendments, and for keeping us legal regarding IRB issues. She also worked endlessly with subject recruiting, scheduling, testing and conflict resolution, ensuring that I could complete the testing of my subjects in a timely fashion. Her humor kept me smiling throughout this project.

I would also like to express my sincere appreciation to Hazel Huang, Mark Cook, Yanjun Li, Calvin Hwang, Chuck Trunick, Don Blocksom and all those who have provided support, encouragement, and assistance towards the completion of this project. Without their unending willingness to provide time, patience, and morale support, this study would have never been completed. To the study subjects, without your commitment and participation in this project, I would still be recruiting.

I would also like to acknowledge the many contributions from the flow lab. Anne “Bunny” Cotleur and Dr. Karen Kelly spent numerous hours advising and assisting me in fine tuning a flow cytometry protocol in whole blood and cell culture for the measurement of TLR2 and TLR4. Also, I would like to thank Cathy Shemo, Sage O’Bryant and Moneen Morgan for their willingness to come to the lab on Saturday mornings to assist in running Friday’s samples.

Finally, I would like to express my deepest appreciation and gratitude to my husband, Christopher J. Melin. Without his support, understanding and commitment to our family while I pursued my professional goals, this accomplishment would have never been achieved. I wish to clarify the amount of commitment my husband devoted to our
family during the course of this study. At the end of the 2007-2008 school year, my oldest daughter, Alyssa, developed anorexia nervosa, in which she required hospitalization and numerous partial hospitalizations over the next several years due to relapses. Chris gave up his vacation time and sick time to assist me in getting my daughter, his stepdaughter, to hundreds of counseling, nutrition and medical follow-up visits during this time, as well as, assistance with obtaining the food, food preparation, supervision of eating and activity restriction supervision until I was able to pick up full responsibility at the end of my data collection. For anyone that has not dealt with someone with a life threatening addiction, this means 24/7 supervision and is all consuming. Anorexia is well known to have the highest mortality rate of any psychiatric disorder. In fact, females between the ages of 15 and 24 are 12 times more likely to die from anorexia than all other causes of death, according to the National Eating Disorders Association in America. This has been an extremely difficult time for our entire family and my daughter’s suicide ideations produced anxiety attacks beyond belief. Although Alyssa is now weight restored, her struggle with AN continues to be a daily battle and she is far from recovery. She is moving forward in her life pursuits and this truly is my biggest accomplishment. To my dearest Alyssa, you have given up numerous nights of one-on-one mother daughter time over the course of your life; I cannot thank you enough for your patience and understanding and unconditional love. You are truly amazing.

The last 2 years have been extremely difficult from a professional and personal perspective. I am deeply indebted to the post doctoral fellows Karen R. Kelly,
Jacob M. Haus, and Thomas P.J. Solomon for their support and guidance throughout the project. Dr. Kelly was a tremendous mentor and source of information both professionally and personally. At times it was hard to keep up with her, but she assisted me on every aspect of the study, from cell culture work to development of new protocols to helping me stay focused when life was overwhelming. Dr. Haus was equally an invaluable source of information from project development to statistical analysis. Dr. Solomon, thank you for all your work on the development of the ROS protocol. All three of the post doctoral fellows assisted me on the intricacies of the project; their attention to detail was phenomenal.

I would also like to thank Dr. Ken Sparks at Cleveland State University for persuading me to continue my academic pursuits. Without his confidence and encouragement, I would have stopped after the Masters program. I extend my sincere appreciation to all of those who helped me during the duration of this project that I may have missed, this was a collaborative effort and I could not have.
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<td>AEX</td>
<td>Aerobic Exercise Training</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
</tr>
<tr>
<td>AS160</td>
<td>Akt Substrate of 160 kDa</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CD14CD16</td>
<td>Cluster of differentiation 14 and 16, (Human)</td>
</tr>
<tr>
<td>CRP</td>
<td>C reactive protein</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunoabsorbant assay</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>HMW</td>
<td>High molecular weight</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>IKKα</td>
<td>Inhibitor of κB alpha</td>
</tr>
<tr>
<td>IKKβ</td>
<td>Inhibitor of κB beta</td>
</tr>
<tr>
<td>IKK</td>
<td>IkappaB kinase</td>
</tr>
<tr>
<td>IKKB</td>
<td>IKK beta (I-Kappa-B kinase-beta)</td>
</tr>
<tr>
<td>IKKγ</td>
<td>Inhibitor of κB gamma</td>
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<tr>
<td>IRAK</td>
<td>IL-1 receptor-associated kinases</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>IRS</td>
<td>Insulin receptor substrate</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinases</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated Protein Kinase</td>
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<tr>
<td>MNC</td>
<td>Mononuclear cell</td>
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<tr>
<td>NEMO</td>
<td>NF-κB essential modulator</td>
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<td>NFκB</td>
<td>Nuclear factor kappa B</td>
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<tr>
<td>NGT</td>
<td>Normal Glucose Tolerance</td>
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<td>OGGT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>PKB</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor kappa B</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
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<tr>
<td>TBK</td>
<td>TANK-binding kinase</td>
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<tr>
<td>T2DM</td>
<td>Type II diabetes mellitus</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll like receptor</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TRAF</td>
<td>TNF receptor associated factor</td>
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<tr>
<td>TRAM/TICAM2</td>
<td>TIR domain-containing protein</td>
</tr>
<tr>
<td>TRIF</td>
<td>TIR-domain-containing adapter-inducing interferon-β</td>
</tr>
<tr>
<td>VO₂max</td>
<td>Maximal oxygen consumption</td>
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<td>WAT</td>
<td>White adipose tissue</td>
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CHAPTER I

INTRODUCTION

The number of U.S. adults that are obese has increased dramatically over the past two decades. The prevalence of obesity appears to stem from two primary causative factors, chronic overnutrition and reduced energy expenditure or sedentary lifestyle. Obesity, defined as having a body mass index (BMI) greater than 30 kg/m² (Consitt, 2009), is now known to be associated with a low-grade inflammation emanating from white adipose tissue (WAT) that results in chronic activation of the innate immune system (Weisberg, 2003) and which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes (Bastard, 2006). It is now well accepted that obesity is a proinflammatory state strongly associated with hyperglycemia and insulin resistance (Ciaraldi, 1981; Kolterman, 1980). In obesity, WAT is characterized by an abnormal adipokine production, which may exert their effects locally but can also exert systemic effects on other organs. Recent data has indicated that WAT is infiltrated by macrophages and may be a major source of locally-produced pro-inflammatory cytokines. Several factors derived not only from adipocytes but also from infiltrated macrophages likely contribute to the pathogenesis in insulin resistance. Most of them are up-regulated during obesity, including leptin, TNF-α, IL-6 and resistin (Bastard, Maachi, Lagathu, Kim, Caron, Vidal, Capeau, Feve, 2006). Conversely, expression and plasma levels of adiponectin, an insulin sensitizing agent, are down-regulated during obesity. Although leptin acts mainly at the level of the central nervous system to regulate food intake and energy expenditure, there is a relationship between leptin and the low grade
inflammatory state in obesity, suggesting that leptin could exert peripheral biological effects. Leptin could modulate TNF-\(\alpha\) production and macrophage activation (Loffreda, 1998). IL-6 synthesis by human adipose tissue increases during obesity and may induce hepatic C reactive protein synthesis. Both TNF-\(\alpha\) and IL-6 can alter insulin sensitivity by triggering different key steps in the insulin signal pathway. Resistin has been shown to induce insulin resistance in animal models, however, its role in the control of insulin sensitivity in humans’ remains controversial. Adiponectin inhibits liver neoglucogenesis and promotes fatty acid oxidation in skeletal muscle. It also has been implicated in counteracting the pro-inflammatory effects of TNF-\(\alpha\) on the endothelial wall (Bastard, Maachi, Lagathu, Kim, Caron, Vidal, Capeau, Feve, 2006).

Hyperglycemia has been shown to increase reactive oxygen species (ROS) generation from peripheral blood mononuclear cells (MNC) in obese individuals (Gonzalez, Rote, Minium, & Kirwan, 2006b). ROS induced oxidative stress is a known activator of nuclear factor kappa B (NF\(\kappa\)B), a proinflammatory transcription factor that promotes tumor necrosis factor alpha (TNF-\(\alpha\)) gene transcription (Barnes & Karin, 1997; Evans, Goldfine, Maddux, & Grodsky, 2002; Mohanty, et al., 2000). TNF-\(\alpha\) has been identified to inhibit insulin signaling (Hotamisligil & Spiegelman, 1994), reduce glucose transporter type 4 (GLUT4) levels (Stephens, 1991), and produce insulin resistance (Hotamisligil, Murray, Choy, & Spiegelman, 1994). With chronic activation of the innate immune system (Barnes & Karin, 1997; Esposito, Marfella, et al., 2002; Hotamisligil & Spiegelman, 1994), plasma concentrations of C-reactive protein (CRP)
and Interleukin 6 (IL-6), both acute phase proteins, are increased in obese subjects. Additionally, toll-like receptors 2 and 4 (TLR2, TLR4) are also elevated which in turn activates NFκB; all of these causal factors are implicated in the progression to low grade inflammation seen in obesity (Gonzalez, et al., 2006b; Mohanty, et al., 2000). The pathogenesis of obesity is multifactorial, however, elevations in MNC derived TNF-α and other proinflammatory cytokines in response to hyperglycemia provide some mechanistic insights for inflammation and insulin resistance in obesity.

MNC are immune mediators of inflammation that are known to be elevated in diseases such as obesity and insulin resistance. TLR2 and TLR4 are predominantly found on the plasma membrane of MNCs and play a key role in the innate immune response. Devaraj et al showed increased TLR2 and TLR4 expression, intracellular signaling, and TLR-mediated inflammation in monocytes with significant correlation to hemoglobin A1C levels in type 1 diabetic patients (Devaraj, 2008), while Creely et al demonstrated increased TLR2 expression in adipose tissue from type II diabetes mellitus (T2DM) patients (Creely, 2007), while others have demonstrated that TLRs are inducible in adipose tissue and linked with downstream NFκB activation and cytokine release (Vitseva, 2008).

With the increased prevalence of obesity and insulin resistant disorders, research has attempted to elucidate the potential mechanisms driving these disease processes, with the hope of ultimately providing cost effective interventions. Aerobic exercise training (AEX) has been well documented to improve glucose tolerance and insulin action in
patients who are obese (Goodpaster, Kelley, 2003), insulin resistant (Rodgers, 1988) and in patients with T2DM (Bruce, 2004) in the absence of weight loss. Arciero et al demonstrated that a moderate exercise training program (50 minutes, 60-65% VO$_{2\text{max}}$) for 10 days in obese people resulted in a significantly greater improvement in glucose disposal than did a low-calorie diet that induced more than 2.5 times the negative energy balance (Arciero, 1999). Others have shown significant improvements in insulin sensitivity and/or glucose tolerance after short term training protocols (10-15 days), however, results have been inconsistent. Regardless of the inconsistencies in exercise protocols or glucose intolerance of subjects, moderate and high intensity exercise protocols that result in ~1000 kcal/week energy expenditure have been suggested to be more effective in producing significant metabolic changes than low intensity regimens or diet alone (Andersen, 1991; Fletcher, 1992; Gregg, Gerzoff, Caspersen, Williamson, & Narayan, 2003; Hickman et al., 2004; Suzuki, et al., 2005).

Despite the numerous studies demonstrating the benefits of lifestyle intervention in obesity and/or insulin resistant states, there are no studies to date that have examined the effects of short term aerobic exercise on toll like receptor regulation in humans. In order to develop appropriate treatment programs in obese patients, we need to understand how exercise affects insulin resistance, oxidative stress and inflammatory cytokine secretion in this disease process. More studies are needed before lifestyle modification guidelines can be established. The rationale for the proposed research is that, once an effective exercise intervention is developed and once it is known how this intervention
regulates metabolism it can then be implemented in treatment and ultimately prevention of obesity and its associated diseases.

**Statement of the Problem**

It is estimated that 65% of the U.S. adult population is now overweight or obese, with a worldwide prevalence of 40-60% (Tjepkema, 2006). Inflammatory activity is implicated in the pathogenesis of obesity induced insulin resistance and metabolic dysfunction. Overnutrition and a sedentary lifestyle are the biggest environmental contributors to obesity-induced insulin resistance.

**Purpose of the Study**

The purpose of this investigation was to examine the effects of a short term (7 day) aerobic exercise training program on insulin resistance, cell signaling, oxidative stress and inflammatory cytokine secretion in 13 sedentary obese (BMI = 30 – 40 kg/m²) adult men and women.

**Significance of Study**

The exercise protocol used in this study does not produce weight loss and therefore, outcomes can be attributed to the effects of exercise alone without the complication of weight loss. To date, the effects of exercise on TLR cell surface expression have not been studied in an impaired population.

**Specific Aims and Hypotheses**

Hypothesis testing will be accomplished by pursuing the following three specific aims:
1. To identify the effects of short-term aerobic exercise training on insulin resistance in obese adults. The working hypothesis is that short-term aerobic exercise training will reduce fasting glucose, and increase glucose disposal in obese adults. The approach used to test this hypothesis will involve measuring glucose and insulin obtained after administration of an oral glucose tolerance test (OGTT) at basal (fasting) and glucose stimulated (120 minutes) time points both pre and post exercise. We expect that the exercise intervention will reduce fasting glucose, and increase insulin-stimulated glucose disposal.

2. To identify the effects of short-term aerobic exercise training on cell signaling and oxidative stress (from monocyte-derived TLR2, TLR4 expression and ROS) in obese adults. The working hypothesis is that short-term aerobic exercise training will decrease TLR2 and TLR4 expression and ROS production obtained in MNCs from obese adults. The approach used to test this hypothesis will involve measuring surface expression of TLR2 and TLR4 by flow cytometry and oxidative stress by chemiluminescence, from basal and glucose stimulated MNCs obtained before and after the 7 day intervention. We expect that the exercise intervention will reduce cell signaling and oxidative stress from MNCs in obese adults. Secondarily, we expect that the decrease in oxidative stress will be associated with the reduction in peripheral insulin resistance.

3. To identify the effects of short term aerobic exercise training on inflammatory cytokine secretion (from monocyte-derived TNF-κ, IL-6) in obese adults. The approach used to test this hypothesis will involve measuring ex vivo cytokine
secretion by ELISA, all from basal and glucose stimulated MNCs obtained before and after the 7 day intervention. The working hypothesis is that short-term aerobic exercise training will decrease proinflammatory cytokine (IL-6, TNF-α) secretion in MNCs obtained from obese adults. We expect that the reduction in proinflammatory cytokine secretion will be associated with a reduction in peripheral insulin resistance.
CHAPTER II
LITERATURE REVIEW

Economic and Health Consequences of Obesity

Within the USA, data suggest that 64.5% of the adult population is now overweight or obese, with a worldwide prevalence of 40-60% (Tjepkema, 2006). In 2006, the per capita increase in spending attributable to obesity was 36% for Medicare and 47% for Medicaid (Finkelstein, Trogdon, Cohen, & Dietz, 2009). The annual medical burden of obesity has risen to almost 10 percent of all medical spending amounting to $147 billion per year in 2008 (Finkelstein et al., 2009). If this trend continues, cost can be $344 billion per year by 2018. In addition to the tremendous financial burden that obesity and its associated risks place on our health care system, it can more importantly impact both the quality and duration of life. Epidemiological studies show an increase in mortality associated with obesity. Individuals who are obese (BMI > 30 kg/m²) have a 50 - 100 percent increased risk of premature death from all causes compared to individuals within the BMI range of 20 to 25 (Allison, 1999). Additionally, an estimated 300,000 deaths a year may be attributable to obesity (Allison, 1999).

Prevalence

The United States has experienced substantial increases in obesity cutting across all ages, racial/ethnic groups, and genders. Disparities in obesity prevalence exist in many segments of the population based on race and ethnicity, gender, age, and socioeconomic status. Obesity is particularly common among minority groups and those
with a lower family income. Within racial groups, gender disparities exist, although not always in the same direction. In addition to racial/ethnic and gender disparities, the prevalence of obesity also varies by age, affecting both genders equally with advancing age.

It is estimated that 7 out of 10 adults are reported to be sedentary and when combined with a chronic excess of calories, this combination has contributed to the worldwide epidemic of obesity (Manson, 2004). In obese populations at risk for developing insulin resistance, it is now well established that increased physical activity levels and long term weight loss reduce the prevalence of insulin resistance and prevent or delay the progression toward T2DM (Knowler, 2002; Kraus et al., 2002). Since insulin resistance is a key element in the pathogenesis of obesity, overwhelming evidence supports a role for weight loss, achieved through diet and/or exercise in the treatment of obesity. Although the pathogenesis of obesity is considered to be multifactorial and not fully understood, the following literature review will attempt to explain the leading theories of pathogenesis and the role of exercise as a therapeutic intervention.

Pathophysiology of Obesity

Obesity and the associated increase in adiposity are the consequences of chronically excessive energy intake and/or inappropriately low energy expenditure. Obesity is considered to be an insulin-resistant state characterized by chronic subclinical inflammation (Diehl, Li, Lin, & Yang, 2005; Harrison, 2007; Perlemuter, 2007) that likely involves complex heterogeneous mechanisms such as nutrient overload, increased fatty acid flux, ER stress, secretion of adipocytokines, and most likely a decline in the
number and function of insulin receptors as well as postreceptor defects. The origin of this inflammation resides, in part, in adipose tissue itself. Since the etiology of obesity is multifactorial, the exact mechanisms of obesity induced insulin resistance will differ across individuals and populations.

**Inflammation, Obesity and Insulin Resistance**

Insulin resistance can be defined as a condition in which normal concentrations of insulin produce an inadequate metabolic response by insulin sensitive tissues (skeletal muscle, liver, and adipose tissue) (Kahn, 1978). The hallmarks of impaired insulin sensitivity in these three tissues are decreased insulin-stimulated glucose uptake into skeletal muscle, impaired insulin-mediated inhibition of hepatic glucose production in liver, and a reduced ability of insulin to inhibit lipolysis in adipose tissue. Insulin resistant states can be further divided into subcategories: those due to decreased sensitivity to insulin, those due to a decrease in the maximal response of insulin, or a combination of these two conditions (Kahn, 1978).

The concept of adipose tissue being an inflammatory state was first introduced by Hotamisligil* et al.* (Hotamisligil, Shargill, & Spiegelman, 1993). This study showed that increased expression of TNF-α by adipocytes in white adipose tissue (WAT) of obese animals, and the neutralization of TNF-α by a TNF-α soluble antibody led to an improvement of insulin sensitivity in these animals. These observations demonstrated the existence of a strong link between TNF-α and the development of insulin resistance associated obesity. Subsequently, many others have also provided evidence to show that
Chronic activation of proinflammatory pathways within insulin target cells can lead to obesity-related insulin resistance (de Luca & Olefsky, 2008; Shoelson, Herrero, Laura, Naaz, Afia, 2007; Shoelson, Lee, & Goldfine, 2006). Consistent with this, elevated levels of the proinflammatory cytokines TNF-α, IL-6, and C-reactive protein (CRP) have been shown in individuals with insulin resistance and diabetes. Although the liver and the lymphoid organs are the major sites of production of inflammatory mediators, recent studies have implied that macrophages infiltrating the adipose tissue contribute to dysregulated adipocytokine production and are the link to various metabolic disorders (Furkawa, S., 2004). Cytokines can be proinflammatory such as TNF-α (Corvera et al., 2006; Crespo, 2001; del Aguila, Claffey, & Kirwan, 1999; Hotamisligil et al., 1994; Hotamisligil et al., 1996; Hotamisligil & Spiegelman, 1994), IL-6 (Dandona, 2001; Wellen, & Hotamisligil, 2005), leptin, and resistin or anti-inflammatory, such as adiponection (Angulo, 2007; Diehl et al., 2005) and IL-10 (Burdin, 1997; Dang et al., 2006), and they can be produced by virtually every nucleated cell in the body. Interestingly, monocyte (MNC)-derived levels of these inflammatory cytokines along with other markers such as NFkB (Barnes & Karin, 1997), TLR2 (Dasu, Devaraj, Zhao, Hwang, & Jialal, 2008), TLR4 (Dasu et al., 2008; Palsson-McDermott & O'Neill, 2004) and serum concentrations of acute phase reactants (C-reactive protein, fibrinogen, α1-acid glycoprotein, amyloid A, sialic acid) have also been shown to be predictors of inflammation, insulin resistance, obesity and T2DM (Bugianesi, Vanni, Gambino, Cassader, Baldi, et al, 2005; Steinberg, 2007).
Deposition of fat is a strong predictor of hyperinsulinemia and/or insulin resistance. Different fat depots have different metabolic characteristics and thus pose different contributions to the pathogenesis and complications of obesity. Adipose tissue not only stores excess calories but also actively secretes various substances such as fatty acids, hormones and cytokines that can function in an endocrine or paracrine fashion. WAT is a very heterogeneous tissue composed of several cell types: mature adipocytes, preadipocytes, endothelial cells and immune cells, macrophages and lymphocytes called the stroma vascular fraction. Due to this heterogeneity, the cellular origin of different secreted inflammatory factors by whole adipose tissue is controversial (Fried, 1998). Thus, the mixture of adipokines secreted by adipose tissue in a given pathophysiological state can have important effects on systemic insulin sensitivity. The events that potentially link obesity to the initiation of inflammation and the induction of tissue and systemic insulin resistance are detailed below in Figure 1 (Schenk, 2008). Increased intramyocellular triglyceride content was shown to more closely correlate with muscle insulin resistance and thus to be a better predictor of impaired insulin action than visceral adiposity (Bugianesi, Marchesini, 2005). Visceral fat produces greater TNF-α and FFA (but less adiponectin) than subcutaneous fat, while subcutaneous fat is known to produce large amounts of leptin (Schenk, 2008).
Figure 1. Events linking obesity to the initiation of inflammation and the induction of tissue and systemic insulin resistance (Schenk & Horowitz, 2007)

The accumulation of triglycerides ectopically has been termed the overflow hypothesis (Danforth, 2000). This hypothesis suggests that insulin resistance results from the inability of adipose tissue to expand to accommodate excess calories. Once the adipocyte’s capacity to store triglycerides is exceeded, fat overflows to other tissues (muscle and liver), where the intracellular triglyceride metabolism interferes with glycogen synthesis in muscle and augments hepatic gluconeogenesis (Bugianesi, Marchesini, 2005).

As the adipose tissue bed expands during the progression to obesity, the enlarging adipocytes become relatively hypoperfused, creating regional areas of microhypoxia (Hosogai, 2007; Wang, 2007). Hypoxic adipose tissue displays enhanced activation of the JNK1 and IKK/NF-κB pathways as well as increased expression of genes involved in
inflammation and ER stress (Hosogai, 2007; Wang, 2007). Activation of these pathways leads to cytokine release by adipose tissue and subsequent recruitment of macrophages into the adipose tissue (Cinti, 2005; Strissel, 2007) where these macrophages can also release proinflammatory cytokines inducing insulin resistance in neighboring adipocytes via paracrine effects. Adipose tissue microhypoxia may exacerbate the proinflammatory nature of these newly recruited macrophages further activating hypoxia-sensitive pathways and amplification of the inflammatory state. Thus, subcutaneous fat, as expansion of visceral fat has a uniquely adverse effect on insulin sensitivity (Boden, 2006).

Cytokine levels are absent or barely detectable in most healthy tissues. However, as physiologic and/or pathologic stimuli are introduced, cytokine levels increase. The inflammatory event is usually initiated by production of pro-inflammatory cytokines (IL-6 and TNF-α), followed by a delayed release of anti-inflammatory cytokines (IL-10 and IL-1ra) thereby limiting the total duration of the proinflammatory event and attenuating the proinflammatory response (Mackinnon, 1999a). However, chronic exposure of proinflammatory cytokines as seen in obesity, leads to sustained macrophage activation, a hallmark of chronic inflammation, and thus sets the stage for inflammatory damage.

**Tumor Necrosis Factor Alpha (TNF-α)**

TNF-α is involved in systemic inflammation and is also involved in the acute phase response. As previously mentioned, TNF-α production has been shown to be
elevated in obese subjects (del Aguila et al., 1999; Gonzalez, Minium, Rote, & Kirwan, 2005, 2006; Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995), diabetic subjects (Gonzalez et al., 2005; Gonzalez, Minium et al., 2006; F. Gonzalez, Rote, Minium, & Kirwan, 2006; Hotamisligil et al., 1995; Hotamisligil et al., 1994; Hotamisligil et al., 1996; Hotamisligil et al., 1993; Hotamisligil & Spiegelman, 1994; Wellen, and Hotamisligil, 2005), and in insulin resistant states (Hotamisligil et al., 1994; Hotamisligil et al., 1993; Hotamisligil & Spiegelman, 1994). Definitive links have been established between TNF-α action and the suppression of insulin action in cells (Figure 1), whole animals, and humans (Wellen, &Hotamisligil, 2005) demonstrating that TNF-α impairs insulin signaling by serine phosphorylation at IRS-1 (Hotamisligil et al., 1996) and that downstream functions such as GLUT 4 uptake and expression are impaired (Goodyear, 1998; Goodyear, Hirshman, Valyou, & Horton, 1992). Furthermore, TNF-α can activate Jun N-terminal kinase (JNK) and inhibitor kappa beta kinase beta (IKKβ ) making cells resistant to the actions of insulin (Kim, Fillmore, Chen, Yu, Morre, Pypaert, et al, 2001; Kim, 2006).

Although many of the physiological roles of TNF-α overlap with IL-1 and IL-6, there are important points of distinction. TNF-α is mainly produced by monocytes and macrophages, however, it can also be produced by T lymphocytes, Kupffer cells, neural and endothelial cells (Cohen, 1996). Most cells express TNF receptors and soluble TNF receptors attenuate the powerful inflammatory reactions induced by TNF-α.
There are two TNF-α cell surface receptors in humans that are present in all types of human cells and in plasma in soluble forms (sTNF-R1 and sTNF-R2), Figure 2. TNF-R1 is expressed in most tissues, can be fully activated by both membrane bound and soluble forms of TNF and is responsible for differentiation and apoptosis. TNF-R2, however, is found only in immune cells, responds to the membrane bound form of TNF and mediates metabolic actions of TNF-α. Activation of TNF-α leads to overexpression of TNF-R2 in adipose tissue and to increased levels of its soluble form in human obesity. Additionally, sTNF-R2 levels correlate with BMI, fasting insulin and with insulin resistance, while there are no such correlations for sTNF-R1 (Moldoveanu, 2001).

**Figure 2.** TNF-α Signaling Pathways (Palladino, 2003).
Adiponectin is a potent TNF-α inhibitor and in vitro and experimental animal studies have proven the importance of this mediator in counteracting inflammation and insulin resistance. Anti-inflammatory effects of adiponectin are exerted both by suppressing TNF-α synthesis and by induction of IL-10 or IL-1ra (Tilg H, 2006). Adiponectin acts directly on hepatocytes to inhibit fatty acid synthesis and uptake while stimulating fatty acid oxidation (Yamauchi et al., 2002; Yamauchi et al., 2003), therefore, enhancing hepatocyte insulin sensitivity (Stefan & Stumvoll, 2002). In humans, various treatments (diet and exercise) that improve obesity, T2DM, and dyslipidemia have been shown to increase adiponectin levels and reduce TNF-α (Esposito et al., 2003). Although several studies have looked at the effects of exercise on TNF-α, results have been mixed. Combined data in master’s athletes and younger individuals have shown nonsignificant increases in MNC-derived TNF-α release immediately after exercise. In contrast, Straczkowski et al. provided evidence supporting a decrease in MNC-derived TNF-α and sTNF-R2 levels after 12 weeks of bicycle ergometry (70% maximal heart rate, for 30 min, 5 days/week) while sTNF-R1 remained unchanged (Staczkowski et al., 2001). Rivier et al. also found decreases in MNC-derived TNF-α production below baseline during the post-exercise period (Rivier et al., 1994). Additionally, Riviera reported that in vivo or in vitro administration of glucocorticoids suppresses the LPS-induced release of TNF-α by blood MNCs (Rivier et al., 1994).

Overall, proinflammatory cytokines have been accepted as key players in the progression of subclinical inflammation, however, their roles and functions once
dysregulated are only beginning to be clarified. Thus it would be important to determine whether the inflammatory cytokine profiles can be modulated by moderate exercise.

**Interleukin-6 (IL-6)**

Among the adipocytokines, the inflammatory regulator interleukin-6 (IL-6) has emerged as one of the potential mediators that link obesity-derived chronic inflammation with insulin resistance. Although adipose tissue is only responsible for a small amount of total body glucose disposal (4-5%) compared to skeletal muscle (80-85%), it plays a very important role in human glucose homeostasis (Yuen, 2009). Adipose tissue contributes up to 35% of circulating IL-6 from both adipocytes and macrophages within the adipose tissue bed (Yuen DYC, 2009). Plasma IL-6 plasma concentrations are known to be elevated in diseases such as obesity and in patients with T2DM; the systemic effects of which have been best demonstrated in the liver. IL-6, however, displays pleiotropic functions that are tissue-specific and environment-dependent. In contrast to its role in liver, IL-6 is believed to be beneficial for insulin-regulated glucose metabolism in muscle. Additionally, the effects of IL-6 are apparently influenced by whether it released acutely or chronically; with chronic production associated with insulin resistance.

Pederson, *et al* (Bente Klarlund Pedersen, Akerstrom, Nielsen, & Fischer, 2007) defined myokines as cytokines and other peptides that are produced, expressed, and released by muscle fibers and that exert either paracrine or endocrine effects. Therefore, skeletal muscle can function as an endocrine organ by secreting myokines and thus linking exercise, metabolism, and immune function (Bruunsgaard, 2005). IL-6 is a pleiotropic cytokine produced by various immune cells (T and B lymphocytes, natural killer cells
and monocytes) as well as a long list of nonimmune cells (adipose, vascular smooth muscle cells, skeletal muscle, chondrocytes, astrocytes and glial cells) (Moldoveanu, 2001).

Characteristicly, we know that IL-6 is down regulated by the inhibitory action of glucocorticoids on macrophages (Moldoveanu, 2001), it is elevated in patients with metabolic disease (Pedersen et al., 2007), plasma concentrations of IL-6 increase exponentially (up to 100 fold) in response to exercise (Febbraio & Pedersen, 2002; Steensberg et al., 2000) and it declines in the postexercise period (Febbraio & Pedersen, 2002; Suzuki, Yamada, Totsuka, Sato, Sugawara, 2002) with values normalizing within 6 hours or less (Moldoveanu, 2001). It would seem paradoxical that working muscle would release an insulin inhibitory factor when insulin action is enhanced in the post-exercise period (Pedersen et al., 2007). Ostrowski et al (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999; Ostrowski, 2000) reported an exercise-induced increase in plasma IL-6 is followed by an increased counter anti-inflammatory response (IL-1ra and IL-10), similarly, infusion of IL-6 into healthy donors has been show to mimic the exercise response of IL-1ra and IL-10. Both exercise and IL-6 have been shown to suppress TNF-α release in humans (Starkie, Ostrowski, Jauffred, Febbraio, & Pedersen, 2003). Given the evidence implicating TNF-α in the pathogenesis of human insulin resistance (del Aguila et al., 1999; Hotamisligil et al., 1994; Hotamisligil et al., 1996; Hotamisligil et al., 1993; Hotamisligil & Spiegelman, 1994; Kirwan, Haugel-de Mouzon et al., 2002), exercise induced IL-6 release can therefore protect against TNF-α induced
insulin resistance. Steensberg et al demonstrated a nineteen fold increase in IL-6 from baseline after prolonged (5 hr, 40-50%) single limb exercise in humans (Steensberg et al., 2000). Pederson et al also found marked increases in circulating IL-6 levels after prolonged exercise, which were correlated to exercise intensity, duration, muscle mass recruited, and endurance capacity, however, independent of associated muscle damage (Pedersen et al., 2007). Recent research has demonstrated that IL-6 mRNA is upregulated in contracting skeletal muscle (Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998) and that the transcriptional rate of the IL-6 gene is markedly enhanced by exercise (Hiscock, 2004; Steensberg et al., 2000).

Although IL-6 plays a role in endogenous glucose production during muscle contraction in humans, its action on the liver is totally dependent on other contraction-induced factors (Febbraio, Hiscock, Sacchetti, Fischer, & Pedersen, 2004). At physiological concentrations at rest, acute IL-6 infusion does not impair whole body glucose disposal, net peripheral glucose uptake, or EGP in healthy young humans (Akira, 1993; Steensberg et al., 2003). In T2DM patients plasma insulin decreases in response to IL-6 infusion without a corresponding increase in EGP (Akira, 1993). Carey et al demonstrated that IL-6 may increase glucose infusion rate and glucose oxidation without changes in EGP during a hyperinsulinemic euglycemic clamp in healthy humans (Carey, 2006). This suggests that IL-6 may have an insulin sensitizing effect in conditions where EGP is completely suppressed. The effect of IL-6 on insulin stimulated glucose metabolism is likely to occur in peripheral tissues, whereas IL-6 does not influence EGP. Several studies in healthy humans (Akira, 1993; van Hall et al., 2003) and T2DM patients
(Akira, 1993) have shown that recombinant IL-6 infusion increases lipolysis and fat oxidation. In vivo experiments by Carey et al demonstrated that IL-6 may increase basal and insulin stimulated glucose uptake via an increased GLUT4 translocation (Carey, 2006). Recently it has been implied that a link exists between IL-6 and AMP-activated protein kinase (AMPK), since both act as energy sensors. IL-6 was shown by Kelley et al to enhance AMPK activity in both skeletal muscle and adipose tissue (Kelly, 2004). Nonetheless, discrepancies exist in results for IL-6 secretion and modulation, since both visceral and subcutaneous WAT of obese and nonobese subjects release this cytokine.

IL-6 is also an important mediator of the acute phase response in the liver, and controls the expression of CRP and fibrinogen. It is generally regarded as the best marker of the clinical disease course and severity of the inflammatory response (Woods, Vieira, Keylock, 2006). One third of IL-6 in humans is produced by adipose tissue during non-inflammatory conditions. Visceral adipose tissue produces three times more IL-6 than subcutaneous adipose tissue (Fried, 1998), and IL-6 and TNF-α released from adipose tissue have been shown to directly induce insulin resistance in model systems (Bugianesi, Marchesini, 2005; del Aguila et al., 1999; Fernandez-Real, Richart, Gutierrez Broach, Vendrell, et al, 2001; Hotamisligil et al., 1993; Senn, 2002). Exogenous IL-6 has also been shown to induce hepatic insulin resistance in vitro and in vivo (Klover, 2005) via impaired insulin signaling in hepatocytes (Senn, 2002). Additionally, elevated IL-6 correlates with severity of inflammation, as indicated by the highly sensitive C-reactive protein serum concentration (Pickup, 2000). Lastly, a human polymorphism in the IL6 gene, which causes decreased IL-6 levels, is associated with increased insulin sensitivity
(Fernández-Real, 2000). IL-6 levels have been shown to decrease in parallel with weight loss (Kopp et al., 2003) and improvement of insulin resistance has been observed after bariatric surgery (Angulo, 2006). Due to the numerous variations in exercise protocols, sample collection times and methodologies, more research is needed, in particular, with disease populations as to the effects of exercise on IL-6.

**Glucose Metabolism**

Glucose is central to all of metabolism. It is the universal fuel and the source of carbon for the synthesis of most other compounds. Historically, glucose metabolism has been used to measure whole body insulin sensitivity, however, different organs and metabolic pathways respond to insulin diversely (Belfiore, 1998). The hyperinsulinemic-euglycemic clamp technique (DeFronzo, & Andres, 1979) remains the gold standard for quantitative measurement of whole body insulin resistance, however, it is time and labor intensive for subjects and staff, therefore, insulin sensitivity was assessed with an insulin sensitivity index (Matsuda M, 1999) derived from the OGTT. Additionally, the homeostasis model assessment of insulin resistance (HOMA-R) was used as it has been validated in large epidemiological studies in a wide range of glucose tolerant patients (normal glucose tolerant, impaired glucose tolerant, T2DM patients) and is an appropriate index to reflect hepatic insulin resistance (Matthews et al., 1985).

Many tissues, including the liver, manifest insulin resistance. Although insulin resistance may develop simultaneously in multiple organs, the severity of insulin resistance can vary considerably between tissues. Interventions that improve insulin resistance are organ specific (exercise training for peripheral insulin resistance,
metformin for hepatic insulin resistance, weight reduction for both), therefore, ideally it is important to quantitate which organs are resistant to insulin, as well as the magnitude of insulin resistance in each organ.

Glucose tolerance is determined by the balance between insulin secretion and insulin action (Goodyear, 1998). In the fasted or postabsorptive state, most of the total glucose disposal occurs in insulin-independent tissues (50% brain, 25% liver and gastrointestinal tissues) (McCullough, 2002). Glucose utilization is precisely matched by glucose release from the liver and approximates 2.0 mg/kg/min (Bugianesi, Marchesini, 2005). Normal glucose homeostasis is dependent on three highly regulated processes: 1) insulin secretion by pancreatic β cells, 2) stimulation of glucose uptake by liver, gut and peripheral tissues, and 3) suppression of hepatic glucose output or production (EGP) (Bugianesi, Marchesini, 2005; McCullough, 2002). Skeletal muscle is the primary site for glucose disposal via insulin dependent pathways, accounting for approximately 75% of whole-body insulin-stimulated glucose uptake (Henriksen, 2002; Houmard et al., 1993).

In skeletal muscle and adipose tissue, an increase in insulin promotes glucose uptake by activating a complex cascade of signaling events. In brief, binding of insulin to the insulin receptor leads to downstream tyrosine phosphorylation of protein substrates that then engage and activate PI3K. This leads to downstream signaling through PKB/Akt and PKC-λ/ζ, which results in GLUT4 translocation from its intracellular pool to the plasma membrane and glucose transport into the cell (Boden, 2006; Donnelly, 2005; Feldstein, 2004). In adipose tissue, insulin inhibits the release of fatty acids from
adipocytes by decreasing the activity of hormone-sensitive lipase and adipose triglyceride lipase (ATGL) is therefore antilipolytic. In the liver, insulin decreases the release of glucose from the liver by inhibiting hepatic glycogenolysis and the expression of key gluconeogenic enzymes (Boden, 2006).

**Fatty Acid Metabolism**

Glucose that is not oxidized or stored as glycogen is metabolized to acetyl CoA, which then enters the lipogenic pathway. Acetyl CoA is catalyzed to form malonyl CoA, which in turn inhibits carnitine palmitoyltransferase 1 (CPT-1), the enzyme responsible for long chain fatty acid transport into the mitochondria (Boden, 2006; Donnelly, 2005). The net effect is that malonyl CoA blocks fatty acid entry into the mitochondria and β-oxidation. These events increase the accumulation of long chain fatty acids and their derivatives (triglycerides and ceramides) contributing to apoptosis and lipotoxicity (Boden, 2006; Donnelly, 2005; Feldstein, 2004).

Under normal hepatic fatty acid metabolism, long chain FFA dissociate from albumin in the space of Disse and cross the liver cell plasma membrane. In the cell, FFA are bound to liver fatty acid binding protein (L-FABP) (Zimmerman, 2002). Under conditions of normal flux, FFA largely undergo oxidation in the mitochondria or are esterified to triglyceride, phospholipids and cholesteryl esters. Under increased FFA flux into the hepatocyte, or impairment of mitochondrial β-oxidation, FFA are directed towards triglyceride synthesis. When the hepatocyte’s capacity to assemble and/or export triglyceride-rich VLDLs is exceeded, hepatocellular steatosis results. This increases the substrate for hepatocellular lipid peroxidation, and may also produce direct cytotoxic
effects. Increased FFA flux activates peroxisome proliferator activated receptor \( \alpha \) (PPAR\( \alpha \)), a nuclear receptor that increases the transcription of fatty acid response genes (L-FABP, CPT-1, CYP4A1, which then initiates the microsomal \( \omega \)-oxidation of FFA and production of dicarboxylic fatty acids (DCFA) (McCullough, & Edmison, 2007). DCFA are preferentially catabolized in the peroxisomes; their presence in urine and the circulation is an indication fatty acid overload.

There is an increased rate of hepatic mitochondrial free fatty acid \( \beta \)-oxidation in obesity, which increases the delivery of electrons to the respiratory chain. Mitochondria are the main site of ROS formation in the cell and ROS formation is increased whenever the electron flow through the respiratory chain is hindered (Evans et al., 2002). ROS production generates lipid peroxidation products that react with mitochondrial DNA and proteins to partially block the flow of electrons in the respiratory chain (McCullough & Edmison, 2007). This imbalance in electron flow may cause over reduction of respiratory chain components, which react with oxygen to generate more ROS and perpetuate this vicious cycle (Boden, 2006; Donnelly, 2005; Feldstein, 2004).

In healthy individuals after an overnight fast, the rate of EGP is proportionate to the requirements of the brain. Basal EGP is variable, and mainly dependent upon the amount of lean body mass and the degree of peripheral insulin utilization (Goodyear, 1998). Insulin released into the portal vein after glucose or meal ingestion suppresses EGP and failure of the liver to recognize this signal results in hepatic insulin resistance. Hyperinsulinemia is a potent inhibitor of EGP and so, normal EGP in the presence of
fasting hyperinsulinemia indicates postabsorptive hepatic insulin resistance. Patients with T2DM have a ~40% increase in EGP which explains the observed excessive postprandial hyperglycemia. Marchesini et al demonstrated less suppression of EGP with these patients after insulin administration (Marchesini, 2001). The ability of insulin to suppress serum FFAs is reduced in normal subjects with as little as 10% liver fat (Seppala-Lindroos A, 2002). These findings have also been confirmed during clamp studies in T2DM patients; higher plasma FFA values were observed as the level of insulin resistance increased (Kelley, McKolanis, Hegazi, Kuller, Kalhan, 2003).

Skeletal muscle data from obese human studies found an abnormal metabolic phenotype in that, despite chronic exposure to high fasting lipid levels, glucose was the preferred substrate for ATP production (D. E. Kelley, Goodpaster, Wing, & Simoneau, 1999). This resulted in increased intramyocellular lipid accumulation and ultimately contributed to defective insulin signaling and the development of peripheral insulin resistance (Goodpaster, Watkinsa & Kelley, 2000). More recently, Reyna et al reported increased expression and protein content of TLR4 in skeletal muscle of obese and T2DM humans which was associated with insulin resistance (Reyna, 2008). Shi et al (Shi et al., 2006) have shown that TLR4 signaling is important to fatty acid induced skeletal muscle insulin resistance. This study also demonstrated that TLR4 is activated in adipocytes and macrophages and that the capacity of the macrophages is blunted in the absence of TLR4. Their data suggest that TLR4 is a molecular link between lipids and inflammation and that the innate immune system participates in the regulation of energy balance and insulin resistance. Additionally, Dasu et al demonstrated that (Dasu, 2010) T2DM subjects had
significantly increased TLR2, TLR4 mRNA, and protein in monocytes compared with control subjects (P < 0.05). Increased TLR2 and TLR4 expression correlated with BMI, homeostasis model assessment–insulin resistance (HOMA-IR), glucose, A1C, and free fatty acid (FFA). In this comprehensive study, they make the novel observation that TLR2 and TLR4 expression and their ligands, signaling, and functional activation are increased in recently diagnosed T2DM subjects and contribute to the proinflammatory state.

**Insulin Signal Transduction**

The action of insulin depends on a cascade of events following the interaction of insulin with its receptors. Insulin signaling at the target tissue is vital for normal growth and development and for macronutrient metabolism (Goodyear et al., 1996). Insulin binding to the extracellular α-subunit initiates a cascade of tyrosine phosphorylations in the β-subunit of the insulin receptor on the plasma membrane, allowing insulin receptor substrate (IRS)-1 adaptor protein to bind and undergo tyrosine phosphorylation (Hotamisligil et al., 1996; Shoelson, Herrero, Naaz, & Afia, 2007; Shoelson et al., 2006; Steinberg, 2007; Sykiotis, 2001). IRS1 is the predominant isoform in skeletal muscle, while IRS2 is the predominant isoform in the liver (Steinberg, 2007). The phosphorylation of IRS proteins induces stimulation of phosphatidylinositol-3-kinase (PI3K), which results in downstream activation/phosphorylation of Akt/ protein kinase B (PKB), and ultimately translocation of the glucose transporter 4 (GLUT4) from the intracellular pool to the plasma membrane (Goodyear, Giorgino, Sherman et al., 1995; Hotamisligil et al., 1996) increasing glucose uptake (Goodyear et al., 1992). Activation
of Akt/PKB also results in phosphorylation and inactivation of the enzyme, glycogen synthase kinase-3 (GSK-3) (Hotamisligil et al., 1996). Inhibition of GSK-3 thus activates glycogen synthase and enhances glycogen synthesis (Shulman, 2000). Insulin signaling involves two major pathways: the PI3K pathway and the mitogen activated protein kinase (MAPK) pathway. The metabolic effects of insulin are mediated primarily through the PI3K pathway (Saltiel & Kahn, 2001).

**Insulin Resistance**

Several theories have been postulated for peripheral insulin resistance, but the most probable mechanism is cytokine-induced serine rather than tyrosine phosphorylation of insulin receptor substrate protein 1 (IRS-1) (Steinberg, 2007; Sykiotis, 2001) which is the earliest and most pronounced defect in the insulin-signaling cascade (Goodyear, Giorgino, Sherman et al., 1995; Herman, 2006; Hotamisligil et al., 1996; Paz, 1997). Similarly, an IRS-2 abnormality in the hepatocyte is also a likely mediator of hepatic insulin resistance (Hotamisligil et al., 1996; Shoelson, Herrero, Laura, Naaz, & Afia, 2007; Shoelson et al., 2006; Steinberg, 2007; Sykiotis, 2001).

The accumulation of FFA in the liver, or the FFA esterification product, diacylglycerol, results in hepatic insulin resistance via PKC-induced IRS-2 serine phosphorylation, inhibition of tyrosine phosphorylation and inactivation of PI3K which reduces insulin’s inhibitory effect on gluconeogenesis, and contributes to hyperglycemia (Hotamisligil et al., 1996; Wellen & Hotamisligil, 2003). The following cytokine-regulated serine/threonine kinases have been implicated in the inhibition of IRS1 signaling at various residues: protein kinase C (PKC), Ikappa B kinase-β (IKK-β),
and c-jun terminal amino kinase (JNK) (Steinberg, 2007). Additionally, the suppressor of cytokine signaling (SOCS) proteins directly interact with both the insulin receptor and IRS proteins via distinct mechanisms involving both proteasomal degradation and direct binding to tyrosine residues, which in turn inhibits insulin signaling (Shi et al., 2006). Table 1 suggests the most likely progression of insulin resistance (Bugianesi & Marchesini, 2005). It is now generally accepted, that hepatic steatosis itself will cause hepatic insulin resistance (Kim, Fillmore, Chen, Yu, Morre, Pypaert, et al., 2001; Kim, Ellmerer, Van Citters, German, 2003).

Table 1. Most Likely Progression of Insulin Resistance (Bugianesi & Marchesini, 2005)

<table>
<thead>
<tr>
<th>Stage</th>
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<tr>
<td>Peripheral insulin resistance</td>
</tr>
<tr>
<td>Reduced ability of insulin to suppress lipolysis</td>
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<tr>
<td>Reactive hyperinsulinemia</td>
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<tr>
<td>Influx of free fatty acids to the liver</td>
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<tr>
<td>Increased hepatic triglycerides</td>
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<tr>
<td>Hepatic insulin resistance superimposed on peripheral resistance</td>
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**Insulin-Mediated Glucose Uptake and GLUT4**

Glucose transport, the rate-limiting step in glucose metabolism, is mediated by specific carrier proteins, of which GLUT4 is the major glucose transporter in skeletal muscle and adipose tissue (Goodyear, 1998; Goodyear, Giorgino, Balon, Condorelli, & Smith, 1995; Goodyear et al., 1992; Goodyear et al., 1996). GLUT4 is localized in the
intracellular space in the basal state, but is translocated to the plasma membrane under postprandial conditions, whereas, GLUT1 is primarily localized at the plasma membrane in the liver and responsible for basal glucose uptake (Wang, 2007; Wojtaszewski et al., 2000; Wojtaszewski, Hansen, Kiens, & Richter, 1997; Zierath, 2002). Glucose transport can be activated in skeletal muscle by two separate and distinct pathways: one stimulated by insulin and the second by muscle contractions (Goodyear, 1998; Goodyear et al., 1992). Insulin stimulated glucose uptake is markedly diminished in T2DM (Goodyear, Giorgino, Balon et al., 1995; Goodyear et al., 1992). The degree of fat infiltration and the amount of visceral fat is inversely related to glucose uptake (Kelley, McKolanis, Hegazi, Kuller, Kalhan, 2003). Nuclear magnetic resonance studies have suggested that an increase in intracellular fatty acid metabolites leads to impaired activation of IRS-1 tyrosine phosphorylation, resulting in decreased PI3-kinase activity and decreased glucose transport (Shulman, 2000). Exercise has been shown to increase GLUT4 translocation in controls (Holloszy, 1986) and T2DM patients (Dela, Ploug, Handberg, Petersen, Larsen, Mikines, Galbo, 1994; Henriksen, 2002; Herman, 2006; Houmard et al., 1991; Houmard et al., 1993; Ivy, 1997b).

**Akt Substrate of 160 kDa (AS160)**

Insulin and contraction are potent stimulators of GLUT4 translocation and increase skeletal muscle glucose uptake. A Rab GTP-ase activating protein (GAP), called Akt Substrate of 160 (AS160), is the most distal insulin signaling event that has been linked to GLUT4 glucose transporter translocation to the plasma membrane in response to insulin in adipocytes (Kane, Liu, Asara, Lane, Garner, Lienhard, 2002;
Welsh, 2005). The mechanism involved in the function of AS160 in GLUT4 trafficking is not known, however, the Rab GTPases are known to be key players in many vesicle formation, fusion and trafficking events. AS160 phosphorylation has been shown to be increased by insulin on six sites in vivo, five of which conform to the PKB substrate consensus sequence (RXRXX[pS/pT]) (Kane, Liu, Asara, Lane, Garner, Lienhard, 2002; Welsh et al., 2005). Under basal conditions, AS160 exists primarily in an unphosphorylated state and retains GLUT4 vesicles intracellularly through the activity of its GAP domain. Mutants of AS160, which lack these PKB sites, block GLUT4 exocytosis, but not endocytosis, in response to insulin at a step prior to the docking and fusion of GLUT4 vesicles at the plasma membrane (Kane, Liu, Asara, Lane, Garner, & Lienhard, 2002; Sano, 2003; Zeigerer, McBrayer, & McGraw, 2004). Interestingly, phosphorylation of AS160 has been reported to be impaired in skeletal muscle of T2DM subjects, consistent with the known defect in insulin-stimulated PI3K (phosphoinositide 3-kinase) activation in these subjects (Karlsson et al., 2005). These results strongly implicate AS160 in the mechanism by which insulin promotes GLUT4 translocation to the plasma membrane. Although AS160 is unlikely to be the only route of regulation, other PKB substrates, including the phosphoinositide 3-phosphate 5-kinase, PIKfyve are also most likely involved (Berwick et al., 2004).

AS160 has been found to interact with the cytosolic tail of IRAP (insulin-responsive aminopeptidase), a known component of GLUT4 vesicles, and, indeed, this may play a part in the mechanism by which it associates with GLUT4 vesicles in the basal state (Larance, 2005). Insulin has been reported to promote AS160
dissociation from the GLUT4 vesicles, which would be expected to lead to an increase in the GTP-bound conformation of its target Rab(s) (Eguez, 2005; Larance, 2005). Taken together, the data suggest that, in the basal state, AS160 binds to GLUT4 vesicles, negatively regulating its target Rab(s), and that, in response to insulin, it is inactivated by PKB phosphorylation, causing it to dissociate from the GLUT4 vesicles, resulting in the activation of the target Rab(s) that is necessary for the translocation step. At present, the target Rab for AS160 is not known.

**Oxidative Stress, Mitochondrial Dysfunction and ROS Production**

Oxidative stress and metabolic inflammation upregulate the expression pro-inflammatory cytokines, including TNF-α, monocyte chemoattractant protein-1 (MCP-1) and IL-6, as well as activating stress-sensitive kinases, such as c-Jun N-terminal kinase (JNK), phosphokinase C isoforms, mitogen-activated protein kinase and inhibitor of kappa B kinase. The JNK pathway (specifically JNK-1) appears to be a regulator that triggers the oxidative-inflammation cascade that, if chronically left unchecked, can cause abnormal glucose metabolism and insulin resistance (Lamb & Goldstein, 2008). The series of events triggered by the interaction between metabolic inflammation and oxidative stress constitutes an ‘oxidative inflammatory cascade’, a delicate balance driven by mediators of the immune and metabolic systems, maintained through a positive feedback loop. Interventions aimed at modulating this oxidative-inflammatory cascade may improve glucose metabolism and insulin resistance thereby slowing the development and progression to T2DM.
There is significant evidence that hyperglycemia results in the generation of reactive oxygen species (ROS). In fact, diabetes is typically accompanied by increased production of free radicals and/or impaired antioxidant defenses, indicating a central contribution for ROS in the onset, progression, and pathological consequences of diabetes (Baynes, 1996; Evans et al., 2002). Abdul-Ghani and DeFronzo have provided evidence that implicates defects in mitochondrial oxidative phosphorylation to insulin resistance. They imply that insulin resistance causes an increase in FFA into the liver, a subsequent increase in mitochondrial FFA oxidation and oxidative stress which damages mitochondrial DNA and eventually leads to mitochondrial DNA depletion (Abdul-Ghani, 2008).

Increased FFAs provide substrate for a number of pathways, such as microsomal cytochrome P450 lipoxygenases, and peroxisomal and mitochondrial β oxidation (McCullough AJ Edmison J, 2007). Mitochondrial β-oxidation is thought to account for the majority of FFA oxidation and free radical/ROS production. ROS can be produced by three mechanisms: 1) lipid peroxidation resulting in hepatocyte death, release of byproducts, and activation of hepatic stellate cells and NFκB, 2) expression of cytokines (TNF-α and IL-6), and 3) expression of chemokines and inflammatory enzymes. Elevated levels of TNF-α have been shown to exacerbate the insulin resistant state (Saghizadeh, Ong, Garvey, Henry, & Kern, 1996) by increasing oxidative stress, apoptosis, and perpetuating the inflammatory state by activating NFκB. NFκB can amplify further TNF-α production, thus setting up a vicious cycle of hepatic
inflammation. TNF-\( \alpha \) itself has been shown to impair insulin signaling directly in addition to decreasing the expression GLUT4 (Goodyear, 1998; Goodyear et al., 1996; Herman, 2006; Hood, 2001). Lastly, 3) induction of the Fas ligand (a TNF-\( \alpha \) transmembrane protein important in immunoregulation) can produce ROS (Saghizadeh et al., 1996). In the absence of an appropriate compensatory antioxidant response (decreased glutathione peroxidases and thioredoxin reductases), imbalance occurs leading to the activation of stress sensitive signaling pathways such as NF\( \kappa \)B (Brownlee, 2001; Evans et al., 2002; van den Berg, 2001).

Increased fat storage within hepatocytes can also lead to excess ROS generation. Insulin directly inhibits liver gluconeogenesis and glycogenolysis (Michael, 2000), or indirectly via its effect on substrate availability. Insulin can also influence glucose metabolism indirectly by changes in FFA generated from visceral fat, the so called ‘single gateway’ hypothesis (Bergman, 1997). Because visceral fat is less sensitive to insulin than subcutaneous fat, postprandially there is minimal suppression of lipolysis. The resulting adipocyte-derived FFA flux into the liver from the portal vein can stimulate glucose production, thus providing a signal for both insulin action and insulin resistance in the liver.

Increased reactive oxygen species (ROS) production in the mitochondria reportedly results from increases in circulating FFA and hyperglycemia which induces electron leakage from the proton gradient across the inner mitochondrial membrane. When the gradient exceeds a threshold, complex III electron transfer is blocked and
superoxide formation results along with (Brownlee, 2001) activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Hyperglycemia results in simultaneous decreases in nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (Brownlee, 2001; Evans et al., 2002; Rolo & Palmeira, 2006). The loss of antioxidant reducing equivalents results in increased vulnerability to ROS associated oxidative stress. Hyperglycemia induced overproduction of superoxide significantly inhibits glucose-6-phosphate dehydrogenase (Nishikawa, 2000a), the rate limiting enzyme of the pentose phosphate pathway that is required for providing reducing equivalents to the antioxidant defense system. The product, NADPH, is the cell’s principal reductant and is required for providing reducing equivalents to the glutathione peroxidase-glutathione reductase system (Brownlee, 2001; Nishikawa, 2000a).

The hexosamine pathway is an additional pathway of glucose metabolism that may mediate some of the effects of glucose toxicity. Under usual metabolic conditions, 2-5% of glucose entering cells is directed into the hexosamine pathway (Brownlee, 2001; Evans et al., 2002; Rolo & Palmeira, 2006), however, during hyperglycemia much of the excess glucose is shunted into the hexosamine pathway. It has been suggested that hyperglycemia-induced overproduction of superoxide is the trigger that drives each of these pathways (Baynes, 1996; Brownlee, 2001; Choi, Benzie, Ma, Strain, & Hannigan, 2008; Evans et al., 2002; Gonzalez et al., 2006b; Nishikawa, 2000a; Oghara, 2004; Rolo & Palmeira, 2006; Scott & King, 2004).

Paradoxically, exercise also potently activates ROS production and downstream NF-κB signaling in skeletal muscle (Powers, 1999). As an adaptive response to training,
antioxidant enzymes (superoxide dismutase and glutathione peroxidase) are upregulated in response to chronic exercise and are believed to be fiber specific and greater in highly oxidative muscles (Ji, 1999). Although aerobic exercise training is known to decrease insulin resistance and increase fatty acid oxidation, the current body of literature is lacking with respect to the immunoregulation of TLR2 and TLR4 after a short term exercise intervention in the obese population.

**Hyperglycemia and Oxidative Stress**

There are few published studies that have monitored changes in markers of oxidative stress during acute hyperglycemia induced by oral glucose loading, and different labs have used different markers and methodologies. However, treatment of diabetic subjects with antioxidants or aerobic exercise training is reported to be associated with lowered oxidative stress mediated by improved glycemic control (Scott & King, 2004; West, 2000). Sampson et al (Sampson, Gopaul, Davies, Hughes, & Carrier, 2002) observed the acute effects of hyperglycemia (OGTT) on oxidative stress in T2DM patients using plasma F2-isoprostanes as a marker of lipid peroxidation. F2-isoprostanes are considered to be a global marker of oxidative stress produced by the peroxidation of arachidonic acid (Milne, Sanchez, Musiek, & Morrow, 2007) F2-isoprostanes can increase in urine and plasma due to increased oxidation, increased release of membrane-bound oxidized phospholipids, or a combination of both (Milne et al., 2007). The mean (SD) plasma glucose concentration at baseline was 10.7 mg/dl, increased to 19.9 mg/dl at 60 min, and peaked at 21.1 mg/dl at 90 min post glucose. F2-isoprostanes increased significantly ($p < 0.01$) at 90 min. and were correlated inversely with the
90-min plasma total antioxidant status \( r = -0.498, p = 0.025 \), however, no decrease in plasma antioxidant status or the ratio of reduced (GSH) to oxidized (GSSG) glutathione in lymphocytes at 90 min was seen compared to baseline levels. GSH is an endogenous antioxidant that is important in intracellular redox balance, and a decrease in the ratio of (GSH) reduced to oxidized (GSSG) forms of glutathione reflects increased oxidative stress or decreased recycling of GSH from GSSG (Norgren, 2005). In a subsequent study by Sampson et al (Sampson et al., 2002), they found that plasma 8 epi-PGF\(_{2\alpha}\) increased during OGTT induced acute hyperglycemia in T2DM patients, establishing direct evidence of free radical mediated oxidative damage. Gonzalez et al (Gonzalez et al., 2006b) reported that MNCs exhibit increased ROS production in response to acute hyperglycemia, which, in turn, activates NF\(\kappa\)B (Gonzalez, Rote, Minium, & Kirwan, 2006a).

**Macrophages/Monocytes**

Monocytes and macrophages are antigen presenting phagocytes derived from a premonocyte precursor in bone marrow. MNCs circulate in the blood and continuously enter tissue to localize and differentiate into macrophages during injury, inflammation and infection (Woods, 2006). MNCs are critically involved in the early stages of the innate immune response primarily via phagocytosis of microorganisms but are also involved in the later adaptive response via cytokine production and antigen presentation to T cells (Mackinnon, 1999b). The MNC/macrophage kills ingested microorganisms by releasing proteases from lysosomes and by generating molecules such as oxygen radicals and nitric oxide that are toxic to microorganisms (Woods, 2006). There are two types of
monocytes in human blood: 1) classical monocytes, characterized by high-level expression of the CD14 cell surface receptor (CD14++ monocytes) and 2) non-classical, pro-inflammatory monocytes with low-level expression of CD14 and additional co-expression of the CD16 receptor (CD14+CD16+ monocytes). The CD14+CD16+ monocytes are more mature and develop from the CD14++ monocytes. Cytokine production in response to TLR ligation is one of the prominent features of monocytes and macrophages (Ziegler-Heitbrock, 2007).

Resident tissue macrophages can be locally activated during an inflammatory response or they can be recruited by blood chemokines to the host tissue. Activation status and microenvironment determines the macrophage response. Macrophages can be classically activated as with LPS exposure or alternatively activated by exposure to cytokines. Once classically activated, they can produce IL-1, IL-6, and TNF-α (Mackinnon, 1999a; Mosser, 2003). Conversely, alternative activation produces substances such as IL-10 and IL1ra, transforming growth factor beta (TGFβ) and vascular endothelial growth factor that tend to resolve inflammation and promote wound healing and repair (Woods, Vieira, Keylock, 2006).

Recently, it has been shown that peripheral blood mononuclear cells (MNC) migrate into WAT and activate adipocyte TNF-α production in obese humans (Weisberg, 2003) and that the major source of adipose tissue TNF-α in the obese is from MNC-derived macrophages. The number of macrophages present in adipose tissue is directly correlated with both adiposity and adipocyte size (Frantuzzi, 2005). These
macrophages have been shown to significantly contribute to the chronic inflammatory obese state (Bouloumie, 2005) as demonstrated by increased MNC-derived NFκB (Gonzalez et al., 2006a; Gonzalez, Rote, Minium, O'Leary, & Kirwan, 2007; Weisberg, 2003) and a concomitant increase in the expression of NFκB-modulated genes such as TNF-α (Diehl et al., 2005; Fantuzzi, 2005; Gonzalez, Minium et al., 2006; Hotamisligil & Spiegelman, 1994; Manigrasso et al., 2005) and IL-6 (Akira, 1993; Cohen, 1996). These cytokines can directly inhibit insulin signaling, alter glucose transport proteins in adipocytes and therefore exacerbate insulin resistance (Crespo, 2001; Diehl et al., 2005; Hotamisligil et al., 1994; Hotamisligil et al., 1996; Hotamisligil & Spiegelman, 1994). Christiansen et al. (Christiansen, 2005) established that adipose tissue secretes monocyte chemoattractant protein 1 (MCP-1). Troseid et al. (Troseid, 2004) confirmed that MCP-1 is decreased after 12 weeks of supervised combined endurance/strength training (treadmill walking/jogging, 80% MHR, 3 x week, 45–60 min, and 15–20 reps, 2-3 sets, large muscle groups, 3 x week) in 20–75 year old males with the metabolic syndrome. These results suggest that exercise decreases monocyte migration, macrophage accumulation and thus subsequent inflammation.

Exercise and Inflammation

Recent epidemiological studies indicate that individuals who maintain a physically active lifestyle are less likely to develop age related diseases such as cardiovascular disease, obesity and T2DM (Fleschner, 2005). Similarly, low cardiorespiratory fitness and physical inactivity have been found to be independent
predictors of all cause mortality in men with low fitness levels who were normal weight, overweight, class I obese (Church, 2005) or that have T2DM compared to normal weight men who were physically fit (Wei, 2000). Other studies have shown that physically active individuals are less susceptible than sedentary individuals to viral and bacterial infections (Febbraio, 2007; Gleeson, 2000, 2007), suggesting that exercise improves overall immune function. Unfortunately, due to the abundant variability in exercise protocols, human genetic variability and the numerous systems affected by exercise, elucidating the exact mechanism by which exercise promotes health benefits has proven to be a challenge in human studies. There is evidence to support a variety of mechanisms, including an increase in innate immune function (Woods, Keylock, & Keylock, 2002), a decrease in chronic inflammation (Esposito, DiPalo, et al, 2003; Fernandez-Real, 2003; Gleeson, 2000, 2007; Goldhammer, 2005; Petersen & Pedersen, 2005) an increase in stress resistance (Fleschner, 2005), a decrease in adiposity (O'Leary et al., 2006) and other systemic changes such as improved lipid profiles (Kirwan, 2007; Schenk & Horowitz, 2007), and decreased insulin resistance (DiPietro, Dziura, Yeckel, & Neufer, 2006; Esposito, DiPalo, et al, 2003; Fletcher, 1992; Goodpaster, Kelley, 2003; Kirwan et al., 1990; Kirwan et al., 2000; Kirwan, Kohrt et al., 2002; Kirwan et al., 1990; Kirwan & Ming, 2002; Weiss et al., 2006; Wojtaszewski et al., 2000). This section will focus on the potential mechanisms involved in the effects of exercise on the innate immune system and its role in regulating the inflammatory process.

The innate immune system provides the first line of defense in response to invading pathogens. When tightly regulated, the inflammatory response is beneficial but
when chronically activated it can lead to tissue destruction. The inflammatory response may be acute, or it can be low grade and chronic, as seen in chronic infections and autoimmune diseases. Chronic low level inflammation is defined as two to fourfold elevation in circulating levels of proinflammatory and anti-inflammatory cytokines and acute phase proteins (Bruunsgaard, 2005), as well as minor increases in neutrophil and natural killer cell counts.

There is convincing evidence from both human and animal models indicating that moderate exercise can reduce acute inflammation. Zielinski, et al (Zielinski, 2004) demonstrated that daily strenuous exercise decreased macrophage and neutrophil numbers. Starkie, et al (Starkie, 2003), has also shown that exercise may modulate the inflammatory response. They administered an LPS challenge to young males under two conditions; after exhaustive exercise or after administration of IL-6. Both the exercise and IL-6 groups had significantly decreased plasma TNF-α compared to the controls suggesting that exercise induced IL-6 can block TNF-α production.

Both epidemiologic and longitudinal data suggest that increasing physical activity is an effective means of reducing chronic low level inflammation in conditions such as obesity and metabolic syndrome. Additionally, the presence of inflammatory indices (Duncan, 2003; Schmidt, 1999) at baseline were shown to predict the development of T2DM (Barzilay, 2001; Pradhan, 2001). Exercise and diet are commonly prescribed treatments to improve insulin action in skeletal muscle in obese, insulin-resistant individuals and T2DM patients. A single bout of exercise increases skeletal muscle glucose uptake via an insulin-independent mechanism that bypasses the typical insulin
signaling defects associated with these conditions (DeFronzo, Kraemer, 1987; Holloszy, 1986). However, this insulin sensitizing effect is transient and disappears after 48 hours (Hawley, 2007). In contrast, repeated exercise bouts have resulted in persistent increases in insulin action in skeletal muscle from obese and insulin resistant individuals (DeFronzo, Kraemer, 1987) The molecular mechanism(s) for the enhanced glucose uptake with exercise training have been attributed to the increased expression and/or activity of key signaling proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle. Exercise-induced changes in inflammatory markers could also contribute to the improvement of insulin sensitivity after physical training. The role of exercise in reducing the risk of insulin resistance and T2DM has also been supported by several aging studies. Rodgers et al (Rodgers, 1988) found reductions in both areas under the curve (36% glucose, 32% insulin) after 7 days of intense exercise in 10 men with abnormal glucose tolerance. Similarly, many others have confirmed these results (Cononie, 1994; Houmard, Cox, MacLean, & Barakat, 2000; Ivy, 1997a; Kasapis & Thompson, 2005; Kirwan, Kohrt et al., 2002; Kirwan et al., 1990; Pishon, 2003; Ryan, 2000). Cononie et al also demonstrated reductions in fasting plasma insulin levels in response to an oral glucose challenge after 7 consecutive days of exercise (50 minutes, 70% VO_{2max}) in 60-80 year old men and women, providing evidence that regularly performed, vigorous exercise can be effective in decreasing insulin resistance and improving glucose tolerance within 7 days (Cononie, 1994). Arciero et al (Arciero, 1999) studied the effects of a 10-day low-calorie diet or exercise training on insulin secretion and action in middle aged obese men and women with abnormal glucose
tolerance. They found a 56% increase in glucose uptake after exercise training compared to a 19% increase in the low calorie diet group suggesting that short-term exercise is more effective than diet in enhancing insulin action in individuals with abnormal glucose tolerance. You et al found similar results in obese postmenopausal women; exercise combined with diet, but not diet alone was associated with significant reductions in IL-6, TNF-α and CRP. Houmard et al found that 7 days of aerobic exercise training (1 h/day, 75% VO$_{2\text{max}}$) was associated with increased insulin sensitivity, enhanced insulin-stimulated PI 3-kinase activity and substantial improvements (2.8 fold higher) in muscle GLUT4 protein content (Houmard et al., 1993). A subsequent 12 month aerobic exercise study (45-90 min at ~90-90% MHR, 4-5 days/wk), by Kirwan et al provided further evidence that insulin stimulated IRS-1 associated PI-3 kinase activity is greater in skeletal muscle from trained subjects compared with untrained subjects (Kirwan et al., 2000) and that PI3-kinase activation was correlated with both glucose uptake and VO$_{2\text{max}}$. Collectively, these results are consistent with the hypothesis that regular exercise training leads to improvements in glucose uptake through enhanced insulin signaling at the level of PI3-kinase.

O’Leary et al examined the effects of chronic exercise training (12 weeks, 5 days/wk, 60 min/day, treadmill/cycle ergometry at 85% HR$_{\text{max}}$) on glucose metabolism, abdominal adiposity, and adipocytokines in the obese elderly. Interestingly, visceral fat (VF), subcutaneous fat, and total abdominal fat were all reduced through training;
however, TNF-α remained unchanged (O'Leary et al., 2007). Insulin resistance was reversed by exercise (P < 0.01) and correlated with changes in VF (r = 0.66, P < 0.01).

Numerous studies have evaluated the effects of exercise on C reactive protein (CRP), an inflammatory marker produced by the liver as part of the acute phase response (Kasapis & Thompson, 2005). In all of these studies, the average CRP in the more physically fit individuals was significantly lower than in the less active or sedentary groups (Kasapis & Thompson, 2005). Three recent studies further demonstrate that physical fitness, assessed by maximum oxygen uptake VO_{2max} are inversely associated with CRP in middle aged healthy adults. Jankord and Jemiolo (Jankford, 2004) found that active older men had significantly lower levels of IL-6 and higher levels of IL-10 than less active older men. This finding suggests the possibility of a dose response, such that greater levels of fitness were associated with higher anti-inflammatory profiles. Pischon et al (Pishon, 2003) also demonstrated an inverse relationship between physical activity and inflammatory markers; although the effects of exercise in this study were also mediated by reductions in adiposity. Both Adamopoulos et al (Adamopoulos s, 2001) and Goldhammer et al (Goldhammer, 2005) found reductions in inflammatory markers in cardiac patients after 12 weeks of intense aerobic exercise training. However, these findings were seen in the absence of significant weight reductions.

**AMP-Activated Protein Kinase (AMPK)**

There is indication that physical exercise may stimulate lipid oxidation and inhibit lipid synthesis in the liver through the activation of the AMPK pathway. AMP-activated protein kinase (AMPK) is a key regulator of energy metabolism during exercise, and so
AMPK is activated by an increase in the AMP/ATP ratio, and AMPK activity is enhanced by physiological processes that induce metabolic stress (decreased ATP production, ischaemia, hypoxia) or increased consumption (exercise). Once activated, AMPK acts to increase ATP-generating cellular events while turning off energy-consuming processes to restore energy balance. In the liver, the activation of AMPK by exercise (Hardie, 2004) or changes in adiponectin levels (Kadowaki et al., 2006) suppress expression of sterol regulatory element-binding protein 1 (SREBP-1) (Zhou, 2001), carbohydrate response element binding protein (ChREBP) (Kawaguchi, 2002), and acetyl-CoA carboxylase (ACC), the enzyme responsible for malonyl-CoA production (Hardie, Scott, Pan, & Hudson, 2003), resulting in decreased de novo lipogenesis, inhibition of hepatic gluconeogenesis and increased FFA oxidation. Within skeletal muscle, exercise induced AMPK activation enhances glucose uptake via direct non-insulin-dependent GLUT-4 translocation, and increases pyruvate oxidation, while in adipose tissue, hormone-sensitive lipase activation is suppressed, resulting in decreased lipolysis (Towler & Hardie, 2007).

**Toll Like Receptor 2 (TLR2)**

Other players involved in immunity are the toll like receptors. Toll like receptors recognize pathogen-derived molecules and induce down stream activation of inflammatory pathways. Activation of these receptors on cells of the innate immune system leads to the production of cytokines, chemokines, and the up-regulation of cell surface molecules. TLRs are expressed in multiple tissues. However, the predominant site of TLR expression is on cells of the innate immune system, especially monocytes.
TLR2 recognizes and signals bacterial lipoproteins, peptidoglycans, and lipoteichoic acid from gram-positive bacterial cell walls. Ligand induced activation of TLR2 and TLR4 leads to common downstream activation of TRAF6 via the adapter molecule, MyD88 (Akira, 1993). This cascade of events culminates in activation of NFκB and induction of TNF-α and other proinflammatory cytokines. Dasu (Dasu et al., 2008) suggested that high glucose increased TLR2 and TLR4 expression via PKC-α and PKC-δ, respectively, by stimulating NADPH oxidase in human monocytes. Devaraj previously reported that hyperglycemia induced monocytic release of IL-6 via induction of PKC-α and PKC-β (Devaraj, 2008).

**Toll Like Receptor 4 (TLR4)**

TLR4 has also been implicated in the progression of subclinical inflammation in various diseases and is expressed on virtually all human cells including peripheral blood mononuclear cells (PBMNC). TLR4 is the signaling receptor for the endotoxin lipopolysaccharide (LPS); the major component of the outer membrane of gram-negative bacteria. Most TLR4 cell surface expression, occurs at low levels on monocytes and at even lower levels on other cell types (Palsson-McDermott & O'Neill, 2004; Schumann RR, 1990). In macrophages, lipid A activation of TLR4 was found to trigger secretion of proinflammatory mediators, such as TNF-α and IL1-β, and also to activate the production of co-stimulatory molecules required for the adaptive immune response, whereas, in mononuclear and endothelial cells, lipid A stimulates tissue factor production. Palsson-McDermott *et al* established that LPS toxicity is associated with the
lipid A component while immunogenicity is associated with the polysaccharide components (Palsson-McDermott & O'Neill, 2004). They also observed LPS to be biologically effective at very low concentrations (lower pg/ml range). Thus, LPS mediates a variety of proinflammatory and toxic functions, which are desirable for clearing local infections, but when overproduced can cause damage.

The TLR4 receptor complex is a potent activator of the downstream transcription factor NFκB (F. Kim et al., 2007) and secretion of pro-inflammatory cytokines and proteins such as TNF-α and inducible NO synthase (iNOS). The key product of TLR signaling in antigen presenting cells is the production of inflammatory cytokines and proteins, which makes them prime targets for recognition by the innate immune system (Palsson-McDermott & O'Neill, 2004) and implicates their role in mediating whole body inflammation and development of chronic disease. While the exact mechanism is not clearly defined at the present time, antibodies to TLR4 have been reported to inhibit the LPS-induced cytokine production by PBMC.

There is evidence to suggest that TLR’s may be involved in the link between sedentary lifestyle, inflammation and disease. Although TLR4 has also been implicated as a mediator of diet-induced obesity and inflammation in peripheral insulin sensitive tissues (F. Kim et al., 2007; J. K. Kim, 2006) the mechanism involved remains unclear.

**TRL4 and Exercise**

Although only a small number of studies have looked at the effects of exercise training on TLR expression, both acute aerobic and chronic resistance exercise have resulted in decreased monocyte cell surface expression of toll like receptors. Lancaster et
was the first to report a decrease in monocyte (CD14+) TLR expression in vivo (Lancaster et al., 2005) after a single bout of prolonged cycling (1.5 hr @ 65% VO\textsubscript{2} max) in the heat (34\textdegree C). Additionally, upregulation of IL-6 by CD14+ monocytes following activation with TLR ligands was found to decrease (P < 0.05) in the post exercise period. Subsequent studies found no significant effect of temperature on CD14+ TLR4 expression. McFarlin et al. found that acute resistance exercise did not alter plasma inflammatory cytokines (IL-6 and TNF-\(\alpha\)) or cell surface expression of CD14+/TLR4 in trained versus untrained (65-85 years old) postmenopausal women (McFarlin, 2004) despite a significant difference in TLR4 expression (124%) between groups. This study did not exercise train their subjects, rather subjects were chosen by predetermined physical activity criteria which could describe differences from previously reported results. Flynn et al observed that after 10 wks of resistance training (3 sets, 9 exercises, 10 repetitions at 80% of the 1-RM); in this same population, TLR4 and CD14 mRNA expression were lower compared to sedentary controls (Flynn, McFarlin, Phillips, Stewart, & Timmerman, 2003). There were no changes noted in plasma samples. Follow-up studies by this group found mixed results, in young versus old sedentary males; physical activity was associated with lower CD14+CD16+ monocyte percentage and LPS-stimulated TNF-\(\alpha\) production, however, no significant exercise training-induced changes were observed in monocyte TLR4 expression (Timmerman, 2008). Interestingly, McFarlin found that training status, not age, influenced TLR4 cell-surface expression and LPS-stimulated inflammatory cytokine production, with active subjects demonstrating lower expression (McFarlin et al., 2006). Stewart et al had subjects
perform 12 weeks (3 days/wk) of combined endurance (20 min) and resistance exercise (eight exercise, two sets), and found significant improvements in CD14+/TLR4 expression and LPS-stimulated IL-6 (Stewart, 2005). No changes were observed in LPS-stimulated TNF-α or TLR2 expression. To date, these are the only published studies in which the TLR4 expression has been examined following a period of exercise training. Because TLR4 activation results in downstream NFκB activation, it is a prime target to determine whether lifestyle modifications, such as moderate aerobic exercise, can modulate inflammatory cytokine profiles.

**LPS Binding Protein and Cluster of Differentiation Group 14 (CD14)**

The first host protein believed to be involved in LPS recognition is LPS-binding protein (LBP) (Schumann et al., 1990). LBP is an acute phase protein, produced in the liver, which circulates in the plasma where it recognizes and forms a high-affinity complex with the lipid A moiety of LPS, as free molecules, fragments, or bound to the outer membrane of intact bacteria (Palsson-McDermott & O'Neill, 2004). LBP appears to aid in LPS docking at the LPS receptor complex by initially binding LPS and then forming a ternary complex with CD14, thus enabling LPS to be transferred to the LPS receptor complex composed of TLR4 and MD-2 (Hailman, 1994; Tobias, 1995). MD-2 is a glycoprotein that acts as an extracellular adaptor protein in the activation of TLR4 by LPS; it is encoded by the human gene Lymphocyte antigen 96 and is essential for LPS signaling to occur (Schromm, 2001; Visintin, Mazzoni, Spitzer, & Segal, 2001). It is unclear as to how all these molecules interact with each other and, particularly, how TLR4 interacts with CD14-LPS to transduce the signal. CD14 is found in two forms:
soluble CD14 (sCD14), which occurs in plasma and assists in transmitting LPS signaling in cells lacking membrane-bound CD14, such as endothelial and epithelial cells, and membrane bound (mCD14) which is attached to the surface of myeloid cells (Palsson-McDermott & O'Neill, 2004). Upon priming with LPS, TLR4 recruits at least 4 different adapter proteins: 1) myeloid differentiation factor 88 (MyD88), MyD88 adaptor-like protein TIRAP, TIR-containing adapter molecule TRIF, and TRIR-related adapter molecule TRAM), however, the specificity of recruitment is not clear (Fan, 2003; Palsson-McDermott & O'Neill, 2004). According to Palsson et al, the TLR4-mediated response to LPS can be divided into two categories: an early MyD88-dependent response and a delayed MyD88-independent response (Palsson-McDermott & O'Neill, 2004).

**MyD88-Dependent Signaling**

The current working model for the early MyD88-mediated signaling pathway in response to LPS can be found in a thorough review by Palsson-McDermott and O’Neill (2004). In brief, circulating LBP recognizes LPS in the plasma, brings LPS to CD14 on the cell membrane and forms the tertiary complex (LPS: LBP:CD14). The LPS: LBP:CD14 complex then transfers LPS to the TLR4 accessory protein MD2 thereby assisting the loading of LPS onto the LPS receptor complex. This final complex is composed of dimerized TLR4 receptors and two molecules of the extracellular adapter MD-2, and ultimately induces activation and homodimerization of TLR4, recruiting MyD88 and Mal to the receptor complex. Subsequent signals activated by TLR4 can be subdivided into those that occur early and are dependent on MyD88 (and Mal) opposed to those which occur later and are independent of MyD88, and use the adapters TRIF and TRAM.
LPS signaling was observed to lead to early NF-κB activation (Flynn et al., 2003; McFarlin et al., 2006; Palsson-McDermott & O'Neill, 2004). TRL4 is known to activate the NFκB pathway and all three MAPK pathways: ERK, JNK/SAPK and p38 (Palsson-McDermott & O'Neill, 2004) which are shared with the IL-1 pathway. More importantly, it is the activation of the NF-κB pathway that links innate and adaptive immune response by production of inflammatory cytokines (IL-1, IL-6, TNF-α), chemokines and induction of costimulatory molecules such as CD80, CD86, CD14 + and CD40 (Flynn et al., 2003; McFarlin et al., 2006; Palsson-McDermott & O'Neill, 2004). Palsson-McDermott confirmed the activation of NF-κB to be initiated by the assembly of a protein complex known as the signalosome, made up of inhibitory binding protein κB kinase (Al-Khalili et al.) α and IKKβ, together with a scaffolding protein named IKKγ, also known as NEMO. Subsequent phosphorylation of a set of inhibitory binding proteins κB (IκB) results in their ubiquitination and degradation, releasing NFκB to translocate into the nucleus (Palsson-McDermott & O'Neill, 2004).

**Lifestyle Modification**

Sedentary lifestyle significantly and adversely impacts metabolic profiles (Haque, 2002). The combination of diet and exercise has a synergistic beneficial effect on metabolic profiles, and therefore, the primary lifestyle modification strategies for obese patients are weight loss and physical activity (Angulo, 2002; Day, 2003). Although there are many studies demonstrating the benefits of lifestyle intervention in T2DM, insulin resistance and obesity, there is little information on the impact of short term...
exercise on immunoregulation of TLRs in obese humans (Alam et al., 2004). When exercise was added to a low calorie diet in obese men, insulin levels obtained via an oral glucose tolerance test were approximately 2 fold better than with diet alone (Rice B, 1999). Data from the Diabetes Prevention Program extended these findings, showing that a low-calorie, low-fat diet with moderate physical activity such as brisk walking for 150 min per week reduced the incidence of diabetes by 58%. This reduced incidence of diabetes was greater than that achieved with the insulin sensitizing drug metformin alone (Knowler, 2002).

The intensity of exercise needed to show improvement in metabolic profiles has been studied by several investigators. O’Donovan evaluated the effects of 24 weeks of moderate intensity exercise (cycling 3 x week, 60% VO₂max, 400 kcal expenditure), versus high-intensity exercise (cycling 3 x week at 80% VO₂max, 400 kcal), versus no exercise, on insulin sensitivity, triglycerides and glucose concentration (O’Donovan G, 2005). The overall energy expenditure achieved per workout session appeared to be more important than the intensity of exercise. This is supported by two recent studies performed in obese patients. Daily exercise at 70% VO₂max (80% heart rate maximum) on a treadmill to achieve 700 kcal energy expenditure (~60 min) resulted in an 8% body weight loss and was associated with significant reductions in abdominal obesity, visceral fat, waist circumference and insulin resistance. O’Leary et al also demonstrated that daily aerobic exercise for 50-60 min (starting at 60-65% maximum heart rate and increasing to 80-85% maximum heart rate, ~ 70% VO₂max ) over 4 weeks improved visceral fat content and this correlated with improved glucose metabolism and loss of
insulin resistance (O'Leary et al., 2006). These encouraging results were seen with only 3% weight loss over time. As a result, both moderate and high intensity exercise protocols that result in ~1000 kcal/week energy expenditure have been suggested to be more effective in producing significant metabolic changes than low intensity regimens or diet alone (Fletcher GF, 1992; Gregg et al., 2003; Houmard et al., 2004; Kraus et al., 2002; O'Donovan G, 2005).

Summary

In summary, although there is overwhelming evidence to support the beneficial role of exercise, independent of weight loss, in the management of obesity, gaps remain in our current knowledge in respect to the progression of obesity, highlighting the need to further explore the role of aerobic exercise on immunoregulation and the physiological mechanisms that link inflammation and metabolism. Because of the role of toll-like receptors in innate immunity and the numerous opportunities for disease reduction, additional research is warranted to identify the mechanisms by which short-term aerobic exercise training regulates toll-like receptor expression, oxidative stress and downstream inflammatory cytokine production in diseased populations. The rationale for the proposed research is that, once an effective exercise intervention is developed and once it is known how this intervention affects immunoregulation and metabolism it can then be implemented as a cost effective therapeutic intervention in treatment and ultimately, prevention of obesity and obesity associated diseases.
CHAPTER III

METHODOLOGY

Overview

This research study involved 13 sedentary obese (BMI = 30 - 40 kg/m²) men and women (4 male, 9 female) between the ages of 20 to 70 years old to determine the effects of 7 consecutive days of aerobic exercise training on insulin resistance/sensitivity, cell signaling, oxidative stress and inflammatory cytokine secretion. This study used the oral glucose tolerance test (OGTT) for the measurement of insulin sensitivity. The OGTT provides classification based on response to a glucose load (ADA, 2007). Although it was not my intention to categorize the participants into groups at the start of the study, after reviewing the results of the OGTT’s, it was determined that the sample consisted of three different groups: normal glucose tolerant, impaired glucose tolerant (IGT) and type II diabetes mellitus (T2DM). This finding is not unexpected however, as the sample consisted of obese sedentary adults. Insulin resistance is a major metabolic feature of obesity.

Participant Recruitment and Initial Screening

13 obese/sedentary participants were recruited from the Departments of Gastroenterology/Hepatology at the Cleveland Clinic, or by Epic query and/or a recruitment flyer. Through initial telephone screenings (Appendix A), information regarding, alcohol consumption, physical activity status, current medical conditions, health status and medications were obtained. The study was briefly explained and participants who met the criteria received a complete medical screening and written
consent was obtained. Please see inclusion and exclusion criteria listed below for specific details (Appendix B).

**Medical Screening**

A health and physical examination including a 12 lead resting electrocardiogram (ECG) was performed on all study subjects prior to participation in the study and lasted approximately one hour. Additionally, a complete blood profile including glycosylated hemoglobin, lipid profiles, liver, renal and hematological functions were also performed in the Clinical Research Unit (CRU). An exercise stress test was performed to evaluate cardiovascular function and was reviewed by a cardiologist to determine medical clearance prior to participation in the study. A urine pregnancy test was administered to all females between the ages of 20 - 60 years old. Women that were pregnant were excluded from the study.

**Informed Consent**

All participants provided written, informed consent in accordance with Cleveland Clinic, and Kent State University (KSU) guidelines for the protection of human subjects (Appendix C).

**Body Composition and Physical Fitness Assessment**

Height without shoes was measured to the nearest 1 centimeter. Body weight was measured to the nearest 0.1 kilogram with the subject wearing their underclothing and a hospital gown on the morning of the oral glucose tolerance test (OGTT). Waist circumference was measured midway between the lower rib margin and the iliac crest to
the nearest 0.1 cm to estimate abdominal adiposity. Hip circumference was measured at the widest girth near the buttocks.

**Dual Energy X-Ray Absorptiometry (DEXA)**

Additionally, all participants were scanned by dual energy x-ray absorptiometry (DEXA) to determine percent total body fat and percent truncal fat with the Lunar iDXA model scanner, serial # ME+200042, Version 11.4 (GE Healthcare, Madison, Wisconsin). This is a whole-body scan that takes approximately 10-12 minutes with the participant in the supine position. Percent fat was determined using a dual energy X-ray fan beam that sweeps across the body in a fan-shaped pattern emitting alternating high, 140kVp, low, 100kVp X-rays. Measurements of fat mass, lean tissue and bone mineral content were determined by the different attenuation characteristics associated with the two levels of X-ray in each type of tissue. Truncal fat content was defined as the area between the dome of the diaphragm (cephalad limit) and the top of the greater trochanter (caudal limit).

**Waist to Hip Ratio (WHR)**

Three measurements were performed using a tape measure to assess the circumference of the hips at the widest part of the buttocks, just under the ribs (natural waist) and at the level of the umbilicus to provide both natural and umbilical WHR ratios.

**Physical Fitness Assessment**

Maximal oxygen consumption (VO$_{2\text{max}}$) during an incremental treadmill test was performed as a measure of physical fitness. The VO$_{2\text{max}}$ test was performed via the Jaeger OxyCon Pro/Delta system, Version 4.6, (Hoechberg, Germany) using the Modified Bruce
protocol (Whaley, 2006) The speed was set between 2 and 4 miles/hr, and the incline of
the treadmill increased 2 – 3 % every 3 min until fatigue. Inspired air volumes were
measured from pressure changes detected from the pneumotach. At least two of the
following criteria were required to assure a maximum test: plateau in VO₂, heart rate
(HR) within 10 beats/min of age-predicted maximum, and/or a respiratory exchange ratio
> 1.0 (preferably 1.1). Measurements of VO₂max and maximal heart rate (HRmax) were
then used to determine the appropriate exercise training intensity. Postintervention
VO₂max testing was performed on the morning of day 8 or day 9, following the last day of
exercise training (day 7).

**Basal Metabolism**

Basal substrate metabolism was performed using an indirect calorimeter (Vmax
Oncore, Viasys Sensormedics, Yorba Linda, CA) to assess substrate oxidation and
calculated according to Frayn (Frayn, 1983). The molar ratio of oxygen consumed to
carbon dioxide produced was used to derive a measure of the relative amounts of
substrate that are being oxidized [respiratory quotient (RQ)]. Resting energy expenditure
(REE) was calculated using the modified Weir equation (Weir, 1949):

$$\text{REE (kcal/day)} = [\text{VO}_2 (3.941) + \text{VCO}_2 (1.11)] \times 1.4$$

**Intervention**

All study participants received an OGTT at baseline testing and on day 8 after the
week of exercise and refrained from alcohol and caffeine ingestion and strenuous
exercise in the 24 hrs preceding the test. Measurement of insulin sensitivity is often of
interest in clinical investigation of metabolic diseases because of its key role in these
diseases. The OGTT is widely used for glucose tolerance classification (ADA, 2007) and can provide clinical information such as pancreatic responsiveness and peripheral target cell responsiveness (glucose uptake). Before the baseline OGTT, participants received nutritional counseling and were provided with a sample 250 gram carbohydrate diet menu. Both OGTT’s were performed after an overnight fast of ~12 hours and after indirect calorimetry. All studies were performed pre and post exercise intervention from blood samples collected at fasting and 2 hrs post glucose ingestion. All participants also underwent body composition assessment on the day prior to, or the same day of the OGTT.

**Control Period**

To control for prior diet and physical activity, all participants were counseled by a registered dietitian to consume a metabolically balanced diet (55% carbohydrate, 30% fat, 15% protein) during a 3 day baseline period. Participants were required to refrain from alcohol and caffeine containing products. Baseline food intake was assessed from diet history including multiple replicate 24-hour recalls to estimate usual intake, customary eating pattern, and food frequency questionnaires to verify initial information. Physical activity questionnaires were also assessed to determine physical activity over the last 12 months and last week, respectively.

**Questionnaires**

Study participants were required to complete 5 questionnaires prior to study participation to assess physical activity over the last 12 months and last week, respectively. The questionnaires took approximately 30 minutes to complete and are
described below:

1. Medical History (current and past medical information checklist) (Appendix D)
2. Seven Day Physical Activity Recall Questionnaire (Appendix E)
3. Minnesota Leisure Time Physical Activity Questionnaire (Appendix F)
4. 24 Hour Diet Record Instructions (Appendix G)
5. 24 Hour Participant Daily Intake Form (Appendix H)

Caloric needs were determined using indirect calorimetry and an activity factor of 1.3. Substrate oxidation was determined using indirect calorimetry with the Vmax Encore, Model, E29N (Viasys Sensormedics, Yorba). During the last 24 hours of this period, participants were instructed to ingest 250 grams carbohydrate to ensure adequate glycogen stores prior to the OGTT. During post exercise testing, participants replicated their baseline (Pre Ex) food diary.

**Exercise Training**

All exercise training sessions were supervised daily by myself and/or a post doctoral fellow in our lab and were conducted in the Walker Building at the Cleveland Clinic. Exercise training consisted of walking and running on a treadmill, walking on an elliptical and stationary cycling on a cycle ergometer. Participants trained for 7 consecutive days, 50-60 minutes at 80-85% heart rate maximum (MHR). The participants wore HR monitors (Polar Electro Inc., Woodbury, NY) during each training session to provide visual feedback of their individualized target HR. Each training session included a brief standardized warm-up and cool-down period and a series of stretching exercises. A typical exercise session began with a 5-10 minute warm-up
period, 10 minutes of cycle ergometry, 30-40 minutes of treadmill walking/jogging with an appropriate grade, 10 minutes of cycle ergometry, and concluded with a 5-10 minute cool-down period. Participant compliance with the exercise training was 100% with the exception of one participant who missed one training day due to renal calculi. Dates and times of exercise sessions were documented, as well as exercise intensity, duration, HR and blood pressure (BP), through the use of training logs.

Figure 3 depicts subject flow through the study.

![Study Schematic](image-url)

**Figure 3.** Study Schematic
Analytic Methods

Specific Aim 1

To identify the effects of short-term aerobic exercise training on insulin resistance/sensitivity in obese adults.

Oral glucose tolerance test (OGTT). Insulin sensitivity was assessed by means of the OGTT performed at baseline and post exercise intervention (day 8). Fasting baseline blood samples (8 x 5 ml each) were drawn from the antecubital vein (non-dominant arm) for glucose and insulin determination. A 75 g glucose beverage was ingested over 10 minutes. Blood samples (5 ml each) were drawn for glucose and insulin every 30 min at 30, 60, 90, 120 and 180 minutes after the glucose load with the exception of a larger blood draw (8 x 5 ml each) at the 120 minute time period. The two larger blood draws at fasting and 120 minutes post glucose load were aliquoted out for MNC isolation, ROS and cytokine analysis.

American Diabetes Association classification criteria (Appendix I) was used to determine subject eligibility and glucose tolerance with normal glucose tolerance (NGT) defined as a 2 hour glucose stimulated value < 140 mg/dl, impaired glucose tolerance (IGT) as a 2 hour value of 140-199 mg/dl, and T2DM defined as a value ≥ 200 mg/dl. Upon completion of the test, subjects were fed a high CHO snack. Plasma glucose concentrations and ROS assays were run immediately from the blood samples collected. All participant samples were measured in duplicate in the same assay. Insulin sensitivity was estimated by \( IS_{OGTT} \) by the following formula (Matsuda, 1999):

\[
IS_{OGTT} = \frac{10000 \times GPA \times I_0 \times I_{120}}{(G_0 - 83.3) \times (I_0 + 3.5)}
\]
\[ IS_{OGTT} = \sqrt{(FPG \times FPI) \times (MG \times MI)} \]

where,

FPG = Fasting plasma glucose
FPI = Fasting plasma insulin
MG = Mean glucose (at time points 0, 30, 60, 90, and 120 minutes)
MI = Mean insulin (at time points 0, 30, 60, 90, and 120 minutes)

Plasma glucose concentrations were measured by the oxidase method (YSI, Yellow Springs, OH) while plasma insulin samples were stored at -80\(^\circ\) C until analysis by double-antibody RIA (Linco Research, St. Charles, MO).

**Specific Aim 2**

To identify the effects of short term aerobic exercise training on monocyte-derived oxidative stress (ROS) and TLR2 and TLR4 expression in obese adults.

**Measurement of TLR2, TLR4 by flow cytometry in whole blood.**

Measurement of MNC derived TLR2/TLR4 surface expression was performed on blood collected at fasting and at 2 hr post glucose ingestion. Monoclonal antibodies for CD14-FITC (fluorescein isothiocyanate, Biolegend, San Diego, CA #301804), TLR2-APC (phycoerythrin, E-Bioscience, San Diego, CA, # 12-9922), TLR4-PE (E-Bioscience, San Diego, CA, #12-9917-73) and Pac Blue CD16, Biolegend, San Diego, CA, # 302021) plus 100 \(\mu\)L of sodium heparin treated whole blood aliquots were placed into 12 x 75 mm polystyrene tubes (Becton Dickinson, San Jose, CA, #352054) and
incubated in the dark at room temperature for 20 minutes. Erythrocytes were lysed with 3 mL FACS Lysing Solution (BD San Jose, CA, #349202) and incubated another 20 min at room temperature. 10 min into this incubation, the tubes were inverted to insure that all erythrocytes were exposed to the lysing buffer. The tubes were then centrifuged at 600 g for 5-7 min at room temperature, the supernatant was discarded, the pellet was resuspended in 3 mL PBS, 1% BSA, 0.05% azide, centrifuged again at 600 g for 5-7 min, at room temperature, the supernatant discarded, and followed by 300 μL 0.5% formaldehyde fixation (EMS, methanol free). Quality control checks were also performed each day by analysis of standard sized polystyrene beads (Invitrogen, Carlsbad, CA, #L-14815) to ensure that photomultiplier tube (PMT) settings on the cytometer did not change from the previous data analysis session. Primary gates were established for the monocytes based on forward and side light scatter. Secondary gates were set to identify CD 14+ and CD16 cells within the monocyte band. Mean fluorescence intensity (MFI) for TLR2 and TLR4 was then determined within the CD14+ cell band. All analyses were performed using a FAC Vantage (Becton Dickinson, San Jose, CA) equipped with three lasers: a 488 nm air-cooled argon ion laser, HeCd UV laser (325nm) and HeNe laser (633nm), data acquisition with Cell Quest V.3.3, (Becton Dickinson, San Jose, CA) and analyzed with FlowJo V6.4.1 (Tree Star, Inc., Ashland, OR).

**Measurement of reactive oxygen species (ROS) generation.** MNCs were isolated by Ficoll density gradient centrifugation using PMN Isolation Medium (MATRIX, Hudson, NH, # 10688-00-0) from fasting and at 2 hr post glucose ingestion
OGTT (Appendix H). The cells were then washed twice with Hank’s buffered saline solution, and reconstituted to a concentration of $4 \times 10^5$ cells/mL in Hank’s buffered saline solution. Respiratory burst activity of MNCs was measured by detection of superoxide radical via chemiluminescence. Duplicate cuvettes containing 500 $\mu$L of MNC (400 cells/$\mu$L) were placed into a two-channel lumi-aggregometer. Fifteen $\mu$L of 10 mM luminol followed by 1 $\mu$L of 10 mM formylmethionyl leucine phenylalanine (fMLFP) was added to each cuvette. Chemiluminescence was recorded in mV by computer software. This method was developed by Thusu and Dandona (Thusu K, 1998) and is similar to that published previously by Dr. Kirwan’s lab (Gonzalez et al., 2006b).

In this assay system, measurement of superoxide radical release has been shown to be linearly correlated with the ferricytochrome C method (Tosi, 1992). In addition, superoxide dismutase, catalase, and diphenylene, a specific inhibitor of nicotinamide adenine dinucleotide phosphate oxidase, have been shown to inhibit chemiluminescence in a dose-dependent fashion. The specific inhibitory effect of diphenylene iodonium on nicotinamide adenine dinucleotide phosphate oxidase has been established by Hancock and Jones (Hancock JT, 1987). As previously validated, the variation of ROS generation by MNCs in humans using this method varies by < 8% over a 2 week period.

**Specific Aim 3**

To identify the effects of short-term aerobic exercise training on monocyte-derived inflammatory cytokine expression (TNF-$\alpha$, and IL6) in obese adults.
Measurement of IL-6 and TNF-α. Cells were cultured as described in Appendix I. After a 24 hour incubation, cell supernatants were collected for IL-6 and TNF-α analysis after centrifugation at 10,000 g for 2 min and then stored at -80°C until analysis. In addition, plasma IL-6 (ALPCO Diagnostics, Salem, NH, #45-IL6HU-E01) and TNF-α (Invitrogen, Camarillo, CA, #KHC3012) concentrations were measured by ELISA. All samples were measured in duplicate in the same assay.

Statistics

Sample Size

Participant sample size for the primary outcome variable (insulin sensitivity) was calculated a priori using G*POWER (Faul et al, Kiel University, Germany) with the following input parameters: two tailed paired t-test, α-error probability = 0.05, Power (1- β error probability) = 0.95, and Effect size dz = 2.44. Effect size was estimated using previous data from our laboratory in 14 T2DM subjects that showed 7 days of aerobic exercise training resulted in a ≈ 60% increase (Pre: 1.64 ± 0.32, Post: 2.63 ± 0.67 mg/kg/min; p< 0.0001 in insulin sensitivity. Participant sample size was estimated to be n = 6 with a calculated actual power of 0.98 (critical t = 2.78). The total number of participants was selected based upon how many participants could reasonably be completed within a six month recruitment window. It was determined that a total sample size of 13 participants would be adequate to detect any significant changes in the primary outcome variable as well as subsequent secondary analyses.
Statistical Analysis

For clarity, I want to state that the study was not set up to examine different levels of insulin resistance (NGT, IGT, T2DM) in an obese population. It was proposed to determine the effects of short term exercise on insulin resistance and inflammation in obese adults. However, after reviewing the OGTT results, it was determined that the sample was not homogeneous and consisted of three subgroups (NGT, IGT, T2DM). Additionally, I realize that I did not hypothesize anything related to these subgroups and that the study is not powered to examine these differences, however, from a pure academic interest to examine the origin of any significance, I decided to include analyses from these subgroups.

Statistical analyses were carried out using StatView for Windows, Version 5.0 (SAS Institute, Cary, NC) with statistical significance accepted at P < 0.05. All values are expressed as means ± SEM. **Specific Aim 1:** Mean differences in the primary outcome variable (insulin sensitivity), as well as all descriptive data (i.e. body composition, VO\textsubscript{2max}), were explored using a paired $t$-test for pooled data and with a 3 x (group: NGT, IGT, T2DM) x 2 (time: Pre Ex, Post Ex) mixed design with repeated measures analysis of variance (RM ANOVA) on the second factor (time). **Specific Aim 2:** To identify the effects of short term AEX on TLR2/TLR4 cell surface expression and oxidative stress, mean differences were explored using a paired $t$-test for pooled data, and a 3 x (group: NGT, IGT, T2DM) x 2 (time: Pre Ex, Post Ex ) mixed design with RM ANOVA on the second factor (time). **Specific Aim 3:** To identify the effects of short term AEX on MNC derived inflammatory cytokine secretion (TNF-$\alpha$, IL-6) pooled data
was analyzed using a paired $t$-test and between group TNF-$\alpha$ comparisons were analyzed with the use of a 3 x 2 factor (group x time) mixed and nested design RM ANOVA for time, a 2 x 2 factor (group x time) mixed design RM ANOVA for time was used for between group IL6 comparisons. Both OGTT time points (0 and 120 minutes) were stimulated with LPS pre and post intervention. Bonferonni post hoc tests were applied to significant group x time interactions in order to detect the source of significance. All $t$-tests were 1 tailed.

**Appendices to Other Specific Methods**

Appendix A: Telephone Screening Script  
Appendix B: Inclusion / Exclusion Criteria  
Appendix C: Informed Consent  
Appendix D: Past Medical History Questionnaire  
Appendix E: 7 Day Physical Activity Recall  
Appendix F: Minnesota Leisure Activity Questionnaire  
Appendix G: 24 Hour Diet Record Instructions  
Appendix H: 24 Hour Participant Daily Intake Form  
Appendix I: Monocyte Isolation from Whole Blood and Cell Cultures  
Appendix J: ADA Diabetes Classification Criteria  
Appendix K: Graded Exercise Stress Test/VO$_{2\max}$ Form  
Appendix L: Exercise Training Heart Rates  
Appendix M: Rating of Perceived Exertion  
Appendix N: Additional Statistical Analyses
CHAPTER IV

RESULTS

Baseline demographics and basic physiological measurements are presented in Table 2. A total of 13 obese men and women completed the entire study. For clarity, on some of the data sets, there are 14 participants noted. This additional participant (720-0209) started the study with her measure of insulin sensitivity being the euglycemic hyperinsulinemic clamp and with a different cell culture protocol for outcome measures. She is included in body composition, exercise and calorimetry data sets, as these protocols were not changed. No ROS, TLR2, TLR4, TNF-α, or IL6 data is available for her. Further stratification demonstrated (4 Caucasian males, and 10 females: 4 Hispanic, 1 African American and 5 Caucasians).

Body Composition

**Body Mass Index (BMI):** As Table 2 illustrates, the study participants were obese with pre-treatment body mass index (BMI) values of 34.4 ± 1.1 kg/m². The BMI and weight for each participant remained stable over the 7 day period with the post treatment BMI 34.4 ± 1.1 kg/m². All data are means ± SEM (standard error of measurement).

**Body Fat Percentage:** Percent total body fat was measured by dual energy x-ray absorptiometry (DEXA) pre and post intervention. 1 tailed paired t-tests for pooled data revealed significant differences (P = 0.02) between pre exercise % body fat (46.2 ± 1.4) and post exercise % body fat (45.7 ± 1.4). RM ANOVA for group differences
determined significance (P = 0.04) between groups, specifically between IGT, NGT (P = 0.0225) and IGT, T2DM (P = 0.0002).

**Fat free mass:** Paired *t*-tests revealed FFM was found to significantly increase with 7 days of aerobic exercise (P = 0.031). RM ANOVA for group differences determined significance (P = 0.011) between IGT, NGT (P < 0.0001) and IGT, T2DM (P < 0.0001) respectively.

Table 2: *Pooled Participant Characteristics Pre- and Post 7 Days Aerobic Exercise*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>58.71 ± 3.21</td>
<td>58.71 ± 3.18</td>
<td>0.366</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.04 ± 1.78</td>
<td>166.04 ± 1.78</td>
<td>0.270</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>94.87 ± 3.58</td>
<td>94.97 ± 3.45</td>
<td>0.366</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.38 ± 1.13</td>
<td>34.42 ± 1.10</td>
<td>0.336</td>
</tr>
<tr>
<td>DEXA, Fat %</td>
<td>46.2 ± 1.43</td>
<td>45.74 ± 1.36</td>
<td>0.020</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>43.64 ± 1.92</td>
<td>43.25 ± 1.79</td>
<td>0.062</td>
</tr>
<tr>
<td>DEXA FFM (kg)</td>
<td>51.23 ± 2.76</td>
<td>51.73 ± 1.36</td>
<td>0.031</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95 ± 0.03</td>
<td>0.95 ± 0.03</td>
<td>0.270</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>112.08 ± 2.94</td>
<td>111.36 ± 2.84</td>
<td>0.007</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>118.69 ± 2.18</td>
<td>118.16 ± 2.26</td>
<td>0.003</td>
</tr>
</tbody>
</table>

n = 14 (4 males, 10 females), values are expressed as means ± SEM. % Body fat determined by dual-energy X-ray absorptiometry.

Bloodwork to include a comprehensive metabolic profile, complete blood count with white blood cell differential, thyroid and lipid profiles were collected only at the outset of the study. No data is available for post exercise comparison.

**Waist to Hip Ratio (WHR):** Paired *t*-tests found no difference in WHR at baseline or post intervention measurement (P > 0.05) for the pooled group, however, RM ANOVA for group differences revealed significance between groups (P = 0.0051); post
hoc analysis determined differences between IGT, NGT (P = 0.0001) and IGT, T2DM (P < 0.0001). Significant differences were found for pooled data between baseline and post measurement waist circumference (P = 0.007). RM ANOVA for group waist circumference differences were determined to be significant (P = 0.036) between all three groups, IGT, NGT (P = 0.0002) and IGT, T2DM (P < 0.0045) and NGT, T2DM (P = 0.0365). An exercise effect on waist circumference was also determined to be significant (P = 0.0111). No group by exercise interaction was found (P > 0.05). Significant differences were also found for pooled data between baseline and post measurement hip circumference (P = 0.003). Although no group differences were found in baseline group hip circumference (P > 0.05), an exercise effect was determined to be significant (P = 0.018) with differences noted between IGT, T2DM (P < 0.0165) and NGT, T2DM (P = 0.0295).

**Exercise Training**

As depicted in Table 3, paired t-tests determined significant differences (P < 0.05) in pooled data for all the observed variables (SBP<sub>Rest</sub>, DBP<sub>Rest</sub> and VO<sub>2</sub>) with the exception of HR<sub>Rest</sub>, although a trend toward significance is noted (P = 0.071). After RM ANOVA, no group differences were found in any of the variables at baseline, however, significant differences (P > 0.05) were observed pre and post intervention in VO<sub>2max</sub> for T2DM subjects (2010.3 ± 148.3 ml/min, p = 0.035). As Appendix K demonstrates, most subjects were able to exercise in the target HR range of 80-85% HR<sub>max</sub> during each exercise session and over the 7 day exercise period, with the exception of subjects 720-1309 and 720-2009 who were 77.1% and 78.5% HR<sub>max</sub>, respectively. Both
participants had increases in orthopedic issues over the 7 days of exercise and could not be encouraged to maintain exercise intensity secondary to pain.

Table 3:  *Pooled Data for Resting and Peak Exercise*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR&lt;sub&gt;rest&lt;/sub&gt; (bpm)</td>
<td>83.5 ± 4.46</td>
<td>77.14 ± 2.42</td>
<td>0.071</td>
</tr>
<tr>
<td>SBP&lt;sub&gt;rest&lt;/sub&gt; (mm Hg)</td>
<td>134.0 ± 3.16</td>
<td>125.14 ± 2.72</td>
<td>0.006</td>
</tr>
<tr>
<td>DBP&lt;sub&gt;rest&lt;/sub&gt; (mm Hg)</td>
<td>82.29 ± 3.20</td>
<td>76.43 ± 2.15</td>
<td>0.027</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2max&lt;/sub&gt; (ml/min)</td>
<td>2029.4 ± 178.4</td>
<td>2210.5 ± 171.4</td>
<td>0.010</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2max&lt;/sub&gt; (ml/kg/min)</td>
<td>21.13 ± 1.37</td>
<td>23.04 ± 1.17</td>
<td>0.004</td>
</tr>
<tr>
<td>HR&lt;sub&gt;max&lt;/sub&gt; (bpm)</td>
<td>51.23 ± 2.76</td>
<td>45.74 ± 1.36</td>
<td>0.031</td>
</tr>
</tbody>
</table>

n = 14 (4 males, 10 females), values are expressed as means ± SEM. HR<sub>rest</sub>, resting heart rate, SBP<sub>rest</sub>, resting systolic blood pressure, DBP<sub>rest</sub> resting diastolic blood pressure, VO<sub>2</sub>, maximal aerobic capacity, HR<sub>max</sub>, maximal heart rate.

**Metabolic Profiles**

**Caloric Intake:** All participants were counseled by a registered dietitian to consume a metabolically balanced diet (55% carbohydrate, 30% fat, 15% protein) during a 3 day baseline period. After dietary counseling, subjects recorded their customary food intake over the 3 days and followed the 250 grams CHO guideline to ensure adequate carbohydrate consumption prior to the OGTT. Participants replicated the same diet they consumed on the evening prior to both OGTTs (pre and post exercise). The indirect calorimetry results are listed in Table 4.
Table 4: *Pooled Data for Resting Metabolic Rate (RMR)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMR VO₂ (ml/min)</td>
<td>229.43 ± 11.88</td>
<td>226.96 ± 11.98</td>
<td>0.037</td>
</tr>
<tr>
<td>RMR VCO₂ (ml/min)</td>
<td>198.93 ± 11.15</td>
<td>187.71 ± 9.80</td>
<td>0.060</td>
</tr>
<tr>
<td>RMR NPRQ</td>
<td>0.87 ± 0.02</td>
<td>0.83 ± 0.01</td>
<td>0.010</td>
</tr>
<tr>
<td>RMR COX (g/min)</td>
<td>147.12 ± 22.14</td>
<td>109.23 ± 15.09</td>
<td>0.021</td>
</tr>
<tr>
<td>RMR FOX (g/min)</td>
<td>46.85 ± 7.65</td>
<td>61.19 ± 7.71</td>
<td>0.030</td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>1613.76 ± 89.86</td>
<td>1597.62 ± 78.02</td>
<td>0.450</td>
</tr>
</tbody>
</table>

n = 14 (4 males, 10 females), values are expressed as means ± SEM. RMR VO₂, resting metabolic rate oxygen consumption, RMR VCO₂ resting metabolic rate carbon dioxide expired, RMR NPRQ, non-protein respiratory quotient, RMR COX resting metabolic rate carbohydrate oxidation, RMR FOX, resting metabolic rate fat oxidation, REE, resting energy expenditure.

**Resting Metabolic Rate (RMR):** Fasting indirect calorimetry measures were performed before and after the exercise intervention (Table 3), however, urine was only collected at the outset of the study, therefore, only non-protein respiratory quotients and resting energy expenditures (via modified Weir equation) were calculated, likewise, no data is available for protein oxidation. Expired air was continuously sampled for 20 min via an automated system (Vmax Encore, Viasys) in a semi-dark, thermoneutral environment (22 ± 1°) under a ventilated hood. Paired t-tests for pooled data (P = 0.01) as well as RM ANOVA for group differences indicated statistical significance in NPRQ (P < 0.009) following training (Figures 4 and 5, respectively). Additionally, no differences were found in REE (P > 0.05).
Figure 4: NPRQ, non protein respiratory quotient following 7 days of aerobic exercise, n = 14 (4 males, 10 females), values are expressed as means ± SEM.

Figure 5: Substrate Oxidation following 7 days of aerobic exercise. RMR, Resting Metabolic Rate, n = 14 (4 males, 10 females), values are expressed as means ± SEM. CHO Ox, carbohydrate oxidation, Fat Ox, fat oxidation.
Carbohydrate Oxidation (CHO ox): Combined study data confirmed improvements in C\textsubscript{OX} after exercise training (P = 0.021). No differences were found in C\textsubscript{OX} (P > 0.05) between groups, however, a significant exercise training effect on C\textsubscript{OX} (P = 0.0019) was observed, Figure 6.

Figure 6: Carbohydrate oxidation after 7 days aerobic exercise, n = 14 (4 males, 10 females), values are expressed as means ± SEM. CHO\textsubscript{OX}, carbohydrate oxidation, NGT, normal glucose tolerance, IGT, impaired glucose tolerance, T2DM, type 2 diabetes mellitus. Derived by using repeated measures analysis of variance, RM ANOVA.

Fat Oxidation (FO\textsubscript{OX}): Pooled data for FO\textsubscript{OX} was also found to be statistically significant (P = 0.031) following training (Figure 7). Figure illustrates that no group differences were found after RM ANOVA (P > 0.05), however, a trend towards statistical significance was observed (P= 0.0772).
**Figure 7:** Fat oxidation after 7 days aerobic exercise, n = 14 (4 males, 10 females), values are expressed as means ± SEM. FatOx, fat oxidation, NGT, normal glucose tolerance, IGT, impaired glucose tolerance, T2DM, type 2 diabetes mellitus. Derived by using repeated measures analysis of variance, RM ANOVA.

**Oral Glucose Tolerance Test (OGTT) glucose responses.** A 75 gram OGTT was performed on each participant at baseline to determine diabetes classification criteria (Appendix I), and then repeated after the exercise intervention. There were significant differences in fasting plasma glucose values between groups (P = 0.046), with post hoc differences noted between IGT and T2DM (P = 0.011) and NGT and T2DM (P = 0.004). In NGT individuals, there was a steady rise in plasma glucose values from baseline (i.e., immediately after the 75 gram glucose drink was completely ingested) to 60 minutes (Figure 8), while in IGT and T2DM individuals, plasma glucose continued to rise until 90 minutes. This is due to early inefficient suppression of hepatic glucose output and impairment in 1st phase insulin response as demonstrated in Figures 8 and 9.
Table 5: OGTT Plasma Glucose Response after 7 Days AEX

<table>
<thead>
<tr>
<th>Time Point</th>
<th>NGT</th>
<th>IGT</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Ex 0</td>
<td>92.10 ± 0.60</td>
<td>99.30 ± 3.46</td>
<td>123.70 ± 9.07</td>
</tr>
<tr>
<td>Post Ex 0</td>
<td>89.80 ± 5.50</td>
<td>101.84 ± 5.87</td>
<td>110.67 ± 4.16</td>
</tr>
<tr>
<td>Pre Ex 30</td>
<td>121.50 ± 7.50</td>
<td>153.00 ± 21.00</td>
<td>189.20 ± 11.85</td>
</tr>
<tr>
<td>Post Ex 30</td>
<td>149.00 ± 2.00</td>
<td>157.60 ± 12.20</td>
<td>186.00 ± 15.50</td>
</tr>
<tr>
<td>Pre Ex 60</td>
<td>143.50 ± 3.50</td>
<td>173.40 ± 13.10</td>
<td>236.50 ± 17.11</td>
</tr>
<tr>
<td>Post Ex 60</td>
<td>145.50 ± 22.5</td>
<td>170.20 ± 21.64</td>
<td>216.83 ± 10.01</td>
</tr>
<tr>
<td>Pre Ex 90</td>
<td>119.50 ± 3.50</td>
<td>177.80 ± 13.16</td>
<td>255.20 ± 12.14</td>
</tr>
<tr>
<td>Post Ex 30</td>
<td>126.50 ± 13.50</td>
<td>153.00 ± 10.85</td>
<td>234.00 ± 9.02</td>
</tr>
<tr>
<td>Pre Ex 120</td>
<td>110.00 ± 7.00</td>
<td>163.60 ± 5.36</td>
<td>253.50 ± 17.37</td>
</tr>
<tr>
<td>Post Ex 120</td>
<td>105.50 ± 2.50</td>
<td>138.80 ± 7.71</td>
<td>221.17 ± 12.98</td>
</tr>
</tbody>
</table>

Plasma glucose in mg/dL, time in minutes, n = 13, NGT = 2, IGT = 5, T2DM = 6.

Figure 8: OGTT Glucose Response
**OGTT Insulin Responses:** In response to the 75 gram OGTT, insulin increased to 60 minutes in NGT, while it continued to rise longer in IGT and T2DM. In T2DM individuals, insulin continued to rise past 120 minutes (Figure 9). There were also significant differences in fasting plasma insulin values between groups (P = 0.0368), with post hoc differences noted between NGT and IGT (P = 0.0481), IGT and T2DM (P = 0.0327) and NGT and T2DM (P = 0.0010).

Table 6: *OGTT Plasma Insulin Response after 7 Days AEX*

<table>
<thead>
<tr>
<th>Time Point</th>
<th>NGT</th>
<th>IGT</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Ex 0</td>
<td>11.37 ± 2.07</td>
<td>19.12 ± 1.98</td>
<td>30.80 ± 5.67</td>
</tr>
<tr>
<td>Post Ex 0</td>
<td>10.14 ± 0.97</td>
<td>17.77 ± 1.71</td>
<td>27.57 ± 5.24,</td>
</tr>
<tr>
<td>Pre Ex 30</td>
<td>61.23 ± 28.20</td>
<td>150.00 ± 20.47</td>
<td>99.03 ± 22.18</td>
</tr>
<tr>
<td>Post Ex 30</td>
<td>85.32 ± 18.56</td>
<td>127.52 ± 20.10</td>
<td>86.93 ± 34.62</td>
</tr>
<tr>
<td>Pre Ex 60</td>
<td>102.45 ± 26.31</td>
<td>184.97 ± 28.29</td>
<td>163.93 ± 46.46</td>
</tr>
<tr>
<td>Post Ex 60</td>
<td>129.58 ± 26.71</td>
<td>166.98 ± 22.52</td>
<td>160.55 ± 71.78</td>
</tr>
<tr>
<td>Pre Ex 90</td>
<td>58.44 ± 1.53</td>
<td>187.21 ± 28.62</td>
<td>215.87 ± 66.41</td>
</tr>
<tr>
<td>Post Ex 30</td>
<td>93.21 ± 39.88</td>
<td>124.20 ± 24.41</td>
<td>284.33 ± 160.76</td>
</tr>
<tr>
<td>Pre Ex 120</td>
<td>41.74 ± 5.3</td>
<td>200.01 ± 37.94</td>
<td>300.90 ± 104.54</td>
</tr>
<tr>
<td>Post Ex 120</td>
<td>72.44 ± 29.48</td>
<td>119.80 ± 31.87</td>
<td>353.24 ± 219.95</td>
</tr>
</tbody>
</table>

Plasma insulin in (IU/mL), time in minutes, n = 13, NGT = 2, IGT = 5, T2DM = 6.
Figure 9: OGTT Insulin Response

Table 7: Metabolic Profiles Pre- and Post 7 Days Aerobic Exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG, mg/dl</td>
<td>109.42 ± 5.69</td>
<td>104.06 ± 3.55</td>
<td>0.096</td>
</tr>
<tr>
<td>Gluc&lt;sub&gt;120&lt;/sub&gt;, mg/dl</td>
<td>197 ± 17.83</td>
<td>172 ± 15</td>
<td>0.002</td>
</tr>
<tr>
<td>FPI, µU/ml</td>
<td>21.11 ± 9.55</td>
<td>18.49 ± 5.69</td>
<td>0.088</td>
</tr>
<tr>
<td>Ins&lt;sub&gt;120&lt;/sub&gt;, µU/ml</td>
<td>173.47 ± 90.82</td>
<td>117.72 ± 55.31</td>
<td>0.007</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.58 ± 1.133</td>
<td>4.53 ± 0.665</td>
<td>0.070</td>
</tr>
<tr>
<td>Matsuda IS</td>
<td>1.83 ± 0.326</td>
<td>2.07 ± 0.228</td>
<td>0.037</td>
</tr>
<tr>
<td>tAUC Glu&lt;sub&gt;120&lt;/sub&gt;</td>
<td>21613.27 ± 1519.32</td>
<td>20444.77 ± 1229.50</td>
<td>0.035</td>
</tr>
<tr>
<td>tAUC Ins&lt;sub&gt;120&lt;/sub&gt;</td>
<td>14965.59 ± 6188.67</td>
<td>12155.23 ± 4106.99</td>
<td>0.014</td>
</tr>
<tr>
<td>iAUC Glu&lt;sub&gt;120&lt;/sub&gt;</td>
<td>8482.50 ± 1090.25</td>
<td>7957.38 ± 1035.77</td>
<td>0.107</td>
</tr>
<tr>
<td>iAUC Ins&lt;sub&gt;120&lt;/sub&gt;</td>
<td>11475.31 ± 6907.37</td>
<td>9171.62 ± 5038.61</td>
<td>0.022</td>
</tr>
</tbody>
</table>

n = 13 (4 males, 9 females), values are expressed as means ± SEM. FPG, fasting plasma glucose, FPI, fasting plasma insulin, Gluc<sub>120</sub>, plasma glucose at 120 min, Ins<sub>120</sub>, plasma insulin at 120 min, IR, insulin resistance, tAUC, total area under the curve, baseline corrected, iAUC, incremental area under the curve above baseline.
Figure 10: Matsuda Index of insulin sensitivity after 7 days aerobic exercise, $n = 13$ (4 males, 9 females), values are expressed as means ± SEM. AU, arbitrary units. Derived from paired $t$-tests.

Figure 11: Matsuda Index of Insulin Sensitivity after 7 days aerobic exercise, $n = 13$ (4 males, 9 females), values are expressed as means ± SEM. RM ANOVA revealed significance between groups, $P=0.003$, exercise $= 0.5193$, and an Ex * Group $= 0.0097$ interaction.
**Insulin Sensitivity:** RM ANOVA revealed significant differences between groups (P = 0.0003) and an exercise * group interaction (P = 0.0097). Post hoc analysis determined differences between all three groups: IGT, NGT (P < 0.0001), IGT, T2DM (P = 0.0233), and IGT, NGT (P < 0.0001).

Paired t-tests established no significant difference in pooled data for HOMA-IR, although a trend towards significance was noted (P = 0.076). RM ANOVA for group data HOMA-IR calculations found significant differences between groups (P = 0.0325), specifically, between IGT, T2DM (P = 0.0046), and NGT, T2DM (P < 0.0013). Sample 720-0209 was not included in the data set as her measure of insulin sensitivity was not OGTT derived.

![HOMA IR](chart.png)

*Figure 12:* HOMA-IR, n = 13 (4 males, 9 females), values are expressed as means ± SEM. No significant difference was found in HOMA-IR (P = 0.076)
Flow Cytometry Data for TLR2 CD14: There were no significant differences in fasting or glucose stimulated whole blood TLR2 CD14 values for pooled or group data (P > 0.05). Average pooled TLR2 CD14 values, in mean fluorescent intensity (MFI) arbitrary units (AU), for the 4 conditions can be found in Table 7 and the average group TLR2 CD14 values in Table 8. Sample number 720-1309 was not available secondary to hemolysis, samples 720-1609 and 720-1709 were not available secondary to prolonged collection of blood sample.

Table 8: Pooled Flow Cytometry Data for TLR2 CD14

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Post Ex Fasting</th>
<th>Pre Ex Gluc$_{120}$</th>
<th>Post Ex Gluc$_{120}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.523 ± 0.640</td>
<td>2.323 ± 0.419</td>
<td>2.519 ± 0.496</td>
<td>2.673 ± 0.785</td>
</tr>
</tbody>
</table>

n = 10 (2 male, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. Pre Ex, pre exercise baseline (fasting), Gluc$_{120}$, 2 hour oral glucose tolerance test (OGTT). Post Ex, pose exercise. Flow cytometry was analyzed from whole blood. (p > 0.05).

Table 9: Group Flow Cytometry Data for TLR2 CD14

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Post Ex Fasting</th>
<th>Pre Ex Gluc$_{120}$</th>
<th>Post Ex Gluc$_{120}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td>1.990 ± 0.000</td>
<td>2.207 ± 0.000</td>
<td>2.475 ± 0.000</td>
<td>1.347 ± 0.000</td>
</tr>
<tr>
<td>IGT</td>
<td>2.746 ± 1.773</td>
<td>2.117 ± 0.317</td>
<td>3.024 ± 0.679</td>
<td>3.982 ± 1.872</td>
</tr>
<tr>
<td>T2DM</td>
<td>2.551 ± 0.966</td>
<td>2.499 ± 0.902</td>
<td>2.197 ± 0.968</td>
<td>2.243 ± 0.977</td>
</tr>
</tbody>
</table>

n = 10, n for NGT = 2, IGT = 2, T2DM = 6, values are expressed as mean fluorescence intensity (MFI) means ± SEM. Pre Ex, pre exercise baseline (fasting), Gluc$_{120}$, 2 hour oral glucose tolerance test (OGTT). Post Ex, post exercise. Normal glucose tolerance (NGT), Impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), Flow cytometry was analyzed from whole blood (p > 0.05).
Figure 13: Flow cytometry analysis for pooled monocyte derived TLR2 CD14 data. n = 10 (2 male, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.

Figure 14: Flow cytometry analysis for group monocyte derived TLR2 CD14 data. n = 10, n for NGT = 2, IGT= 2, T2DM = 6, values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.
Flow Cytometry Data for TLR2 CD14 CD16: There were no significant differences in fasting or glucose stimulated whole blood TLR2 CD14 values for pooled or group data (P > 0.05).

Average pooled TLR2 CD14 CD16 values, MFI, for the 4 conditions can be found in Table 9 and the average group TLR2 CD14 values in Table 10. Sample number 720-1309 was not available secondary to hemolysis, samples 720-1609 and 720-1709 were not available secondary to prolonged collection of blood sample, no data available.

Table 10: Pooled Flow Cytometry Data for TLR2 CD14 CD16

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre Ex Fasting</th>
<th>Post Ex Fasting</th>
<th>Pre Ex Gluc_{120}</th>
<th>Post Ex Gluc_{120}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.994 ± 0.757</td>
<td>2.908 ± 0.548</td>
<td>2.882 ± 0.745</td>
<td>3.671 ± 0.839</td>
</tr>
</tbody>
</table>

n = 10 (2 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM.

Table 11: Group Flow Cytometry Data for TLR2 CD14 CD16

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre Ex Fasting</th>
<th>Post Ex Fasting</th>
<th>Pre Ex Gluc_{120}</th>
<th>Post Ex Gluc_{120}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td>2.915 ± 0.000</td>
<td>3.403 ± 0.000</td>
<td>3.190 ± 0.000</td>
<td>2.413 ± 0.000</td>
</tr>
<tr>
<td>IGT</td>
<td>3.553 ± 2.482</td>
<td>3.040 ± 0.553</td>
<td>4.040 ± 1.010</td>
<td>4.878 ± 2.115</td>
</tr>
<tr>
<td>T2DM</td>
<td>2.734 ± 0.950</td>
<td>2.719 ± 0.982</td>
<td>2.226 ± 1.182</td>
<td>3.382 ± 1.146</td>
</tr>
</tbody>
</table>

n = 10, n for NGT = 2, IGT= 2, T2DM = 6, values are expressed as mean fluorescence intensity (MFI) means ± SEM. Pre Ex, pre exercise baseline (fasting), Gluc_{120}, 2 hour oral glucose tolerance test (OGTT), Post Ex, post exercise, Normal glucose tolerance (NGT), Impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), Flow cytometry was analyzed from whole blood. (p > 0.05).
Figure 15: Flow cytometry analysis for pooled monocyte derived TLR2 CD14 CD16 data. n = 10 (2 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.

Figure 16: Flow cytometry analysis for group monocyte derived TLR2 CD14 CD16 data. n = 10, n for NGT = 2, IGT = 2, T2DM = 6, values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.
**Flow Cytometry Data for TLR4 CD14:** There were no significant differences in fasting or glucose stimulated whole blood TLR4 CD14 values for pooled or group data (P > 0.05).

Average pooled TLR4 CD14 values, in MFI, for the 4 conditions can be found in Table 11 and the average group TLR2 CD14 values in Table 12. Sample numbers 720-1609 and 720-1709 were not available secondary to delayed sample processing.

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Post Ex Fasting</th>
<th>Pre Ex Gluc120</th>
<th>Post Ex Gluc120</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.111 ± 0.303</td>
<td>3.654 ± 0.427</td>
<td>3.824 ± 0.440</td>
<td>4.044 ± 0.310</td>
</tr>
</tbody>
</table>

n = 11 (3 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. (p > 0.05).

*Figure 17:* Flow cytometry analysis for pooled monocyte derived TLR4 CD14. n = 11 (3 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.
Figure 18: Flow cytometry analysis for group monocyte derived TLR4 CD14. n = 11, n for NGT = 2, IGT = 4, T2DM = 5, values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.

Flow Cytometry Data for TLR4 CD14 CD16: There were no significant differences in fasting or glucose stimulated whole blood TLR4 CD14 CD16 values for pooled or group data (P > 0.05).

Average pooled TLR4 CD14 CD16 values, MFI, for the 4 conditions can be found in Table 13 and the average group TLR2 CD14 values in Table 14. Sample numbers 720-1609 and 720-1709 were not available secondary to delayed sample processing.
Table 13: *Group Flow Cytometry Data for TLR4 CD14*

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Pre Ex Gluc₁₂₀</th>
<th>Post Ex Fasting</th>
<th>Post Ex Gluc₁₂₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td>2.934 ± 0.581</td>
<td>3.778 ± 0.358</td>
<td>2.188 ± 0.125</td>
<td>3.967 ± 0.177</td>
</tr>
<tr>
<td>IGT</td>
<td>3.597 ± 0.450</td>
<td>3.046 ± 0.395</td>
<td>4.684 ± 0.670</td>
<td>4.530 ± 0.577</td>
</tr>
<tr>
<td>T2DM</td>
<td>2.891 ± 0.518</td>
<td>4.309 ± 0.813</td>
<td>3.623 ± 0.556</td>
<td>3.783 ± 0.376</td>
</tr>
</tbody>
</table>

n = 11, n for NGT = 2, IGT = 4, T2DM = 5, values are expressed as mean fluorescence intensity (MFI) means ± SEM. Pre Ex, pre exercise baseline (fasting), Gluc₁₂₀, 2 hour oral glucose tolerance test (OGTT), Post Ex, post exercise, Normal glucose tolerance (NGT), Impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), Flow cytometry was analyzed from whole blood. (p > 0.05).

Table 14: *Pooled Flow Cytometry Data for TLR4 CD14 CD16*

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Pre Ex Gluc₁₂₀</th>
<th>Post Ex Fasting</th>
<th>Post Ex Gluc₁₂₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.657 ± 0.829</td>
<td>5.478 ± 0.841</td>
<td>4.886 ± 0.544</td>
<td>5.546 ± 0.632</td>
</tr>
</tbody>
</table>

n = 11 (3 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. (p > 0.05).

Table 15: *Group Flow Cytometry Data for TLR4 CD14 CD16*

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex Fasting</th>
<th>Pre Ex Gluc₁₂₀</th>
<th>Post Ex Fasting</th>
<th>Post Ex Gluc₁₂₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td>3.447 ± 1.572</td>
<td>6.183 ± 0.947</td>
<td>1.254 ± 0.887</td>
<td>4.280 ± 3.027</td>
</tr>
<tr>
<td>IGT</td>
<td>4.577 ± 0.681</td>
<td>4.383 ± 0.782</td>
<td>6.453 ± 0.754</td>
<td>5.806 ± 0.724</td>
</tr>
<tr>
<td>T2DM</td>
<td>4.922 ± 1.630</td>
<td>6.379 ± 1.514</td>
<td>4.521 ± 0.687</td>
<td>5.135 ± 0.795</td>
</tr>
</tbody>
</table>

n = 11, n for NGT = 2, IGT = 4, T2DM = 5, values are expressed as mean fluorescence intensity (MFI) means ± SEM. Pre Ex, pre exercise baseline (fasting), Gluc₁₂₀, 2 hr oral glucose tolerance test (OGTT). Post Ex, post exercise. Normal glucose tolerance (NGT), Impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), Flow cytometry was analyzed from whole blood. (p > 0.05).
Figure 19: Flow cytometry analysis for pooled monocyte derived TLR4 CD14CD16 data. n = 11 (3 males, 8 females), values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.
Figure 20: Flow cytometry analysis for group monocyte derived TLR4 CD14CD16 data. n = 11, n for NGT = 2, IGT = 4, T2DM = 5, values are expressed as mean fluorescence intensity (MFI) means ± SEM. P > 0.05.

**Oxidative Stress (ROS)**

Table 16: ROS Pre- and Post 7 Days Aerobic Exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (mV·min⁻¹)</td>
<td>0.26 ± 0.07</td>
<td>0.24 ± 0.05</td>
<td>0.48</td>
</tr>
<tr>
<td>2 HR&lt;sub&gt;Glucose&lt;/sub&gt; (mV·min⁻¹)</td>
<td>0.60 ± 0.23</td>
<td>0.22 ± 0.05</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Δ&lt;sub&gt;OGTT&lt;/sub&gt; (Glucose – Fasting)</td>
<td>0.34 ± 0.19</td>
<td>0.01 ± 0.06</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

n = 11 (3 males, 8 females), values are expressed as means ± SE.

There were no significant differences noted in fasting respiratory burst activity of MNC derived ROS for pooled data (p = 0.48), however, there were clinical significance observed at the 2 HR<sub>Glucose</sub> time point (p = 0.05) and in the difference between glucose stimulated and fasting time points (p = 0.04). There were no significant differences in fasting, 2 hour glucose stimulated, or the difference between glucose stimulated and fasting time points for group data (P > 0.05). Sample numbers 720-1609 and 720-1709 were not available secondary to delayed sample processing.
Figure 21: Respiratory burst activity of monocyte derived ROS measured by detection of superoxide radical via chemiluminescence for pooled data. \( n = 11 \) (3 males, 8 females), values are expressed as mV·min\(^{-1}\) means ± SEM. \( P > 0.05 \).

![Graph showing ROS levels in different conditions](image)

Figure 22: Respiratory burst activity of monocyte derived ROS measured by detection of superoxide radical via chemiluminescence for group data. \( n = 11 \), \( n \) for NGT = 2, IGT = 4, T2DM = 5, values are expressed as mV·min\(^{-1}\) means ± SEM. \( P > 0.05 \).

**Cytokine Secretion**

There were no significant differences noted in fasting MNC derived IL-6 for pooled data \( (p = 0.43) \), however, there was clinical significance observed at the 2 hour glucose stimulated time point \( (p = 0.05) \). No significant differences in IL-6 group data were observed for T2DM subjects at any time point \( (p > 0.05) \), however, significance was noted at the 2 hr OGTT time point in IGT subjects \( (p = 0.0011) \). Additionally, there were no significant differences in TNF-\(\alpha\) pooled or group data for any time point \( (p > 0.05) \).
(See Table 16, Figures 23, 24, and 25). Sample number 720-0809 was not run because the subject biked greater than 10 miles into the clinic for his post exercise OGTT. Samples 720-1109 and 720-2609 (both NGT subjects) were not run because a decision was made secondary to funding not to purchase another IL6 kit. It was determined that we would benefit from data collected from the IGT and T2DM subjects more so than the NGT subjects. Lastly, sample 720-0209 was not available; a different cell culture protocol was used when this subject came through the study.

Table 17: **Cytokine Secretion** Pre- and Post 7 Days AEX (Pooled Data)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre 7 Day</th>
<th>Post 7 Day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 Fasting (pg/mL)</td>
<td>1670.93 ± 143.07</td>
<td>1651.84 ± 145.89</td>
<td>0.43</td>
</tr>
<tr>
<td>IL-6 2 HR Glucose (pg/mL)</td>
<td>1505.04 ± 159.21</td>
<td>1267.30 ± 191.82</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>TNF-α Fasting (pg/mL)</td>
<td>1639.53 ± 209.63</td>
<td>1904.75 ± 389.72</td>
<td>0.24</td>
</tr>
<tr>
<td>TNF-α 2 HR Glucose (pg/mL)</td>
<td>1212.55 ± 209.11</td>
<td>1590.56 ± 392.69</td>
<td>0.17</td>
</tr>
</tbody>
</table>

n = 10 for IL-6 (3 males, 7 females), n = 13 for TNF-α (4 males, 9 females), values are expressed as means ± SEM. Subjects #720-0809, 720-1109, and 720-2609 were not run.
Figure 23: ELISA analysis of monocyte derived IL-6, pooled data, n = 10, 4 IGT, 6 T2DM, values are expressed as means ± SEM. Subject #720-0809, and both NGT subjects 720-1109, 720-2609, were not run. Significance noted at 2 hr OGTT (p = 0.05).

Figure 24: ELISA analysis of monocyte derived IL-6, n = 10, 4 IGT, 6 T2DM, values are expressed as means ± SEM. Subject #720-0809, and both NGT subjects 720-1109, 720-2609, were not run. Significance noted at 2 hr OGTT in IGT (p = 0.0011).
Figure 25: ELISA analysis of monocyte derived TNF-α for pooled data. n = 13 (4 males, 9 females), values are expressed as pg/mL means ± SEM. (P > 0.05).

Figure 26: ELISA analysis of monocyte derived TNF-α for group data. n = 13 (4 males, 9 females), values are expressed as pg/mL means ± SEM. P > 0.05.
CHAPTER V
DISCUSSION

The combined effects of a sedentary lifestyle and poor dietary choices contribute to inflammatory changes and, in those individuals who are genetically predisposed, obesity-related insulin resistance. The prevalence of obesity has reached epidemic proportions globally (Tjepkema, 2006), posing major public health issues, since obesity is a major contributor to the overall burden of chronic disease. Although the relationship between obesity, insulin resistance and other obesity related diseases is well-recognized, the mechanisms involved remain relatively poorly understood. Aerobic exercise training can be used as an inexpensive, non-pharmacological treatment for the metabolic and inflammatory impairments found in obesity, pre-diabetes and type II diabetes mellitus (T2DM) to improve whole-body insulin sensitivity and glucose tolerance. Additionally, we now know that contracting muscle secretes chemical mediators that effectively combats chronic inflammation, whereas, inactive muscle does not secrete these protective mediators. Therefore, the purpose of the study was threefold: 1) to identify the effects of short term aerobic exercise on whole body insulin resistance and to determine the effect of training on mononuclear cell (MNC) derived markers of inflammation, specifically, 2) toll like receptors (TLR2, TLR4), and reactive oxygen species (ROS), and lastly, 3) downstream inflammatory cytokine secretion of tumor necrosis factor alpha and interleukin 6 (TNF-α and IL-6) in sedentary obese adults. It was hypothesized that 7 consecutive days of aerobic exercise would significantly reduce insulin resistance (increase insulin sensitivity), oxidative stress, and cytokine secretion. The principal
finding in this investigation was that sedentary, obese adults can significantly improve insulin sensitivity (Matsuda IR, p = 0.037) after 7 consecutive days of supervised aerobic exercise. Although statistical significance was not obtained in fasting plasma glucose or insulin in this time frame, significance was obtained at Glucose$_{120}$ (p = 0.002). It can be predicted with reasonable certainty that with continued exercise training clinical significance can also be achieved in fasting plasma glucose and insulin in a relatively short time frame.

**Effects of Aerobic Exercise on Insulin Sensitivity**

Previous studies in humans and animals have shown that exercise training by itself generally improves β-cell sensitivity and reduces insulin secretion, thereby improving insulin action (Kirwan, Kohrt, Wojta, Bourey, & Holloszy, 1993; Mikines, Farrell, Sonne, Tronier, & Galbo, 1988). Insulin resistance has been shown to be partially reversed with increased physical activity in obese populations (Cononie, 1994; DeFronzo, Sherwin, & Kraemer, 1987). Kirwan *et al* demonstrated significant improvements in insulin action, peripheral insulin sensitivity and responsiveness and enhanced suppression of hepatic glucose production in T2DM patients after 7 days of vigorous exercise training (Kirwan, Solomon, Wojta, Staten, Holloszy, 2009). Winnick reported mixed results after a similar exercise protocol; improvement in peripheral, but not hepatic insulin sensitivity (Winnick, 2008). Kelley *et al* recently found a significant increase in high molecular weight (HMW) adiponectin and a significant decrease in leptin after 7 days aerobic exercise (Kelly, 2011) and that HMW adiponectin was positively correlated with basal fat oxidation after 7 days aerobic exercise, suggesting this
time period is sufficient to favorably alter adipokine secretion, as well as modify metabolism and improve insulin sensitivity independent of changes in body weight or composition (Haus, 2011; Kelly, 2011).

Previous work has shown that in order to achieve the desired metabolic improvements, an appropriate exercise stimulus must be applied. Subjects in the current study were able to exercise within the target heart rate range of 80-85% $HR_{max}$ ($\approx 70\% VO_{2max}$) for 50-60 minutes per day for 7 consecutive days with the exception of two subjects. These two subjects averaged 77.1 and 78.5 % $HR_{max}$ and were limited in reaching their $HR_{max}$ secondary to orthopedic issues. While others have shown greater improvements in metabolic profiles at greater exercise intensities (Houmard et al., 2004), it is noteworthy to mention that significant improvements were achieved at moderate exercise intensities without the risk of injury in this sedentary obese elderly population.

Peripheral glucose uptake by skeletal muscle is determined by the interaction of three factors: delivery of glucose to the skeletal muscle (blood flow), transport of glucose in the skeletal muscle cell, and the rate of glucose phosphorylation after it has entered the skeletal muscle cell. Although the current investigation clearly does not attempt to determine the mechanisms responsible for changes in peripheral glucose uptake that were observed, it appears that any of these factors, either singly or interactively, contributed to the observed increase in peripheral glucose uptake. Muscle glycogen utilization has also been shown to increase with 7 days of aerobic exercise (two times greater at 70% $VO_{2peak}$ compared to 50 % $VO_{2peak}$), suggesting that this effect may also be partially accountable for the improved insulin sensitivity that was observed (Dela, Mikines, von Linstow,
Secher, & Galbo, 1992; DiPietro et al., 2006). Exercise training also has been shown to increase Glut 4 protein levels and non-oxidative glucose metabolism in humans with T2DM (Dela, Ploug, Handberg, Petersen, Larsen, Mikines, Galbo, 1994). Thus it appears likely that a combination of these factors could also have contributed to the increase in insulin-mediated glucose uptake observed in the current study.

A number of previous studies have investigated the effect of aerobic exercise training on insulin sensitivity in obese humans with impaired glucose tolerance. These studies have demonstrated that chronic aerobic exercise training resulted in improved glucose tolerance during glucose clamp conditions and ultimately improved insulin sensitivity. Although these studies exhibit the efficacy of aerobic exercise to improve whole body insulin sensitivity in impaired glucose states, the experimental design that was employed often produced decreased body fat or increases in aerobic power. Both of these factors can separately improve insulin sensitivity independent of the effect of exercise training making it difficult to determine the sole effect of exercise training on insulin sensitivity. To resolve this problem, a number of studies as well as the current study have utilized short term aerobic exercise training 1-10 days in duration. Rogers et al (Rodgers, 1988) and Kang et al both reported that seven days of aerobic exercise training, utilizing an identical training protocol as the current study, resulted in improved glucose tolerance and insulin action, interpreted as reduced plasma glucose and insulin levels during the post exercise OGTT. Arciero et al showed that 10 days of aerobic exercise training in humans with impaired glucose tolerance or mild T2DM resulted in increased whole-body glucose disposal during hyperglycemic clamp conditions (Arciero,
With a shorter training period, there are usually minimal, if any, changes to either body fat levels or aerobic power permitting the independent determination of the effect of exercise on glucose tolerance, insulin sensitivity, and other inflammatory parameters. This study, however, showed significant improvements in both aerobic power, VO$_{2\text{max}}$ ($p = 0.004$) and DEXA body fat % ($p = 0.02$) in the absence of weight loss or change in BMI. Although these studies have been significant in developing our understanding of the independent effect of aerobic exercise training on whole body glucose tolerance and insulin sensitivity in humans with impaired glucose tolerance, they do not provide information on the relative contributions of changes in inflammatory parameters that may be responsible for improving whole body glucose metabolism in response to exercise training. This is an area that has yet to be fully investigated.

**Effects of Aerobic Exercise on Substrate Oxidation**

Kelley *et al.* performed numerous euglycemic insulin clamp studies to measure substrate fluxes across groups of subjects (lean, obese, IGT, T2DM) with different degrees of insulin sensitivity using stable isotope infusions and indirect calorimetry with relatively short duration (40-60 min, 40-50% VO$_{2\text{max}}$) exercise bouts (Kelley, 2000). They found lean subjects derived $\approx 1/3$ of their energy during exercise from Fat$_{OX}$ and the other $\approx 2/3$ from CHO$_{OX}$ (approximately equally derived from glycogenolysis and plasma glucose). In insulin resistant groups, the major differences were a higher dependence on FAT$_{OX}$ and a lower contribution from muscle glycogen. Goodpaster *et al* demonstrated that the increase in FATox during exercise in insulin resistant subjects, compared with normal persons, is more specifically derived from intramyocellular lipids (Goodpaster,
In particular, that intramyocellular Fat\textsubscript{OX} was \(\approx 50\%\) higher in obese subjects than in lean subjects. Paradoxically, highly trained athletes also have high muscle triglyceride content but they are able to mobilize these stores more readily because their muscles are very insulin sensitive and their oxidative enzymes are highly expressed. It is now known that endurance exercise training promotes mitochondrial biogenesis in skeletal muscle and enhances muscle oxidative capacity. Peroxisome proliferator-gamma coactivator-1 alpha (PGC-1\(\alpha\)) is now recognized as the master regulator of mitochondrial biogenesis. Mitochondrial adaptations lead to a metabolic preference of the mitochondria to a greater reliance on lipid rather carbohydrate metabolism at submax exercise intensities (Tarnopolsky et al., 2007). Higher mitochondrial content has been shown to be protective against ROS induced damage due to lower respiration per mitochondria (Sen, 1995).

Pooled data from this study confirmed improvements in CHO\textsubscript{OX} (\(p = 0.021\)) and Fat\textsubscript{OX} (\(p = 0.031\)) after exercise training. No between group differences were observed in either substrate oxidation, however, exercise training was found to independently improve CHO\textsubscript{OX} (\(p = 0.019\)). A trend towards significance was observed in Fat\textsubscript{OX} (\(p = 0.0772\)) in group data. Improvements in Fat\textsubscript{OX} may also be related to increased AMP-activated protein kinase (AMPK) activity, the molecular fuel sensor. As a result of AMPK activation during muscle contraction, acetyl-CoA carboxylase activity decreases while malonyl-CoA decarboxylase increases and these two enzymes remain in this state for some time into the post exercise period, permitting enhanced fatty acid oxidation.
AMPK is also beneficial in that it can inhibit early steps in the formation of diacylglycerol and triglycerides and enhance peripheral glucose uptake (Goodyear, 1998).

**Effects of Aerobic Exercise on Cell Surface Expression TLR2 and TLR4**

No exercise effect was found on either MNC derived TLR2 or TLR4 for CD14 or CD14 CD16 populations for pooled or group data. However, the study was not powered to examine differences between groups. Alternatively, 7 days may be an insufficient time frame for inflammatory adaptations to occur in this population. The fact that the aerobic training protocol used in the study did not affect TLR4 is somewhat surprising, considering a number of studies using a similar intensity of exercise that have reported reductions in TLR4 cell surface expression. Although data from Flynn’s group demonstrated that exercise training induced a lowering of CD14+ cell surface expression of LPS stimulated TLR4 and IL-6 in older healthy sedentary adults, these subjects followed a 12 wk (3 days/wk) combined endurance (20 min) and resistance training protocol (8 exercise, two sets), suggesting that 7 days may not be a sufficient time frame for changes in inflammatory parameters in impaired populations.

Oliveira and Gleeson recently examined the effects of 1.5 hr cycling at 75% VO$_2$(peak) in young healthy males on human monocyte TLR2 and TLR4 expression and how long it takes for TLR expression to return to pre-exercise values. They observed that TLR4 expression decreased (P < 0.05) by 32 and 45% at 0 and 1 h post-exercise, respectively, compared with pre-exercise values but had returned to baseline values by 4 hr post-exercise. They did not observe any statistically significant changes in TLR2 expression after exercise. Simpson examined expression of classic and pro-inflammatory
monocyte subsets following 45min of treadmill running at 75% VO$_{2\max}$ from moderately trained male subjects before, immediately after and 1h after post exercise. They found TLR2 expression was 12% lower on CD16(+dim) monocytes POST (p<0.05), whereas TLR4 expression on total monocytes was 12% and 22% lower at 1H (Simpson et al., 2009)

All of the data supporting a decrease in the TLR response to exercise have been generated in healthy older or younger subjects and it is likely that in older, impaired, glucose tolerant subjects may need a larger stimulus to modify the TLR response and alter cytokine release. Based on results from the present study, it would seem that a greater exercise training period may be required to elicit a more favorable response. Differences in study design, subject fitness and overall health, sample collection and measurement may provide some answers to the discrepancies found between studies.

**Effects of Aerobic Exercise on Oxidative Stress (ROS)**

It is well established that contracting skeletal muscles produce free radicals and other reactive oxygen species in skeletal muscle (Powers, 2011). ROS are considered harmful in excess or if chronically activated because they can lead to oxidative stress and cell damage as they play a prominent role in the pathogenesis of several diseases. T2DM is characterized by hyperglycemia, increased ROS generation and inflammation. Mohanty *et al* previously showed that ROS generation by mononuclear cells (MNC) increases to a peak of the basal level at 2 hr post OGTT (Mohanty et al., 2000). Another study by Dasu’s group demonstrated that high glucose also induces TLR expression, activity, and inflammation via NF-κB followed by cytokine release in *vitro* and in *vivo*
(Dasu MR, 2010). Gonzalez et al reported similar findings; hyperglycemia increased MNC derived ROS production (Gonzalez et al., 2006b) and NFκB activation (Gonzalez et al., 2006a) in women with polycystic ovary syndrome (a disease that often presents with insulin resistance and chronic low level inflammation). To elucidate the relationship between inflammation and glucose induced ROS generation, the current study investigated the effects of exercise after a glucose challenge (OGTT) on ROS generation by MNC’s. The results of this study did not indicate any significant change in fasting ROS (p= 0.48) pre to post exercise, however, both the 2 hr. glucose stimulated condition (p = 0.05) and the difference between glucose stimulated and fasting time points (p = 0.04) were significantly diminished indicating an exercise training effect on MNC generated ROS. Differences in time points when ROS was measured may provide explanation for differences found between studies. The current study examined ROS production in response to an OGTT 24 hours after the last exercise bout.

It is now clear that ROS play important roles as second messengers in cell signaling pathways that regulate both normal physiological and pathological signaling. Although progress has been made in understanding the role of ROS as signaling molecules in skeletal muscle, many unanswered questions remain. A fundamental question remains: “How does ROS production promote an anabolic response in some conditions (exercise training) and a catabolic response (disuse) in others? Several potential explanations exist for this dichotomy, including differences in the oxidant species produced, divergence in the sequential pattern of ROS production (acute vs. chronic activation), inconsistency in ROS levels produced and/or differences in the
cellular locations of ROS production (MNC vs. skeletal muscle). If exercise reduces ROS generation, oxidative stress, and redox signaling pathways, does this imply a possible reduction in ROS damage, or is this indicative of redox stimulated exercise adaptations? Certainly with chronic exercise there is an upregulation of the antioxidant defense systems. A methodological limitation in redox biology investigations has been the problem of quantifying the different levels of ROS in vivo due to the short half-lives and the high reactivity of ROS. The half-life of singlet oxygen has been estimated to $10^{-6}$ to $10^{-5}$ sec (Stahla, 2002). Further methodological development and research into ROS generation and exercise adaptations will help to answer questions regarding redox signaling and relevance to human exercise.

**Effect of Aerobic Exercise on TNF-α**

Although chronic inflammatory diseases comprise a very heterogeneous group of chronic diseases, all systemic chronic inflammatory diseases share common characteristics, including elevated circulating levels of cytokines, such as TNF-α and IL-6 under basal conditions. While regular exercise is often recommended as an important component of therapy for many chronic diseases and there is a vast knowledge base established regarding the effects of exercise on immune function and cytokines in healthy individuals, it is unfortunate that more research has not been conducted in patients with chronic inflammatory diseases in which inflammation is known to be dysregulated. The impact of exercise on immune activation in diseases with an underlying inflammatory burden is less clear and the few studies that have been conducted present conflicting data. Flynn (Flynn et al., 2003) reported decreased
mitogen stimulated cytokine production, Gielen (Gielen, 2003) noted reduced IL-1β and TNF-α protein in skeletal muscle, while Rabinovich et al. reported increased plasma TNF-α levels in COPD patients ( p<0.005) after 8 weeks of moderate-intensity constant-work-rate exercise (11 min at 40% of pretraining peak work-rate) compared to controls, without exercise-induced upregulation of the TNF-α gene in skeletal muscle; no changes were observed in sTNFRs or IL-6 levels after exercise training (Rabinovich et al., 2003). Despite significant decreases in visceral fat in older obese men and women after 12 wks of exercise training (same exercise intensity as current study), data from Kirwan’s group revealed no change in MNC-derived TNF-α (O’Leary et al., 2006). Similar results were also obtained after 7 days of exercise training (p = 0.24, 0.17 for fasting and 2 hr OGTT) in the current study, suggesting that exercise without muscle damage has no effect on TNF-α.

Low to moderate intensity training in healthy elderly people has been shown to reduce resting levels of pro-inflammatory markers such as monocytes, CRP and IL-6 (Nicklas, 2008; Timmerman, 2008); however, as the initiation of the acute-phase response develops, exercise in this population was not followed by a fully developed systemic response, suggesting that anti-inflammatory cytokines, soluble receptors and receptor antagonists restricted the magnitude and duration of the inflammatory response to exercise. Strenuous exercise or exercise that induces skeletal muscle damage seems to elicit the most prominent changes in immune and inflammatory responses (Gleeson, 2007; Mackinnon, 2000). TNF-α and IL-1β do not seem to increase in short
periods of moderate intense exercise although conflicting results have been documented. This is probably counterbalanced by the release of cytokine inhibitors (IL-1ra, sTNF-r1 and TNF-r2) and the anti-inflammatory cytokine IL-10 in healthy subjects.

The effect of training on inflammatory markers also seems to be dependent on the intensity of exercise, training status (Gleeson, 2007), age and disease pathology. For example, T2DM is associated with an overproduction of TNF-α by the adipocyte, whereas in autoimmune diseases, macrophages and T cells are the principal source (Abbas, 2007). Studies conducted in this area show mixed results. Because TNF-α is an inflammatory cytokine produced by a variety of cells and only limited information is available regarding MNC derived TNF-α, distinguishing the effects of exercise on TNF-α content has been difficult.

**Effect of Aerobic Exercise on IL-6**

The role that IL-6 plays in obesity and insulin resistance remains controversial even after many years of research. Circulating levels of IL-6 are increased in obesity (Bastard, Jardel, Bruckert, et al, 2000), consistent with the view that obesity is characterized by a state of chronic low-grade inflammation (Wellen, & Hotamisligil, 2005). For this reason, it has been proposed that IL-6 contributes to the pathogenesis of insulin resistance in these disease states (Senn, 2002). However, based on basal studies, it is suggested that TNF-α and not IL-6 is the driving force behind insulin resistance. It is possible that selective secretion of IL-6 may even inhibit TNF-α induced insulin resistance. Muscle contractions are known to induce release of IL-6 but not TNF-α into
the circulation in both young and elderly humans (Pedersen & Febbraio, 2008). IL-6 has strong anti-inflammatory effects. IL-6 administration in humans induces the induction of IL-1ra, preventing signal transduction of IL-1. Furthermore, IL-6 stimulates release of soluble TNF receptors, but not IL-1β and TNF-α (Tilg, 2006). Early mediators of inflammatory activity are TNF-α and IL-6, which are tightly linked. TNF-α induces production of IL-6, which in turn inhibits TNF-α gene expression. Therefore, muscle derived IL-6 may mediate the beneficial metabolic effects of exercise and contribute by inhibiting TNF-α production and ultimately insulin resistance.

Although fat mass in kg, (p = 0.06) was not significantly reduced in this study, a trend towards significance was observed as well as a corresponding significant increase in DEXA fat free mass, kg (p = 0.03). The major source of systemic IL6 at rest is adipose tissue, and thus by reducing fat mass this could potentially reduce fasting plasma IL6. Following exercise, the high circulating levels of skeletal muscle IL-6 are followed by an increase in IL-1ra and IL-10, and the latter two anti-inflammatory cytokines can be induced by IL-6. Therefore, by increasing fat free mass, this could potentially increase skeletal muscle derived IL6 and inducing an anti-inflammatory environment. The 2 hr OGTT response was significantly decreased (p = 0.05) indicating better metabolic regulation to glucose and glucose induced inflammation. Other explanations of IL-6 response to exercise include genetic variants in the IL-6 gene (-174 G/C) which significantly modify changes in IL-6 serum concentrations in response to long-term exercise training programs, (Oberbach et al., 2008).
Conclusion

Postprandial hyperglycemia is an important risk factor for insulin resistance and the underlying mechanisms include glucose-mediated inflammation, the innate immune response and oxidative stress. In conclusion, this investigation demonstrates that seven days of aerobic exercise training is of sufficient length to produce changes to insulin sensitivity in obese humans with varying degrees of insulin resistance. Furthermore, these changes are associated with reductions in ROS and improvements in insulin sensitivity of peripheral tissues. However, because there were no changes in innate response, it is likely that a longer training duration greater than seven days might be required to produce these responses in obese sedentary humans. Therefore, there is an urgent need to understand the molecular mechanisms of exercise in regulating metabolic dysregulation especially in glucose-mediated activation of inflammatory pathways. Once elucidated, these findings could lead to development of more precise therapeutic interventions both preventatively and in the treatment of chronic diseases.

Limitations

Measurements were restricted to TLR2/TLR4, ROS, TNF-α, and IL-6. Although these are key factors in the regulation of inflammation, other proteins may be involved. Inflammation involves complex and coordinated immune responses to tissue damage. In the absence of tools to routinely assess inflammation within living tissues, measurements of humoral factors such as cytokines and other inflammatory mediators or markers can provide predictive clinical information and insights into disease mechanisms. ELISAs
were used in this study and provided measurement of single proteins in each sample, limiting the amount of information which could be obtained. Multiplex technologies which detect large numbers of proteins in a limited volume may have provided additional information to address the complexity of the inflammatory response.

**Delimitations**

The findings of the present investigation are delimited to 13 sedentary obese adult subjects. Thus, the data obtained from these investigations may not be fully representative of the overall human population. These data were obtained from a mononuclear cell (MNC) model of inflammation. The metabolic responses to other physiological stressors may vary significantly. The results of this study are delimited to 7 days post aerobic exercise, 24 hours post *ex vivo* endotoxin (LPS) induced MNC secretion of TNF-α and IL-6. The data collected in this study may vary significantly at other time points.
APPENDICES
APPENDIX A

TELEPHONE SCREENING SCRIPT
Appendix A

Telephone Screening Script

Effects of 7 Days Aerobic Exercise on Insulin Sensitivity, Oxidative Stress and Inflammatory Cytokine Secretion in Obesity

Subject Name: ___________________________ Date ____________

Hello, my name is _______________________ and I am calling from the Cleveland Clinic in regards to your interest in a research study involving the effects of exercise on insulin resistance, inflammation and Fatty Liver Disease. Is this correct?

YES \(\rightarrow\) Continue \quad NO \(\rightarrow\) Thank you for your time

This is a follow up call to see if you are still interested in being a research participant. If you are still interested then I will give you some additional information about the research study and I will also need to ask you a few questions. The questions are arranged so that if you are not eligible to participate you will be screened out as we go down the list of questions. This will save your time and avoid any inconvenience. All information collected and discussed during this conversation will be kept strictly confidential. It will take about 15 minutes to complete this initial screening. Do you have the time to continue now or would another time be more convenient for you?

YES \(\rightarrow\) Continue

NO \(\rightarrow\) Is there a more convenient time I can call you back? When?______________

First, I need to verify your contact information. (Verify contact info against what we have.)

1. Do you still live at (address)? ________________________________

2. Do you have an alternate telephone number you’d like to be contacted at (such as a cell or (work/home) phone)? __________________________

Now we need go over your health information to see if you qualify for the study and then I will briefly explain the study to you. Again, all health and personal information will remain confidential.
3. Date of Birth ___/___/___    Age _____ (must be 18-70 yrs)  
   Male/Female

4. Height ___________ inches _________ cm

5. Weight __________ lbs __________ kg  
   (At this point calculate BMI → must be 26 - 40 kg/m²)

6. Have you had any large fluctuations/changes in your weight over the last 6 months?  YES  NO

   NO → Continue to 7  
   YES → How much? ______________  If > 2 kg change, I’m sorry you are not eligible to participate, large weight changes are part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

7. Do you currently smoke?  YES  NO

   NO → Continue to 7a.  
   YES → I’m sorry you are not eligible to participate, smoking is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

7a. Have you been a non-smoker for at least 5 years?  YES  NO

   YES → Continue to 8.  
   NO → I’m sorry you are not eligible to participate, smoking in the past 5 yrs is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

8. Do you use alcohol?  YES  NO

   YES → Continue to 8a  
   If NO → Continue to 9.

8a. How often? ________________________  8b. What type?

**If more than 5/wk:** I’m sorry you are not eligible to participate, alcohol use is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO
9.  Do you exercise?  YES  NO

YES→ Continue to 9a  
NO → Continue to 10.

9a.  How often? ________________________  

9b.  Duration?

**If more > 20 min of ex, > 2 times/wk:** I’m sorry you are not eligible to participate, participants in this study must be sedentary. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

10.  Do you have diabetes?  YES  NO

YES → Continue to 10a.
NO → Continue to 11.

10a.  Is your diabetes currently being treated?  YES  NO

NO → Continue to 11.
YES→ What is your current treatment routine?______________________________

If current treatment consists of insulin, thiazolidinediones, metformin, acarbose, activity > 20 min, 2X/week, abnormal diet→ I’m sorry you are not eligible to participate, your (medication/activity level/diet) is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

11.  Has anyone in your family had Diabetes?  YES  NO

No → Continue to 12.
Yes → Who? _________________________

12.  Do you have, or have you ever had any of the following conditions?

No → Continue to 13.
YES→ I’m sorry you are not eligible to participate, _____ condition is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES  NO

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Disease/Failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke or Aneurism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacemaker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Disease/Cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung or Respiratory Disease</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Kidney Disease  Yes _____     No _____

Epilepsy  Yes _____     No _____

Thyroid Disease  Yes _____     No _____

Bleeding or Clotting Disorders  Yes _____     No _____

Immune Disorder  Yes _____     No _____

Cancer or Malignancy (in the past 5 yrs)  Yes _____     No _____

If yes, what kind: ______________________________

***Except for localized squamous or basal cell cancer treated with excision

Mental illness  Yes _____     No _____

Severe Depression  Yes _____     No _____

Claustrophobia  Yes _____     No _____

High blood cholesterol > 260 mg/dl  Yes _____     No _____     Don’t Know____

High blood triglycerides > 400 mg/dl  Yes _____     No _____

Hepatitis B or Hepatitis C virus  Yes _____     No _____     Don’t Know____

13. Have you had any operations/surgeries?     YES     NO

NO → Continue to 14.

YES → describe

Surgery     Date     Reason

_________________________________________________________________________

_________________________________________________________________________

_________________________________________________________________________

14. Are you currently taking any prescription medication?     YES     NO

No → Continue to 15.

Yes → describe

Medication     Dose/Time     Reason

_________________________________________________________________________

_________________________________________________________________________

(exclusions: amiodarone, methotrexate, perhexilene maleate, estrogens, tamoxifen, nifedipine, diltiazem, chloroquine, S-adenosylmethionine (SAM-e), Betaine, prednisone or other immunosuppressants)
15. Are you taking any over-the-counter medications or supplements such as a daily vitamin, herbals, cold medicine or allergy pill, laxatives, Aspirin or pain killer?  
YES   NO  

No → Continue to 16.  
Yes → describe  

<table>
<thead>
<tr>
<th>Name</th>
<th>Dose/Time</th>
<th>Reason</th>
</tr>
</thead>
</table>

(exclusions: vitamin E, fish oil or omega -3 fatty acid)  

16. Are you allergic to any medications or drugs?  
YES   NO  

NO → Continue to 17.  
YES → Specify allergy  

17. Do you have any other types of allergies?  
YES   NO  

NO → Continue to 18.  
YES → Specify allergy  

18. (Females Only) Are you postmenopausal?  
YES   NO  

18a. Are you on Hormone Replacement Therapy?  
YES   NO  

NO → Continue to 19.  
YES → I’m sorry you are not eligible to participate, HRT is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  
YES   NO  

19. (Females Only) Are you pregnant or nursing?  
YES   NO  

NO → Continue to 20.  
YES → I’m sorry you are not eligible to participate, pregnancy or nursing is part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  
YES   NO
20. Do you have any pieces of metal implanted or imbedded in your body such as, metal fragments in the eye, schrapnel, spinal nerve stimulator, dental braces, cochlear implant, or other medical implant incompatible with the magnetic field environment in magnetic resonance imaging?  

YES       NO

NO → Continue
YES → I’m sorry you are not eligible to participate, metallic implants or fragments are part of the exclusion criteria for this particular study. Would you mind if we held onto your name in the event you qualify for a future study?  YES     NO

From the initial answers you have provided, you are a likely candidate to qualify for the current study. Would you like to hear some additional information about participating in the study?

YES → Continue
NO → Thank you for your time

The purpose of the study is to evaluate the effect of 7 days of aerobic exercise on insulin resistance, oxidative stress and inflammation in obese patients. Obesity has been linked to insulin resistance and T2DM. Accumulation of fat can cause problems with controlling blood sugar levels and can affect fat metabolism as well. The outcomes of this study will provide valuable information on how exercise affects insulin resistance, oxidative stress & inflammation (cytokine secretion) and will provide new data for the development of future therapeutic interventions that target obesity & T2DM.

Before you begin the study you will need to come to the Clinical Research Unit (previously called the General Clinical Research Center, GCRC) at the Cleveland Clinic to complete the initial study paper work. At this time we will review the study again, in detail, answer any questions you may have, and have you sign an Informed Consent and HIPPA forms. After this is completed the study involves four separate phases that will take approximately 2 weeks to complete.

The first phase is the SCREENING phase, which will be performed over 1 to 3 days depending on your schedule. As part of the screening you will complete a series of questionnaires, a physical examination, medical history, resting electrocardiogram (ECG) and blood samples. These are the preliminary screening tests. If the results from these tests look ok, you will be eligible to progress to the next phase of the study.

Then we will measure your metabolic rate. This involves lying down while a clear plastic canopy is placed over your head. We will measure the oxygen and carbon
dioxide in your breath. We are then able to determine what source of energy your body prefers (carbohydrates, fats, or proteins) & to calculate your metabolic rate. The indirect calorimeter allows us to determine carbohydrate & fat oxidation rates only, therefore, we will need to collect a fasting urine sample in order to determine protein oxidation. The next test is an Oral Glucose Tolerance Test used to detect Diabetes. This test involves having you drink an orange flavored sugar drink and we will draw a small amount of blood every thirty minutes for 3 hours.

The second phase of the study will include BODY COMPOSITION ASSESSMENTS AND METABOLIC TESTS. We need to control your activity level, sleep and diet before we do the metabolic tests. In order to do this you will have to stay overnight at the Clinical Research Unit before the insulin/glucose test. The body composition and metabolic tests will all be performed on these days.

We will use 2 separate tests to measure your body composition. One of these tests will measure your percent body fat using a Dual Energy Absorptiometry (DEXA) scan: During this test, you will lay on your back on a table for 10-15 minutes while a scanner passes over your body. This will allow us to measure your total percent body fat.

The final body composition test is the waist to hip ratio test. We will perform three measurements using a tape measure to assess the circumference of your hips at the widest part of your buttocks. Then two more measurements will be performed just under the ribs and another measurement at the level of the belly button.

The last measurement during this phase is the exercise capacity test. This test will be used to evaluate your current level of physical fitness. You will be encouraged to walk on a treadmill for as long as you can, safely. During this test you will breathe into a mask and a tube (pneumotach) is connected to the mask so that all of the air you breathe in and out, will pass through the pneumotach and into a machine alongside the treadmill. The machine allows us to measure your oxygen consumption and provides a measure of your maximum oxygen capacity, called your VO$_{2\text{ max}}$, or maximum exercise capacity. During this test your heart’s activity will be monitored with an electrocardiogram (ECG) and blood pressure measurements will be taken frequently. You will walk on a treadmill, with the incline progressively increased, until fatigue, breathlessness, and/or symptoms indicate to the medical staff, or yourself, that you should stop the exercise. The test will be repeated during the post-testing period (approximately 9 days after the first VO$_{2\text{ max}}$ test) to evaluate the effects of the exercise program.

The third phase of the study involves the 7 days of exercise and diet monitoring. You will exercise for 50-60 minutes each day. The exercise will consist of walking/jogging on a treadmill and pedaling a stationary bike. The exercise sessions
will be supervised and conducted at the Cleveland Clinic Research Unit or the Fitness Center in the Walker Building. You will be instructed to keep your heart rate within a prescribed limit (85% of your heart rate maximum) and you will be provided with a heart rate monitor and watch to make it easy to check your heart rate while exercising.

The last phase of the study involves the post exercise program testing. At the end of the 7 days of exercise, you will be admitted to the CRU as an inpatient overnight. During this time, we will again control your diet and activity levels. You will then repeat the tests performed at the beginning of the program.

The metabolic tests (indirect calorimetry & the oral glucose tolerance test, OGTT) will take ≈ 4 hours. The OGTT measures your body’s ability to respond to sugar (glucose) or the ability to clear sugar/glucose from the blood. Insulin produced by your pancreas normally responds to glucose in your diet so the OGTT also measures your body's sensitivity to insulin. Blood samples are drawn every 30 min for 3 hours during the OGTT for the measurement of insulin and glucose. You will be asked not to restrict carbohydrate intake in the days or weeks before the test, & more specifically to consume a diet of at least 250 grams of carbohydrate prior to the metabolic testing. A sample diet will be provided & can be modified to your tastes.

I know this is a lot of information. I will send you copies of the Informed Consent for this study in the mail. The Informed Consent describes the study in more detail and you can read through it at your convenience. Do you have any questions at this time?

We will need to review the information you’ve provided. Someone will contact you within the next few days to follow-up and set up your first appointment. In the mean time, I will mail you a copy of the Informed Consent. You can read through it and make notes of any questions you may have for (name) when they contact you.

Here is our contact information: (screener’s name / telephone #). Thank you very much for your time and for your interest in these studies.
APPENDIX B

INCLUSION/EXCLUSION CRITERIA
Appendix B

Inclusion/Exclusion Criteria

**Inclusion criteria:**

Male and female patients between 20 to 70 years old.

A BMI between 30-40 kg/m$^2$.

Verbal and written fluency in English.

**Exclusion Criteria:**

Morbidly obese patients, defined by a BMI $\geq$ 40 kg/m$^2$ and lean patients, defined by a BMI $<$ 26 kg/m$^2$.

Evidence of Type 1 currently being treated by insulin.

Hypertriglyceridemic (>400 mg/dl) and hypercholesterolemic (>260 mg/dl) subjects will also be excluded, and referred for treatment.

Subjects must be weight stable (<2 kg weight change in past 6 months), and sedentary (< 20 min of exercise, 2 times per week).

History of past excessive alcohol drinking for a period longer than 2 years at any time in the past 10 years or current consumption of alcohol (>1 drink/day or >5 drinks/week).

Positive testing for hepatitis B surface antigen, hepatitis C virus antibody, or RNA of hepatitis C virus of DNA of hepatitis B virus.

Patients taking medications known to cause steatosis, including amiodarone, methotrexate, perhexilene maleate, estrogens, tamoxifen, nifedipine, diltiazem, or chloroquine.
Chronic (greater than 30 days) use of immunosuppressive medications including steroids in doses equivalent to 10 mg of prednisone or higher, 30 days prior and anytime during the course of the study.

Other causes of liver disease suspected by history, family interview, serologic tests including antinuclear, anti-smooth muscle, and anti-mitochondrial antibodies; iron transferrin saturation, ceruloplasmin, alpha-1 anti-trypsin levels; or evidence of cardiac failure.

Patients with cirrhosis defined by stage 4 fibrosis on liver biopsy, or if the patient shows unequivocal clinical evidence of portal hypertension, such as thrombocytopenia, splenomegaly, or esophageal varices.

Patients who are taking medications or dietary supplements shown to exert some benefits in improving fatty liver disease, including: Vitamin E, Betaine, fish oil, S-adenosylmethionine (SAM-e), thiazolidinediones, metformin and acarbose.

History of clinical manifestations of significant metabolic, hematological, pulmonary, ischemic or unstable heart disease, gastrointestinal, neurological (including recent cerebral hemorrhage), renal, urological, endocrine, ophthalmologic (including retinal hemorrhage) or immune mediated diseases that in the investigator’s opinion would interfere or alter the outcome measures, or impact subject safety.

Patients who have had a malignancy diagnosed and/or treated within the past 5 years, except for localized squamous or basal cell cancers treated by local excision, and those who have been adequately treated.
Severe psychiatric or neuropsychiatric disorders including severe depression, history of suicidal ideations, or suicide attempt(s).

Pregnant or nursing women.

Patients that have any metal in their body, such as a fragment in their eye, aneurysm clip, spinal nerve stimulator, or a pacemaker, dental braces, cochlear implant, or other medical interventions incompatible with the magnetic field environment in magnetic resonance imaging.
Appendix C

Informed Consent

KSU/Cleveland Clinic
Informed Consent to Participate in a Research Study

Study title: Effects of Short Term Aerobic Exercise Training on Insulin Resistance and Inflammatory Cytokine Secretion in Nonalcoholic Fatty Liver Disease (NAFLD)

The purpose of the informed consent is to provide you with information to help you decide whether you wish to participate in this research study. Your decision is completely voluntary and will not affect your medical care if you choose not to participate. It is important for you to understand the research risks and benefits, be able to ask questions and understand alternatives. Please carefully review this document.

This is a research study, not standard treatment
Your participation is completely voluntary
You must carefully weigh the risks and benefits of participating in this research

1. INFORMATION ON THE RESEARCH

Why Are You Being Asked To Take Part In This Research?

You are being invited to participate in a research study because you have Nonalcoholic Fatty Liver Disease (NAFLD) which is a condition of excess fat accumulation in your liver caused by being overweight and/or obese and lack of regular exercise. Before you can decide whether or not to volunteer for this study, you must understand the purpose of this study, how this study may help you, any risks to you, and what is expected of you. This information gathering process is called informed consent. The study is being conducted through the Departments of Gastroenterology and Hepatology, at the Cleveland Clinic.

Why Is This Study Being Done?

The purpose of the study is to evaluate the effects of a short term exercise (7 consecutive days) intervention on insulin resistance and inflammation in Nonalcoholic Fatty Liver Disease (NAFLD), a disease that is the result of excess fat accumulation in your liver.
How Many People Will Take Part In The Study?

Approximately 12 sedentary overweight/obese adults will participate in this study.

What Is Involved In The Study?

Phase 1 (Screening)

These tests may be performed over 1 to 3 days depending on your schedule. The order of these tests may vary. As part of the screening you will complete a physical examination, medical history, resting electrocardiogram (ECG, a non-invasive measure of the electrical activity of your heart), blood sample (13 cc, or 2½ teaspoons), and an exercise stress test (all procedures are described below).

Medical History and Physical (approximately 1 hour):

You will have a medical history and physical exam, and a resting electrocardiogram.

Questionnaires (30 minutes): You will be asked to complete 5 questionnaires, described below:

Medical History (a checklist about current and past medical information)

NMR procedure Screening Form (a checklist about any metal that may be implanted into your body)

Minnesota Leisure Time Physical Activity (a checklist of 60 physical or recreational activities you may have participated in over the last 12 months)

Seven Day Physical Activity Recall (9 questions about your sleep and physical activity in the past week)

24 Hour Diet Record (you will be asked to record the amount and time of foods and beverages consumed over a 24–hour period of time, then repeat for a total of 3 days)

Urine Pregnancy Test (if you are female between the ages of 40 and 60 years olds)

Exercise Stress Test (approximately 1 hour):

This test evaluates whether your heart is healthy and safe for exercise. You will be encouraged to exercise as hard as you can, but safely. During this test, adhesive pads will be attached to your chest and leads (wires) will be connected to a machine that measures the electrical activity in your heart. The procedure is generally painless. Males that are
particularly “hairy” in the chest area may need to have their chests shaved in order to ensure proper conductivity of the leads.

Next, you will walk on a treadmill, with the incline (slope/angle) progressively increased, until fatigue, breathlessness, and/or symptoms indicate to the medical staff, or yourself, that you should stop the exercise.

**Phase 2 (Diet / Weight Stabilization and Pre-Program Testing)**

If the cardiologist from this research study considers your heart healthy to exercise, you will be eligible to participate in the study. These tests will be performed once before and once after the diet and exercise program. During this period we will control your diet and activity levels. To control for diet and physical activity, you will be instructed on how to follow a weight maintenance diet for a 2-week period and to maintain your normal daily activities. During the last day of this period, you will be admitted as an inpatient and reside in the CRU for an overnight stay (arrive before 5 pm). Also during this time you will be asked to complete a series of tests that include: (1) **Body Composition Assessment**, (2) **Exercise Capacity Test**, and (3) **Metabolic Tests**.

1. **Body Composition:**

**Waist to Hip Ratio (WHR):** To determine if you have a healthy waist to hip ratio, we will perform three measurements using a tape measure to assess the circumference of your hips at the widest part of your buttocks. Then two other measurements will be performed just under the ribs (natural waist) and at the level of the belly button (umbilicus).

The accumulation of body fat in specific body regions has been linked to health problems. For example, people with "apple-shaped" bodies (more weight around the waist) face more health risks than people with "pear-shaped" bodies who carry more weight around the hips.

**Computer Tomography (CT) scan:** We will measure the amount of fat distributed around your waist using a CT scan: This test is similar to an x-ray and involves a small amount of radiation exposure. During this test you will lie on your back and a picture will be taken of your abdomen. This picture will allow us to measure the fat inside your abdomen. This test will take about 1 hour.

**Proton-Nuclear Magnetic Resonance Spectroscopy (NMR):** We will also measure the amount of fat within your liver using Proton-Nuclear NMR: This is similar to the CT scan but uses a different piece of equipment that does not involve radiation. You will lie on your back and separate pictures will be taken of your liver. This will allow us to measure the amount of fat within the liver. This test will take about 1 hour. This
particular test is unlike a typical NMR in that it uses different methods to look at the body’s internal organs. This test is being done for research purposes. It is not a diagnostic imaging examination.

**Dual Energy Absorptiometry (DEXA):** This test is used to measure the amount of fat distributed throughout your whole body and will allow us to measure your percent body fat. During this test, you will lay on your back on a table for 10-15 minutes while a scanner passes over your body. This test also involves a very small amount of radiation exposure.

**2. Weight Stabilization Diet:**

Before beginning the exercise program you will be provided with instruction on how to follow a weight maintenance diet for two weeks. In the last three days of the diet and activity standardization period you will be asked to eat approximately the same amount of calories as your normal everyday diet with only two exceptions. You cannot drink alcohol 48 hours prior to any of the metabolic tests and cannot eat or drink foods that contain caffeine for at least 12 hours prior to testing. Common foods containing caffeine include: coffee, tea, soda and chocolate. If you have questions on foods containing caffeine, please ask one of the team members prior to your diet standardization period. In addition, we ask that you refrain from any structured exercise for 24 hours prior to testing and limit exercise to your normal everyday activities.

**3. Exercise Capacity Test:**

This test is used to evaluate your current level of physical fitness. You will be encouraged to walk on a treadmill for as long as you can, safely. During this test you will breathe into a mask with a tube attached (pneumotach) so that all of the air you breathe in and out will pass through the pneumotach and into a machine alongside the treadmill. The machine allows us to measure your oxygen consumption and provides a measure of your maximum oxygen capacity ($\text{VO}_2\text{max}$), or maximum exercise capacity. During this test your heart’s activity will be monitored with an ECG (as described above) and blood pressure measurements will be taken frequently. You will walk on a treadmill, with the incline progressively increased, until fatigue, breathlessness, and/or symptoms indicate to the medical staff, or yourself, that you should stop the exercise. The test will be repeated during the post-testing period (≈ 9 days after the first test.) to evaluate the effects of the exercise program. The pictures below show what a typical test looks like.
4. Metabolic Tests

**Oral Glucose Tolerance Test (approximately 4 hours):**

You will be asked to come to the Clinical Research Unit (Unit M51) on the morning of the test. You will need to be fasted at the time of your arrival, which means nothing to eat or drink (except for sips of water) for at least 10 hours before the test. This is a standard 2-hour test that is used to detect diabetes mellitus. An indwelling plastic catheter (needle) will be placed in a vein in your arm. This needle will remain in place for about 3 hours. Once the needle is in place, an initial blood sample will be taken. This sample will tell us your fasting blood glucose (sugar) level, calcium, phosphorus, cholesterol and triglyceride (fats) levels, your complete blood count, and your thyroid (a gland in your neck) function.

Once this sample has been obtained, you will drink an orange flavored beverage that contains a known amount of glucose (sugar). Over the next 3 hours, we will measure your body's tolerance to the glucose (oral glucose tolerance test, OGTT). Blood glucose and insulin levels will be measured again at ½, 1, 1½, 2 and 3 hours. The amount of blood that will be obtained for this test will be approximately 70 cc (or 14 teaspoons). During this test you will complete questionnaires to assess your diet and activity levels over the previous 12 months.
Indirect Calorimetry (approximately ½ hour):

This test will calculate your resting metabolic rate (the amount of energy that your body requires over 24 hours while at rest). We will place you in a hospital bed or a reclining chair in the CRU. Once you are comfortable, a clear plastic hood with two tubes will be placed over your head. One tube allows fresh air to flow in while another tube sends the air that you breathe out into an instrument that measures oxygen and carbon dioxide. We will ask you to breathe normally under the hood for approximately 15-20 minutes. A computer then analyzes your oxygen and carbon dioxide readings and calculates your resting metabolic rate. This will allow us to determine your body’s fuel preference. The following photo shows the test device:

![Indirect Calorimetry](image)

Urine collection: Urine will be collected from 04:00 am until completion of the calorimetry measures. Total volume and time of urine collection are recorded and analyzed for urea nitrogen and subsequent protein oxidation.

Phase 3 (Exercise Intervention)

You will meet with a member of the research staff at the CRU for approximately 1 hour once before you begin your exercise program to familiarize yourself with the facility, the exercise equipment and the goals of the study (exercise prescription). You will also have time during the study or after the study is completed to ask any questions about continuing your exercise program or to share any “likes” or “dislikes” of the study.

Exercise Intervention:

During the 7-day program you will exercise for 7 consecutive days for 50-60 minutes each day. The exercise will consist of walking/jogging on a treadmill or pedaling a stationary bike. Most of the exercise sessions will be conducted at the Fitness Center in
the Walker Building, while the exercise stress test, and maximum aerobic capacity tests will be conducted at the CRU at the Cleveland Clinic.

You will be instructed to keep your heart rate within a prescribed limit (85% of maximum) and you will be provided with a heart rate monitor and watch to make it easy to check your heart rate. You should notify the investigators if there is any change in your medications (prescription, over-the-counter, herbal products) during the study period.

**Phase 4 (Post-Program Testing)**

At the end of the 7-day Exercise Program you will be admitted as an inpatient to the CRU overnight. During this time we will again control your diet and activity level. You will then repeat the tests performed at the beginning of the program.

**How Long Will You Be In The Study?** You will be in the study for \( \approx 2 - 3 \) weeks.

**Study Schematic:**

```
<table>
<thead>
<tr>
<th>Health and Physical</th>
<th>Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting 12 lead ECG</td>
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<tr>
<td>Graded Exercise Test</td>
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<tr>
<td>Blood Chemistries</td>
<td></td>
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<tr>
<td>Nutritional Counseling</td>
<td></td>
</tr>
<tr>
<td>Diet/Activity Questionnaires</td>
<td></td>
</tr>
</tbody>
</table>

| OGGT, Urine, CT               | Baseline Testing   |
| Body Composition              |                    |
| NMR, DEXA, WHR                |                    |
| Indirect Calorimetry          |                    |
| VO_{2max}                     |                    |

| Aerobic Exercise Training     | Intervention       |
| Diet/Activity Questionnaires  |                    |

| OGGT, Urine, CT               | Post Intervention testing |
| Body Composition              |                         |
| NMR, DEXA, WHR                |                         |
| Indirect Calorimetry          |                         |
| VO_{2max}                     |                         |
2. RISKS AND DISCOMFORTS

What Are The Risks Of The Study?

Your participation in this study may involve the following risks:

Venipuncture/catheter placement (blood sampling): When a needle is inserted into a vein there will be some temporary pain and possible bruising. Infection is very rare as your skin is cleansed prior to needle insertion and only sterile needles are used. Some people also feel dizzy when they have their blood drawn.

Pregnancy Test (Urine HCG): There are no known risks related to testing your urine.

Oral Glucose Tolerance Test: The sugar drink tastes very sweet and it can make some people feel nauseated or even vomit.

Exercise Testing on the Treadmill: You might experience fatigue or shortness of breath. You might also feel like your heart is pounding very fast or very hard and experience dizziness or chest pain. You could stumble and fall off the treadmill. If you have any of these experiences, tell the research team. Also, when the mask is on your face, this might feel uncomfortable.

Indirect Calorimetry: For some people, the plastic canopy over their heads makes them feel claustrophobic or anxious. This feeling is temporary and will go away when the canopy is removed. The canopy is “see through” and does not restrict movement.

Cumulative Radiation Exposure: This research study involves exposure to radiation from CT and DEXA scans. The amount of radiation exposure is equivalent to 3% of the exposure doses for clinical determinations, and is within the guidelines recommended by the Food and Drug Administration for radioactive research.

CT Scan: This technology uses radiation to take a picture of whatever is being scanned. The amount of radiation you will be exposed to in the CT scan is about what you receive naturally over the course of a year in the general environment. Such doses of radiation may be potentially harmful, but the risks are so small that they are difficult to measure. If you have already had many x-rays, you should discuss the potential added risk from more radiation with the researchers before agreeing to be in the study.

DEXA Scan: You will be exposed to a small amount of radiation. The amount is similar to that which a person living in Ohio, would be exposed to from natural sources over a 2-month period. Such doses of radiation may be potentially harmful, but the risks are so small that they are difficult to measure. If you have already had many x-rays, you should discuss the potential added risk from more radiation with the researchers before agreeing
to be in the study.

**NMR Scan:** Because the NMR machine is a large magnet, it can move iron-containing objects in the room during your examination. Unexpected movement of such objects could possibly harm you. Precautions have been taken to minimize such an event from happening. Loose metal objects, like pocketknives or key chains, are not allowed in the NMR room. If you have a piece of metal in your body, such as a fragment in your eye, aneurysm clips, ear implants, spinal nerve stimulators, or a pacemaker, dental braces, or a cochlear implant, you cannot have an NMR. You must inform the research team if you have any of these implants in your body. In addition there is a small risk of a claustrophobic reaction during the procedures.

**ECG:** A small amount of adhesive might remain on your skin when the pads are removed or the adhesive might pull on hair in the area. The adhesive is easily removed with alcohol or soap and water.

**Questionnaires:** You might find it boring or time-consuming to complete the questionnaires. Also, some of the questions might be stressful. You don’t have to answer any question that makes you feel uncomfortable.

**Exercise Program:** You might find it taxing or burdensome to have to come to the Cleveland Clinic for 7 consecutive days to do the exercise program. You might have to make special arrangements with work or family to have the time off to participate. The exercise might make you tired and cause temporary muscle soreness. You could also feel dizziness, shortness of breath or have chest pain, abnormal blood pressure or abnormal heart rhythms while exercising. You could stumble and fall off the treadmill or bike. If you have any of these experiences, tell the research team immediately.

Additionally, there may be risks that we do not know about.

3. BENEFITS

**Are There Benefits To Taking Part In The Study?**

Benefits include obtaining knowledge of current health status as determined by medical history and physical examination, assessment of current physical fitness status, improved knowledge of exercise and access to personal data collected during the study. Your glucose tolerance may improve as a result of participating in this study and your liver fat may be reduced, but these changes cannot be guaranteed.

Societal benefits may include new knowledge about the immediate effects of exercise on control of blood sugar. Information from this study may help us learn the most effective exercise protocol to prevent the development of, and/or help, in the treatment of insulin
resistance or type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD).

4. ALTERNATIVES

What Other Options Are There?

This is a research project. You may decline to participate. The procedures described in this consent form are not needed for your medical care and they do not treat or cure diabetes, obesity, and/or fatty liver disease. We do not guarantee that you will lose weight if you participate in this research study.

5. PRIVACY AND CONFIDENTIALITY

Will Your Information Be Kept Private?

The medical and research information recorded about you will be used within the Cleveland Clinic and/or disclosed outside the Cleveland Clinic as part of this research. Tests and procedures done solely for this research study may be placed in your medical record to indicate your participation in this study. Upon completion of the study, you may have access to the research information if contained in the medical record.

Your access to research information about you will be limited while the study is in progress. Preventing this access during the study keeps the knowledge of study results from affecting the reliability of the study. This information will be available should an emergency arise that would require your treating physician to know this information to treat you best.

Your research information may be disclosed to the National Institutes of Health, the research study Sponsor and its agents, the Cleveland Clinic research review staff, the U.S. Food and Drug Administration, and other outside collaborators or laboratories that are participating in this study, if any, that are listed as follows: Department of Health and Human Services Agencies, National Committee for Quality Assurance. The Cleveland Clinic also may use and disclose this information for treatment and payment reasons. The Cleveland Clinic must comply with legal requirements that mandate disclosure in unusual situations. Otherwise, the information recorded about you as part of this research will be maintained in a confidential manner. It is possible that information disclosed about you outside the Cleveland Clinic could be re-disclosed and no longer protected by federal privacy laws.

Your research information may be used and disclosed indefinitely, but you may stop the use and disclosure of personal information at any time by writing to John Kirwan, PhD at Cleveland Clinic, 9500 Euclid Avenue, Cleveland, Ohio 44195. If you do so, any
information previously disclosed cannot be withdrawn. The Cleveland Clinic will not use or disclose the information collected in this study for another research purpose without your written permission; unless the Cleveland Clinic Institutional Review Board gives permission after ensuring that appropriate privacy safeguards are in place. The Institutional Review Board is a committee whose job is to protect the safety and privacy of research subjects.

If you choose not to sign this consent form, you will not be permitted to participate in this research study.

6. RESEARCH RELATED INJURIES

What Happens If An Injury Occurs?

If physical injury occurs due to your involvement in this research, medical treatment is available, but your medical insurance must pay the cost of treatment. Such medical treatments that are not covered by your medical insurance shall not be paid by the sponsor, the National Institutes of Health. Compensation for lost wages and/or direct or indirect losses are not available. The Cleveland Clinic will not voluntarily provide compensation for medical expenses or any other compensation for research-related injuries. Further information about research-related injuries is available by contacting the CCF Institutional Review Board at (216) 444-2924.

7. COSTS

What Are The Costs?

There is no cost to you or your insurance company for participation in this study.

The sponsor will pay for the procedures and extra study specific tests that are not routine and only being performed because you are participating in this study. The Cleveland Clinic will not pay for the costs of procedures, tests, visits and hospitalizations in connection with this study.

For your participation in this study you will be compensated $150.00.
8. VOLUNTARY PARTICIPATION

What Are Your Rights As A Participant?

Taking part in this study is completely voluntary. You may choose to leave the study or decline participation at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

9. QUESTIONS

Whom Do You Call With Questions Or Problems?

If you have any questions about the research or develop a research-related problem, you should contact:

John Kirwan, PhD at 216-444-3412
Jacob Haus, PhD 216-445-4864
Amy Patrick-Melin, MEd 216-444-8460
After hours: Call GI Fellow at 216-444-2000 pager #25555

If you have questions about your rights as a research subject, you should contact the Cleveland Clinic Institutional Review Board at (216) 444-2924.

Cleveland Clinic Clinical Research Unit (CRU) study participants may also contact the CRU Research Subject Advocate (RSA), Carmen Paradis, MD at 216-445-2767 or 216-444-2200 and ask the operator to page beeper #28848 with regard to questions about study participation and research subject protections.
10. SIGNATURE

I have read the informed consent (the above information) and I have had the informed consent verbally explained to me. I have had all my questions answered to my satisfaction. I understand that my participation is completely voluntary and that I may decline my participation at any time during the course of the study. Signing this form does not waive any of my legal rights. I understand that a copy of this consent will be provided to me. By signing below, I agree to take part in this research study.

____________________________________  __________________________________________
Subject Signature                   Date

____________________________________
Printed name of Subject

Statement of Person Conducting Informed Consent Discussion

I have discussed the above points with the subject. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

____________________________________  __________________________________________
Signature of person obtaining consent  Date

____________________________________
Printed name of person obtaining consent
APPENDIX D

PAST MEDICAL HISTORY QUESTIONNAIRE
Appendix D

Past Medical History Questionnaire

Study Title: Effects of Short Term Exercise Training on Insulin Resistance, Oxidative Stress and Inflammatory Cytokine Secretion in Obesity

The following questions are designed to obtain a thorough medical history. The information you provide will help us to make the best determination about your eligibility for a particular study. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide, will be kept confidential and will not be shared with any unauthorized person or organization unless you specifically request us to do so.

Name: ____________________________________________

Date Completed: _________________________________
Name: ____________________________________________
Street Address: ____________________________________________
City, State, Zip: ____________________________________________
Telephone number: Home (     )__________ Work (     )______________
Date of Birth: __________   Age: _____
   mm-dd-yy
Sex:   M _____ F _____
Personal Physician's Name: ___________________________ Phone___________
          Address:_________________________________________________
                           ____________________________________________
Height: estimated _____ in    measured _____ in    Date measured___________
Weight: estimated _____ lb    measured _____ lb    Date measured___________

Education

Last grade completed in elementary or high school: ____________

Education completed since leaving elementary or high school:

______ None
______ Vocational School
______ Community or Junior College
______ Four-Year College
______ Graduate School
______ Professional School
**Occupation**

Current occupation or if retired what was your occupation at retirement:

__________________________

**Marital Status**

- Married
- Divorced
- Widowed
- Single, never married
- Separated

**Living Situation**

- Alone
- With family member(s)
- With non-family members(s)

**Do you have any pets?** No _____ Yes _____ (please describe)

__________________________

**Race (optional)**

__________________________

**Personal Health History**

Have you ever been hospitalized or had surgery? Yes _____ No _____

Please list all hospitalizations and surgeries to the best of your recollection

<table>
<thead>
<tr>
<th>Disease/Operation</th>
<th>Duration</th>
<th>Age when</th>
<th>Hospitalized</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
List any disease or illness you have had not listed above (e.g., mumps, measles, broken bones, etc.)

____________________________________________________________________________________

____________________________________________________________________________________

Are you allergic, sensitive, or intolerant of any foods or nutritional supplements, products, or substitutes? Yes___ No___

If yes, please describe: ________________________________________________________________

Are you allergic, sensitive, or intolerant of any medications? Yes___ No___

If yes, please describe: ________________________________________________________________

Are you allergic, sensitive, or intolerant of local anesthetic (pain killing medications; e.g., Novocaine, Lidocaine or Xylocaine)?
Yes___ No___

If yes, please describe: ________________________________________________________________

Have you ever received an injection of a local anesthetic (pain killing medication; e.g., Novocaine, Lidocaine, or Xylocaine). Yes___ No___

Are you allergic, sensitive, or intolerant of latex? Yes___ No___

Are you allergic, sensitive, or intolerant of any kind of tape or adhesive? Yes___ No___

Are you currently seeing a doctor or other health care provider for any reason?

Yes______ No______

If yes, please explain:

____________________________________________________________________________________

____________________________________________________________________________________
Do you have, or have you ever had any of the following conditions?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteration of your ability to remember</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurring headaches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent changes in your vision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numbness of an arm or leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakness of an arm or leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulty in speaking or slurred speech</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fainting or dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulty in walking (staggering)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung or Respiratory Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatism or arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding or clotting disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk for infectious diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(AIDS, IV drug use, blood transfusions, hemophilia, hepatitis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin: rashes, lumps, moles, itching, eczema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose, sinuses: frequent colds, sinus trouble</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nose-bleeds, deviated septum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck lumps, swollen glands, pain or stiffness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Breasts: lumps, nipple discharge, pain or discomfort
Yes ____  No ____

For women: Date of last mammogram ___________

High blood cholesterol
Yes ____  No ____
Date of last reading ______  Value ______

Stomach: chronic indigestion, ulcer, hiatal hernia, heartburn, trouble swallowing, vomiting.
Yes ____  No ____

Intestine: constipation, diarrhea, change in bowel habits, irritable bowel disorder, colitis, polyps.
Yes ____  No ____

Rectum: hemorrhoids, bleeding, polyps
Yes ____  No ____

Liver, gallbladder: hepatitis, gallstones
Yes ____  No ____

Urinary: frequent urination, urgency, burning, pain, blood in urine, infection, kidney stones
Yes ____  No ____

Incontinence: Loss of bladder or rectal control
Yes ____  No ____

Do you use adult undergarment products (e.g. Depends)?
Yes ____  No ____

Have you ever had any form of cancer, skin or other?
Yes ____  No ____
If yes, what kind: ______________________________

Do you have diabetes mellitus (high blood sugar)?
Yes ____  No ____
If yes, when and what kind of treatment did/do you receive:
Insulin _____  Diet _____  Pills _____  No treatment _____

Is there a family history of diabetes mellitus?
Yes ____  No ____

Have you ever had or been told that you had high blood pressure?
Yes ____  No ____
If yes, when and what kind of treatment or medicine did/do you receive:
Do you have any chronic illnesses?  
Yes _____  No _____
If yes, please explain:

List all the prescribed medications you are currently taking:

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Reason for Medication</th>
</tr>
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</tbody>
</table>

List all the over-the-counter medications you are currently taking:

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Reason for Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

Do you have any problems with constipation or diarrhea?  Yes _____  No _____
If so, how do you usually remedy it?

For Females Only

Do you or have you ever had menstrual problems, vaginal discharge, irregular bleeding, sexually transmitted illness.

Yes _____  No _____
If yes, please explain:

Age of menopause (if applicable): ________________
Type of menopause: _____ natural    _____ surgical    _____ other

Estrogen replacement: Yes _____ No _____

If yes, please explain:

______________________________________________________________

Last PAP smear: ______________________________________________

Number of pregnancies: _________________________________________

Number of births: _____________________________________________

Type of delivery: ______________________________________________

Your age at time of birth(s):_____________________________________

Date of Hysterectomy (if applicable): ______________________________

Reason: _______________________________________________________

**For Males Only**

Have you ever had prostate problems, hernias, testicular pain, lumps, discharge from or sores on penis, sexually transmitted illness.

Yes _____ No _____

If yes, please explain:

______________________________________________________________

**Dietary Information**

Are you currently taking any vitamins, minerals or health food supplements at least once per week on a regular basis? Yes _____ No _____

If yes, please describe:

_____________________________________________________________
Would you be willing to stop your vitamins, minerals or health food supplements if needed while participating in a study?  Yes _____ No _____

Are you currently following a special diet (i.e., vegetarian, diabetic, low fat, lactose free)?

Yes _____  No _____  If yes, what kind?__________________________________________

Has this diet been prescribed by your health care provider?  Yes ____ No _____

If accepted for a study, are you willing to follow a diet that may vary from your current food intake?

Yes _____ No _____

Have you had a weight loss or gain in the last 6 months?  Yes _____ No _____

If yes, how much?   _____lbs.  Gain _____ Loss _____ (check one)

How do you describe your appetite?               Poor _____ Fair _____ Good _____

When required during the study, would you be willing and able to eat only the items provided by the research team?

Yes _____ No _____

Do you have any food allergies/intolerance?  Yes _____ No _____

If so, please explain:

Do you drink caffeinated beverages? (coffee, tea, soda)  Yes _____ No _____

If yes, how many caffeinated beverages do you drink in an average day?  _______/day
If required during a study, would giving up caffeine cause any problems for you?

Yes _____ No _____

Do you drink alcoholic beverages? Yes _____ No _____

If yes, how many alcoholic beverages do you consume in an average week? _____/wk

Can you forego drinking alcoholic beverages for the duration of a research study?

Yes _____ No _____

Do you have a problem drinking milk or eating dairy products?

Yes _____ No _____

If yes, what happens?

________________________________________________________________________________

**Exercise History**

Do you participate in a regular exercise program? Yes _____ No _____

If yes, please describe the exercise that you usually participate in (e.g., walking, running, weightlifting).

________________________________________________________________________________

________________________________________________________________________________

If you are not currently participating in a regular exercise program, have you participated in one in the past? Yes _____ No _____

If yes, when was the last time you participated in the exercise on a regular basis?

Could you please describe the type of activity that you performed (e.g., walking, running, weightlifting).

________________________________________________________________________________
How often did you exercise (days/week)? ______

At what intensity did you exercise?  Light ______
     Moderate ______
     Hard ______

On days that you did exercise, how long did you usually exercise for (hours)?______

Have you ever participated in competitive sports (e.g., football, basketball)? Yes _____
   No _____

If yes, when was the last time that you participated in this sport? ______

Could you please list the sport that you played and briefly describe your role (e.g.,
distance runner, outfielder, linebacker).

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
APPENDIX E

7 DAY PHYSICAL ACTIVITY RECALL
Appendix E

7 Day Physical Activity Recall

Now we would like to know about your physical activity during the past 7 days. But first let me ask you about your sleep habits.

On the average, how many hours did you sleep each night during the last five weekday nights (Sunday-Thursday)?
  _______ hours

On the average, how many hours did you sleep each night last Friday and Saturday nights?
  _______ hours

Now I am going to ask you about your physical activity during the past 7 days, that is the last 5 weekdays, and last weekend, Saturday and Sunday. We are not going to talk about light activities such as slow walking, light housework, or unstrenuous sports such as bowling, archery, or softball. Please look at this list which shows some examples of what we consider moderate, hard, and very hard activities (Interviewer: hand subject card No. 9 (and allow time for the subject to read it over). People engage in many other types of activities, and if you are not sure where one of your activities fits, please ask me about it.

First, let’s consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour
  _______ hours

Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Probe: Can you think of any other sports, job, or household activities that would fit into this category?)
  _______ hours

Now, let’s look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.
  _______ hours
Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

______ hours

Now, let’s look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

______ hours

Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Probe: Can you think of any other sports, job, or household activities that would fit into this category?)

______ hours

Compared with your physical activity over the past 3 months, was last week’s physical activity more, less, or about the same?

_____ 1 More  _____ 2 Less  _____ 3 About the same

Interviewer: Please list below any activities reported by the subject which you don’t know how to classify.

<table>
<thead>
<tr>
<th>Activity (brief description)</th>
<th>Hours: Workday</th>
<th>Hours: Weekend day</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

Initials:______
Examples of Activities in Each Category

**Moderate activity**

Occupational tasks:
1) delivering mail or patrolling on foot;
2) house painting; and
3) truck driving (making deliveries, lifting and carrying light objects)

Household activities:
1) raking the lawn;
2) sweeping and mopping
3) mowing the lawn with a power mower; and
4) cleaning windows

Sports activities (actual playing time): 1) volleyball; 2) Ping-Pong; 3) brisk walking for pleasure or to work (483 km/hr (3 miles/hr) or 20 min/km (mile)); 4) golf, walking and pulling or carrying clubs; and 5) callisthenic exercises

**Hard activity**

Occupational tasks: 1) heavy carpentry; and 2) construction work, doing physical labor

Household tasks: 1) scrubbing floors

Sports activities (actual playing time): 1) tennis doubles; and 2) disco, square, or folk dancing

**Very hard activity**

Occupational tasks: 1) very hard physical labor Digging or chopping with heavy tools; and 2) carrying heavy loads such as bricks or lumber

Sports activities (actual playing time): 1) jogging or swimming; 2) singles tennis; 3) racquetball; and 4) soccer
APPENDIX F
MINNESOTA LEISURE TIME ACTIVITY QUESTIONNAIRE
Appendix F

Minnesota Leisure Time Activity Questionnaire

Name_______________________________ Date________________

Listed below are a series of Leisure Time Activities. Related activities are grouped under general headings. Please read the list and check ‘Yes’ in column 2 for those activities which you have performed in the last 12 months, and ‘No’ in column 1 for those you have not. Do not complete any of the other columns.

<table>
<thead>
<tr>
<th>Did you perform this activity</th>
<th>Total # of Days = _________</th>
<th>Avg # / mo</th>
<th>Times per occasion</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td>J</td>
<td>F</td>
<td>M</td>
</tr>
</tbody>
</table>

SECTION A: Walking and Miscellaneous

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking for pleasure</td>
<td>35</td>
</tr>
<tr>
<td>Walking to and from work</td>
<td>40</td>
</tr>
<tr>
<td>Walking during work break</td>
<td>35</td>
</tr>
<tr>
<td>Using stairs instead of elevator</td>
<td>80</td>
</tr>
<tr>
<td>Cross-country hiking</td>
<td>60</td>
</tr>
<tr>
<td>Back-packing</td>
<td>70</td>
</tr>
<tr>
<td>Mountain climbing</td>
<td>80</td>
</tr>
<tr>
<td>Bicycling to work and/or for pleasure</td>
<td>40</td>
</tr>
<tr>
<td>Ballroom &amp;/or Square Dancing</td>
<td>55</td>
</tr>
</tbody>
</table>

SECTION B: Conditioning Exercises

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home exercise</td>
<td>45</td>
</tr>
<tr>
<td>Health club</td>
<td>60</td>
</tr>
<tr>
<td>Jogging and walking</td>
<td>60</td>
</tr>
<tr>
<td>Running</td>
<td>80</td>
</tr>
<tr>
<td>Weight Lifting</td>
<td>30</td>
</tr>
</tbody>
</table>

Initials: ___
<table>
<thead>
<tr>
<th>Did you perform this activity</th>
<th>Total # of Days = _________</th>
<th>Month of Activity</th>
<th>Avg # / mo</th>
<th>Times per occasion</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td>J</td>
<td>F</td>
<td>M</td>
<td>A</td>
</tr>
</tbody>
</table>

**SECTION D: Water Activities**

- Water skiing
- Sailing
- Canoeing-pleasure
- Canoeing-competition
- Canoeing-camping
- Swimming (at least 50ft) in pool
- Swimming at the beach
- Scuba diving
- Snorkeling

**SECTION D: Winter Activities**

- Snowskiing: downhill
- Snowskiing: x-country
- Ice or roller skating
- Sledding / Tobogganing

**SECTION E: Sports**

- Bowling
- Volleyball
- Table tennis
- Tennis, singles
- Tennis, doubles
- Softball
- Badminton
- Paddleball
- Racquetball
- Basketball – non-game
- Basketball – game-play
- Touch football

Intials: _____
<table>
<thead>
<tr>
<th>Did you perform this activity</th>
<th>Total # of Days =</th>
<th>Month of Activity</th>
<th>Avg # / mo</th>
<th>Times per occasion</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
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<td>O</td>
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<td>N</td>
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<tr>
<td>D</td>
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<td></td>
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<tr>
<td>Hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Section E: Sports (Continued)**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity Code</th>
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</thead>
<tbody>
<tr>
<td>Handball</td>
<td>120</td>
</tr>
<tr>
<td>Squash</td>
<td>120</td>
</tr>
<tr>
<td>Soccer</td>
<td>70</td>
</tr>
<tr>
<td>Golf – with cart</td>
<td>70</td>
</tr>
<tr>
<td>Golf – with pull cart</td>
<td>50</td>
</tr>
</tbody>
</table>

**SECTION F: Lawn and Garden Activities**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mowing lawn with riding mower</td>
<td>25</td>
</tr>
<tr>
<td>Mowing lawn pushing power mower</td>
<td>45</td>
</tr>
<tr>
<td>Mowing lawn pushing hand mower</td>
<td>60</td>
</tr>
<tr>
<td>Weeding and cultivating garden</td>
<td>45</td>
</tr>
<tr>
<td>Spading, digging, filling in garden</td>
<td>50</td>
</tr>
<tr>
<td>Raking Lawn</td>
<td>40</td>
</tr>
<tr>
<td>Snow shoveling by hand</td>
<td>60</td>
</tr>
</tbody>
</table>

**SECTION G: Home Repair Activities**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpentry outside</td>
<td>30</td>
</tr>
<tr>
<td>Painting inside of house (includes paper hanging)</td>
<td>45</td>
</tr>
<tr>
<td>Carpentry outside</td>
<td>60</td>
</tr>
<tr>
<td>Painting outside of house</td>
<td>50</td>
</tr>
</tbody>
</table>

**SECTION H: Fishing and Hunting**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishing from river bank</td>
<td>35</td>
</tr>
<tr>
<td>Fishing in stream with wading boots</td>
<td>60</td>
</tr>
<tr>
<td>Hunting pheasants or grouse</td>
<td>60</td>
</tr>
<tr>
<td>Hunting rabbits, prairie chickens, squirrels, raccoon</td>
<td>50</td>
</tr>
<tr>
<td>Hunting large game: deer, elk, boar</td>
<td>60</td>
</tr>
</tbody>
</table>

Initials: ______
<table>
<thead>
<tr>
<th>Did you perform this activity</th>
<th>Total # of Days = _________</th>
<th>Avg # / mo</th>
<th>Times per occasion</th>
<th>Intensity Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td>J</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>M</td>
<td>J</td>
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<td></td>
<td>Hr</td>
<td>Min</td>
<td></td>
</tr>
</tbody>
</table>

SECTION I: Other Activities (please list)

| Iniials:______ |
APPENDIX G

24 HOUR DIET RECORD INSTRUCTIONS
Appendix G

24 Hour Diet Record Instructions

DIRECTIONS
Start with your first meal of the day. Record all foods, beverages, and supplements that you consume (except water) during the next 24 hours. Record the time of day that you consume each item.

Record each item right after you consume it, rather than later in the day.

If possible, list separately the different foods that compose one food item. Example: ham sandwich with ham, mayonnaise, and lettuce.

If possible, specify the brand name, and how the item was prepared. Example: broiled, steamed, fried, poached, toasted, grilled, baked, or raw.

If you eat at a restaurant, write the name of the restaurant.

Include side items like gravy, jams, sauces, salads dressing, butter, margarine, sugar, and milk on cereal. Include alcohol-containing beverages.

YOU MAY HAVE WATER on the evening prior to and the morning of your Glucose Tolerance Test! Water is actually encouraged. Do not consume flavored, or carbonated water on the evening prior to or on the morning of your Glucose Tolerance Test.

Describe amounts as accurately as possible. Record amounts in terms of dimensions, weight, or portion size. See below for some tips:

**Meats, poultry and fish**
*Record in ounces, or measure the dimensions.*

*Example: Beef, 3 oz.*
*Example: Beef, 1 piece, 2” x 3” x 1”*

**Beverages**
*Record in fluid ounces or measuring cup sizes.*

*Example: Cola, 12 oz. can*  
*Example: Coffee, 1 cup*

**Cereals, fats, & many snack items**
*Record in ounces, or in measuring cup or measuring spoon sizes.*

*Example: Cole slaw, 1/2 cup*  
*Example: Margarine, 1 tsp.*

**Fruits and vegetables**
*Record in number of items or in measuring cup sizes.*

*Example: Cole slaw, 1/2 cup*  
*Example: Apple, 1 item.*
APPENDIX H

24 HOUR PARTICIPANT DAILY INTAKE FORM
Appendix H

24 Hour Participant Daily Intake Form

Subject ______________________   ID# ________________   Date ______________

Exercise Day __________   Recall For Intake Consumed On ______________

The following questions apply to the last 24 hours:

1. Did you consume any caffeinated beverages?   _____ Yes       _____ No
   If yes, please specify beverage and portion.
   BEVERAGE:                                PORTION (oz):

   ______________________________________
   ______________________
   ______________________________________
   ______________________

2. Did you consume any alcoholic beverages?   _____ Yes       _____ No
   If yes, please specify alcoholic beverage and portion.
   BEVERAGE:                                PORTION (oz):

   ______________________________________
   ______________________
   ______________________________________
   ______________________

3. Besides the beverages/ fluids that were provided to you for the study diet, did you
   consume any other beverages/ fluids?   _____ Yes       _____ No
   If yes, please specify beverage/ fluid and portion.
   BEVERAGE:                                PORTION (oz):

   ______________________________________
   ______________________
   ______________________________________
   ______________________

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4. Did you eat any non-study foods?  
   _____ Yes  _____ No
   If yes, please specify food item and portion.
   **FOOD:**
   **PORTION (cups/ oz):**
   ____________________________________  ____________________________________
   ____________________________________  ____________________________________

5. Did you take any medications or supplements?  
   _____ Yes  _____ No
   If yes, please specify medication/ supplement/ vitamin.
   **DESCRIPTION:**
   **AMOUNT/ DOSE:**
   **REASON:**
   ____________________________________  ____________________________________  ____________________________________
   ____________________________________  ____________________________________  ____________________________________

6. Did you consume the following study foods and fluids?

<table>
<thead>
<tr>
<th>MEAL</th>
<th>MENU</th>
<th>YES</th>
<th>NO</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

7. Additional Comments:
   ____________________________________
   Initials: __________
APPENDIX I

MONOCYTE ISOLATION PROTOCOL FROM WHOLE BLOOD AND CELL CULTURES
Appendix I

Monocyte isolation protocol from whole blood and cell cultures

1) Take 20mL blood and pour into 50 mL conical vial
2) Add 20mL PBS (room temp)
3) Underlay 10 mL Histopaque 1077 (Sigma # 10771-500) (room temp)
4) Centrifuge 45 min @ 1200 rpm (18 ºC)
5) Remove MNC layer with spinal needle- MNC layer is the interlayer between the saline and plasma layer
6) Transfer MNC to a clean 50mL conical vial
7) Bring volume up to 50 mL with PBS
8) Mix by pipetting in and out 8 x
9) Spin 10 min @ 1200 rpm (18 ºC)
10) Gently suction off supernatant, leaving cell pellet
11) Pipelette 10 mL of PBS in and “suck/ squat” to mix cells
12) Take 20 uL of cells + 50 uL trypan blue + 30 uL PBS into eppendorph
13) Mix thoroughly and add to hemocytometer
14) To the 10 mL cells suspension (step 15), bring volume up to 50mL and spin 10 min @ 1200 rpm (18 ºC)
15) Count cells while 2rd spin is going on
16) After spin, gently suction off all PBS and re-suspend cells in appropriate volume of RPMI media + (do not add LPS yet!!) so that there are 1 million cells (1 x 10⁶) in each well
17) Take at least 1 mL for NO LPS condition (if there are a lot of cells you may be able to take 2 mL)
18) To the remaining cell suspension add appropriate volume of LPS (1ng/mL) for TLR4 determination- 1uL of reconstituted LPS = 1ng, so take what you need from aliquots and add to RPMI and then plate, or 100 ng/ml peptidoglycan (PGN) for TLR2 determination.

CELL COUNTING:
1) Count all 4 square corners and middle square- Take AVERAGE of all squares = cell number
2) Total cells = cell # * 5 (dilution factor) * 105

EXAMPLE:
Average cell count = 200
200 *5 * 105 = 1, 000, x 106 cells in 10mL or 100 x 105 cells in 1 mL.
For a final concentration of 1 x 10⁶, then resuspend cells in 10 mL of RPMI + media

RPMI + media = Hepes- 400uL, Pen/strep- 400uL, Serum TCH- 800 uL
APPENDIX J

ADA DIABETES CLASSIFICATION CRITERIA
Appendix J

ADA Diabetes Classification Criteria

<table>
<thead>
<tr>
<th></th>
<th>FPG</th>
<th>2 hr OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NGT</strong></td>
<td>&lt; 100 mg/dl</td>
<td>&lt; 140 mg/dl</td>
</tr>
<tr>
<td></td>
<td>&lt; 5.6 mM</td>
<td>&lt; 7.8 mM</td>
</tr>
<tr>
<td><strong>IGT</strong></td>
<td>≥ 100 and &lt; 126</td>
<td>≥ 140 and &lt; 200</td>
</tr>
<tr>
<td></td>
<td>mg/dl ≥ 5.6</td>
<td>mg/dl ≥ 7.8</td>
</tr>
<tr>
<td></td>
<td>&lt; 7.0 mM</td>
<td>&lt; 11.0 mM</td>
</tr>
<tr>
<td><strong>T2DM</strong></td>
<td>≥ 126 mg/dl</td>
<td>≥ 200 mg/dl</td>
</tr>
<tr>
<td></td>
<td>&gt; 7.0 mM</td>
<td>≥ 11.0 mM</td>
</tr>
</tbody>
</table>

NGT = Normal Glucose Tolerance

IGT = Impaired Glucose Tolerance

T2DM = Type 2 Diabetes Mellitus

APPENDIX K

GRADED EXERCISE STRESS TEST OR VO$_{2\text{MAX}}$ TEST
Appendix K

Graded Exercise Stress Test or $\text{VO}_{2\text{max}}$ Test

Name_______________________________________  Date_____________________

Weight_________  Age_________  ID #_____________________

Medications____________________________________________________________

Physician__________________________  Technician_________________________

Supine:     HR_________  BP_________  Age-predicted HR$_{\text{max}}$_________

Standing: HR_________  BP_________  85% APMHR_________

<table>
<thead>
<tr>
<th>SPEED GRADE (MPH) (%)</th>
<th>TIME (MIN)</th>
<th>HEART RATE</th>
<th>BLOOD PRESSURE</th>
<th>RPE</th>
<th>TIME IN RECOVERY</th>
<th>HEART RATE</th>
<th>BLOOD PRESSURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min.</td>
<td></td>
<td></td>
<td></td>
<td>Immediate</td>
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<tr>
<td></td>
<td>4 min.</td>
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<tr>
<td></td>
<td>6 min.</td>
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<tr>
<td></td>
<td>8 min.</td>
<td></td>
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<tr>
<td></td>
<td>10 min.</td>
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<tr>
<td></td>
<td>12 min.</td>
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<tr>
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<td>14 min.</td>
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<td>16 min.</td>
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<td></td>
<td>18 min.</td>
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<td></td>
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Reason for Termination ____________________________________________________

Duration of test_________  HR$_{\text{max}}$_________  BP$_{\text{max}}$_________  RPE$_{\text{max}}$_________

Comments______________________________________________________________

_______________________________________________________________________

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APPENDIX L

EXERCISE TRAINING HEART RATES
**Appendix L**

**Exercise Training Heart Rates**

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>HR (bpm)</th>
<th>% HR&lt;sub&gt;max&lt;/sub&gt;</th>
<th>%VO&lt;sub&gt;2max&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>720-0209</td>
<td>139</td>
<td>83.5</td>
<td>71.7</td>
</tr>
<tr>
<td>720-0609</td>
<td>122</td>
<td>82.7</td>
<td>70.4</td>
</tr>
<tr>
<td>720-0809</td>
<td>147</td>
<td>80.2</td>
<td>66.5</td>
</tr>
<tr>
<td>720-1109</td>
<td>130</td>
<td>81.5</td>
<td>68.5</td>
</tr>
<tr>
<td>720-1309</td>
<td>116</td>
<td><strong>77.1</strong></td>
<td><strong>61.6</strong></td>
</tr>
<tr>
<td>720-1509</td>
<td>142</td>
<td>84.4</td>
<td>73.2</td>
</tr>
<tr>
<td>720-1609</td>
<td>132</td>
<td>84.3</td>
<td>72.9</td>
</tr>
<tr>
<td>720-1709</td>
<td>133</td>
<td>85.0</td>
<td>74.1</td>
</tr>
<tr>
<td>720-1809</td>
<td>151</td>
<td>83.7</td>
<td>72.1</td>
</tr>
<tr>
<td>720-2009</td>
<td>135</td>
<td><strong>78.5</strong></td>
<td><strong>63.8</strong></td>
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<tr>
<td>720-2209</td>
<td>141</td>
<td>83.2</td>
<td>71.2</td>
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<td>720-2609</td>
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<td>84.8</td>
<td>73.7</td>
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<td>720-2709</td>
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<td>80.8</td>
<td>67.4</td>
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<tr>
<td>720-2809</td>
<td>154</td>
<td>81.1</td>
<td>68.0</td>
</tr>
</tbody>
</table>

n = 14 (4 males, 10 females), values are expressed as means ± SEM. HR, heart rate, bpm, beats per minute, VO<sub>2max</sub>, maximal aerobic capacity.
APPENDIX M

RATING OF PERCEIVED EXERTION
Appendix M

Rating of Perceived Exertion (Borg, 1970)

6
7 Very, Very Light
8
9 Very Light
10
11 Fairly Light

12
13 Somewhat Hard
14
15 Hard

16
17 Very Hard
18
19 Very, Very Hard
20 I’m definitely done!
APPENDIX N

ADDITIONAL STATISTICAL ANALYSES
## Appendix N

### Additional Statistical Analyses

<table>
<thead>
<tr>
<th>RM ANOVA</th>
<th>Pooled P</th>
<th>Group P</th>
<th>Post Hoc NGT/IGT P</th>
<th>Bonferroni IGT/T2DM P</th>
<th>NGT/T2DM P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.076</td>
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Sample Means for TNF-\(\alpha\)

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RM ANOVA for TNF-\(\alpha\)

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RM ANOVA for IL-6

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