The influence of acute resistive exercise on inflammatory markers in the blood of obese, postmenopausal women.

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The influence of acute resistive exercise on inflammatory markers in the blood of obese, postmenopausal women.

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Abbreviations

IL-6 – Interleukin-6
rhIL-6 – recombinant human Interleukin-6
TNF-α – Tumor Necrosis Factor-α
IL-1β – Interleukin-1β
CRP – C-Reactive Protein
RE – Resistive Exercise
1-RM – 1-Repetition Maximum
PRE – Pre-exercise time point
PO – Post-exercise time point
2H – 2 hour post-exercise time point
24H – 24 hour post-exercise time point
LPS – Lipopolysaccharide
BMI – Body Mass Index
IRS – Insulin Receptor Substrate
mRNA – messenger Ribonucleic Acid
pg – picogram
ng – nanogram
TLR – Toll-Like Receptor
APMHR – Age-Predicted Maximum Heart Rate
GXT – Graded Exercise Test
ECG – Electrocardiogram
ACC – Acclimation
ELISA – Enzyme-linked immunosorbant assay
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CHAPTER I
INTRODUCTION

Background

The prevalence of obesity, defined as an excessive accumulation of fat at least 30 percent above recommended body weight (Center for Disease Control and Prevention (a), 2005) in the United States is steadily increasing and has reached pandemic levels (CDC (c), 2006). Over 60 million people, or approximately 34% of persons over the age of 20 years, are now classified as “obese” in the United States (CDC (b), 2007). Obesity is a major risk factor for the development of conditions such as coronary artery disease, hypertension, several types of cancer, and type 2 diabetes (Bastard et al., 2006; Lukaszewicz, 2007; Van Gaal et al., 2006). Researchers have examined the effects of obesity on mortality and cancer rates and have found that adiposity, rather than physical activity level, was a better predictor of mortality, regardless of level of fitness (Hu et al, 2004). Calle and associates (2003) reported that the higher the degree of obesity, the stronger the association with an increased risk of death from various types of cancer, including esophageal, colorectal, pancreatic and hepatic. Along with negative consequences on individual health status, obesity is also a major contributor to the growing cost of healthcare in the U.S. An estimated $92.6 billion, or 9.1% of total U.S. expenditures, was spent on healthcare related to obesity (CDC (b), 2005) in 2002. While genetics are believed to play a role in the development of obesity, physical inactivity, poor nutrition and the presence of a positive energy balance are crucial lifestyle factors that can be modified to prevent obesity and the accompanying diseases. While in a positive caloric balance, the excess energy is stored as adipose tissue for future use. With continued accumulation of excess body fat, however, normal metabolic processes can become impaired (Garg, 2006; Trayhurn et al., 2001).
Through a wide array of mechanisms, excessive accumulation of adipose tissue can lead to diseases of the endocrine and cardiovascular systems. Patients will often exhibit difficulties in metabolizing lipids and carbohydrates (Ferrannini et al., 2004) before progressing to more advanced stages of disease. The disruption in substrate homeostasis can lead to symptoms associated with the metabolic syndrome (MS), which is characterized by the presence of usually three or more risk factors such as hypertension, abdominal obesity, high triglyceride levels, low HDL levels, and high fasting levels of blood glucose (Chew et al., 2006). High fasting blood glucose levels result from impaired glucose tolerance (IGT), which is defined as a significantly elevated blood glucose level after fasting, following a high carbohydrate meal, or after ingestion of a glucose test solution, but not high enough to be diagnostic of diabetes mellitus (American Diabetes Association, 2007; Shaw et al, 1999). If left untreated, IGT can continue to worsen until the patient develops clinical type 2 diabetes. When diet and exercise can not stabilize blood glucose levels, oral anti-diabetic agents or supplemental insulin treatments may be necessary.

Inflammatory cytokines, especially interleukin-6 (IL-6) (Rotter et al., 2003) and tumor necrosis factor-α (TNF-α) (Stephens et al., 1997), are believed to play a role in the progression of insulin resistance. Cytokines are proteins or polypeptides involved in cellular communication. TNF-α, Interleukin-1β (IL-1β) and IL-6 are cytokines that have been characterized by their role in the initiation and control of the inflammatory response due to injury, infection, or other stress, including exercise. While TNF-α and IL-1β have a predominantly pro-inflammatory role in the body, IL-6 is pluripotent because of its ability to serve as an anti-inflammatory agent, such as after an exercise bout, and also as a pro-inflammatory marker, such as in the case of metabolic syndrome (Fasshauer et al., 2004). When the stimulation and secretion of TNF-α and IL-6 is chronically unabated, harmful
Recent research supports the association of elevated inflammatory cytokine levels with the pathophysiology of diabetes mellitus and atherosclerosis (Nilsson et al., 1998; Saghizadeh et al., 1996). Despite its possible role as a marker of systemic inflammation, IL-6 also has the ability to act as an anti-inflammatory agent by blunting the production of TNF-α and IL-1β within the body (Pedersen, 2003, Phillips et al., 2008).

Aerobic exercise of varying intensity and duration is capable of generating a mild, systemic inflammatory response to the exercise bout as evidenced by increased blood levels of IL-6, IL-1β and TNF-α. Blood concentrations of IL-6 have been shown to significantly increase following continuous one-legged exercise (Steensberg et al, 2000), following one-legged exercise with and without glycogen depletion (Keller et al, 2005), and following a marathon race (Ostrowski et al, 1999). Ostrowski and associates (1999) also reported significant increases in TNF-α following the 26.2-mile race. Although significant increases in circulating cytokines have been recorded following strenuous aerobic exercise, the elevations are transient and return to baseline levels within 2 to 24 hours (Weinstock, 1997). The physiological effects of exercise-induced IL-1β and TNF-α are not yet known, however, these cytokines have been shown to stimulate production of IL-6 (Kandere-Grzybowska et al, 2003). Scientists have suggested that IL-6 may actively suppress the production of TNF-α and thus protect against impaired glucose tolerance and whole-body inflammation (Pedersen, 2003). Furthermore, aerobic exercise may also influence the production, secretion and expression of inflammatory cytokines from immune cells within the blood (Kurokawa et al, 2007; Weinstock et al, 1997).

Similar to aerobic exercise, resistive exercise (RE) can produce transient increases in contraction-induced cytokine production (Bautmans et al, 2005; McKarlin et al, 2004; Rhind et al, 2001; Weinstock, 1997). While the benefits of chronic RE have been well-documented,
the effects of an acute bout of RE on contraction-induced cytokine production are not well understood. Dionne and associates (2004) reported that 6 months of RE increased the fat free mass (FFM) and decreased the body fat percentage of women aged 55 to 70 years. Maintaining a high level of energy expenditure through exercise will also increase resting energy expenditure (REE), which is especially important in elder populations, and may prevent age-associated weight gain. Scientists reported that REE was significantly decreased in elder populations between 60 and 70 years of age, regardless of body composition (Alfonzo-Gonzalez et al., 2006). Researchers reported that the decrease in REE was greater than predictive equations had calculated for those specific age groups (Alfonzo-Gonzalez, 2006). Furthermore, increased levels of physical activity in elder men aged greater than 65 years, measured in minutes per week, reduced the risk of falling accidents and increased quality of life (Chan et al., 2007).

Resistive exercise can have profound effects on circulating cytokines, as well as cytokine expression in biologically active tissues. Greiwe and colleagues (2001) found significant reductions in TNF-α mRNA and protein expression following 3 months of resistive training in frail, elder men and women (ages 81 ± 1 year). Investigators have reported variable results regarding the effects of a single session of RE on serum cytokine levels. While some researchers have observed little or no change in circulating TNF-α in response to a single session of resistive exercise (McFarlin et al., 2004), IL-6 levels have been shown to increase significantly in elder individuals following resistive exercise at 80% 1-RM (Bautmans, 2005; Phillips, 2008). The 1-RM is defined as “the greatest resistance that can be moved through the full range of motion in a controlled manner with good posture” (ACSM, 2006). Despite the absence of significant changes in plasma levels of IL-1β and TNF-α, some have reported immune cells, specifically monocytes, will greatly increase production of
inflammatory cytokines in response to mitogen stimulation following an acute bout of resistive exercise (McFarlin, 2004, Rhind et al, 2001; Weinstock et al, 1997). In the presence of an endotoxin, such as lipopolysaccharide, monocytes and other white blood cells are capable of secreting large quantities of pro- and anti-inflammatory cytokines into the circulation (Akiyama et al, 1985). The immunocreativity, or the ability of cells to respond to an attack on the immune system, determines the amount of cytokine production from the white blood cells, especially monocytes. Physical activity and exercise can decrease the immunoreactivity of monocytes and attenuate the release of pro-inflammatory cytokine release into the circulation (Starkie et al, 2003). Researchers have also reported that the mitogen-stimulated production of inflammatory cytokines is in fact suppressed following acute RE when cytokine production was expressed per monocyte (Phillips, 2008). This report highlights the role RE might play in the control and regulation of whole-body inflammation, and possibly metabolic disease pathways that are related to systemic inflammation. Many obese individuals are unable to participate in aerobic activities due to cardiopulmonary and/or orthopedic limitations. Resistive exercise can be performed in the prone, supine and seated positions, which allows obese individuals with certain limitations to increase their physical activity and possibly attenuate the effects of uncontrolled, systemic inflammation. Resistive exercise could possibly serve as a viable method of disease prevention for those populations who are unable to participate in other exercise modalities.

**Purpose**

The effects of a single bout of moderate-to-high intensity RE on serum cytokine levels and immunoreactivity in elder, postmenopausal women have been investigated (McFarlin, 2004; Phillips, 2008), however, this study assessed the effects of a single, high-
intensity resistive exercise session on plasma IL-6 and cytokine production from LPS-stimulated, whole blood cultures in obese, postmenopausal women, as well as the effects of RE on plasma cortisol levels. We also sought to determine if the effects of exercise on plasma cytokine production and LPS-stimulated production are related to the stress hormone cortisol. Specifically, we measured circulating levels of IL-6 and cortisol in plasma, as well as the LPS-stimulated production of TNF-\(\alpha\) and IL-1\(\beta\) from diluted whole blood samples. Circulating levels of cytokines were compared between exercise and non-exercise control groups.

**Hypothesis**

- A single bout of intense resistive exercise will decrease cytokine production in LPS-stimulated, whole blood cultures, as compared with non-exercising control participants when expressed as cytokine production per monocyte.

- The immunoreactivity of exercising participants will increase compared to non-exercising controls following stimulation with LPS.

- Plasma IL-6 will increase immediately following a single bout of resistive exercise versus a non-exercise control group

- Plasma IL-6 concentration in the exercise group will return to baseline levels within 2 to 24 hours

- Resistive exercise will generate leukocytosis in the EX group

- Plasma cortisol concentration will increase following the exercise session, while values for CON participants will decrease throughout the morning according to the normal diurnal pattern of cortisol appearance.
• Cortisol will contribute to the anti-inflammatory environment following resistive exercise by inhibiting TNF-α and IL-1β production from stimulated whole blood cultures.

**Significance**

This study will contribute to the expanding body of knowledge related to the metabolic responses to resistive exercise and the response of inflammatory-related cytokines associated with metabolic diseases. Increasing age is associated with a systemic inflammatory response, which may be blunted or reversed with daily physical activity and exercise. Many elder individuals are unable to perform aerobic exercise due to orthopedic conditions or other complications. Resistive exercise may be an effective training modality that could generate similar anti-inflammatory benefits as aerobic training in the reduction of cardiovascular risk and the increase in glucose metabolism, as well as generating increases in fat free mass.
CHAPTER II
REVIEW OF LITERATURE

Cytokines

Cytokines have been described as “a vast array of relatively low molecular weight, pharmacologically active proteins that are secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect)” (McDermott, 2001). Cytokines belong to a family of proteins involved in cell-to-cell signaling and are widely involved in the inflammatory process, as well as nutrient availability and uptake (Northoff et al, 1991). Various tissues throughout the body have been shown to produce inflammatory cytokines (Febbraio, 2002), including adipose tissue, brain tissue, immune cells, and contracting skeletal muscle tissue. Macrophage and monocyte production of IL-6, TNF-α (Weisberg, 2003) and IL-1β (Martin and Wesche, 2002) has been observed and it is thought that chronic elevations of these cytokines within the circulation, as well as within local tissues, have a negative effect on metabolism (Lazar, 2005). IL-6 is capable of acting on several different tissues and types of cells (Febbraio, 2002) as a regulatory protein for controlling and possibly reducing inflammation. TNF-α is also capable of acting on various tissues and cells, but its principal action is to intensify systemic inflammation (Cantor, 2005). IL-1β reduces activity of lipoprotein lipase within adipose tissue, thereby increasing plasma lipid levels (Guyton and Hall, 2006).

During sustained physical activity and exercise, skeletal muscle is capable of generating large amounts of IL-6. Production of IL-6 in contracting skeletal muscle varies depending upon the mode, intensity and duration of the exercise utilized. Researchers have investigated both aerobic and resistive exercises to determine patterns of IL-6 expression (Fischer, 2006; Keller et al., 2001; Steensberg et al., 2003a; Ostrowski et al., 2000).
Researchers have theorized that IL-6 acts as a chemical messenger that signals for hepatic glycogenolysis during periods of decreased nutrient availability (Keller et al., 2001). With regard to nutrient availability, plasma IL-6 was significantly greater in a glycogen-depleted state than control trials following 3 hours of two-legged knee extensor exercise (Keller et al., 2001). The theory connecting IL-6 with nutrient availability could partially explain the chronically elevated plasma levels seen in patients with metabolic disorders.

**Cytokines and Obesity-Related Diseases**

Cytokines have also been studied in relation to obesity and insulin resistance by researchers such as Kern and associates (2001), who reported a trend for increasing IL-6 expression in adipose tissue with increased BMI, although the differences were not significant between lean (BMI > 25 kg\(\text{m}^{-2}\)) and obese (30 kg\(\text{m}^{-2}\) < BMI < 40 kg\(\text{m}^{-2}\)) participants. Kern (2001) did observe that plasma IL-6 concentrations were strongly related to BMI, increasing four-fold from lean participants (0.73 ± 0.23 pg\(\text{ml}^{-1}\)) to the most obese group (2.86 ± 0.61 pg\(\text{ml}^{-1}\)) with a BMI > 40 kg\(\text{m}^{-2}\), a trend also seen by Engeli and colleagues (2003). Serum IL-6 is also elevated in patients with type 2 diabetes (Kado et al., 1999), but contrary to the effects of TNF-\(\alpha\), IL-6 has been shown to improve whole body glucose disposal (Pickup et al., 2000) when acutely elevated after an exercise bout (Starkie et al., 2001; Stouthard et al., 1995). In fact, IL-6 directly inhibits the expression of TNF-\(\alpha\) and acts as the primary stimulant of the hepatic acute-phase protein, C-reactive protein (CRP) (Pue et al., 1996), which induces anti-inflammatory cytokine production from monocytes and suppresses pro-inflammatory cytokine production from tissue macrophages (Pue, 1996). Secreted by macrophages, T cells (Cantor and Haskins, 2005) and monocytes (Kurokawa, 2007), TNF-\(\alpha\) is thought to decrease insulin sensitivity by causing phosphorylation of serine
residues on the insulin receptor substrate-1 (IRS-1) (Hotamisligil et al., 1996; De Aguila et al., 1999) and on IRS-2 (De Aguila et al., 1999). By phosphorylating the insulin receptor complexes, TNF-α converts IRS-1/2 into an inhibitory protein for insulin receptor tyrosine kinase activity (Hotamisligil et al., 1994), which is necessary for insulin-mediated glucose transport. In addition, it has been suggested that elevated TNF-α levels could result in insulin resistance through the inhibitory effect of TNF-α on lipoprotein lipase (LPL) and the stimulatory effect on adipocytes to release non-esterified free fatty acids (Kern et al, 2001).

Inhibition of LPL results in decreased lipid deposition within adipocytes, which, when accompanied by stimulation of free-fatty acid release from adipocytes, leads to increased blood lipid concentrations in the blood and could contribute to the development of insulin resistance. Similar to chronically elevated serum IL-6, serum TNF-α is also correlated to adiposity (Kondo et al., 2006) and could contribute to insulin resistance through the disruption in insulin signaling mentioned previously (De Aguila et al., 1999). Kern and associates (2001) also examined the relationship between TNF-α and obesity and found a 7.5-fold increase in TNF-α expression in adipose tissue biopsies from obese (1.21 ± 0.36 pg/μg) versus lean (0.16 ± 0.006 pg/μg) participants, and that TNF-α expression was inversely correlated with insulin sensitivity. Although the effects of TNF-α and IL-6 on insulin resistance are well documented, little is known about a potential role of IL-1β. IL-1β has been observed to behave in a fashion similar to TNF-α in regard to decreasing the ability of body tissues to utilize glucose (Spranger et al, 2003; Martin and Wesche, 2002). IL-1β is a pro-inflammatory cytokine that is produced by monocytes and macrophages (Martin and Wesche, 2002), and exerts its biological function by binding to its type I receptor and activating the inhibitor-KB kinase/nuclear factor-KB (IKK/NF-KB) pathway and the three types of MAPKs: ERK, JNK, and p38MAPK (Martin and Wesche, 2002). A recent report
suggested that IL-1β could also play a role in the development of insulin resistance (Spranger et al., 2003). In fact, the authors reported that individuals with detectable circulating levels of IL-1β and elevated levels of IL-6 had an increased risk of developing type 2 diabetes, compared with individuals with increased concentrations of IL-6 but undetectable levels of IL-1β (Spranger et al., 2003). Furthermore, IL-1β concentration is elevated in the non-diabetic offspring of diabetic individuals and is correlated with the metabolic syndrome (Salmenniemi et al., 2004). Juge-Aubry and associates (2004) reported that the expression of both IL-1β and its receptor are elevated in the visceral adipose tissue of obese subjects. Despite early evidence of its association with metabolic disorders, the mechanism through which the overproduction of IL-1β alters the metabolic function of insulin in adipocytes remains unclear. However, researchers have recently found that IL-1β significantly inhibits insulin-induced glucose transport in adipocytes by decreasing IRS-1 expression and that ERK pathway is involved in the inhibitory action of IL-1β on insulin signaling (Jager et al., 2007).

Along with their role in diabetes mellitus, IL-1β, TNF-α and IL-6 are critical components of atherogenesis because they are released at the site of injury on arterial walls and initiate the inflammatory response, which in turn can stimulate plaque formation within the cardiovascular system (Ross, 1999; Smith et al., 1999). Plasma IL-6 concentrations have even been shown to predict total and cardiovascular mortality (Ridker et al., 2000). IL-6 also induces hepatic production of the acute phase proteins, such as CRP, which assist in the control of the inflammatory response. One study suggested that IL-6 decreases lipoprotein lipase activity, which results in increased macrophage uptake of lipids (Yudkin et al., 2000). A positive relationship between serum concentrations of IL-6 and CRP was reported in normal subjects and those with chronic, stable angina (Ikonomidis et al., 1999). In addition to its effects on insulin sensitivity, TNF-α has been shown to increase activation of endothelial
and smooth muscle NF-κB, which, in turn, induces vascular adhesion molecules and cytokines, resulting in inflammatory and foam cell accumulation (Bhagat et al., 1997). IL-1β has also been implicated in the progression of cardiovascular disease. DeLorenzo and associates (2007) investigated a group of young women (ages 20 – 30 years) known as “normal-weight, obese” (NWO) patients and found significantly elevated circulating levels of IL-1β in the NWO (15.0 ± 3.1 pg*mL\(^{-1}\) vs. 5.0 ± 2.6 pg* mL\(^{-1}\)) compared to non-obese women of the same age. DeLorenzo (2007) defined NWO patients as having a BMI within normal limits (18 < BMI < 25 kg*m\(^{-2}\)) but whose body fat is above 30%. In fact, the circulating levels of IL-1β found in NWO patients were approximately the same levels found in traditionally defined obese women (15.0 ± 3.1 pg*mL\(^{-1}\) vs. 19.0 ± 4.1 pg*mL\(^{-1}\)). While the direct link between IL-1β and atherosclerosis remains unclear, Ruan and colleagues (2006) have investigated the effects of direct exposure to IL-1β on vascular smooth muscle cells (VSMCs). Researchers reported that following exposure to relatively small amounts of IL-1β (5 ng*mL\(^{-1}\)), VSMCs began rapid uptake of LDL cholesterol and increased cholesterol ester accumulation within the muscle cells (Ruan, 2006). Researchers believe this mechanism, which is similar to the action of TNF-α, could disrupt cholesterol-mediated LDL receptor feedback regulation, permitting intracellular accumulation of unmodified LDL and causing foam cell formation (Ruan, 2006). Whether classified as pro- or anti-inflammatory, cytokines are capable of exerting powerful effects on the cardiovascular and the glucoregulatory system. Without any method of controlling cytokine production and release, chronically elevated serum concentrations have been shown to lead to heart disease and metabolic syndrome. Scientific research is currently directed at determining the most efficient method of reversing, or at least managing, the effects of whole-body inflammation resulting from excess adiposity and physical inactivity.
**Interleukin-6**

Specialized immune cells, particularly monocytes and macrophages, are capable of producing IL-6, as well as various tissues throughout the body, including adipose tissue, brain tissue, and skeletal muscle (Febbraio, 2002; Febbraio, 2004; Pedersen et al, 2005; Pedersen, 2001; Weisberg, 2003). Transient increases in plasma IL-6 have been linked to improvements in glucose tolerance in patients (Pickup et al., 2000; Starkie et al., 2001; Stouthard et al., 1995), but also to systemic inflammation related to excessive amounts of adipose tissue and cardiovascular disease (Engeli, 2003; Kado, 1999; Kern, 2001). IL-6 exerts its effects on tissues throughout the body by binding its membrane-bound receptor (IL-6r) (Taga et al, 1989) or as a receptor-bound soluble complex (sIL-6r) (Mullberg et al, 1993; Mullberg et al, 1994). Once bound to either the membrane-based receptor, or the soluble receptor complex, IL-6 is able to bind with glycoprotein-130 (gp-130) and finally exert its effects on individual cells within the tissues. The effects of IL-6 binding have been observed through signaling via the Jak-STAT pathway, through which the IL-6r–gp130 complex activates members of the Janus-activated protein kinases (Jak), Jak1, Jak2 and Tyk2 (Heinrich et al, 1998). Jak proteins then phosphorylate and activate the signal transducer and activator of transcription (STAT)-3 pathway, which is present in numerous cell types (Heinrich, 1998). As a result of STAT-3 signaling, IL-6 induces transcription of a family of proteins termed the suppressors of cytokine signaling (SOCS) (Starr et al, 1997). The IL-6-induced reduction in insulin signaling appears to be due to stimulation of SOCS-3, which can directly inhibit the insulin receptor by preventing the phosphorylation of the tyrosine residues associated with the insulin-receptor substrate (Senn et al, 2002). While IL-6 has been observed to decrease glucose uptake in cells, it has also been shown to increase insulin-like activity by increasing glucose disposal in cells (Carey et al, 2006; Chen et al, 1999; Jee et al, 2002; Wei et
Researchers reported an increase in glucose transport across cell membranes after treatment with IL-6, which they thought was due to the activation of the phosphatidylinositol-3-kinase (PI3-K)/acute transforming retrovirus thymoma (Akt) signaling pathway (Jee, 2002; Wei, 2001). The Akt pathway was shown to increase glucose transport by increasing phosphorylation of serine residues on insulin receptor substrates (Chen, 1999; Jee, 2002). Despite contradictory results, it has generally been accepted that short-term, contraction-induced increases in IL-6 can be beneficial for metabolic function, whereas chronically elevated levels of IL-6 can contribute to metabolic dysfunction and cardiovascular disease.

**IL-6 and Exercise**

Muscle tissue is a major production site for cytokines (Febbraio et al, 2002; Febbraio et al, 2004; Pedersen et al., 2001; Weisberg et al., 2003), and is stimulated to release cytokines in response to an exercise stress. It was once hypothesized that circulating blood leukocytes were the sole source of IL-6 following exercise (Nehlsen-Canarella et al., 1997) but studies have shown that IL-6 mRNA does not in fact increase within muscle tissue as a result of exercise (Hoffman-Goetz, 2000). Another theory, developed from animal models, reported that IL-6 was produced within the liver during an exercise bout. Rat livers were shown to produce IL-6 in response to stimulation with corticosterone (Liao, 1995a) and adrenaline (Liao, 1995b). A human study by Febbraio and colleagues (2002) has shown that IL-6 is actually removed from the circulation in the liver, and not produced there during exercise. Contracting skeletal muscle has been shown to produce increases in plasma IL-6 as well as increased muscle mRNA for IL-6 (Ostrowski et al, 1998). Resting levels of IL-6 mRNA are often not detectable, but significant increases in IL-6 mRNA have been reported following
both eccentric and concentric exercise (Starkie, 2001), which highlights the fact that IL-6 release is more directly related to muscle contraction than muscle damage from exercise (Starkie, 2001). Exercise studies utilizing one exercising leg and one resting leg have determined that the exercising limb does in fact release IL-6 into the circulation and that the resting leg did not contribute to the increase in plasma IL-6 (Steensberg et al., 2001a). To further understand which type of cells are actually producing IL-6, Keller and associates (2001) took muscle biopsies from exercising and resting muscles and found that that transcription rate within the nuclei increased after the onset of exercise. This study confirms that muscle cells, or myocytes, can produce very large amounts of IL-6 when subjected to an exercise stimulus (Keller, 2001).

Both aerobic and resistive exercises have been utilized to study the role of muscle tissue in the production and release of IL-6. Significant increases in plasma IL-6 have been observed following continuous one-legged extensions (Steensberg et al., 2000), following a marathon race (Ostrowski et al., 1999), following two hours of intense resistive training with and without glycogen depletion (Nieman, 2004) and following one-legged extensions with and without glycogen depletion (Keller et al., 2005), which highlight the role of exercise in the production of IL-6. With regard to nutrient availability, plasma IL-6 was significantly greater in a glycogen-depleted state (10.1 ± 1.3 ng*L⁻¹) than control trials (6.3 ± 0.7 ng*L⁻¹) following 3 hours of two-legged knee extensor exercise (Keller et al., 2001), whereas Nieman and associates (2004) reported no significant difference in plasma IL-6 between glycogen-depleted participants (6.61 ± 1.48 pg*mL⁻¹) and participants with normal levels of glycogen (7.72 ± 2.19 pg*mL⁻¹) following two hours of intense resistive training. Steensberg and colleagues (2000) explained that the increased arterial concentrations (0.74ng·L⁻¹ at rest to 14.13 ng·L⁻¹) of IL-6 were solely the result of contraction-induced IL-6 production during
the 5-hour exercise bout. Another study by Steensberg’s team (2002) reported significant increases in arterial IL-6 concentration and IL-6 mRNA in muscle tissue biopsies following 180 minutes of two-legged knee-extensor exercise and similar increases in IL-6 mRNA within exercising muscle tissue were reported by Nieman’s team (2003).

Infused IL-6

A variety of hormonal factors could influence the role of increased plasma IL-6 during an exercise session, so investigators have studied the effects of IL-6 infusion on glucose and fatty acid metabolism in an attempt to isolate the consequences of IL-6 production. Van Gaal and associates (2003) infused eighteen young, healthy men with either saline solution, a low dose of recombinant human IL-6 (rhIL-6), or a high dose of rhIL-6 and found that the men receiving infused IL-6 had significant changes in fatty acid metabolism and fatty acid turnover versus the control group receiving the saline infusion. Of particular interest, arterial fatty acid concentration and fatty acid turnover, as well as arterial glycerol and glycerol turnover were all elevated in the infusion group, but without a concomitant increase in catecholamines, glucagon or insulin (Van Gaal, 2003). Furthermore, the increase in arterial fatty acid was seen without the presence of hypertriglyceridemia, which highlights the beneficial role of IL-6 as a mediator of lipolysis within the body. A similar study by Steensberg and colleagues (2003b) demonstrated that similar infusions of IL-6 do not have any effect on glucose metabolism, as measured by whole-body glucose uptake and leg muscle glucose uptake. Neither glucose uptake nor glucose disposal was adversely affected in Steensberg’s study. Although elevated IL-6 concentrations have been found in patients with impaired glucose tolerance and type 2 diabetes, acute elevations in plasma IL-6 do not appear to negatively affect systemic glucose utilization or localized
glucose uptake (Steensberg, 2003a). These findings have been further reinforced with a study performed by Krogh-Madsen and associates (2006) in which 9 healthy, young men underwent consecutive infusions of IL-6, TNF-α and saline solution. Insulin-mediated glucose disposal was measured during an euglycemic hyperinsulinemic clamp procedure. Researchers found that while TNF-α inhibited glucose disposal, the IL-6 infusion had no impact on insulin-mediated glucose uptake (Steensberg, 2003a).

**Age and IL-6**

While most studies have investigated the effects of exercise and cytokine infusion on young adults, few studies have measured cytokine responses in elder men and women. Similar to their younger counterparts, elder participants also have significant increases in serum IL-6 following a single bout of resistance exercise (Bautmans et al, 2005). In Bautman’s project (2005), a group of twelve elder men and women (69 ± 5 years) showed elevated plasma IL-6 concentrations (1.90 ± 1.50 ng*L⁻¹ baseline vs. 2.29 ± 1.37 post-exercise ng*L⁻¹) following a session of high intensity resistance exercise. Another study demonstrated that elder men are also capable of increasing IL-6 expression following exercise. Ten elder and ten young men participated in one hour of strenuous eccentric cycling and both groups generated significant increases in plasma IL-6 immediately following the exercise, and for four hours following the exercise session (peak IL-6 at 4H post-exercise: 4.35 ± 1.7 vs. 5.05 ± 3.17 pg*mL⁻¹, respectively) (Toft et al, 2002). Despite the dramatic increase in circulating cytokine levels following exercise, researchers reported small and non-significant increases in markers of muscle damage, in particular creatine kinase. While IL-6 concentrations are related to muscle damage, researchers believe that muscle
damage is not directly related to the IL-6 response to exercise, but could have a role in the impaired muscle repair mechanism (Toft, 2002).

**IL-6 as an Anti-Inflammatory Mediator**

With several different cytokines and hormones responding to the exercise stimulus, researchers are now beginning to understand how these different molecules interact within the body. Steensberg and associates (2003) infused twelve healthy, young men with recombinant-human IL-6 (rhIL-6) for three hours at a rate of 30 μg*hour⁻¹ in order to duplicate plasma IL-6 levels similar to those found after high intensity exercise. Six similarly aged men completed the same trial but with a saline infusion rather than receiving rhIL-6. Arterial samples were collected during the infusion and for three hours following the infusion. Samples were measured for circulating TNF-α, IL-6, IL-10, IL-1 receptor antagonist (IL-1ra), C-Reactive Protein (CRP), cortisol, lymphocytes and neutrophils. IL-6 levels in the infusion group ranged from 125 to 166 pg*mL⁻¹, which was slightly higher than concentrations reported by Keller (100 pg*mL⁻¹) and associates (2003) and by Ostrowski (80 pg*mL⁻¹) and colleagues (1997). IL-10 and IL-1ra were significantly elevated in the infusion group for the entire duration of the study. TNF-α release was blunted by the rhIL-6 infusion compared to circulating levels of the control participants. Cortisol was also significantly increased for the first two hours of the infusion, before returning to normal levels. Neutrophils were elevated from the second hour of infusion to the sixth hour, while lymphocytes remained near control values and even decreased below control levels for hours three through six. Interestingly, CRP was unchanged for six hours during the infusion until becoming significantly elevated at hours six and twenty-four. This study is crucial to our understanding of the interaction between inflammatory cytokines within the body and
solidifies the hypothesis that IL-6 is an anti-inflammatory marker. The increase in plasma IL-10 and IL-1ra, known for their ability to reduce TNF-α cell surface receptor expression and inhibit the effects of IL-1α and IL-1β, respectively, signifies that IL-6 may play a major role in the attenuation and regulation of inflammation and injury. Also, the increase in cortisol concentration, which was seen in other studies (Bethin et al, 2000; Stouthard et al, 1995), resulted in increased numbers of neutrophils in the blood. Cortisol increases neutrophil concentration within the blood by inhibiting the cell’s ability to bind to the plasma membrane of other cells in the body, and thus inhibits neutrophil infiltration into the tissues (Cronstein et al, 1992). Increased levels of neutrophils are also seen in exercise and this effect is likely mediated by the increase in plasma IL-6 following exercise (Steensberg et al, 2003).

*Tumor Necrosis Factor-α*

Characterized exclusively as a pro-inflammatory cytokines, TNF-α is secreted by blood mononuclear cells, especially macrophages, T cells (Cantor and Haskins, 2005) and monocytes (Kurokawa, 2007), and is capable of exerting its deleterious effects on a variety of tissues, including adipose tissue, liver tissue and skeletal muscle (de Aguila, 1999; de Alvaro et al, 2004; Feinstein et al, 1994; Lang et al, 1992; Hotamisligil et al, 1993). Insulin receptor substrate (IRS)-1 and IRS-2 are docking proteins that are crucial in the initiation of the insulin cascade through phosphatidylinositol 3-kinase (PI 3-kinase) (Cheatham et al, 1995; Sun et al, 1993) and mitogen-activated protein kinases (MAPK) p42MAPK and p44MAPK (Cheatham, 1995). Both PI 3-kinase and MAPK are activated by insulin via tyrosine phosphorylation and have been shown to be involved in the regulation of glucose uptake and glycogen synthesis, respectively (Cheatham, 1995). Researchers reported that TNF-α
decreased PI-3-kinase activity at IRS-1 and IRS-2 in cultured myotubes exposed to TNF-α (de Aguila, 1999). Furthermore, TNF-α was shown to impair insulin-induced p42MAPK and p44MAPK tyrosine phosphorylation in cultured muscle cells. The p44 and p42 MAPK are two common isoforms found in muscle cells involved in the regulation of glycogen synthesis. Authors elaborated further, stating that their study demonstrated the extent to which TNF-a downregulates p42MAPK and p44MAPK (81%) is greater than TNF-α impairment of IRS-1-mediated PI 3-kinase activation (54%) (de Aguila, 1999). With this study, de Aguila and associates (1999) suggest that the intracellular components involved in insulin-mediated glycogen synthesis may be more sensitive to the effects of TNF-α than the upstream molecules of the insulin signaling pathway involved in glucose uptake. Similar results were found in cultured adipocytes by Stephens and colleagues (1997), although the mechanism behind the insulin resistance was contradictory to the results in de Aguila’s project (1999). Stephens (1997) reported that the insulin resistance observed in adipocytes following 72 to 96 hours of exposure to TNF-α was due not to decreases in IRS-1 phosphorylation, but due to significant decreases in IRS-1 protein content (>80%), as well as decreases in protein content of GLUT-4, which is a crucial, insulin-dependent glucose translocator within cells; TNF-α also appeared to decrease the amount of available insulin receptor within the cell membrane (Stephens, 1997). Despite the decrease in protein content, and thus available receptors and receptor substrates, remaining IRS-1 complexes were able to phosphorylate on tyrosine residues in a normal fashion, although the rate of phosphorylation decreased to a similar degree that the protein content was decreased following treatment with TNF-α (Stephens, 1997). Furthermore, GLUT-4 translocation was also executed in the presence of TNF-α, but the degree of translocation had decreased due to the diminished amount of available GLUT-4 protein (Stephens, 1997).
Data concerning TNF-α production in response to acute exercise has been contradictory. Serum concentrations of TNF-α have been shown to increase in response to acute bouts of exercise (Febbraio et al., 2002; Borst, 2004, Ostrowski et al, 1999; Kraemer et al, 1999; Neiman et al, 2004). Weinstock and associates (1997) found increased plasma levels of TNF-α following an hour-long, exhaustive treadmill run (mean duration 68 minutes), while Haahr and colleagues (1991) found no changes in TNF-α expression after 60 minutes of cycling at 75% VO_{2max}. Ostrowski’s research (1997) team reported 2-3 fold increases in plasma levels of TNF-α in 10 young men immediately following the Copenhagen marathon and for three hours following completion of the race. Other studies have also shown minimal or no change in serum TNF-α following exercise. Steensberg and colleagues (2003) reported no changes in arterial concentrations of TNF-α after 180 minutes of two-legged knee extensor exercise. Smith and associates (2000) found no significant change in blood concentrations in young men following high-intensity eccentric resistance exercise. Another study by Ostrowski and associates (1999) found modest, yet still significant, increases in plasma TNF-α following an intense 26-mile race and similar findings have been reported following only 20 minutes of cycling at 75% VO_{2max} (Goebel et al, 2000). Interestingly, Smith and colleagues (2000) found no significant change in plasma TNF-α following a high intensity bout of eccentric resistive exercise, and similar findings were confirmed by Hirose and associates (2004) following a single session of eccentric elbow flexor exercises. Recent studies by Phillips and associates (2008) and Starkie and associates (2005) have reported decreases in circulating TNF-α following resistive exercise, while Steensberg and colleagues (2003) also reported no changes in arterial TNF-α following a prolonged (180 minutes) session of knee-extensor exercise. Changes in TNF-α concentration are also present within
muscle tissue during and after an exercise session. Thirty strength-trained men completed two hours of resistive exercise with muscle biopsies before and after the exercise bout, and the expression of TNF-α mRNA was significantly increased, regardless of nutrient supplementation, following exercise (Neiman et al, 2004). Although predominantly produced by immune cells, TNF-α has recently been reported to be expressed within skeletal muscle in a fiber type-dependent manner (Plomgaard et al, 2005). In Plomgaard’s study (2005), muscle biopsies of the triceps, vastus lateralis and soleus muscles revealed the presence of TNF-α within the muscle fibers. As mentioned, TNF-α was expressed in a fiber type-dependent manner, with type II fibers containing soluble TNF-α, as well as TNF-α mRNA. TNF-α was not detected in type I muscle fibers. Plomgaard and associates (2005) hypothesized that the fiber-dependent expression, as well as the age-dependent expression, of TNF-α could explain the role the cytokine plays in age-related muscle wasting that is present in frail, elder patients.

**LPS-Stimulated TNF-α Production**

Immune cells within the blood are capable of producing pro- and anti-inflammatory cytokines in response to different stimuli to the immune system. Research into the differentiation of white blood cells has revealed that monocytes are a major producer of IL-6, TNF-α and IL-1β, along with many other proteins in the cytokine family (Xing et al, 2003). While other subtypes of white blood cells produce and release cytokines into the blood stream, monocytes are considered the subset most capable of generating large amounts of cytokines. In fact, on a per cell basis compared to monocytes, neutrophils produce less than 1.5% the amount of cytokines (Xing, 2003). TNF-α is one cytokine that is produced in large quantities by peripheral blood mononuclear cells (PBMCs) in response to
mitogen stimulation by lipopolysaccharide (LPS), a component of the cell membrane of gram-negative bacteria. The cytokine response to mitogen-stimulation with LPS has been thoroughly investigated and researchers have found monocytes to be extremely reactive to an endotoxin stimulus as well as the stimulus of exercise (Starkie et al., 2005). Researchers report that following 90 minutes of cycling exercise, PBMCs produced significantly less TNF-α (expressed per PBMC) immediately post- and 2 hours post-exercise than pre-exercise values following stimulation with LPS (Starkie, 2005). Further research into the effects of aerobic exercise on LPS-stimulated cytokine production revealed that after only a 5-km run, TNF-α production from PBMCs was significantly decreased (Drenth et al, 1998). Similar exercise-induced decreases were also found after a 6-hour endurance run (Drenth et al, 1995). Finally, researchers have reported that moderately intense resistive exercise is capable of reducing LPS-stimulated TNF-α production by PBMCs in elder women (Phillips, 2008). Whole blood samples were obtained immediately following and 2 hour following one hour of intense (80% 1-RM) resistive exercise and stimulated with LPS ex vivo. LPS-stimulated TNF-α levels were significantly reduced on a per monocyte basis in the exercising participants compared to non-exercise controls in the study (Phillips, 2008).

**Infused TNF-α**

Patients with type 2 diabetes present elevated levels of plasma TNF-α (Feingold et al, 1992), skeletal muscle (Saghizadeh, 1994) and adipose tissue (Hotamisligil, 1996). The presence of excessive amounts of TNF-α is thought to disrupt normal insulin signaling pathways at the cellular membrane, which contributes to the decrease in glucose uptake and can lead to hyperlipidemia. Plomgaard and associates (2005a) investigated the influence of TNF-α on whole body glucose disposal and reported that following a one-hour infusion,
TNF-α significantly reduced the rate of whole body glucose disposal, without the presence of significant changes to the rate of glucose appearance from peripheral tissues. Researchers also demonstrated that within skeletal muscle tissue, TNF-α infusion resulted in significantly increased rates of serine phosphorylation on IRS-1, with concomitant decreases in tyrosine phosphorylation (Plomgaard, 2005a). Furthermore, Plomgaard (2005a) reported that TNF-α infusion resulted in impaired insulin action of Akt-substrate 160 (AS-160), which is a vital step in GLUT-4 exocytosis and could provide another mechanism through which TNF-α inhibits glucose uptake. Further research utilizing TNF-α infusion has revealed additional effects on metabolism that could contribute to chronic diseases such as type 2 diabetes.

Plomgaard and colleagues (2008) subjected ten healthy, young men to a 4-hour infusion of recombinant human TNF-α (rhTNF-α) and found that as plasma TNF-α increased (from 0.7 ± 0.04 pg*ml⁻¹ to approximately 16.7 ± 1.8 pg*ml⁻¹) plasma concentrations of IL-6 were increased and peaked during the fourth hour of infusion (9.2 ± 1.0 pg*ml⁻¹), whole body glucose turnover was decreased, although muscle glucose utilization was unchanged, and finally, whole body fatty acid release was increased, as measured by palmitate turnover. Despite increases in fatty acid secretion within the body, muscle fatty acid metabolism was unchanged during the infusion, resulting in a systemic release of fatty acids into the circulation (Plomgaard, 2008).

Age and TNF-α

Although the majority of the projects investigating acute exercise responses of TNF-α have involved young, trained men, researchers have begun to investigate the effects of aging on cytokine production. Bautmans and associates (2005) found no change in plasma TNF-α in elder men and women (69 ± 5 years) following an intense bout of resistive
exercise after six sessions of weight training acclimation or following six weeks of resistive exercise training. Despite not seeing any significant change in plasma levels following exercise, which may have resulted from a less intense workload, other researchers have reported elder individuals with the ability to exercise at a sufficient intensity to elicit an increase in plasma TNF-α. McFarlin and associates (2004) found a significant increase in plasma TNF-α of postmenopausal women (ages 65 to 80 years) following an intense resistive exercise workout (3 sets, 9 exercises, 10 repetitions at 80% 1-RM). Interestingly, McFarlin’s team (2004) reported those women presenting the lowest levels of Toll-like receptors (TLRs) also produced the smallest amount of TNF-α following the exercise bout, regardless of training status. TLRs are essential for the cellular response to a pathogen and play a role in the immunoreactivity of white blood cells. Phillips and colleagues (2008) also subjected postmenopausal women (mean age 72 ± 6.2 years) to an intense resistive exercise bout and reported a trend toward a significant difference between exercising women and non-exercising control participants.

**Interleukin-1β**

Also produced by monocytes and macrophages (Martin and Wesche, 2002), IL-1β exerts its biological function by binding to its type I receptor and activating the inhibitor-K kinase/nuclear factor-KB (IKK/NF-KB) pathway and the three types of MAPKs: ERK, JNK, and p38MAPK (Martin and Wesche, 2002). Animal models have shown the effects of IL-1β on insulin-producing, pancreatic β-cells. Emanuelli and colleagues (2004) subjected harvested rat β-cells to small amounts of IL-1β (10 ng/ml) and found that IL-1β decreases insulin-induced tyrosine phosphorylation of the IR and IRS proteins as well as phosphatidylinositol 3-kinase (PI3K) activation, which is a crucial component of insulin
signaling and is one of the major events following insulin-stimulated IRS tyrosine phosphorylation. IRS tyrosine activation results from the recruitment of the PI3K p85 regulatory subunit to phosphotyrosine residues on IRS proteins that activate the catalytic (Emanuelli et al, 2004). Further research into the interaction between IL-1β and insulin signaling revealed that IL-1β is capable of acting through the SOCS-3 pathway (Emanuelli, 2004). Apparently, IL-1β upregulates SOCS-3 mRNA within pancreatic cells, which leads to dysfunctional insulin signaling and eventual cell death (Emanuelli, 2004). Exercise studies have reported variable results, which may be the result of various exercise regimens and intensities. Ostrowski and associates (1999) found slightly elevated plasma concentrations following an intense 26.2-mile run, but suggested that the modest increases were due to the local production of IL-1β and the rapid clearance from the circulation. Ostrowski’s team reported that small increases in IL-1β mRNA were found both in muscle tissue and blood mononuclear cells following the two-hour race, despite the fact that plasma levels did not increase significantly.

IL-1β and Exercise

Research concerning the patterns of production and release of IL-1β are also similar to TNF-α because there several contradictions in the literature regarding its expression in response to an acute exercise bout. Ostrowski and colleagues (1997) found elevated plasma concentrations immediately following a marathon race, and for one-half hour during the recovery period where levels began to return to baseline. Other researchers have found IL-1β to be elevated during (0.19 pg*mL⁻¹), immediately after (0.59 pg*mL⁻¹), two hours after (0.20 pg*mL⁻¹) and 24 hours following a 3-hour exercise session of cycling and up-hill walking, compared to baseline (0.04 pg*mL⁻¹), in untrained men (Moldoveanu et al, 2000).
Although not exhibiting any change with substrate availability, IL-1β was elevated following two hours of intense resistance training in young men (Neiman et al, 2004). Contrary to the research completed by others, Hirose and associates (2004) found no significant changes in plasma IL-1β after a single and multiple bouts of eccentric muscle contractions of the elbow flexors. Because of such discrepancies, further studies in the area are necessary to determine patterns of release during acute exercise. A meta-analysis by Suzuki and associates (2002) reported that the majority of exercise studies, whether they investigated aerobic or resistive exercise, found plasma IL-1β rose most significantly 2 h after exercise, but that there was an accompanying increase in plasma IL-1ra which would block the bioavailability of IL-1β in the circulation. A further study by Suzuki and colleagues (2003) found insignificant decreases immediately following a marathon race, but did find elevated urine levels of IL-1β. The authors believed that despite the systemic increase in cytokine concentration, there was an overwhelming antioxidant and anti-inflammatory response that prevented significant exercise-induced oxidative stress (Suzuki et al, 2003). Past studies involving cytokine production following high intensity, eccentric muscle contraction have revealed similar results. Smith and associates (2000) had found significant decreases in plasma IL-1β following eccentric phases of the bench press and leg curl exercises. In fact, plasma levels reached their lowest levels following exercise at 6 hours, 25 hours, and 120 hours post exercise (Smith, 2000). The authors believed that although there was a significant decrease, the normal increase in circulating cytokines could have been present, though undetectable due to powerful removal processes within the body (Smith, 2000). Recent results from Phillips and colleagues (2008) support these findings, showing insignificant increases in plasma IL-1β immediately post exercise and 2-hours post exercise. IL-1β plays a role in the
inflammatory response to exercise and in diseased states, but further research is needed to elucidate the exact function of IL-1β production and release.

**LPS-Stimulated IL-1β Production**

Similar to trends seen in TNF-α, IL-1β is produced by PBMCs and released into the circulation following antigen stimulation with LPS (Xing, 2003), as well as in response to the exercise stimulus (Drenth, 1998). Interestingly, IL-1β has been reported to increase in response to short duration exercise (Drenth, 1995). Following a 5-km run, Drenth and associates (1995) reported a two-fold increase in LPS-stimulated IL-1β production 3 hours into the recovery period. Conversely, Drenth and colleagues (1998) reported that following the 6-hour endurance run mentioned previously, participants showed decreased IL-1β production following LPS-stimulation. Researchers believed that a possible link between the increased plasma IL-6 could explain the down-regulation of LPS-induced IL-1β production (Drenth, 1998). Similar observations have been made by researchers investigating the effects of resistive exercise on LPS-stimulated cytokine production. Phillips and associates (2008) found that ex vivo IL-1β production from stimulated whole blood cultures was reduced following a single session of intense resistive training in elder, postmenopausal women. It has been hypothesized that the contraction-induced, plasma IL-6 elevations could cause the blunted immunoreactivity of stimulated whole blood cultures and thus inhibits an immune response that generates significant amounts of pro-inflammatory IL-1β (Phillips, 2008).

**IL-1β Infusion**

With such variable sites of action and production, it was surprising that IL-1β infusion had been used so infrequently in humans. Virtually the only method of generating
elevations in circulating levels of IL-1β had been to induce the elevations through intense physical activity. Mouse and rat models have been used frequently, but the aim of such studies had been to investigate the effects of IL-1β on neurodegeneration (Turrin et al, 2006), on leukocyte-induced breakdown of the blood-brain barrier (Kaiser et al, 2006) and neuroprotection in the face of endotoxin insult (Bernardino et al, 2005).

**Cortisol and Metabolism**

Belonging to a class of hormones known as glucocorticoids, cortisol has numerous effects on various tissues throughout the body, including muscle, adipose, hepatic and vascular tissues. Synthesized and released by the adrenal medulla, cortisol controls the catabolism and anabolism of lipids, proteins and glucose (Guyton and Hall, 2006). When presented with a stress stimulus, the adrenals secrete cortisol to increase nutrient availability by stimulating gluconeogenesis in the liver, as well as increasing glycogen storage in the liver for future metabolic needs. Glucose is synthesized from amino acids that are made available from lean tissue depots within the body, namely muscle tissue. Cortisol also stimulates fat tissue to catabolize triglycerides into free fatty acids (FFA) and glycerol. The increase in plasma FFA provides fuel for tissues to oxidize during stress, which can be in the form of exercise, injury or general inflammation. Interestingly, cortisol decreases the utilization of glucose in muscle and adipose tissues. Cortisol reduces glucose metabolism by inhibiting nicotinamide-adenine dinucleotide (NADH) oxidation within the tissue. Without the oxidation of NADH to NAD+, glucose cannot be metabolized and the cells must rely on the fat metabolism for generation of adenosine triphosphate (ATP) for energy. Also of interest, the resulting decrease in glucose utilization is not counteracted by the secretion of insulin. In the case of hypersecretion of cortisol, the condition of “adrenal diabetes” can
occur. One possible explanation of this inability of insulin to neutralize hyperglycemia is the effect of increased FFA causing decreased glucose transport across cell membranes according to the “glucose-fatty acid cycle.” The “glucose-fatty acid cycle”, as described by Randle and colleagues (1964) is an environment where the excessive availability of one nutrient (especially lipids in the form of acyl-CoA) inhibits the enzymes responsible for glucose and glycogen metabolism (phosphofructokinase). Further research has linked fatty acyl-CoA, ceramides and diacylglycerols with disruptions in the insulin signaling cascade (Hulver et al., 2004), namely through the phosphorylation of serine and threonine residues at the insulin receptor substrate-1 (IRS-1). Hepatoctyes within the liver (Oakes et al., 1997) and pancreatic beta-cells (Cnop et al., 2005) are also affected by the elevation of plasma free fatty acids. A lipotoxic environment is created within tissues, which leads to premature apoptosis via excessive stress on the endoplasmic reticulum (Cnop et al., 2005). The inability of insulin to decrease blood glucose levels signals for an increase in insulin production to combat the rising glucose concentrations. One side effect of hyperinsulinemia is an increase in blood triglyceride levels, caused by chronic fat utilization in the liver, which then brings about an increased risk of arterial plaque development and subsequent ischemic heart disease (Guyton et al., 2006). As the tissues lose the ability to utilize glucose, energy must be derived from triglycerides, which are readily available from adipose tissue stores. In addition to its effects on nutrient metabolism, cortisol is capable of influencing the inflammatory process through inhibition of lysosomal activity within the cells. Decreased lysosome activity leads to decreased cell degradation and inflammation. Also, cortisol controls inflammation by inhibiting the production of prostaglandins and leukotrienes, both of which activate numerous inflammatory processes such as white blood cell infiltration. In conjunction with the effects on inflammatory proteins, cortisol suppresses white blood cell proliferation,
especially lymphocytes, in response to cell injury. It has been shown that corticosteroids
given intravenously to humans cause lymphocytopenia, monocytopenia, eosinopenia, and
neutrophilia that reach their maximum effect 4 hours after administration (Rabin et al, 1996).
Glucocorticosteroid infusion, especially in supra-physiological doses, induces cell death of
immature T and B cells, while mature T cells and activated B cells are relatively resistant to
cell death (Cohen and Duke, 1984). In agreement, recent work shows that the percentage of
proliferative lymphocytes expressing early markers of apoptosis do not increase immediately
after or 2 h after intense exercise despite an increase in blood glucocorticoid levels
(Hoffman-Goetz et al, 1998). Finally, cortisol has been known to reduce the production of
interleukins from white blood cells in the body.

One of the major hormonal changes that occur with resistive exercise is the release
of cortisol from the adrenal glands, which has been noted in several studies (Kraemer et al,
1998; Kraemer et al, 1999; Harber et al, 2004; Neiman et al, 2004). The majority of these
studies have investigated the adaptations, both hormonal and cellular, to various types of
resistive training in young participants (Kraemer et al, 1998; Harber et al, 2004; Neiman et al,
2004), but Kraemer and associates (1999) were able to measure cortisol in older men (aged
62 years), as compared to younger men (30 years), before and immediately after an acute
bout of resistive exercise. The older group of men had higher baseline levels of cortisol, and
although no significant difference existed for changes in cortisol between groups, the older
group of men did have significantly elevated serum cortisol immediately following the
exercise session (Kraemer et al, 1999). Another study involving resistive exercise in elder
women reported no overall change in cortisol from pre-exercise levels following moderate
intensity (3 sets of 10 repetitions at the 10-RM) resistive exercise (Copeland et al, 2002).
However, when the women were grouped into quintiles for age, the elder age quintile (57-69 years) did have increased cortisol following the acute session of resistive exercise.

**Leukocytes and Exercise**

When stimulated by an exercise stress, the immune system will generate a systemic response by increasing production and adhesion of leukocyte populations. Neutrophils are activated during exercise and circulating numbers increase rapidly following an exercise session (Tidball et al, 2005). Neutrophils typically have the most rapid onset of infiltration of muscle tissue as well as into the circulation (Fielding et al, 1993). Once neutrophils have entered the muscle cells, they are capable of signaling further white blood cell adhesion and infiltration into the active tissue, especially immature monocytes (Tidball, 2005). Following entrance into body tissues, monocytes differentiate into macrophages and assist neutrophils in the phagocytosis associated with muscular rebuilding (Guyton and Hall, 2006; Tidball, 2005). With the penetration of muscle cells following exercise, circulating number of blood monocytes are typically elevated immediately following exercise and begin a transient decrease in circulating monocytes, which has been seen in several research projects (Degerstrom et al., 2006; Drenth, et al., 1998; Phillips, 2008). Significant elevations of circulating lymphocytes also occur in response to exercise (Drenth, 1998; Pedersen and Hoffman-Goetz, 2000), followed by transient lymphopenia that is resolved 48 hours after the exercise stimulus (Drenth, 1998; Pedersen and Hoffman-Goetz, 2000). It is thought that the lymphocytosis that occurs following exercise is the result of migration of lymphocytes from the vascular pools, as well as from extra vascular pools such as the lungs (Hansen et al, 1991). Specifically, researchers have found that resistive exercise increases the post-exercise expression of CD62L on circulating neutrophils and monocytes (Malm et al, 2000; Miles et
al, 1998) and of CD11b on monocytes (Pizza et al, 2002; Malm, 2000), with associated increases in leukocyte adhesion. Cluster of differentiation (CD) markers are immunohistochemical markers on the cell membrane of white blood cells that identify individual subsets of leukocytes. Each CD marker corresponds to a particular cytokine or subset of leukocyte. However, changes in total leukocytes and subset proportion in general are less significant in resistive exercise than with endurance exercise (Drenth, 1998; Phillips, 2008). In women, the surface density of CD62L was increased by intense resistance, although there was no change in the proportion of neutrophils expressing CD62L, and the expression of VLA-4 (CD49d/CD29) on the various leukocyte subsets remained unchanged. In men, four sets (12 repetitions per set) of bench press and leg curl reduced P-selectin (CD62P) at 24 and 144 hours after exercise (Smith et al, 2000). Some cytokines activate leukocytes, inducing the synthesis of specific adhesion molecules. For example, during the local inflammation that may accompany a bout of vigorous exercise, TNF-α and IL-1 induce the synthesis and secretion of adhesion molecules such as ICAM-1, VCAM-1 E-selectin (Sahnoun et al, 1998). Neilsen and colleagues (2003) reported CD11b expression on lymphocytes and monocytes was moderately increased following a marathon race, compared to pre-race values. A smaller, though still significant, increase was also observed following a half-marathon race, but was restricted to monocytes and granulocytes. Monocyte CD14 expression also increased after a marathon race, whereas non-significant changes were observed following the half-marathon race.
CHAPTER III

METHODS

Participants and Screening

Participants recruited for this project were post-menopausal, obese women (60 - 70 yrs; 30 ≤ body mass index ≤ 40 kg*m$^{-2}$; N = 23) that had not participated in a regular exercise program for the previous six months. The study protocol, including possible risks, was explained to potential participants at recruiting meetings or over the telephone. Those interested in participation signed an informed consent form and completed a medical history survey. Participants had the opportunity to ask questions before they signed the informed consent form approved by the Internal Review Board (IRB) of Texas Christian University (TCU). After signing, participants had their height and weight measured and their body mass index (BMI) calculated to determine whether they met the BMI exclusion criteria (BMI ≤ 30 or BMI ≤ 40 kg*m$^{-2}$). Other exclusion criteria included: use of hormone replacement therapy or selective estrogen receptor modulators, severe arthritis, bedridden within three months of the study, central or peripheral nervous system disorders, previous stroke, use of anti-depressant medications, acute or chronic infection, major affective disorder, HIV infection or auto-immune disorders, metabolic disorders (type I or type II diabetes mellitus), smokers or smokeless tobacco users, regular aerobic training or resistance training within the previous six months, oral steroid use, or surgery within the previous three months. Regular NSAID users were asked to refrain from taking their medication until after the experimental trials on test days. Subjects meeting the exclusionary criteria of the American College of Sports Medicine (ACSM) were not allowed to participate. Interested participants obtained written approval from their personal physician prior to all pre-testing procedures. Participation in the project was completely voluntary.
Pre-Testing Procedures

Following all screening procedures, and provided the participants met all inclusion criteria, body density was estimated via the Jackson & Pollock 7-site skin-fold procedure by a female technician, and percent body fat was estimated from body density using the Siri equation. After obtaining approval from their personal physician, each potential participant was given a medical screening by our study physician. This screening included a review of their medical history form and a physical exam, which included a lower extremity examination to identify musculoskeletal or flexibility limitations, and a screening for dementia.

Group Assignment

Those approved for participation were randomized, matched for age, BMI, and body fat into either an exercise group (EX) or a control group (CON): obese exercise (30 ≤ BMI ≤ 40 kg*m^2; N = 12; EX) or obese control participants (30 ≤ BMI ≤ 40 kg*m^2; N=11; CON).

Submaximal Treadmill Stress Test

Those cleared for participation returned to the lab and completed a submaximal, graded exercise test (GXT) up to 80% of their age-predicted maximum heart rate (APMHR) for use as a pre-screening procedure. Participants completed the GXT under continuous electrocardiogram (ECG) and blood pressure monitoring with a study physician present. During the GXT, participants began by walking slowly on the treadmill and a technician increased the speed of the treadmill every two minutes until the participant reached 80% of
their APMHR or requested to terminate the test. ECG leads were properly placed by a female technician and heart rate was continuously monitored throughout the test using a Polar Heart Rate System.

**Acclimation**

Both groups completed a week-long acclimation period to the following resistive exercises: chest press, “lat” pull-down, seated rows, shoulder press, leg abduction, leg adduction, chest flys, leg press, leg curl, and leg extension. They performed three acclimation exercise sessions on non-consecutive days. On acclimation day 1 (ACC 1), participants were taught proper lifting techniques for each of the ten exercises to minimize the risk of injury. The seat height was recorded for each participant to maximize the comfort level, assure proper form and standardize the workouts. Each subject’s 8-repetition maximum (8-RM) was assessed for each of the 10 resistive exercises. On the second acclimation day (ACC2), participants performed 3 sets of each exercise at 50% of their estimated 1-RM. During the first two sets, participants performed eight repetitions and the last set was completed to volitional fatigue. Eight-RM for all exercises was reassessed on acclimation day 3, in addition to 1-RM assessment for chest press, leg extension, and leg curl. All acclimation and exercise sessions were preceded by 5-10 min of cycle ergometry or walking. All acclimation sessions and resistive exercise sessions were completed in the weight room at the TCU Recreation Center using Nautilus (Vancouver, WA) and Cybex equipment (Medway, MA) under the supervision of trained technicians.
**Experimental Trials**

All participants completed an experimental trial (EX) at least one week after the last acclimation session or a control day (CON). For the experimental trials, participants reported to the lab, after an overnight fast, between 0530-0730 and rested quietly for at least 15 min before the baseline, pre-exercise blood sample was obtained. The EX group then performed 3 sets of the 10 exercises at 80% of their estimated 1-RM while the CON group rested quietly in the lab. During exercise, participants performed 8 repetitions at 80% of their estimated 1-RM during the first two sets and the last set was performed to volitional fatigue. At least 1.5 minutes of rest was given between sets. Following the exercise bout, participants rested quietly in the lab for 2 hours and were given water ad libitum. Approximately 20 mL of blood was obtained from an antecubital vein immediately pre-exercise (PR), immediately post-exercise (within 4 min, PO), 2 hours post-exercise (2H), and 24 hours post-exercise (24H) in EX and at same time points for CON. Due to the nature of certain exercises, the use of venous catheters was not possible in the EX group. However, venous catheters were used in the CON group to decrease the risk of bruising and maximize efficiency of collecting samples. The morning after the experimental trial, participants reported back to the lab after an overnight fast for the 24-hour post-exercise blood sample.

**Blood Sample Treatment and Analysis**

Blood was drawn into pre-chilled, EDTA tubes and room-temperature EDTA and sodium heparin tubes. The pre-chilled tubes were centrifuged (1500 * g, 4°C) for ten minutes and the plasma separated and stored at -80°C until analyzed for serum IL-6 and cortisol. Whole blood from the room temperature sodium heparin tubes was diluted 1:10 in RPMI (Sigma Diagnostics, St. Louis, MO) cell culture medium supplemented with penicillin
(100U/ml) and streptomycin (100 micrograms/ml) using aseptic techniques for subsequent use in the LPS-stimulated cytokine production assay.

**Total and Differential Leukocyte Counts**

Total leukocyte number and a five-part differential count were measured in whole blood from room temperature EDTA tubes, using an ACT-Diff 5 Beckman-Coulter hematology analyzer (Hilach, FL, USA). Leukocytes were classified as neutrophils, lymphocytes, monocytes, eosinophils, or basophils.

**Cytokine Analysis**

Cytokine production was assessed by diluting 0.5 mL of whole blood into 4.5 mL culture medium. Two milliliters of diluted whole blood samples was placed in 24-well flat-bottomed plates and stimulated with the polyclonal activator lipopolysaccharide (LPS from S. enteriditis, final concentration 25 micrograms/mL, Sigma Diagnostics, St. Louis). Following a 24-hour incubation in physiological conditions (37°C, 5% CO₂), the culture plates were centrifuged at 4°C for 10 minutes (800 * g) and supernatants were harvested and stored at -80°C until further analysis. The supernatant cytokine concentrations (TNF-α and IL-1β) from LPS-stimulated whole blood cultures were analyzed using enzyme linked immuno-sorbent assay (ELISA) (Invitrogen, Carlsbad, CA). Supernatants were diluted in assay solution prior to ELISA analysis (TNF-alpha, 1:10; IL-1β, 1:20) so that sample concentrations would fit the standard curve. LPS-stimulated cytokine production was expressed as concentration in supernatant (pg/mL) and per monocyte in each culture well (fg/monocyte). Serum IL-6 concentrations were assayed using high-sensitivity ELISA kits (R&D Systems, Minneapolis, MN).
Cortisol

Blood samples were centrifuged for ten minutes (1500 * g), and plasma was removed before being stored at -80 °C for later analysis by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Samples were run in duplicate to avoid intra-assay variations. Plasma cortisol was expressed in nanomoles per liter.

Statistics

Using SPSS (Chicago, IL) statistical analysis software, a 2-factor analysis of variance (ANOVA) with repeated measures on the within factor was used to determine if main effects or interactions existed within the data. The first factor was group (2 levels: EX and CON) and the second factor (within design) was condition (exercise in EX or time in CON). When the ANOVA detected a significant main effect, the Bonferroni analysis was used post hoc to determine where the differences were located. Mauchly's test of sphericity was employed. Correction for sphericity, using a Huynh-Feldt procedure, was performed when necessary. When the ANOVA detected a time by group interaction (p < 0.05), independent samples t-tests were conducted to determine if significant differences existed between groups. Finally, a one-way, repeated measures ANOVA was run within each group to determine if significant differences existed within the group across time points. Descriptive characteristics between groups were compared using independent samples student T-test with the Bonferroni adjustments. Descriptive information is expressed as mean plus/minus standard deviation (SD) and dependent variables are presented as means plus/minus standard error (SE). Threshold for significance was set at p < 0.05.
CHAPTER IV

RESULTS

Descriptive Statistics

No significant differences existed between the groups in terms of age, weight, height, fat-free mass, fat mass, BMI or BF (p > 0.05). Descriptive data for the two groups appear in Table 1.

Table 1. Physical characteristics of study participants.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>Fat Free Mass (kg)</th>
<th>Fat Mass (kg)</th>
<th>Body Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX (N=12)</td>
<td>65.08 ± 3.42</td>
<td>85.24 ± 9.67</td>
<td>67.79 ± 1.86</td>
<td>32.53 ± 2.15</td>
<td>55.1 ± 5.68</td>
<td>30.14 ± 5.415</td>
<td>35.22 ± 3.2</td>
</tr>
<tr>
<td>CON (N=11)</td>
<td>66.22 ± 4.36</td>
<td>87.46 ± 16.5</td>
<td>63.5 ± 3.16</td>
<td>33.43 ± 3.15</td>
<td>54.83 ± 10.1</td>
<td>32.63 ± 8.61</td>
<td>35.40 ± 3.45</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

Circulating Leukocytes

Due to difficulties in blood sampling, subject 5 was excluded from white blood cell data analysis. A significant group by time interaction was detected (p = 0.00). Resistive exercise increased circulating leukocytes in the EX group compared to the non-exercise, control group. EX participants showed a significant increase in total number of WBC (Figure 1) immediately PO (p = 0.025). Post hoc analysis revealed significant differences between time points within each group. In the exercise group, all time points were significantly higher than 24H (p < 0.008). In the CON group, total leukocyte number at both PO and 24H were significantly lower than 2H (p < 0.016).
We analyzed the change in composition of the total white blood cell count in EX and CON groups. Figures 2-7 illustrate the proportion of neutrophils, lymphocytes and monocytes in both EX and CON groups. A significant time by group interaction was found in proportion of neutrophils (p = 0.00). The percentage of neutrophils increased 2 hours following the exercise session, but it was not significantly different from CON (p = 0.084). Mean differences were detected within groups over time. In the CON group, there was a mean difference between PRE and PO (p = 0.047), and in the EX group between PRE and 2H (p = 0.00), between PO and 2H (p = 0.001), and between 2H and 24H (p = 0.013). We also found that number of circulating neutrophils behaved in a predictable pattern as well. A significant time by group interaction was detected between EX and CON (p = 0.00, F = 8.378), although differences between groups were not significant. Post hoc analysis revealed a time effect for the EX and CON groups. In CON, number of neutrophils was different.
between 2H and 24H (p = 0.006). In the exercise group, significant differences were found between PRE and PO (p = 0.00), between PRE and 2H (p = 0.013), between PO and 24H (p = 0.00) and between 2H and 24H (p = 0.003).

Figure 2. Percent neutrophils in EX and CON groups. # - Significant difference in EX compared to all other time points (p < 0.013). $ - Significant difference in CON vs. PRE (p = 0.047). Values are mean ± SE.

Figure 3. Circulating neutrophils in EX and CON. $ - significant difference in EX vs. PRE (p < 0.013); # - significant difference in EX vs. PO (p = 0.00); & - significant difference in EX vs. 2H (p = 0.003); * - significant difference in CON vs. 2H (p = 0.006). Values are mean ± SE.
Another type of WBC, lymphocytes, was also investigated and only the EX group showed any type of significant change during the experimental trial period. Comparing proportion of lymphocytes, a significant time by group interaction was found (p = 0.00). Exercising participants displayed a lower percentage of lymphocytes at 2H. The difference at 2H was significantly different between EX and CON groups (p = 0.038). The mean of the exercise group at 2H was significantly different from all other time points within the group (p < 0.017). The change in total number of circulating lymphocytes showed a group by time interaction (p = 0.00), but differences between groups did not reach significance. Significant differences were present within the CON group between PO and 2H. (p = 0.012). Differences were also found in EX between PRE and PO (p = 0.02), between PRE and 24H (p = 0.047), between PO and 2H (p = 0.05) and between PO and 24H (p = 0.00).

Lymphocytes Proportion

Figure 4. Percent of total WBCs from lymphocytes in EX and CON. * - Significant difference between EX and CON at time point (p = 0.038). $ - Significant difference in EX vs. 2H (p < 0.017). Values are mean ± SE.
Changes in the percentage of total white blood cells occupied by monocytes were also calculated for the study participants. No significant time by group interaction was found between the groups (p = 0.0625), but a significant time effect for proportion of monocytes was found (p < 0.001). The average within groups decreased from PRE (7.835 ± 0.25) to PO (6.92 ± 0.23) and continued to decrease until 2H (6.74 ± 0.23).

A significant time by group interaction was detected for number of monocytes (p = 0.00). EX had higher numbers of circulating monocytes post-exercise, and the difference was significant compared to CON at PO (p = 0.005). Significant differences were detected over time within each group. Differences were found in CON between PRE and PO only (p = 0.045). Circulating monocyte number was different in EX between PRE and 24H (p = 0.026), between PO and 2H (p = 0.044) and between PO and 24H (p = 0.01).
Figure 6. Percent of total WBCs from monocytes in EX and CON. $ - Significant difference within groups vs. PRE (p < 0.00); # - Significant difference within groups vs. 2H (p = 0.048). Values are mean ± SE.

Total number monocytes per well followed a similar pattern as total circulating monocytes. ANOVA identified a significant time by group interaction (p = 0.00). In the EX group, the number of monocytes per well was significantly increased following the exercise
session compared to the CON group (p = 0.005). A significant time effect was detected for CON (p = 0.031). Significant differences were detected between PRE and PO (p = 0.045) in the CON group. There was also an exercise effect found in the EX (p=0.001) group between Pre and 24H (p = 0.026), between PO and 2H (p = 0.044) as well as between PO and 24H (p = 0.01).

![Monocytes per well](image)

**Figure 8.** Monocytes per well. * - Significant difference between EX and CON at same time point (p = 0.005). $ - Significant difference in CON vs. PRE (p = 0.045). # - Significant difference in EX vs. PRE (p = 0.026). & - Significant difference in EX vs. PO (p = 0.044). Values are mean ± SE.

**Circulating IL-6**

A significant group by time interaction was detected for plasma IL-6 concentration (p = 0.038, F = 3.376). Plasma IL-6 concentration in EX was significantly lower compared to CON at PRE, 2H and 24H (p < 0.05). Resistive exercise resulted in a significant increase (p < 0.001) in plasma IL-6 concentration, which remained elevated at 2H (p = 0.002) before returning to baseline (PRE) at 24H. There was no significant change over time in CON.
Figure 9. Plasma IL-6 in EX and CON. * - Significant difference between EX and CON (p = 0.022). ¥ - Significant difference in EX vs. Pre (p < 0.019). # - Significant difference in EX vs. 24H (p < 0.007). Values are mean ± SE.

**LPS-Stimulated TNF-α**

There was no group by time interaction (p = 0.151, F = 1.838) and no significant changes occurred over time (p = 0.080, F = 2.370) for LPS-stimulated TNF-α production.

Figure 10. LPS-stimulated TNF-α production. Values are mean ± SE.
When TNF-α levels were expressed per monocytes, there were still no significant changes to report between EX and CON (p = .127) or across time (p = 0.069).

![TNF-α*monocyte\(^{-1}\)](image)

Figure 11. EX vs. CON TNF-α produced per monocyte. Values are mean ± SE.

**LPS-Stimulated IL-1β**

No significant group by time interaction was detected (p = 0.518, F = 0.735) and no significant changes were seen between the groups over time (p = .204, F = 1.611) in LPS-stimulated IL-1β production. When cytokine levels were adjusted for changes in number of monocytes per culture well and expressed in femtograms of cytokines produced per monocyte, there was also no significant effect over time (p = 0.071, F = 2.665) and no time by group interaction existed (p = 0.766, F = 0.309).
Figure 12. LPS-Stimulated IL-1β production. Values are mean ± SE.

Figure 13. LPS-Stimulated IL-1β production per monocyte. Values are mean ± SE.
**Plasma Cortisol**

A significant time by group interaction was found for plasma cortisol concentration \( (p = 0.001) \). A significant difference was found between the groups at 24H \( (p = 0.008) \). Significant differences were found over time in EX between PRE and 2H \( (p = 0.00) \), between PRE and 24H \( (p = 0.00) \), between PO and 2H \( (p = 0.006) \) and between PO and 24H \( (p = 0.049) \). Significant differences were also found between time points in CON at PRE and PO \( (p = 0.001) \), between PRE and 2H \( (p = 0.002) \), between PO and 2H \( (p = 0.041) \), between PO and 24H \( (p = 0.049) \) and between 2H and 24H \( (p = 0.00) \).

![Plasma Cortisol](image)

**Correlations**

Pearson-Product correlation analysis was completed to determine where significant correlations existed within the data. In the exercise group, positive correlations were found between plasma IL-6 at 24H and percent body fat \( (p = 0.046) \), between plasma IL-6 at 2H
and BMI (p = 0.029), between plasma IL-6 at PO and BMI (p = 0.022), between plasma IL-6 at PRE and body fat percentage (p = 0.048). In the CON group, significant, positive correlations were found between plasma IL-6 at PO and body fat percentage (p = 0.002). A significant, positive correlation was found between resting IL-6 concentrations across all time points and BMI for all participants (p < 0.013). To examine the relationship between resting plasma IL-6 concentration, BMI and percent BF, we divided all subjects into groups of the highest (N=10) and lowest (N=10) BMI and BF and compared resting plasma IL-6 concentrations between the groups. We also divided all subjects into the highest (N=10) and lowest (N=10) producers of plasma IL-6 at PRE and compared BMI and BF. A significant difference (p = 0.022) was found between resting plasma IL-6 concentrations of participants with the highest (3.12 ± 0.35 pg*mL⁻¹) BF compared to participants with the lowest (1.92 ± 0.23 pg*mL⁻¹) BF percentage.
CHAPTER V

DISCUSSION

We sought to investigate the effects of a single session of high intensity resistive exercise on the inflammatory response in obese, postmenopausal women. The primary finding of the study was that high intensity resistive exercise generates a contraction-induced, increase in plasma IL-6 in obese, postmenopausal women, but the elevated plasma IL-6 response did not generate any significant blunting effect on the immunoreactivity of blood mononuclear cells.

Descriptive Statistics

The twenty-three women who participated in the study did not differ significantly between the exercise (EX) and control group (CON) in terms of physical characteristics. Researchers were able to match participants between groups based on age, body mass index (BMI) and body fat percentage (BF) to reduce the effects of confounding variables such as fat mass and fat-free mass.

Circulating Leukocytes

Exercise has been shown to dramatically increase circulating levels of leukocytes, especially monocytes, neutrophils and lymphocytes (Pizza et al, 2002). The extent of the increase in circulating numbers is related to the duration and intensity of the exercise (Pedersen et al, 2002). The resistive exercise session lasted approximately one hour long and was completed at approximately 80% of each participant’s maximal effort. Of particular importance to researchers was the increase in total number of monocytes per well for the LPS-stimulation procedure. Resistive exercise significantly increased the total number of
monocytes per well that were able to respond to the mitogen stimulation. Similar patterns of monocyte appearance have been reported following eccentric cycling exercise (Malm et al., 2000) and following high intensity eccentric resistive training (Paulsen et al., 2005). Malm and colleagues (2004) also demonstrated similar findings that total leukocytes were significantly increased following an eccentric exercise session. Researchers report that monocytes, lymphocytes and neutrophils all are significantly increased following a single bout of exercise (Malm, 2000; Malm, 2004; Paulsen, 2005; Phillips, 2008). Of particular interest was the study by Paulsen and associates (2005), where monocyte numbers following intense resistive exercise increased immediately post-exercise, decreased one half hour and one hour following exercise, and increased significantly six hours after exercise. Researchers described this change in monocytes during recovery from exercise as a delayed-onset monocytosis and attributed the pattern of change in neutrophils and monocytes to increased release of immature leukocytes from bone marrow stimulated by the exercise session (Paulsen, 2005). Unlike leukocyte changes from our study, levels of circulating white blood cells, neutrophils and monocytes had all returned to baseline values within 24 hours of the exercise session (Paulsen, 2005). It has been argued that changes in circulating leukocytes could be the result of contraction-induced stress hormone and cytokine release from skeletal muscle (Steensberg, 2003a). While changes in numbers and proportion of neutrophils in our study were similar to previous research (Phillips, 2008; Paulsen, 2005), our monocyte data was somewhat different from previous studies. Malm and colleagues (2000) found increased monocyte populations following intense resistive exercise, as did Phillips and associates (2008). Although our exercising participants exhibited increased monocyte levels following the exercise session, this increase was resolved at the 2-hour time point and circulating monocytes decreased below baseline levels by 24H. While neutrophil infiltration into
damaged muscle cells has been a recent controversy within the scientific community (Schneider et al, 2007), some researchers believe that neutrophil infiltration and subsequent release of chemo-attractant agents such as IL-8 stimulates the migration of monocytes and macrophages into damaged muscle tissues. Still others have reported that monocytes and macrophages are capable of infiltrating muscle tissue without the presence of neutrophils within the damaged area (Frenette et al, 2003; Pizza et al, 2005). With or without the presence of neutrophil infiltration, immature monocytes are drawn to active and/or damaged muscle cells and differentiate into macrophages to initiate the repair response following exercise. As monocytes differentiate into macrophages, the number in circulation decreases, as does the number of monocytes capable of producing inflammatory cytokines such as TNF-α and IL-1β. This cycle of infiltration and differentiation is a possible explanation for our reported decreases in circulating monocytes and monocytes per culture well at 2H and 24H following the exercise session.

Exercise-induced muscle damage is also capable of affecting leukocyte populations and inflammation (Frenette, 2003; Paulsen, 2005; Pizza, 2005). Damaged muscle tissue quickly attracts phagocytic white cells to clear cellular debris following intense aerobic or resistive exercise (Tidball, 1995), however, a recent study by Malm and colleagues (2004) suggested that muscle damage is not necessarily related to leukocyte infiltration. Researchers found that following intense, eccentric muscle contractions, participants exhibiting the most severe delayed-onset muscle soreness (DOMS) had the lowest levels of neutrophils in muscle tissue biopsies (Malm, 2004). Macrophage and neutrophil infiltration has also been investigated in response to exercise-induced muscle damage. Although neutrophils have been shown to infiltrate damaged muscle more rapidly than macrophages (Fielding et al. 1993), it has been shown that macrophages actually support angiogenesis and promote
healing following intense exercise rather than increasing muscle damage and DOMS (Malm, 2004). Neutrophil infiltration usually peaks in the hours following exercise, while monocytes follow a slower time course (Malm, 2004), and typically return to baseline values within 24 to 48 hours (Malm, 2004). Muscle damage can also be determined by measurement of plasma levels of creatine kinase (CK) following exercise. Unpublished data by Phillips and colleagues reported that 10 weeks of RE resulted in significantly lower plasma CK levels following acute RE compared to pre-training values. Although we did not measure CK in our study, number of neutrophils was still elevated two hours after the exercise session, and was 13% lower than pre-exercise values at 24H, but the difference was non-significant. The effects of muscle damage associated with acute, high intensity RE were of concern in our study because it could have contributed to the significant difference between plasma levels of IL-6 in EX and CON at PRE, but previous research has shown that the leukocytosis and muscle tissue infiltration is resolved within 72 hours (Malm, 2004). Although the literature supports the idea that leukocytosis and muscle infiltration are resolved 24 hours following exercise, however, damage to muscle tissue and hormone kinetics still remain and could play a role in the changes seen in plasma IL-6 following intense RE. Researchers have shown that plasma IL-6 is still secreted in the hours after exercise during the recovery phase. Pedersen and Bruunsgaard (2003) reported that plasma IL-6 was increased immediately after exercise, as well as four to five hours following RE. The contraction-induced increase in plasma IL-6 is easily seen immediately post-exercise, whereas the delayed increase in plasma IL-6 four hours after exercise is most likely the result of damage to muscle tissue (Pedersen, 2003) and is thought to play an immunoregulatory role in the body.
**Interleukin-6**

Interleukin-6 is produced by contracting skeletal muscle and is released into the circulation during resistive exercise, (Borst, 2004; Ostrowski et al, 1999; Kraemer et al, 1999) and is capable of exerting its effects on various tissues and cell types throughout the body (Febbraio et al., 2002; Neiman et al, 2004). Researchers have hypothesized that IL-6 is the “exercise factor” that could possibly control glucose homeostatis during prolonged physical activity (Febbraio, 2002). Supporting this hypothesis are research studies that report IL-6 inhibits the action of glycogen synthase and activates glycogen phosphorylase activity (Kanemaki et al., 1998). In addition to its possible regulatory role of hepatic glucose production, IL-6 may also influence insulin-dependent glucose uptake in active tissues (Tsigos et al., 1997). Research studies have shown that IL-6 increases basal glucose uptake in cultured adipocytes and that rIL-6 infusion enhanced fatty acid oxidation (Stouthard et al., 1996). IL-6 also has the capability to induce anti-inflammatory cytokine receptor production, especially IL-1ra and soluble TNF-α receptors (Tilg et al., 1997), as well as inducing an increase in CRP production (Tilg, 1997), which has been shown to induce anti-inflammatory cytokine production from monocytes and suppress pro-inflammatory cytokine production from tissue macrophages (Puc, 1996).

In the present study, plasma IL-6 increased from PRE to PO in exercise samples and returned to baseline levels within 24 hours following the exercise session. Of particular interest to researchers was the significant difference between groups at the PRE experimental trial time point. CON participants presented with significantly elevated levels of IL-6 (3.02 ± 0.34 pg*mL⁻¹) compared to the EX participants (1.81 ± 0.19 pg*mL⁻¹). It was thought that a difference in body composition could explain this difference, but the differences between the groups did not reach statistical significance. Researchers postulated
that, despite efforts to randomize participants between groups, the possibility still existed that participants randomized to the EX were already in a healthier state of being than those randomized into the CON group. Differences between the highest and lowest (N = 20) “producers” of IL-6 at PRE were compared based on BF and BMI. Differences between participants with the highest and lowest (N = 20) BMI and BF were compared based on resting plasma IL-6 production. The only significant difference was found between the PRE IL-6 values of the participants with the highest (3.03 ± 0.375 pg*mL$^{-1}$) and lowest (1.92 ± 0.234 pg*mL$^{-1}$) body fat percentages (p = 0.022). Despite a significant difference within this group, half of each of the groups had been randomized to the CON and EX groups. Similar findings were reported by Cartier and associates (2008) who correlated BMI and visceral adiposity with plasma IL-6 concentrations. Middle-aged men with elevated body fat percentages (28.9 ± 0.55) had higher plasma levels of IL-6 (1.23 pg*mL$^{-1}$) compared to non-obese controls (0.58 pg*mL$^{-1}$) with normal body fat percentages (18.9 ± 0.56) (Cartier, 2008).

We observed similar increases in plasma IL-6 as reported by other studies of obese, elder women following intense, resistive exercise (McFarlin, 2004; Phillips, 2008; Toft, 2002). While plasma IL-6 may reach levels between 20 and 100 pg*mL$^{-1}$ following prolonged, endurance exercise (Ostrowski, 1998; Starkie, 2003), resistive exercise studies have reported plasma IL-6 values between 1 and 5 pg*mL$^{-1}$, which is in line with the data we reported. Another point of interest to researchers is the fact that while plasma IL-6 returned to baseline levels in EX participants at 24 hours post exercise, IL-6 levels in CON participants did not change significantly, but remained significantly different from EX at 2H (p = 0.022). Plasma IL-6 concentrations increased from PO to 2H in CON participants, but the difference between time points was not significant (p = 0.43). A possible mechanism for this
phenomenon has been investigated and researchers found that prolonged presence of a
venous catheter can actually increase plasma concentrations of IL-6 taken from the catheter.
The effect of the venous catheter on plasma IL-6 seen in this study is similar to results
reported by Haack and colleagues (2000). Three to four hours of exposure to a catheter can
increase plasma IL-6 in non-exercising participants to levels reported following intense
resistive exercise (4.0 ± 1.5 pg*mL⁻¹) (Haack, 2000). Although this mechanism could
possibly explain the increase in plasma IL-6 concentration during the rest period, it would
not account for the elevated PR value seen in the CON participants. We saw similar results
in our participants, and the presence of the venous catheter may account for the increased
plasma IL-6 at 2H in CON. The fact that plasma IL-6 returned to baseline values the
following morning supports the theory that the increase at 2H was in fact caused by the
catheter. One study has reported that IL-6 follows a circadian pattern of release, and sleep
deprivation can actually affect this rhythm by suppressing plasma concentrations of IL-6
(Vgontzas et al, 2005). In this study, sleep-deprived participants showed lower levels of
plasma IL-6 concentrations than during a normal sleep trial. IL-6 concentration peaks
several times during the day (Vgontzas, 2005), most notably in the evening, and again in the
early morning hours leading up to waking, which is another possible, although unlikely,
explanation for the significant difference at PRE seen between the groups. Another theory
investigated by researchers was the length of time between the acclimation days and the PRE
sample for the experimental trials for the CON group. Specifically, we were concerned that
inadequate time had been given for exercise-induced changes in cytokines and muscle
damage from the ACC days to return to baseline. All participants were given at least three
days between ACC3 and ET, but a significant, negative correlation was found between days
between ACC3 and resting levels of plasma IL-6 (r = -0.461, p = 0.020). Despite the
correlations and possible effects of the most recent exercise session, the literature does not support the theory that plasma IL-6 would remain elevated for 3 days and affect the PRE values of the experimental trial. Toft and colleagues (2002) investigated plasma IL-6 concentrations following intense, eccentric exercise in young and elder men and obtained blood samples immediately post, and for every hour up to 4 hours post exercise, and continued to monitor plasma IL-6 at 24 hours post, 2 days post, and 5 days post exercise. Plasma IL-6 returned to baseline in both groups at the 24 hour time point (Toft, 2002). White blood cell data was also collected on the same timeline in this study, and Toft (2002) reported that lymphocytes and neutrophils were both elevated in the hours following exercise, but returned to baseline levels within one and two days post-exercise, respectively. Despite the similarities between data from Toft and colleagues (2002) and our study, one major difference is the type of exercise model used in the study. Toft (2002) utilized an eccentrically-based, lower body cycling protocol whereas we used a whole body, resistive exercise protocol. Although both exercise sessions will generate significant amounts of contraction-induced IL-6, the kinetics of IL-6 release are different following eccentric exercise compared to concentric exercise (Pedersen, 2003). With the differences between eccentric and concentric contraction-induced IL-6 secretion, and significant correlation between PRE IL-6 and days between trials, researchers cannot ignore the fact that there is a possibility that the ACC3 exercise session caused sufficient muscle tissue damage to elevate the PRE IL-6 values seen in the CON group at the beginning of the experimental trial.

IL-6 is known to induce anti-inflammatory properties, and has particularly potent inhibitory effects on TNF-α and IL-1β secretion. Comparing the situation of systemic sepsis versus exercise, IL-6 is secreted preferentially in response to exercise compared to TNF-α secretion during systemic infection (Suzuki, 2002). Animal models have shown the beneficial
effects of IL-6 using anti-IL-6 treated mice as well as IL-6 knockout mice. IL-6 deficient mice have elevated levels of TNF-α, which suggests that IL-6 is capable of inhibiting TNF-α release within the body (Mizuhara et al, 1994). IL-6 infusions have also demonstrated the anti-inflammatory benefits of IL-6. Infusion with rhIL-6 diminishes the endotoxin-induced release of TNF-α from peripheral blood monocytes (Starbie, 2003). Plasma IL-6 also has the ability to indirectly regulate the increase in lymphocytes following long duration exercise activities through its control of cortisol release following exercise (Steensberg et al, 2001). Unpublished data from Steensberg and colleagues has shown that IL-6 infusion causes a direct increase in plasma cortisol levels. Researchers believed that IL-6 is capable of stimulating the hypothalamic-pituitary-adrenal axis and thereby increasing cortisol secretion (Steensberg, 2001). A trend for significance was also seen at PO between the two groups where the exercise session reduced the effect of the normal diurnal decrease in cortisol seen in CON at PO (p = 0.093). A significant difference in plasma cortisol was also seen between the groups at 24H (p = 0.08), which researchers feel is the result of muscle contraction-induced increases in plasma IL-6. We also analyzed the changes in plasma IL-6 between PRE to PO and plasma cortisol between PRE and 24H for significant correlations. A trend for significance was found (p = 0.057), which illustrates the feedback system between plasma IL-6 and cortisol following exercise. The normal diurnal increase in morning cortisol concentration was blunted at 24H as a result of the previous day’s exercise session. The blunted diurnal rhythm contributed to the significantly decreased numbers of lymphocytes, monocytes and neutrophils compared to baseline values in EX. The reduction in circulating neutrophils, lymphocytes and monocytes coincides with the trend for blunted cortisol release at 24H (p = 0.093). Changes in white blood cell number are also linked to exercise-induced muscle damage (Malm, 2004; Pedersen, 2002). Correlations between catecholamine release
and white blood cell number has been hypothesized (Pedersen, 2002), but elevations in adrenaline return to baseline values within 1 to 2 hours, whereas leukocyte number can remain below baseline levels up to 24 hours following exercise (Pedersen, 2002). Pedersen and associates (2002) believed that the prolonged decrease in white blood cells, especially lymphocytes, is the result of changes in plasma cortisol levels.

Finally, IL-6 is characterized as anti-inflammatory because of its ability to stimulate the release of other anti-inflammatory cytokines and receptors, namely IL-10 and IL-1ra (Steensberg, 2003). Our group hypothesized that the contraction-induced IL-6 release during exercise would have similar anti-inflammatory effects and blunt the release of TNF-α and IL-1β in response to antigen assault by LPS, which has been seen in a similar study with elder, postmenopausal participants (Phillips, 2008). Unfortunately, due to unexpected changes in plasma IL-6 within the CON group, any anti-inflammatory effect of the exercise session was not seen when compared to the EX group, although there was a trend ($p = 0.069$) for a blunting effect in TNF-α production per monocyte in the exercise group relative to the findings of the previous study by Phillips (2008). The EX group showed less TNF-α per monocyte at PO and 2H than CON, although the difference was not significant.

**LPS-Stimulated Cytokine Production**

It is thought that contraction-induced elevations in circulating IL-6 may play a role in the inhibition of TNF-α release from white blood cells, especially monocytes. Early studies showed the effects of plasma IL-6 elevations on LPS-stimulated TNF-α production from peripheral blood monocytes (Schindler, 1990). Researchers reported significant reductions in pro-inflammatory cytokine production following LPS-stimulation of monocytes in the presence of varying concentrations of IL-6 (Schindler, 1990). Suppression of TNF-α by IL-6
was also seen at the transcriptional level (Schindler, 1990). Schindler’s team (1990) used plasma IL-6 concentrations similar to those seen in this study (1 -5 pg*mL$^{-1}$) and still generated an inhibitory effect on LPS-stimulated release of TNF-$\alpha$ and IL-1$\beta$. Similar suppression of pro-inflammatory cytokines has been reported by Starkie and associates (2003) when participants either cycled for 3 hours or received rhIL-6 infusion that elevated plasma IL-6 to levels found in the cycling trial. Following in vivo administration of LPS, the exercise and infusion groups exhibited significantly lower levels of TNF-$\alpha$ compared to control participants (Starkie, 2003). In the present study, researchers hypothesized that the contraction-induced IL-6 production during the resistive exercise session would blunt the immunoreactivity of the circulating monocytes and decrease the amount of LPS-stimulated TNF-$\alpha$ and IL-1$\beta$.

Although there appears to be a trend for diminished TNF-$\alpha$ response in the EX group ($p = 0.069$), the blunting effect does not appear to be as profound as researchers hypothesized relative to previous research (Phillips, 2008). Overall, exercising participants (39.9 ± 4.7 fg*mL$^{-1}$) exhibited lower levels of TNF-$\alpha$ production when expressed as femtograms per monocyte compared to non-exercising, control participants (45 ± 4.6 fg*mL$^{-1}$), but these differences were not significant. Further investigation shows that in the EX group, number of circulating monocytes was decreasing from PO to 2H, as was number of monocytes per culture well. Meanwhile TNF-$\alpha$ remained relatively stable, which gave rise to a slight increase in TNF-$\alpha$ production per monocytes. However, cytokine production in the CON group was elevated at 2H, although not significantly, while number of circulating monocytes and monocytes per culture well remained somewhat stable. This combination led to a rise in TNF-$\alpha$ production per monocytes, although the difference was not significant. When compared to EX, there was a trend for a blunting effect compared to CON, although
the difference was not significant, which was likely due to the high variability found within
the participants. One theory regarding the absence of IL-6-induced suppression in LPS-
stimulated samples is that the non-significant blunting effect of the exercise-induced IL-6 on
monocyte-derived TNF-α was the result of the elevated PRE levels of IL-6 in CON
participants. However, if the lack of LPS-stimulated cytokine production was affected by the
elevated IL-6 in CON participants, researchers should not have seen any tendency for
increases in mitogen-stimulated cytokine production. On the contrary, 2H values for TNF-α
production per monocyte in the CON group showed a tendency to increase (p = 0.069)
despite the fact that the CON group showed significantly higher levels of IL-6 at that time
point compared to EX. The possibility remains that the presence of the venous catheter
could have affected the 2H time point and, according to Haack and colleagues (2000), the
increase in IL-6 might have been localized to the injured vein during the blood sampling and
did not reflect the systemic cytokine profile of the participants. Had a significant time effect
for CON been observed, the tendency for increased IL-6 in CON could have been
attributed to the presence of the catheter, but because the difference between PO and 2H in
CON was non-significant (p = 0.430), researchers feel another mechanism is responsible for
the IL-6 response in CON.

Results for LPS-stimulated IL-1β production were similar to TNF-α following the
exercise session in terms of absolute concentration and when corrected for number of
monocytes per culture well. LPS-stimulated IL-1β production in EX (1985 ± 295.2 pg*mL⁻¹)
appeared to be elevated at PO compared to CON samples (1354 ± 245.53 pg*mL⁻¹),
although this was not a significant difference since there was no time by group interaction (p
= 0.0776), nor was there a significant main effect for group (p = 0.716) or for time (p =
0.716). Similar studies in elder women performing an equally intense exercise routine
reported somewhat higher levels of absolute LPS-induced cytokine production, as well as cytokine production expressed per monocyte. Phillips and associates (2008) reported absolute cytokine production following LPS stimulation between 500 and 800 pg*mL^-1 for TNF-α, compared to our values of 1700 to 2300 pg*mL^-1, and between 3000 and 6000 pg*mL^-1 for IL-1β, compared to our values of 1300 to 2500 pg*mL^-1. Values for cytokine production per monocyte were between 150 and 500 fg*monocytes^-1 for TNF-α, compared to our values of 40 to 60 fg*monocytes^-1, and between 30 and 50 fg*monocytes^-1 for IL-1β, compared to our values of 30 to 55 fg*monocytes^-1 (Phillips, 2008). It was somewhat expected to find higher levels of both absolute cytokine production and cytokine production per monocytes to be higher in the present study because the participants were on average younger and had higher BMI and BF than the participants in the study by Phillips and colleagues (2008). Bruunsgaard and colleagues (1999) investigated the effects of age and BMI on LPS-stimulated cytokine production and found that elder participants have diminished cytokine-producing abilities compared to younger counterparts within the study. Bruunsgaard (1999) also found that those participants with lower BMI values also had lower LPS-stimulated cytokine production. When compared to the study by Phillips (2008), the participants in the present study were younger and had higher BMI, and should have had higher levels of cytokine production. Although this was true for TNF-α, it was not seen in the LPS-stimulated IL-1β samples, or in cytokine production per monocyte. Plasma concentrations of IL-6 were similar between the studies.

Absolute values for EX LPS-stimulated IL-1β were not significantly different from CON samples. No significant differences were found between EX (36.4 ± 4.82 fg*monocytes^-1) and CON (32.49 ± 3.99 fg*monocytes^-1) groups when comparing IL-1β production per monocyte, despite the significant difference in number of circulating
monocytes at PO and CON groups. Previous research (Phillips, 2008) indicated that a significant blunting effect was seen in IL-1β production following an intense bout of resistive exercise in postmenopausal women. Similar to the confounding factors influencing the LPS-stimulated TNF-α production, significantly elevated PRE values of plasma IL-6 may have contributed to the non-significant difference in LPS-stimulated cytokine production between EX and CON, although this theory is unlikely given the slight increases in absolute IL-1β production in the presence of elevated plasma IL-6 at 2H. Furthermore, issues arose with samples obtained and analyzed from two of the CON participants and were not included in the statistical analysis. Samples from two participants were hemolyzed and were not viable for use in cytokine analysis.

**Cortisol**

Serum cortisol concentrations following the intense bout of resistive exercise decreased over time and remained below baseline levels 24-hours after the exercise session. The acute effects of the exercise session closely resemble data seen in similar populations and following similar exercise protocols (Copeland et al., 2002), yet is contrary to established patterns of glucocorticoid release following acute resistive exercise (Kraemer, 1999).

Kraemer and colleagues (1999) reported significant elevations in plasma cortisol following intense resistive exercise by young and elder men.

Cortisol is involved in basic metabolic functions including mobilization of free fatty acids and amino acids from tissue stores in response to exercise (Guyton and Hall, 2006). Cortisol is also known as an anti-inflammatory hormone and capable of reducing capillary permeability, inhibiting leukocyte infiltration into damaged tissues, and reducing lymphocyte reproduction (Guyton and Hall, 2006). The effects of exercise on cortisol have been studied.
extensively (Hackney et al. 1999; Hermann et al., 2006; Nehlsen-Canarella, 1997; Steensberg, 2001) and our results are in line with previous research on resistive exercise in elder populations (Copeland, 2002). Cortisol has been shown to control lymphocyte release in response to an exercise session (Steensberg, 2001) and has been shown to alter the number and function of lymphocytes with regards to glycogen availability (Nehlsen-Canarella, 1997). During the hours following resistive exercise, we saw a significant decrease in the number of circulating lymphocytes, which is intriguing due to the significant decrease in plasma cortisol seen between PO and 2H in EX. Low levels of plasma cortisol would typically be accompanied by an increase in circulating lymphocytes, as cortisol is capable of reducing lymphocyte reproduction. On the contrary, we found significant decrease in number of circulating neutrophils, which is expected because of the regulatory role cortisol exerts on neutrophil population. Elevated plasma cortisol inhibits white blood cell infiltration into tissues, which matches the pattern reported in our study. Neutrophil populations decrease and infiltrate muscle tissues as cortisol levels remain below baseline at 2H and 24H. The effect of prior exercise on plasma cortisol following two sessions of high-intensity and moderate-intensity protocols has been investigated by Hackney and associates (1999). Hackney (1999) reported that plasma cortisol is lower 24 hours after high intensity exercise compared to moderate-intensity exercise and non-exercise control group. Researchers hypothesized that the decrease in plasma cortisol resulting from prior exercise is likely the result of decreased secretion of catecholamines (Hackney, 1999). Hackney (1999) hypothesized that the decreased catecholamine secretion seen 24 hours after exercise down-regulates the secretion of adrenocorticotropic hormone (ACTH). Cortisol release is directly related to ACTH concentrations (Guyton and Hall, 2006) and will also be down-regulated when catecholamine concentrations are diminished (Hackney, 1999). Cortisol concentration
is related to plasma IL-6 (Steensberg, 2003), and this relationship was seen in this study, although IL-6 concentrations were not significantly correlated with plasma cortisol. Cortisol has also been hypothesized as a control mechanism for the diurnal release of inflammatory cytokines from monocytes (Hermann et al., 2006), but the authors reported that this hypothesis only held true with regard to TNF-α and not IL-1β. In a study by Steensberg and associates (2003), a low dose infusion of rhIL-6 (30 μg/hr) resulted in plasma concentrations of IL-6 above 150 pg/mL. Although plasma IL-6 concentrations of this magnitude are achievable following prolonged, intense exercise, they are significantly higher than those seen in the present study (1 – 4 pg/mL). Plasma IL-6 was thought to induce release of cortisol (Steensberg, 2003), and although the there was not an increase in plasma cortisol following exercise as expected by Steensberg’s theory, there was a reduction in the diurnal variation in cortisol in the EX group compared to CON. There was a trend for significance between EX and CON at PO (p = 0.093) and there was a significant difference between groups at 24H (p = 0.08) resulting from the apparent exercise effect of the previous day’s resistive exercise session. In the early morning, cortisol concentrations are high, but decrease throughout the morning, as was seen in the CON group. This normal variation in cortisol was not seen in the EX group and could be a direct result of the significant increase in plasma IL-6 following exercise. An apparent exercise effect was observed until the next morning at 24H when cortisol was still below baseline levels for EX while CON exhibited the normal diurnal increase in the morning. Finally, cortisol may be a factor in muscle-wasting associated with inactivity and bed rest (Paddon-Jones et al., 2006). Researchers found that muscle-wasting was significantly accelerated following 28 days of bed rest in a hypercortisolemic state (Paddon-Jones, 2006). Due to the effects of elevated cortisol, especially the increase in amino acid availability through protein catabolism (Guyton and
Hall, 2006), there could be a protective period following resistive exercise during which cortisol levels fall below baseline in an effort to reduce the effects on damaged muscle tissue and facilitate muscle tissue repair and rebuilding, although the exact mechanism of this effect is not clearly understood (Paddon-Jones, 2006).

**Conclusions**

Resistive exercise has been repeatedly shown to increase circulating levels of anti-inflammatory cytokines in both young and elder populations (Febbraio et al, 2002; Febbraio et al, 2004; Pedersen et al., 2001; Weisberg et al., 2003). Contracting skeletal muscle is capable of generating significant increases in plasma levels of IL-6, which can then in turn influence the state of systemic inflammation. The hypothesized reduction in mitogen-stimulated cytokine production was not seen in the study population, although a tendency for separation between the groups following exercise was observed which was similar to results seen in Phillips’s study (2008). Factors that could have affected the LPS-stimulated cytokine production include the elevated plasma IL-6 at PRE in the CON group, which may have been the result of inadequate recovery time from ACC3 before the experimental trial, as well as the differences in body weight, BMI, and BF percentage compared to the study by Phillips and colleagues (2008). Women in the present study were on average heavier (86.35 ± 13.1 kg), had higher BMI (32.98 ± 2.65 kg*m⁻²) and had higher BF percentages (35.31 ± 3.33) than participants in the previous study (Phillips, 2008) (68.1 ± 11 kg; 26.3 ± 4.6 kg*m⁻²; 32.73 ± 4.6 %, respectively).

Variables that might have influenced study results included the nature of randomization of participants, sample preparation and storage, as well as intensity of the exercise session. While true random group assignment was attempted by researchers, there
were obvious differences between the plasma IL-6 concentrations of EX and CON participants as evidenced by the significant difference between PRE values of plasma IL-6. Despite efforts to maintain viable fluid samples for analysis, three participants were removed from the statistical analysis of leukocyte and LPS-stimulated data due to compromised samples. These samples were not contaminated by any outside agent, but were hemolyzed to the point of forcing researchers to doubt the validity of the concentrations of circulating and LPS-stimulated cytokines within the sample. Lastly, achieving 80% of one’s 1-RM is difficult for even the recreationally trained athlete, but can be especially difficult for sedentary, elder women without any previous experience with resistive exercises. Researchers attempted to reduce this effect by allowing for a one week acclimatization period that would allow participants to become accustomed to the intensity and proper form of the exercises.

Contraction-induced IL-6 production is a possible factor in the suppression and down-regulation of systemic inflammation. Plasma IL-6 has the ability to exert its effects on leukocyte-derived inflammatory cytokine production, immuno-regulatory hormones, and substrate metabolism during an exercise session and in the hours following exercise. Within the cytokine family of molecules alone, there are hundreds of substances that influence the metabolic processes during and after exercise. Cytokine production from skeletal muscle, circulating white blood cells, and immuno-regulatory hormones such as cortisol and the catecholamines contribute to the overall anti-inflammatory effects of exercise. Further research is needed to truly comprehend the interaction between anti-, pro-inflammatory cytokines and diurnal hormone changes that influence the metabolic and inflammatory profile.
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Abstract

The influence of acute resistive exercise on inflammatory markers in the blood of obese, postmenopausal women.

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Background: Contracting skeletal muscle is capable of producing a metabolic response involving the production of large amounts of intracellular messengers known as cytokines. IL-6 is one cytokine known to be released in response to muscular contraction. Plasma IL-6 concentrations have been shown to increase significantly following intense resistive exercise, and muscle-derived IL-6 is capable of reducing the inflammatory response of blood mononuclear cells. Purpose: To examine the effects of an acute bout of resistive exercise on inflammatory markers in the blood, 23 obese, postmenopausal women performed a high intensity resistive exercise session and the effects of contraction-induced IL-6 on LPS-stimulated TNF-α and IL-1β production were measured. Methods: Obese, postmenopausal (65.65 ± 3.89 years) women (N=23) were acclimated to resistive exercise over a three day period. After the acclimation period, participants were randomized into one of two groups: non-exercising control group (CON; N=11) or exercise group (EX; N=12). At least three days after the third acclimation day, participants reported to the lab and either completed a resistive exercise session (EX) at 80% of their estimated 1-RM, or rested quietly in the lab (CON). Blood samples were obtained pre, post, 2 hours-post, and 24 hours-post exercise. Similar time points were used in the CON group. Blood samples were analyzed using ELISA for plasma IL-6 concentrations. Whole blood samples were stimulated with LPS endotoxin and incubated for 24 hours in physiological conditions (37°C, 5% CO₂). LPS-stimulated production of TNF-α and IL-1β were measured in the stimulated supernatants to assess immunoreactivity of blood mononuclear cells. Results: Plasma IL-6 increased significantly following the exercise session (p<0.019), although the difference compared to CON at PO was not significant. Significant leukocytosis occurred following the exercise session. Total white blood cells were increased, as well as numbers of circulating monocytes, neutrophils and lymphocytes immediately following exercise. LPS-stimulated cytokine production was not significantly affected by the exercise session. Plasma cortisol pattern of release was unchanged in the CON group, while the diurnal decrease in cortisol was delayed immediately post-exercise in the EX group. Cortisol remained below baseline values at the 24H sample in EX (p=0.00). Conclusion: Resistive exercise is capable of generating an immune response in elder, postmenopausal women as seen in the significant increase in plasma IL-6 and systemic leukocytosis. Plasma IL-6 exerted its effects on plasma cortisol and mitogen-stimulated, whole blood cultures. The typical decrease in cortisol was not seen in the EX group, likely because of the contraction-induced changes in plasma IL-6. Unfortunately we did not find any significant difference between LPS-stimulated samples, although there was a tendency for significance at PO, indicating that plasma IL-6 was inhibiting pro-inflammatory cytokine release to some degree.
Curriculum Vitae

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