MENOPAUSAL STATUS IMPAIRS RESISTANCE EXERCISE-INDUCED INFLAMMATORY RECOVERY

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ABSTRACT

Purpose: To observe the effects of menopause status on exercise-induced inflammatory responses to acute resistance exercise. Methods: Twenty women aged 18 to 65 years completed a session of submaximal, full body resistance exercise. Subjects were categorized as either pre- or post-menopausal based upon history of menopausal status and follicle stimulating hormone (FSH) levels. On a separate day prior to testing, one repetition maximums (1RM) were determined for the chest and leg press, leg curl, vertical pull down, triceps and leg extension exercise. On the day of testing, subjects performed 3 sets of 10 repetitions at 75% 1RM for all exercises. Blood samples were obtained from the antecubital vein prior to, immediately after, and one hour after the termination of exercise. Changes in plasma concentrations of IL-6, IL-10, IL-13, and TGF-β1 were determined via enzyme-linked immunosorbent assays (ELISA). An independent t-test was used to assess basal group differences. A mixed design, repeated measures analysis of variance (ANOVA) was used to assess within and between group differences over time. Pairwise comparisons were then made from significant main effects using the Bonferroni procedure. Results: Significant (p<0.05) differences over time were found for IL-6 and IL-10. A significant (p<0.05) time by group difference was revealed for IL-10. Both groups displayed a significant one fold change in IL-6 pre to one hour post, and a half fold change immediately after to one hour post exercise.
Pre-menopausal levels of IL-10 significantly increased one-fold from pre to post exercise, whereas post-menopausal levels did not significantly differ over time. Immediately post exercise IL-10 levels significantly differed between pre- (4.66 ± 1.47) and post-menopausal (1.29 ± .97) women. **Conclusion:** Menopausal status influenced the magnitude and time-course of the cytokine response to exercise-induced stress. Post-menopausal women exhibited an impaired capacity to resolve exercised-induced inflammation. This is consistent with several cell and animal models describing the role of estrogen in IL-10 recruitment. Thus, menopausal status, in conjunction with ageing, influenced dynamic inflammatory resolution.
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Menopause encapsulates the life-altering process whereby the loss of ovarian follicles facilitates a number of permanent biochemical and physiologic changes. Though a highly individual process, clinically, menopause can be identified by the cessation of menses in conjunction with systemic changes in inhibin B, anti-müllerian hormone (AMH), follicle stimulating hormone (FSH) and estrogens. Over two million women in the United States transition into menopause yearly, equating to roughly 6,000 individuals daily (United States Census Bureau, 2002). With the vast increase in life expectancy over the last century, women regularly spend 40% or more of their lives in the post-menopausal state (Department of Health and Human Services, 2014).

Though a natural process, menopause is associated with an elevated risk of osteoporosis, cardiovascular disease, gastrointestinal distress, chronic low-grade inflammation and sarcopenia (Abu-Taha et al., 2009; Drake, Clarke, & Lewiecki, 2015; Infantino, 2007; Messier et al., 2011; Rosano, Vitale, Marazzi, & Volterrani, 2007). The risk of onset becomes exponentially confounded in conjunction with
other pre-existing chronic conditions, primarily insulin resistance and obesity (Stefanska, Bergmann, & Sypniewska, 2015). The precise mechanisms underlying menopause-induced disease risk remains unclear. However, evidence thus far appears to point toward the role of estrogen deficiency and inflammation in the post-menopausal state (Weitzmann and Pacifici, 2006).

Estrogens encompass a broad class of steroidal hormones present in both males and females. Ovarian follicles are the primary source of estrogen production in menstruating females, whereas synthesis for males and postmenopausal females occurs in the liver, adipose tissue, and various other extragonadal sites (Simpson, 2003). Several forms of estrogens exist, 17β-estradiol (E2) being the most biologically potent and relevant. Transition into menopause causes variable fluctuations in circulatory E2, until finally levels drop and remain low in otherwise healthy individuals. Other than development and maintenance of reproductive tissues, E2 assumes a number of biological roles including skeletomuscular remodeling, lipid metabolism, immune function, and importantly, inflammatory regulation (Simpson, 2003).

Inflammation is most simply understood as the immune system’s response to any detected stimuli, such as tissue damage, invoked through exercise or stress. Cytokines are a group of small protein molecules involved in cell signaling processes, which are pivotal to proper immune response (Dinarello, 2000). When certain physiological stimuli are detected, such as inflammation or infection, cytokines are secreted into the bloodstream, signaling intercellular communication (Moldoveanu, Shephard and Shek, 2001). Cytokines are involved in a number of
local and systemic immune processes and as such, are vital to maintaining homeostasis in the body.

Under certain chronic physiological stressors, such as metabolic and cardio-pulmonary disorders, the body may overproduce specific cytokines, a condition referred to as low-grade inflammation (Franceschi, Bonafe & Valensin, 2000). Cytokines involved, such as the interleukins 1, 1β and 6 (IL-1, IL-1β and IL-6) and tumor necrosis factor-alpha (TNF-α), are therefore classically termed pro-inflammatory, due to their role in catalyzing the inflammatory process (Dinarello, 2000). However, it is well known that many cytokines are involved in both pro and anti-inflammatory signaling, suggesting that specificity of stimuli and cell type must be taken into account when considering the mechanistic roles (Opal and DePalo, 1999). Moreover, the pleiotropic and biologically redundant nature of many cytokines provides an obstacle in determining the net molecular effect.

Anti-inflammatory cytokines can thus be defined as a sub-group involved in inhibiting or limiting pro-inflammatory cytokine secretion, as well as directly promoting recovery (Opal and DePalo, 1999). Anti-inflammatory cytokines may also participate in paracrine or autocrine signaling with other anti-inflammatory cytokines, and thus, produce an anti-inflammatory effect. A primary example of classic anti-inflammatory cytokine signaling is IL-1 receptor antagonist (IL-1ra), which functions to inhibit the action of IL-1α and IL-1β (Opal and DePalo, 1999). Anti-inflammatory cytokines are defined by the net effect of their immune response involvement, as many carry out pro-inflammatory functions as well.
Inflammation serves as a target for risk reduction treatment due to the association with menopause-induced disease (Sites et al., 2002). Hormone replacement therapy (HRT), more specifically exogenous treatment with \(E_2\), has been the focus of risk reduction and symptomatic treatment in post-menopausal women over the past 30 years. Currently, a number of well-controlled, randomized trials have documented the safety and efficacy of HRT in post-menopausal women (Shah, Borenstein, & Dubois, 2005; Yang, Li, Yuan, & Liu, 2013). Overall, HRT appears to be an effective intervention in the immediate post-menopausal period, with an increasing risk to reward ratio over time (Hodis and Mack, 2013). In certain populations, such as those with pre-existing cardiovascular disease and thrombosis, HRT may cause deleterious effects. Thus, a need to elucidate other means of intervention is warranted.

Exercise, specifically resistance exercise (RE), may offer an alternative means of risk reduction without a significant change in risk to reward ratio over time. RE is the primary lifestyle means of maintaining and improving musculoskeletal mass. RE promotes a number of other positive physiologic adaptations, including increased strength, neuromuscular coordination, proprioception, and improved body composition, metabolic rate, immune function and inflammatory control. Interestingly, these changes have been observed independently of changes in body mass (Phillips et al., 2012; Starkie, Ostrowski, Jauffred, Febbraio & Pedersen, 2003). Thus, exercise programs may present a unique opportunity to reduce risk of disease development, while providing numerous other positive outcomes. Exercise also

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provides a stress model by which biological mechanisms may be further tested and illuminated in order to provide highly specific, novel pharmacologic treatments.

**Purpose**

The purpose of this study was to explore the effects of menopausal status on inflammatory responses to a single bout of RE.

**Hypothesis**

It was hypothesized that post-menopausal women will differ in their anti-inflammatory cytokine response to acute RE when compared to pre-menopausal women. It was also hypothesized that post-menopausal women will have increased baseline pro-inflammatory plasma cytokine levels in comparison to pre-menopausal women.
CHAPTER II.
REVIEW OF LITERATURE

Overview of Cytokines

IL-6

IL-6 is a 26 kDa pleiotropic cytokine produced by multiple cell and tissue types, including hepatocytes, adipocytes, myocytes, endothelial and immune cells (Kishimoto, 1989; Steensberg et al., 2002). IL-6 plays a foundational role in orchestrating the acute-phase inflammatory response via induction and inhibition of other cytokines and receptor complexes. Since its discovery, IL-6 has been the subject of extensive investigation. Chronically elevated concentrations of IL-6, both in the circulation and within specific tissues, has been noted in numerous disease states, including morbid obesity, insulin resistance and aging (Hunter and Jones, 2015). Acutely elevated levels, however, promote cellular recovery and adaptation to stress, as seen in exercise models.

Upon production, IL-6 classically binds to its receptor unit (IL-6r) and associates with glycoprotein 130 (gp130). Subsequently, activation of both the janus and tyrosine kinases (JAK-TYK) and signal transducers and activators of
transcription (STAT) pathways, or Src homology 2-domain tyrosine phosphatases (SHP-2) pathways occurs. Alternatively, IL-6 engages in *trans* signaling via binding to the soluble IL-6 receptor (sIL-6r). The majority of biological IL-6 activity is accounted for by *trans* signaling and subsequent phosphorylation (Heinrich et al., 2003).

IL-6 significantly increases (up to 100-fold) in response to varying modalities and intensities of training and has been extensively researched in the field of exercise physiology and molecular biology (Bruunsgard et al., 1997; Drenth et al., 1995; Ostrowski, Schjerling and Pedersen, 2000; Ullum et al., 1994). Upon elevated secretion into the bloodstream, IL-6 potently suppresses the expression of TNF-α and IL-1β, while simultaneously promoting IL-10 production (Steensberg, Fischer, Keller, Møller, & Pedersen, 2003). There is much debate as to the biological function of elevated IL-6 in response to exercise-induced stress. However, there is a growing body of evidence supporting muscle-derived IL-6 as the primary driver of elevated circulatory concentrations in response to exercise (Pedersen, 2012). Furthermore, it has been posited that IL-6 produced from contracting skeletal muscle promotes glucose homeostasis, among other beneficial adaptations (Suzuki et al., 2011).

**IL-10**

IL-10 is an 18.5 kDa interleukin and receptor family cytokine produced by various leukocytes and other cell types (Lalani, Bhol, & Ahmed, 1997). The primary physiological role of IL-10 is to preserve tissue via the regulation, limitation and inhibition of pro-inflammatory activators during varying phases of an immune response (Maynard and Weaver, 2008). IL-10 is most understood within the context
of the acute phase response, whereby macrophage infiltration is largely responsible for the elevated production of IL-10 (Saraiva & O'Garra, 2010). In the context of exercise-induced stress, it is stimulated by the release of IL-6, which in turn inhibits the release of multiple pro-inflammatory cytokines, chemokines and other factors (Pedersen, 2006).

In response to exercise, elevated extracellular signal-related kinase 1 and 2 (ERK1 & ERK2) pathways are activated, largely from IL-6 induced macrophage stimulation (Goodyear, Change, Sherwood, Dufresne, & Moller, 1996; Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Subsequently, IL-10 then binds to its receptor, which activates phosphorylation of janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2). JAK1 and TYK2 are then able to phosphorylate tyrosine residues on the intracellular domain of the IL-10 receptor unit (Ahmed & Ivashkiv, 2000). Tyrosine residues then dock STAT3, which binds and then is phosphorylated by JAK1 & TYK2. STAT3 then translocates to the nucleus and binds to the promoter region of IL-10 genes, promoting further mRNA transcription and translation of the IL-10 protein. The STAT3-induced IL-10 protein then inhibits production of several proinflammatory mediators, such as TNF-α and IL-1β (Ahmed & Ivashkiv, 2000).

**IL-13**

IL-13 is an interleukin and receptor family cytokine secreted by several leukocytes, but primarily via T-lymphocytes. The primary physiological function of IL-13 is to mediate type-2 cytokine immune signaling, though knowledge of IL-13’s effector functions remains limited in scope (Wynn, 2015). IL-13 is highly similar to
IL-4 in both genetic structure and function. The IL-13 response to exercise is largely unknown.

*TGF-β*

TGF-β is a 25 kDa TGF-β Superfamily cytokine and is secreted by nearly all cell types. The primary physiological function of TGF-β is to regulate immune cells, cell growth, division, and proliferation (Shull et al., 1992). TGF-β exists in three distinct isoforms; TGF-β1, TGF-β2, and TGF-β3. The isoforms are upwards of 70% homologous in function.

Active TGF-β1 is found non-covalently attached to the latency-associated peptide (LAP), forming the small latent complex (SLC) (Sharples, Plowman, Rose, Twardzik, & Purchio, 1987). The LAP can then form disulfide bridges with the latent TGF-β binding protein (LTBP), forming the large latent complex (LLC) (Sharples et al., 1987). The LLC is then secreted from the cell for activation or further processing. Active TGF-β1 can be liberated from the SLC by several laboratory methods, the most common of which is treatment with a strong acid, followed by neutralization and binding to a plate with antibodies sensitive to active TGF-β1 (Taylor, 2009).

Signaling of TGF-β occurs when the LAP or liberated active TGF-β1 bind to their respective receptor (TGF-βRI, II) to form a receptor complex (Travis and Sheppard, 2014). The receptor complex then phosphorylates via Smad-dependent pathways or other non-canonical pathways, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3 Kinase) (Travis & Sheppard, 2014).
Estrogen increases circulating TGF-β, which promotes apoptosis in certain bone cells and inhibits bone resorption (Hughes et al., 1996). Postmenopausal status, hallmarked by reduced levels of plasma E2, also promotes a reduction in TGF-β. Thus, a reduction of TGF-β in postmenopausal women likely facilitates increased bone turnover, which has been associated with a decrease in bone mass (Garnero, Sornay-Rendu, Chapuy & Delmas, 1996; Mirza & Prestwood, 2004).

An experimental animal study conducted on rats found no significant changes in TGF-β1, an isoform of TGF-β1, in response to a 6-week endurance training protocol (Czarkowska-Paczek, Zendzian-Piotrowska, Bartlomiejczyk, Przybylski & Gorski, 2011). There is some evidence to suggest that TGF-β1 may increase acutely in response to high intensity cycling in trained males (Czarkowska-Paczek, Bartlomiejczyk & Przybylski, 2006).

*Inflammation, Disease and Aging*

Elevated basal levels of cytokines have been associated with various disease and aging states (Bradley, 2008). Visser et al. (2002) examined the relationship between cytokine expression and muscle mass and strength in 2746 healthy, White and Black males and females, ages 70-79 years. Participant information and data were obtained from The Health, Aging and Body Composition (Health ABC) study. Baseline levels of IL-6 and TNF-α were tested for association with mid-thigh muscle mass, grip strength and knee-extension strength. A number of covariates such as height, age, weight, race, body mass index (BMI), physical activity and drug use were utilized to control for confounding effects.
Negative associations were found between increased IL-6 and TNF-α levels and decreased muscle cross-sectional area and strength (Visser et al., 2002). The associations remained when adjusted for confounding variables. The study suggested that elevated baseline levels of classical pro-inflammatory cytokines may contribute to age-related muscle decay and decline (Visser et al., 2002).

In another study, Elosua et al. (2005) assessed the relationship between physical fitness and low-grade inflammation in 841 men and women aged 65 years or older. Subject characteristics and data were obtained from the InCHIANTI study, a cross-sectional study on elderly mobility conducted in Tuscany, Italy. Fasted basal levels of IL-1ra, IL-1β, IL-6, sIL-6R IL-10, IL-18 and TNF-α were analyzed. Physical activity and performance were assessed via questionnaire and a 400-meter walk test for time, respectively.

A significant, negative association was found between both physical performance and activity and presence of pro-inflammatory cytokines IL-6 and TNF-α for both males and females (Elosua et al., 2005). The anti-inflammatory cytokines IL-1ra and IL-10 remained constant with increased or decreased levels of fitness and performance (Elosua et al., 2005). Thus, decreased physical activity level may influence chronic inflammation.

These studies support physical activity influencing and predicting levels of inflammation. Given the data on anti-inflammatory response, adaptations to physical fitness and activity may cause an altered cytokine response, rather than a simple increase in anti-inflammatory and decrease in pro-inflammatory activity.
These studies lend support to the hypothesis that aging may be an independent factor in low-grade inflammation.

*Inflammation and the Post-Menopausal State*

Post-menopausal women are at high risk for the development of diseases related to estrogen-deficiency and age, such as osteoporosis and rheumatoid arthritis (Sambrook, Eisman, Champion & Pocock, 1988). Phillips et al. (2012) analyzed the effect of resistance training on low-grade inflammation in 23 obese, postmenopausal women aged 60-70 years. Subjects had a BMI of 30-40 kg/m² and no physical activity for the previous six months. Subjects were randomly assigned to a resistance training (RT) or control (C) group for 12 weeks. Subjects in the training group participated in total body resistance exercise three times per week. Serum levels of TNF-α, IL-6 and IL-10, as well as leptin, adiponectin and C-reactive protein (CRP) were obtained at baseline and immediately, two and 24 hours post exercise.

The intervention resulted in a significant decrease in circulating TNF-α and increase in IL-10, independent of changes in body composition (Phillips et al., 2012). Subjects also exhibited significant decreases in circulating leptin and CRP as a result of the training period. The study suggested that resistance training may improve biomarkers of inflammation and as such, may be an effective intervention in high-risk populations, such as the clinically obese or post-menopausal women.

In another study, Prestes et al. (2009) assessed the effects of resistance training on biomarkers of inflammation and muscle force production in 35 post-menopausal women. Subjects were sedentary, non-obese or overweight, 50-70 years old, and free from cardio-pulmonary disease. Subjects were acclimated to full
body resistance training, tested for 1RM and then participated in a 16-week intervention, which utilized a linear progression protocol. Circulating levels of TNF-\(\alpha\), IL-6, IL-15, as well as leptin and resistin, were measured prior to and immediately 24 and 48 hours post training.

Subjects exhibited a significant decrease in serum levels of leptin, resistin and IL-6 as a result of the training intervention (Prestes et al., 2009). Little to no change was observed in IL-15 and TNF-\(\alpha\) levels. Subjects also exhibited significant increases in both lower and upper extremity strength (Prestes et al., 2009). The study suggested that resistance-training programs may attenuate low-grade inflammation and improve strength in elderly post-menopausal women.

Giannopoulou et al. (2005) examined the effects of diet and/or aerobic exercise on inflammatory cytokines in 33 post-menopausal women, aged 50-70 years, with type-2 diabetes. Subjects were randomly assigned to either a diet alone (DA), exercise alone (EA), or diet and exercise (DE) intervention group. Aerobic fitness was determined via open circuit spirometry. Subjects assigned to the DA intervention were prescribed a hypocaloric diet consisting of 40% fat (30% of which monounsaturated), 40% carbohydrate, and 20% protein with consulting available weekly. Subjects assigned to the EA group performed 60 minutes of submaximal walking at 70% \(V_{O2\text{peak}}\) three to four days per week. Subjects assigned to the DE group followed both the DA and DE protocols simultaneously. Circulating levels of TNF-\(\alpha\) and IL-6, as well as insulin, leptin, resistin, adiponectin and C-reactive protein, were assessed before and after the 14-week intervention period.
Data from the study revealed significant decreases in C-reactive protein levels with all interventions (Giannopoulou et al., 2004). Significant reductions in leptin were observed in the DA and DE interventions. Little to no change in resistin or TNF-α was observed as a result of any of the interventions. The study suggested that light to moderate aerobic exercise, without a weight loss protocol or some type of clinical intervention, might not improve cytokine and other inflammatory markers in post-menopausal women with metabolic syndrome (Giannopoulou et al., 2004). This study stands in contradiction to previous literature in suggesting that resistance exercise interventions may not decrease inflammatory cytokine levels independently of weight loss. However, the results may have been limited due to the moderate exercise intensity.

Malutan, Dan, Nicolae and Carmen (2014) assessed baseline cytokines of women at varying stages of menopause. Subjects were divided into five groups, which consisted of 35 pre-menopausal (20-40 years), 40 pre and peri-menopausal (46-53 years), 40 natural post-menopausal (54-65 years), 35 surgically induced menopausal (no age restrictions), and 25 pre-menopausal women with chronic inflammatory disease (20-40 years). Serum levels of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, IL-20 and TNF-α were obtained and compared between groups. A panel of hormones, including estradiol, were also assessed and compared between groups.

Subjects in both natural and surgically induced menopausal states were found to have significantly increased levels of IL-1β, IL-8 and TNF-α in comparison to healthy, pre-menopausal women (Malutan et al., 2014). It was also found that
women with inflammatory conditions had similar baseline IL-8 levels as post-menopausal women (Malutan et al., 2014).

The results of this study confirm the hypothesis that women in a post-menopausal state have elevated inflammatory markers in comparison to healthy individuals, in part as a result of decreased estrogen production. A significant limitation to this study was the use of regular ELISAs instead of high-sensitivity assays, which decreased the detection of various cytokines in the absence of some type of disease or damage.

Nunes et al. (2016) examined the effect of 16 weeks of randomized no, low or high volume resistance training (RT) on muscle strength, cardio-metabolic parameters and inflammatory markers in 32 overweight, post-menopausal women. The RT protocol consisted of the squat, leg curl, leg extension, bench press, cable row, vertical pull down, triceps extension and bicep curl. Subjects randomized to low volume training completed three sets of 8-12 repetitions at 70% of 1RM with one and a half minutes rest in between sets and exercises. The high volume group completed the same protocol, and also added one additional set per exercise each week until a total of six sets per exercise was reached. Fasting blood samples were obtained before and after the intervention. Inflammatory markers were measured using commercially available ELISA kits. Cardio-metabolic factors were assessed via electrochemiluminescence assays. Body composition was assessed using a 4-site skinfold test.

No baseline differences were found between groups. Both low and high volume RT resulted in significant improvements in % body fat and muscle strength.
Low volume RT alone significantly lowered HbA1c%. High volume RT alone resulted in significant improvements in total cholesterol, lipid profile, waist circumference and waist to hip ratio. No significant changes in inflammatory markers were observed in control, low volume RT, or high volume RT. Delta IL-6% was significantly lower in high volume RT alone over the control such that the treatment appeared to have a protective effect against elevated basal IL-6. Concentrations.

Based on the literature, both age and menopausal status may compromise the ability of the immune system, resulting in systemic low-grade inflammation. However, it appears that certain exercise protocols may effectively mediate, to a certain degree, the increased presence of pro-inflammatory cytokines TNF-α, IL-6 and IL-1β.

*Acute Pro- and Anti-Inflammatory Responses to Exercise*

Exercise induces an acute dynamic stress response in order to facilitate transient recovery and adaptation to future trauma (Flynn, McFarlin & Markofski, 2007). The magnitude of response is based upon intensity, duration, modality, type of muscle contraction and other factors. Ostrowski, Rohde, Asp, Schjerling and Pedersen (1998) evaluated acute cytokine responses to exhaustive exercise in 10 well-trained male endurance athletes, aged 24-37 years, participating in the Copenhagen marathon. VO2\text{MAX} was determined using a progressive treadmill test prior to the marathon. Plasma concentrations of TNF-α, IL-1ra, IL-1β, IL-6 and IL-10, as well as sTNF-r1 and sTNF-2, were obtained at baseline, immediately post-exercise and every 30 minutes after over a four-hour period.
Plasma concentrations of IL-10, IL-6, IL-1ra, TNF-α, sTNF-r1 were significantly increased immediately post exercise (Ostrowski et al., 1998). IL-6 and IL-10 followed a similar time-course, peaking immediately post exercise and showing a marked decrease to near resting levels at the four-hour post-exercise period. TNF-α peaked immediately post exercise, but remained somewhat elevated throughout the entire 4-hour post-exercise period. IL-1ra peaked roughly one and a half hours after exercise, followed by a steady decrease until reaching near-resting levels 4 hours post exercise (Ostrowski et al., 1998).

This study provided a time-course for both pro- and anti-inflammatory cytokines in response to prolonged running. It was found that elevated levels of anti-inflammatory cytokine inhibitors, such as IL-1ra and IL-10, respond to increased concentrations of TNF-α and IL-1β, known pro-inflammatory cytokines. Though the exercise was aerobic in nature, the time course maintains some relevance across varying modalities.

Pereira et al. (2013) assessed the effects of resistance exercise on acute cytokine and osteoprotegerin (OPG) secretion in 24 pre-menopausal women ages 25-45 years. Subjects were assigned to either a control or metabolic syndrome group based upon waist circumference, blood triglyceride levels, fasting blood glucose and blood pressure. Women with three or more abnormal or elevated risk factors were placed into the intervention group. Subjects receiving the treatment went through a familiarization period, were tested for maximal strength, and then trained through a full body resistance exercise protocol at 60% 1RM. Serum concentrations of TNF-α, IL-1a, IL-1β, IL-6, IL-10, IL-12 and OPG were drawn at
baseline, immediately and 60 minutes after the training protocol (Pereira et al., 2013).

Subjects with metabolic syndrome exhibited non-significant, elevated baseline values of IL-6 and IL-1β compared to control subjects (Pereira et al., 2013). TNF-α was also elevated in the metabolic syndrome group, however, there were several outliers. Subjects with metabolic syndrome did not exhibit an altered acute pro-inflammatory response to exercise. However, this was likely a result of the low intensity level prescribed in the training protocol. This study lends support to the use or implementation of resistance exercise protocols in high-risk populations, as it may not provide any additional inflammatory stress on the body. This study was limited by sample size.

Benini, Nunes, Orsatti, Portari & Orsatti (2014) examined sex differences in markers of acute cytokine, heat shock protein and oxidative stress following total body resistance training. Eight young adult men and women, aged 20-30 years, with a minimum of one year of resistance training experience, performed one hour of upper and lower body dynamic movements. Serum concentrations of TNF-α, IL-6 and IL-10, as well as creatine kinase (CK), heat shock protein 60 (HSP 60) and HSP 70, were obtained at baseline with participants in a fasted state and one, four and 24 hours post resistance exercise.

Significant differences were observed between sexes in concentrations of IL-6 and CK (Benini et al., 2014). Women had a delayed IL-6 response in comparison to men, peaking close to four hours post-exercise, with males peaking closer to one hour. Women had a significantly reduced peak IL-6 response (~4.4 pg/mL), when
compared to men (~3.4 pg/mL) (Benini et al., 2014). Non-significant baseline differences were observed for IL-10 and TNF-α.

Thus, there may be sex variability in the response to acute muscle tissue damage. A limitation to this study was the use of birth control by all of the female participants, which alters E₂ rhythm, production and circulation, potentially bearing downstream effects on cytokine expression. This study strengthens the notion that sex specific research regarding cytokine regulation is needed. This study was limited by not being conducted in a controlled environment, decreasing the ability to assess changes throughout the prolonged exercise period and control of extraneous influences.

Resistance exercise provokes an acute inflammatory immune response. Levels of IL-6 are highly elevated (100-fold) in the post-exercise period. TNF-α responses to exercise appear to be sensitive to and dependent on the intensity and duration of the protocol. A distinct anti-inflammatory response is also noted in the post-exercise period, with cascades of both IL-1ra and IL-10, which function to inhibit further inflammation (Pedersen, 2000).

Chronic Adaptations to Resistance Exercise and Inflammation

Gattà, Garnham, Peake and Cameron-Smith (2014) assessed the effects of resistance exercise training on cytokine expression in 16 men, eight young (age=20-25 years) and eight elderly (age=60-70 years). During a familiarization session, participants performed a single bout of isokinetic exercise in order to measure maximum muscle contractility. Both groups participated in 12 weeks of progressive resistance training three days per week on non-consecutive days. Muscle biopsies
were obtained from the vastus lateralis during the familiarization session and after the 12-week intervention period. Expression of IL-4, IL-6, IL-8, IL-10, IL-13 and TNF-α, as well as MCP-1, was analyzed using a multiplex assay kit.

Levels of IL-10 significantly increased as a result of isokinetic exercise in both the pre and post intervention testing (Gatta et al., 2014). However, IL-10 levels did not significantly differ between groups. Prior to the intervention, the elderly group exhibited an elevated IL-6 response to isokinetic exercise, which was largely attenuated by the intervention. IL-4 Levels significantly increased in both groups as a result of the intervention period (Gatta et al., 2014).

Results from this study suggest that age differences in cytokine expression in skeletal muscle are somewhat dependent upon training status. Neither the training period or age distinction accounted for substantive changes in either pro or anti-inflammatory cytokine expression in skeletal muscle (Gatta et al., 2014). Though changes in the expression of anti-inflammatory cytokines occurred, there was little to no variation between age groups. Thus, it may be deduced that aging, as an independent factor, may not result in irregular or impaired anti-inflammatory responses to tissue damage. This study was limited by sample size.

In another study, Lima et al. (2015) analyzed the effect of training modalities on biomarkers of inflammation in 44 individuals with hypertension, aged 60-75 years. Subjects with clinically diagnosed hypertension without thyroid, cardio-respiratory or renal conditions were assessed for VO2\text{MAX} and upper and lower extremity 1RM. Subjects were then randomly assigned to either an aerobic (N=15),
aerobic and resistance exercise (N=15), or control (N=14) group. The intervention period was 10 weeks.

The aerobic protocol consisted of 20-30 minutes of continuous exercise three times per week at 50-70% VO2\text{MAX} for sedentary, and 60-80% VO2\text{MAX} for trained subjects (Lima et al., 2015). The resistance and aerobic protocol consisted of one to two laps of circuit training at 50-60% 1RM with 15 and 20 repetitions for upper and lower extremity exercises, respectively. Subjects performed the same aerobic protocol immediately after circuit training. Plasma concentrations of TNF-\alpha and IL-6 were assessed at baseline and 24 hours after the intervention.

Aerobic training alone resulted in a significant reduction in baseline levels of IL-6 (Lima et al., 2015). The combination aerobic and resistance training resulted in little to no change in plasma concentrations of IL-6. Changes in baseline concentrations of TNF-\alpha were highly variable, yet when controlled for BMI, had a significant negative correlation (Lima et al., 2015). The study as a whole suggests that chronic adaptations to exercise may result in decreased low-grade inflammation. This use of circuit style resistance training may have limited the effect of the combined aerobic-resistance group, given the muscle fiber utilization and lack of overload placed on the muscle tissue.

Suzuki et al. (2000) explored changes in immune function in response to prolonged endurance exercise. Sixteen trained, adult male endurance athletes aged 21-39 years completed a 42.5 km outdoor run. Blood was collected from the antecubital vein immediately prior to and after the race. Changes in plasma concentrations of IL-1\beta, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-\alpha, IFN-\alpha, IFN-
Y, G-CSF, GM-CSF, and TGF-β1 were then assessed from pre- to post-exercise using commercially available ELISA kits. Hematological and blood biochemical data were also assessed.

The prolonged run resulted in significant changes in the plasma concentrations of IL-1ra, IL-2, IL-6, IL-8, and IL-10. IL-1ra, IL-6, IL-8 and IL-10 increased markedly (200, 100, 2.5, and 3.5 fold, respectively), whereas IL-2 decreased 1.5-fold. No significant differences were observed for TGF-β1. The total leukocyte count significantly increased 3-fold, whereas the red blood cell count did not change from baseline. Creatine kinase and myoglobin were both significantly elevated in the post exercise period.

Exhaustive, prolonged exercise results in significant muscle damage. In turn, damaged tissue likely leads to the release of several intermediate and signaling proteins, such as IL-6. Given the intensity of change, it is also likely that IL-6 plays a central, mediating role in both the acute inflammatory response to exercise and the subsequent cascade regulation. Although TGF-β1 appeared to not change in response to exhaustive exercise, the result may have been due to improper sample collection and assay technique as described by Heinemeier, Langberg, Olesen, and Kjaer (2003).

Heinemeier et al., (2003) assessed the role of TGF-β in aerobic exercise-induced collagen production. Six healthy adult males, aged 25 to 28 years, performed one hour of running at approximately seven and a half miles per hour (mph) with a three percent grade. Microdialysate samples were collected from the Achilles tendon, as well as blood samples from the antecubital region prior to,
immediately after and six, 20, and 68 hours after cessation from exercise. Plasma and dialysate concentrations of TGF-β1 were analyzed via ELISA (R & D systems). Latent TGF-β1 was activated using 2.5 M acetic acid, followed by neutralization with 1.2 M sodium hydroxide/0.5 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Microdialysis samples were assessed for collagen turnover with biomarkers procollagen I COOH-terminal propeptide (PICP) and COOH-terminal telopeptide of type I collagen (ICTP).

Plasma concentrations of TGF-β1 corrected for hematocrit increased significantly in response to exercise (15-fold). TGF-β1 from dialysate followed a similar time course, but was insignificant from pre to post exercise. PICP concentration from peritendinous tissue was significantly elevated 68 hours post exercise. No significant changes in ICTP were observed. TGF-β1 may be stimulated in response to mechanical loading of bone, joint, ligament, and skeletal muscle. In response to repeated micro trauma, several factors, including TGF-β1, may be secreted in order to promote bone reformation and muscular adaptation to stress (Heinemeier et al., 2003).

Thus, exercise provokes an acute and chronic immune response. Resting levels of classically pro-inflammatory cytokines are elevated during the acute post-exercise period, with significantly decreased values as a result of an exercise intervention (Gatta et al., 2014). Anti-inflammatory cytokines are also present and elevated during the post-exercise period. However, there is limited understanding of the chronic anti-inflammatory adaptations to a resistance training protocol. Given the complex nature of cytokine secretion, cellular inflammatory adaptations to
exercise may be better understood as a balancing act, rather than changes of degree only.
CHAPTER III

METHODS

Research Design

This study utilized a causal comparative research design.

Independent Variables

The independent variables were menopausal status and time.

Dependent Variables

The dependent variables were IL-6, IL-10, IL-13 and TGF-β1.

Subjects

20 women, 10 pre-menopausal and 10 post-menopausal, were selected based upon exclusionary criteria and convenience to the investigator. Participants were recruited using flyers and regular notifications during lectures at a Midwestern university. All participants received and signed an informed consent form and were free to leave the study at anytime (Appendix A). The Cleveland State University Institutional Review Board (IRB) approved this study (Appendix B).
Delimitations

Exclusion criteria included the following: history of cardiovascular or pulmonary disease, stroke, autoimmune disorders, chronic infection, smokers or smokeless tobacco users, surgery in the past 6 months, inflammatory disease, or pre, type I and type II diabetes. Participants had not engaged in any structured resistance training programs for at least six months prior to beginning the study. Enrolled participants were not currently engaged in any structured high intensity aerobic training. Structured activity was defined as two or more days a week, every week, with progressive improvement in a given activity. Participants reported varying levels of recreational activity, including but not limited to, yoga, Pilates, hiking, swimming, jogging, and walking. The participants were free from muscular-skeletal injury. Some participants reported minor aches and pains, such as elbow tendonitis, but nothing that restricted their ability to exercise.

The allowed upper limit for % body fat was 30% and 35% for the pre- and post-menopausal groups, respectively. The elevated % body fat criteria for the post-menopausal women was to account for age-related physiological changes. Participants had not been taking anti-inflammatory medications, hormone replacements or used hormonal birth control in the past six months.

Menopausal Status

Participants were categorized as either pre- or post-menopausal based upon history of menopausal status and follicle stimulating hormone (FSH) production. Pre-menopausal was defined as having a regular, monthly menstrual cycle during the past six months and a FSH level of less than 20 mIU/ml. Postmenopausal was
defined as not having a menstrual cycle for the past three years, as a result of either surgical or natural menopause, and a FSH level of 30 mIU/ml or greater.

*Anthropometry*

Height was measured upon arrival to the Human Performance Laboratory using a clinically calibrated stadiometer.

*Body Composition*

Body composition was determined using whole body air-displacement plethysmography with the BOD POD system (Life Measurement Instruments; Concord, California). On the day of testing, subjects were instructed to arrive at the Human Performance Laboratory between 7 and 9am after a 10-hour overnight fast. The BOD POD and weight scale calibration occurred prior to each individual test. All subjects wore minimal, form fitting clothing and a swim cap during testing according to device guidelines. During measurement, subjects were instructed to sit still with their hands resting upon their thighs and breathe normally. Whole body density was used to determine percent body fat using the following Siri equation:

\[
% \text{ Body Fat} = \left(\frac{495}{\text{Body Density}}\right) - 450.
\]

*Metabolic Panel*

Total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglycerides (TRG), TC/HDL ratio, non-HDL cholesterol, estimated LDL cholesterol and blood glucose were determined via the Cholestech LDX Lipid Profile GLU Test. Blood samples were obtained from a fingerstick and handled according to the manufacturer’s instructions. Samples were analyzed using an Alere Cholestech LDX® System.
**Blood Sampling**

Blood samples were obtained from the antecubital vein immediately prior to (PRE), immediately after (POST), and one hour (1 HOUR POST) after the termination of exercise. Subjects reported to the Human Performance Laboratory between seven and nine am for the baseline sample. Samples were collected in 6 mL BD Vacutainer® tubes treated with K2 EDTA. Whole blood was then centrifuged for 10 minutes at 1000 x g in 4° Celsius. The plasma was drawn off in 500 μL aliquots and then immediately transferred and stored in a -80°C freezer until the time of analysis.

**Blood Analysis**

For analysis, samples were thawed and centrifuged for 30 seconds at 1000 x g in 4° Celsius for purification and separation. Samples underwent no more than two freeze thaw cycles during the span of the analysis. Plasma concentrations of IL-6, IL-10, IL-13, TGF-β1 and FSH were then assessed using commercially available enzyme-linked immunosorbent assays (ELISA) (IL-6 & TGF-β1, R&D Systems®, Minneapolis, MN; IL-10, eBioscience®, San Diego, CA; IL-13, Thermo Fisher Scientific, Waltham, MA; FSH, Ray Biotech, Norcross, GA). The sensitivities of the assays were 0.11, 0.17, <7, 15.4, and 8.0 pg/mL for IL-6, IL-10, IL-13, TGF-β1, and FSH, respectively. The intra assay coefficients of variation (CV) for each kit were 5.8%, 8.8%, 3.0%, and 5.2% for IL-6, IL-10, TGF-β1, and FSH, respectively. High sensitivity kits were used when available and appropriate.
Strength Assessment

On a separate day, at least two or more weeks prior to the training bout, participants came in to the Human Performance Laboratory for exercise acclimation and completed a 1RM testing battery. The battery consisted of the following: bilateral leg press, seated chest press, knee extension, knee flexion, triceps extension, biceps curl and vertical pull down exercise. All exercises were performed on cable-based stack machines. After a dynamic warm up, subjects began with a submaximal load and progressed until failure. One minute of rest was allotted between each attempt. The last completed load prior to failure was considered the subject’s 1RM. The exercise sequence was the same for each participant.

Resistance Exercise Protocol

On the day of testing, participants arrived in a fasted state and sat quietly for 5 minutes. After the initial blood draw, subjects performed 5 minutes of submaximal walking. The walking speed was selected by the subject, and ranged from two to three miles per hour (mph). After a brief stretching and dynamic warm up period, the subjects performed three sets of 10 repetitions of the supine chest press, bilateral leg press and seated knee extension, prone knee flexion, seated triceps extension, biceps curl and vertical cable pull down exercise machines. Exercise intensity was set at 75% of 1RM. If the subject was not able to complete all of the prescribed repetitions, the load was reduced to the next available weight and the subjected continued until completion. Participants followed a 2/0/2 tempo pattern with approximately 60-second rest intervals between sets.
Statistical Analysis

Both descriptive and inferential statistics were obtained. An independent $t$ test was used to determine baseline differences between groups. A mixed design, two way repeated measures analysis of variance (ANOVA) was used to assess differences between groups in cytokine concentrations across three time periods (Pre, Post, and 1 Hour Post). The Wilks-Shapiro test was used to assess normality of the sample population. Mauchly’s sphericity test was used to assess homogeneity of variance between groups. The Greenhouse-Geisser correction was used in cases where sphericity was violated. When significant main effects for time or time by group interactions were revealed, post-hoc analysis was conducted using the Bonferroni procedure. SPSS (version 24.0) was used for all analyses with .05 used as the level of significance.
CHAPTER IV

RESULTS

Results from the study are displayed in the following tables and figures.

Subject characteristics are displayed in Table 1. Results for IL-6, IL-10, and TGF-β are presented in Figures 1, 2, and 3, respectively. A bivariate correlation matrix is displayed in Table 2. IL-13 concentrations were below the limit of detection at all time points.

Table 1. Subject Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre-Menopausal</th>
<th>Post-Menopausal</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>27</td>
<td>3.0</td>
<td>59</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.4</td>
<td>7.2</td>
<td>164.1</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>60.5</td>
<td>9.1</td>
<td>60.1</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>45.9</td>
<td>5.5</td>
<td>43.1</td>
</tr>
<tr>
<td>Fat %</td>
<td>23.7</td>
<td>4.6</td>
<td>28.3</td>
</tr>
<tr>
<td>Waist to Hip Ratio</td>
<td>0.73</td>
<td>0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>Blood Glucose (mg/dL)</td>
<td>83.3</td>
<td>6.4</td>
<td>89.5</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>167.7</td>
<td>30.5</td>
<td>183.5</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>100.8</td>
<td>30.0</td>
<td>97.0</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>59.8</td>
<td>22.3</td>
<td>66.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>65.7</td>
<td>33.2</td>
<td>80.6</td>
</tr>
<tr>
<td>FFM-Adjusted 1RM Index</td>
<td>7.89</td>
<td>1.52</td>
<td>6.53</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>5.2</td>
<td>2.9</td>
<td>58.0</td>
</tr>
</tbody>
</table>

Note. FFM-Adjusted 1RM index was calculated by dividing an individuals raw total (kg) by their fat free mass (kg).
An independent samples t-test was conducted in order to assess differences in basal characteristics between groups. There were significant differences in age (p<.0001), waist to hip ratio (p<.01), FFM-adjusted 1RM index (p<0.05), and FSH (p<.0001). The post-menopausal group was significantly older than the pre-menopausal group, as well as exhibiting a significantly greater waist to hip ratio, and FSH. The pre-menopausal group was significantly stronger than the post-menopausal group when adjusted for FFM. There was a trend for significance in blood glucose (.13) fat percent (p=.07), such that the post-menopausal group tended to have higher blood glucose levels and body fat percentages.

![Figure 1](image.png)

*Figure 1.* Plasma concentrations of IL-6 prior to, immediately after, and one hour after the cessation of resistance exercise. Asterisk (*) indicates effect of time at the p<0.05 level. Black bars represent pre-menopausal means, whereas grey bars post-menopausal.

There was a significant main effect for time (p < .05), with both groups exhibiting increased plasma concentrations of IL-6 pre to post exercise followed by
a further increase one hour post exercise (see Figure 1). Post hoc analysis revealed a significant increase (p<0.05) in IL-6 pre to one hour post exercise, as well as immediately post to one hour post exercise in both groups. However, there was no significant group by time interaction.

![Figure 2](image)

**Figure 2.** Plasma concentrations of IL-10 prior to, immediately after, and one hour after the cessation of resistance exercise. Asterisk (*) indicates effect of time at the p<0.05 level. Pound (#) signifies group by time effect at the p<0.05 level. Black bars represent pre-menopausal means, whereas grey bars post-menopausal.

There was a significant main effect for time (p < .05) with the pre-menopausal group displaying increased plasma concentrations of IL-10 pre to post exercise followed by a reduction to near basal levels one hour post exercise (see Figure 2). Post hoc analysis revealed a significant increase (p<0.05) in IL-10 pre to immediately post exercise. The repeated measures ANOVA also revealed a significant (p<0.05) time by group effect. Post hoc analysis further identified a significant interaction for IL-10 immediately post exercise between groups, such
that the pre-menopausal group significantly increased while the post-menopausal did not significantly change.

*Figure 3.* Plasma concentrations of TGF-β1 prior to, immediately after, and one hour after the cessation of resistance exercise. Black bars represent pre-menopausal means, whereas grey bars post-menopausal.

TGF-β1 levels increased slightly, but not significantly, pre to post exercise in both groups, followed by an increase in the pre-menopausal group and reduction in the post-menopausal group one hour post. However, no significant effects for time or time by group interactions were observed for TGF-β1 (see Figure 3).
**Table 2.** Correlation matrix of selected study variables.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Fat %</th>
<th>WHR</th>
<th>1RM Score</th>
<th>FSH Post</th>
<th>IL-6 Post</th>
<th>IL-6 1HP</th>
<th>TGFβ 1 1HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-</td>
<td>0.417</td>
<td>0.587**</td>
<td>-0.494*</td>
<td>0.841**</td>
<td>0.294</td>
<td>0.253</td>
<td>-0.433</td>
</tr>
<tr>
<td>Fat %</td>
<td>0.417</td>
<td>-</td>
<td>0.463*</td>
<td>0.052</td>
<td>0.496*</td>
<td>0.101</td>
<td>-0.074</td>
<td>-0.32</td>
</tr>
<tr>
<td>WHR</td>
<td>0.587**</td>
<td>0.463*</td>
<td>-</td>
<td>0.052</td>
<td>0.496*</td>
<td>0.202</td>
<td>0.256</td>
<td>-0.470*</td>
</tr>
<tr>
<td>1RM Score</td>
<td>-0.494*</td>
<td>0.052</td>
<td>-0.089</td>
<td>-</td>
<td>-0.336</td>
<td>0.123</td>
<td>0.088</td>
<td>-0.103</td>
</tr>
<tr>
<td>FSH</td>
<td>0.841**</td>
<td>0.496*</td>
<td>0.655**</td>
<td>-0.336</td>
<td>-</td>
<td>0.401</td>
<td>0.432</td>
<td>-0.518*</td>
</tr>
<tr>
<td>IL-6 Post</td>
<td>0.294</td>
<td>0.101</td>
<td>0.202</td>
<td>0.123</td>
<td>0.401</td>
<td>-</td>
<td>0.721**</td>
<td>-0.527*</td>
</tr>
<tr>
<td>IL-6 1HP</td>
<td>0.253</td>
<td>-0.074</td>
<td>0.256</td>
<td>0.088</td>
<td>0.432</td>
<td>0.721**</td>
<td>-</td>
<td>-0.359</td>
</tr>
<tr>
<td>TGFβ 1 1HP</td>
<td>-0.433</td>
<td>-0.32</td>
<td>-0.470*</td>
<td>-0.103</td>
<td>-0.518*</td>
<td>-0.527*</td>
<td>-0.359</td>
<td>-</td>
</tr>
</tbody>
</table>

*Correlation was significant at p<0.05
**Correlation was significant at p<0.01

Bivariate analysis of study variables revealed a moderate, significant positive correlation (r=.587, p=.006) between age and WHR, as well as a negative correlation (r=-.494, p=.027) between age and FFM-adjusted 1RM score. There was a significant negative correlation (r=-.518, p=.019) between FSH and TGF-β1 levels 1 hour post exercise, as well as a positive correlation (r=.655, p=.002) between FSH and WHR.
The aim of this investigation was to explore if menopausal status influenced the inflammatory response to acute RE-induced stress. This study specifically examined changes in plasma concentrations of IL-6, IL-10, IL-13, and TGFβ1 over time and between groups. Significant time and time by group differences were found. Thus, the null hypothesis was rejected and the research hypothesis was accepted.

**IL-6 Response to RE**

Significant changes in IL-6 concentrations over time were observed. IL-6 has been widely demonstrated to increase significantly upon an acute bout of aerobic exercise (Fischer, 2006). However, data has been less consistent in studies utilizing RE protocols. Prestes et al. (2009) found no acute RE induced changes in IL-6 concentrations in post-menopausal women. The relatively high basal IL-6 concentrations may have masked modest changes as a result of RE. However, several other studies describe significant changes pre to post, with an increase into the recovery period in both post-menopausal women and the general population (Phillips, Flynn, McFarlin, Stewart, & Timmerman, 2010; Phillips et al., 2012;
Ihalainen et al., 2014) The latter studies employed hypertrophy-oriented RE protocols and revealed changes similar as described herein. Thus, factors such as repetition range, rest interval, contraction tempo and relative intensity likely influence stimulation and time course of exercise-induced circulatory IL-6 changes.

**Biological Relevance of Exercise-Induced IL-6**

In order to understand the biological relevance of IL-6 production in response to exercise-induced stress, one must consider the possible sources of origin. Under basal conditions, skeletal muscle IL-6 mRNA is hardly detectable. Upon onset of contracting skeletal muscle, IL-6 mRNA levels increase exponentially up to 12 hours post exercise (Louis, Raue, Yang, Jemiolo, & Trappe, 2007; Steensberg et al., 2002).

Adipose tissue derived IL-6 is also stimulated by exercise, however, there appears to be no activity until well into the recovery phase (Keller, Keller, Marshal, & Pedersen, 2003; Lyngso, Simonsen, & Buluw, 2002). Peritendinous IL-6 concentrations were observed to be seven to eight fold greater than nearby skeletal muscle gastrocnemius concentrations after prolonged running, suggesting a contribution from connective tissue (Langberg, Olesen, Gemmer, & Kjær, 2002). However, given the relative contribution of the medial gastrocnemius as compared to the vastus lateralis in both RE and AE, the effect may be obscured.

Though the specific biological functions of IL-6 in response to acute stressors is poorly understood, several key insights have led to promising new hypotheses oriented toward the role of IL-6 in skeletal muscle adaption and glucose homeostasis. Ikeda et al. (2016) revealed an IL-6 dependent mechanism for
exercise-induced improvements in insulin sensitivity via impaired glucose transporter type four (GLUT4) expression in mice treated with a neutralizing IL-6 antibody. Mauer et al. (2014) also observed the promotion of insulin-sensitivity and inflammatory recovery by IL-6 via the promotion of IL-4 receptor (IL-4R) and STAT3 signaling in myeloid cells. Thus, IL-6, in response to stress, likely promotes glucose homeostasis and adaptation by improving insulin sensitivity and facilitating glycogen storage. However, this model may be more relevant to prolonged AE than hypertrophic RE.

Toth et al. (2011) found satellite cell regulated, IL-6 dependent STAT3 signaling following a 30 minute eccentric RE bout in humans. Begue et al. (2013) reported similar results in rats, finding significantly elevated IL-6 mRNA, as well as STAT3 phosphorylation in response to acute resistance exercise. Serrano, Baeza-Raja, Perdiguerò, Jardí, & Muñoz-Cánoves (2008) found IL-6 knockdown mice to have reduced satellite cell and myoblast proliferatory capacity in response to compensatory muscle hypertrophy. Collectively, accruing evidence points toward cytokine mediation in satellite cell proliferation and STAT3 phosphorylation, leading to downstream myocyte hypertrophy (Snijders et al. 2015).

**IL-10 response to RE**

Several studies have demonstrated a significant increase in IL-10 concentration after prolonged high intensity endurance exercise (Ostrowski et al., 1998; Peake et al., 2005; Zaldivar et al., 2005). However, data regarding changes in plasma IL-10 following acute RE is mixed. Hirose et al. (2004) reported significant increases in IL-10 one hour after performing six sets of five repetitions of an elbow
extensor exercise in untrained young adult males. Izquierdo et al. (2009) found significant post-exercise levels of IL-10 after a seven week RE intervention when adjusted for relative intensity performing five sets of 10 repetitions of a leg press exercise in active young adult males.

Jajtner et al. (2015) found elevated plasma concentrations of IL-10 30 minutes after high volume lower body RE in 30 trained males. In contrast, several acute RE studies have reported no significant changes in IL-10 activity (Fatouros et al., 2010; Forti et al. 2016; Pereira et al., 2013) Interestingly, there appeared to be a trend in elevated post-exercise IL-10 in trained individuals, providing a potential mechanism for training induced improvements to inflammatory recovery and immunity.

The majority of trials examining IL-10 responses to acute RE were included males only. For the limited investigations including women, experimental control of key confounders such as regularity of menstrual cycle, day of testing in relation to menstrual phase, use of hormonal birth control, history of birth control use, history of resistance exercise, and fasting status appeared lacking. Overall, inconsistency across protocols and lack of data with well-controlled female cohorts warrants further experimentation.

Menopausal Mechanisms for Exercise-Induced IL-10 Variation

Significantly elevated concentrations of IL-10 were found in pre-menopausal women immediately post-exercise. Steensberg, Fischer, Keller, Møller, and Pedersen (2003) demonstrated that infusion of IL-6 directly stimulates IL-10 production in humans. Though the dosage was significantly higher than what was observed in
response to RE, it remains possible that IL-6, in conjunction with other physiologic mediators, stimulates IL-10 in response to contracting skeletal muscle.

Recently, estrogen was shown to be a primary regulator of IL-10 production in response acute inflammation in macrophages (Villa, Rizzi, Vegeto, Ciana, and Maggi, 2015). In response to treatment with lipopolysaccharide (LPS), cells treated with estrogen showed increased IL-10 mRNA expression, which further promoted inflammatory resolution in contrast to cells treated with LPS alone. Toniolo et al. (2015) similarly found that post-menopausal women displayed an impaired capacity to polarize or recruit resolutionary macrophages in response to LPS treatment. It was also revealed that 100 nM treatments with estrogen improved the count of CD206+/CD163+ cells, both of which preferentially express IL-10 (Toniolo et al., 2015). However, the authors suggested the role of IL-10 to be potentially pro- rather than anti-inflammatory (Toniolo et al., 2015).

Further research is needed to elucidate the step-wise mechanistic role of IL-10 as an inflammatory mediator. Consistent in both in vitro models is an impaired capacity to resolve acute inflammation via an estrogen-related mechanism. Data herein provides an in vivo model further illustrating this notion. Though the specific mechanism remains unknown, it seems likely that IL-10 facilitates the anti-inflammatory response (AIR) via an estrogen-IL-10-STAT3 signaling axis. A mechanistic model adapted from Villa et al. (2015) is presented in Figure 4.
Figure 4. Schematic overview of proposed mechanism for estrogen-induced IL-10 production.

Limitations

The primary limitation of this study pertained to analysis of circulatory TGF-β1 and IL-13. As presented in the review of literature, TGF-β1 is a complex protein sensitive to varying immune stimuli. Though present at sub-clinical levels in plasma and leukocyte fractions, the largest contributor to TGF-β1 concentrations in whole blood is platelet α-granules (Grainger, Mosedale, & Metcalfe, 2000). Several collection methods have been demonstrated to increase the rate of platelet degranulation, including use of a tourniquet, sodium citrate and EDTA collection tubes, and quantification from serum (Grainger, Mosedale, & Metcalfe, 2000).

Herein, EDTA was used as the anticoagulant and tourniquets were frequently used, introducing variability to the measurement. Several in house assay techniques have been suggested for the measurement of circulatory TGF-β1, though the cost
and feasibility may be restrictive in some research environments. Obtaining a greater understanding of the complex metabolic profile of TGB-β1 may reveal novel techniques and collection methods.

Though well known for its anti-inflammatory effector functions, IL-13 concentrations above the limit of detection have yet to be demonstrated in response to exercise (Peake et al., 2005). This was confirmed by the analysis performed herein. Thus, more sensitive assay techniques are needed in order to determine its potential role in exercise-induced inflammatory mediation.
CHAPTER VI
CONCLUSION

Moderate intensity total body RE resulted in significant changes to the cytokine milieu. Menopause did not alter IL-6 production in response to exercise-induced stress. Pre-menopausal women exhibited enhanced inflammatory resolution post-exercise illustrated by increased IL-10 production. Though mechanistically unclear, recent developments in cytokine biology have elucidated an estrogen-dependent signaling pathway ultimately resulting in stress-induced IL-10 production. Due to comparative limitations, further well-controlled research is needed to confirm and expand upon the results presented herein. Future research would likely benefit from an expanded experimental design including older pre-menopausal and peri-menopausal women. Upon future research, IL-10 may provide a highly specific, novel pharmacologic treatment for estrogen deficient postmenopausal women.
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APPENDIX A

INFORMED CONSENT FOR PARTICIPATION

The effect of menopausal status on cytokine balance after an acute bout of resistance exercise

Introduction
Thank you for considering participation in this project. My name is Chris Axelrod and I am working on my Master’s Thesis at Cleveland State University. This study will be done under the supervision of Dr. Emily Kullman, Assistant Professor of Exercise Science in the Health and Human Performance Department. The purpose of this study is to examine the effect of menopausal status on markers of inflammation in response to full body resistance training. The study will also provide insight into the influence of metabolic health on inflammation in pre and postmenopausal women. The research study will be conducted in the Human Performance Laboratory at Cleveland State University.

Procedures
In this study, we will be determining the effect menopausal status on markers of inflammation in response to a single resistance training session. We will recruit 20 healthy subjects, 10 pre- and 10 post-menopausal, who are not currently involved in a regular resistance-training routine.

Prior to the training session, participants will come to the Human Performance Laboratory at Cleveland state in order to determine loading and receive training on how to perform the exercises correctly. Prior to exercising, participants will be analyzed for body composition. Body composition is a measure of the amount of fat mass and fat-free mass on a person. We will measure body composition using air displacement plethysmography (Bod Pod) in the CSU Human Performance Lab. This procedure takes approximately 10 minutes to complete, and requires you to be dressed in a bathing suit, or form-fitting clothes. Participants will also be analyzed for blood cholesterol and sugar by a fingerstick test. This visit will take approximately 1.5 to 3 hours.
At least two weeks after the initial assessment, subjects will come to the CSU Human Performance Laboratory after an overnight fast. Prior to exercise, the initial blood collection will be performed. You will then participate in the experimental trial, which consists of a resistance training session consisting of 3 sets of 10 repetitions of seven different exercises. These exercises will target all of the major muscle groups, including: chest, back, shoulders, arms, hips and legs. This session, including the body composition measurement, blood draws, and training will take approximately 3 to 4 hours to complete. The total time commitment to the study will be approximately 4 to 5 hours.

In order to measure inflammation, blood will be drawn prior to, immediately after and 60 minutes after the completion of the training session. There will be a total of three blood draws, each requiring ~7 mL of blood, for a total of 21 mL during the testing day. All storage and analysis of blood will be done at the Cleveland State University Human Performance Laboratory by study personnel. There will also be a fingerprick test prior to exercise for blood chemistry analysis. After the final blood draw, snack food (fruit, juice, granola bars) will be available, or you may bring your own food.

**Risks and Discomforts**
Risks of these tests are minimal and do not exceed those of normal exercise. Risks associated with this study include muscle soreness from the completed resistance training exercises. There may be joint discomfort due to the inexperience with resistance training. In regards to drawing blood, the needle stick may hurt. There is also a small risk of bruising, a rare risk of infection, and you may feel lightheaded. The fingerstick test may cause slight soreness at the puncture site.

Because of the moderate cardiovascular strain of moderate intensity resistance training, participants may be subject to, in rare instances, fainting, abnormal blood pressure, fatal heart rhythms, stroke or heart attack. Prior to starting the study, you will be required to complete a health risk appraisal questionnaire, to screen for any underlying medical issues. In the event you are injured as a result of participation in this research, please notify the research team and seek medical attention by your primary care physician. The costs of such medical care will be billed to you or your insurance company. There are no plans to provide compensation for lost wages, direct or indirect losses. Cleveland State University will not voluntarily provide compensation for research related injury.

**Benefits**
The indirect benefits of the study are to help your understanding of how menopausal status and resistance exercise influence inflammation and the role resistance training may play in reducing risk of inflammation-related disease development. There are no guaranteed direct benefits, but this study may help you in improving your fitness level as well as educate you of your current metabolic
Confidentiality
To protect your privacy, your name will not be used in any document of the project. A number will be assigned to each subject in place of a name. The information, however, may be used for a statistical or scientific purpose with your right of privacy retained. Dr. Emily Kullman and Chris Axelrod will be the only witnesses of the information being presented. Data will be stored in the Human Performance Lab PE60B in a locked filing cabinet.

Participation
I understand that participation in this project is voluntary and that I have the right to withdraw at any time with no consequence. I attest and verify that I have no known health problems that could prevent me from successfully participating in the interval protocol treadmill test. If I have any questions about the procedures I can contact Dr. Emily Kullman at (216) 687-4854 or Mr. Chris Axelrod at (216) 470-7379.

I understand that if I have any questions about my rights as a participant, I can contact Cleveland State University's Review Board at (216) 687-3630.

Participant Acknowledgement
The procedure, purposes, known discomforts and risks, possible benefits to me and to others have been explained to me. I have read the consent form or it has been read to me, and I understand it. I agree to participate in this program. I have been given a copy of this consent form. I am at least 18 years of age.

Signature: __________________________ Date:____________________

Witness: __________________________ Date:____________________

Menopausal Status
Please check the box that most accurately describes your current menopausal status.

☐ I have experienced normal, monthly menstrual cycles during the previous six months.

☐ I have not experienced a menstrual cycle in the previous three years as a result of surgical or naturally occurring menopause.

☐ Other
   Please explain:________________________________________________________________________
Dear Emily Kullman,

**RE: IRB-FY2016-10**

*The effect of menopausal status on cytokine balance after an acute bout of resistance exercise*

The IRB has reviewed and approved your application for the above named project, under the category noted below. Approval for use of human subjects in this research is for a one-year period as noted below. If your study extends beyond this approval period, you must contact this office to initiate an annual review of this research.

**Approval Category: Expedited, Category 2**
**Approval Date:** Aug 14, 2015  
**Expiration Date:** Aug 12, 2016

By accepting this decision, you agree to notify the IRB of: (1) any additions to or changes in procedures for your study that modify the subjects’ risk in any way; and (2) any events that affect that safety or well-being of subjects. Notify the IRB of any revisions to the protocol, including the addition of researchers, prior to implementation.

Thank you for your efforts to maintain compliance with the federal regulations for the protection of human subjects. Please let me know if you have any questions.

Sincerely,

Bernie Strong  
IRB Analyst  
Cleveland State University  
Sponsored Programs and Research Services  
(216) 687-3624  
b.r.strong@csuohio.edu
This study has been approved by the Cleveland State Institutional Review Board  IRB-FY2016-10

APPENDIX C

Have you already experienced menopause?

Are you interested in weight lifting, but currently do not have structured routine or program?

We are recruiting for a study observing the effects of menopause on exercise and inflammation.


Volunteers Wanted for Exercise Study

If you are:
Woman between the ages of 40-65, have already undergone menopause and are in general good health, you may qualify!

Volunteers will receive:
A metabolic Panel, body composition test, personal training and exercise testing

Have Questions?

Chris Axelrod 216-470-7379 caxelrod11@gmail.com

or

Dr. Emily Kullman 216-687-4854 ekullman@csuohio.edu
APPENDIX D

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all true statements.

History
You have had:
___ A heart attack
___ Heart surgery
___ Cardiac catheterization
___ Coronary angioplasty (PTCA)
___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
___ Heart valve disease
___ Heart failure
___ Heart transplantation
___ Congenital heart disease

Symptoms
___ You experience chest discomfort with exertion.
___ You experience unreasonable breathlessness.
___ You experience dizziness, fainting, blackouts.
___ You take heart medications.

Cardiovascular risk factors
___ You are a man older than 45 years.
___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal.
___ You smoke, or quite within the previous 6 mo.
___ Your BP is greater than 140/90.
___ You don't know your BP.
___ You take BP medication.
___ Your blood cholesterol level is >200 mg/dL.
___ You don't know your cholesterol level.
___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week).
___ You are more than 20 pounds overweight.

___ None of the above is true.

If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Other health issues
___ You have diabetes
___ You have or asthma other lung disease.
___ You have burning or cramping in your lower legs when walking short distances.
___ You have musculoskeletal problems that limit your physical activity.
___ You have concerns about the safety of exercise.
___ You take prescription medication(s).
___ You are pregnant.

If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a professionally qualified exercise staff to guide your exercise program.

You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.


www.acsm-msse.org/pt/pt-core/template-journal/msse/media/0698c.htm