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## Research Article

# Menopausal Status Impairs Acute Inflammatory Recovery from Resistance Exercise

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ABSTRACT

The purpose of this study was to explore the effects of menopausal status on inflammatory responses to a single bout of resistance exercise (RE). Twenty pre- (N=10) or post- menopausal (N=10) women aged 18-65 years had one repetition maximum (1RM) determined for the chest and leg press, leg and biceps curl, vertical pull down, triceps and leg extension exercises. On a separate day, participants completed a session of full body resistance exercise (RE) during which subjects performed three sets of 10 repetitions at 75% 1RM for the aforementioned exercises. Blood samples were obtained prior to, immediately, and one hour after exercise. Changes in interleukin-6, -10, and transforming growth factor beta (IL-6, -10, and TGF-β1) were determined via enzyme-linked immunosorbent assays (ELISA). IL-6 significantly increased ($P<0.05$) over time in both groups. Immediately post exercise levels of IL-10 were significantly lower ($P<0.05$) in the post-menopausal group. Changes in IL-10 correlated with 17β-estradiol levels ($r2=0.45$, $P<0.001$). Menopausal status impaired inflammatory recovery following acute RE. These changes may be attributed to menopause-induced perturbations to the hormonal milieu.



## Introduction

Menopause encapsulates the life-altering process whereby 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. With the vast increase in life expectancy over the last century, women spend nearly 40% of their lives in the post-menopausal state.

Though a natural process, menopause is associated with an elevated risk of osteoporosis, cardiovascular disease, gastrointestinal distress, chronic low-grade inflammation and sarcopenia [1]. The risk of disease onset increases in the presence of other pre-existing chronic conditions, primarily insulin resistance and obesity [2]. The precise mechanisms underlying menopause-induced disease risk remains unclear. However, evidence thus points toward the role of estrogen deficiency and inflammation in the post-menopausal state [3].

Several forms of estrogens exist, 17β-estradiol (E2) being the most biologically active and relevant. Transition into menopause causes variable fluctuations in circulating E2, until finally concentrations 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 inflammatory regulation [4].

Cytokines are a group of small, ubiquitously expressed and secreted pro- and anti-inflammatory proteins responsible for orchestrating chemical signal transduction [5]. In response to acute, exercise-induced muscle contraction, cytokines are secreted from skeletal muscle into the bloodstream, promoting numerous cellular and physiologic adaptations [6]. This action continues until anti-inflammatory proteins are recruited

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to the injury site to resolve the acute phase response and promote inflammatory recovery [7]. Under certain chronic physiological stressors, such as metabolic and/or cardio-pulmonary disorders, the body may consistently overproduce specific cytokines, a condition referred to as chronic, low-grade inflammation [8].

Inflammation serves as a target for risk reduction treatment due to the association with menopause-induced disease [9]. Resistance exercise (RE) is the primary lifestyle means of maintaining and improving musculoskeletal mass [10]. Also, RE promotes a number of other positive physiologic adaptations, including improvements in strength, neuromuscular coordination, proprioception, body composition, metabolic rate, immune function and inflammatory control [11]. Interestingly, these changes have been observed independently of changes in body mass [12]. Thus, RE programs may present a unique opportunity to reduce risk of disease progression, while providing numerous other health benefits. Exercise also provides a stress model by which biological mechanisms may be further tested and illuminated.

Thus, the purpose of this study was to explore the effects of menopausal status on inflammatory responses to a single bout of RE. It was hypothesized that post-menopausal women will differ in their 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 concentrations in comparison to pre-menopausal women.

## Materials and Methods

### I Subjects

Twenty women, 10 pre-menopausal and 10 post-menopausal, were selected based upon the following exclusionary criteria: history of cardiovascular or pulmonary disease, stroke, autoimmune disorders, chronic infection, smokers or smokeless tobacco users, surgery within 5 years, use of anti-inflammatory medications, hormone replacement, hormonal birth control in the past 6 months, inflammatory disease, pre, type 1 or type 2 diabetes, and a body fat >35%.

Participants had not engaged in any structured resistance and/or aerobic training programs for at least six months prior to beginning the study. Structured exercise was defined as two or more days per week of moderate intensity resistance (>70% one repetition maximum, 1RM) or aerobic (>60% maximal oxygen consumption, VO2MAX) exercise. Enrolled participants were not currently engaged in any high intensity training. Participants reported varying levels of recreational activity, including but not limited to, yoga, Pilates, hiking, swimming, jogging, and walking. The participants were free from musculoskeletal injury.

Participants were recruited using flyers and regular notifications during lectures at local universities. All participants received and signed an informed consent form that was approved by the Institutional Review Board (IRB), and were free to leave the study at any time.

### II Menopausal Status

Participants were categorized as either pre- or post-menopausal based upon history of menopausal status and plasma follicle stimulating hormone (FSH) levels. Pre-menopausal was defined as having a regular, monthly menstrual cycle during the past six months and a FSH level of less than 25 mIU/ml. Post-menopausal was defined as not having a menstrual cycle for the past three years, as a result of natural menopause, and a FSH level of 25 mIU/ml or greater [13].

### III Body Composition

Body composition was determined using whole body air-displacement plethysmography with the BOD POD® system (COSMED USA Inc.; Concord, California). On the day of testing, subjects were instructed to arrive at the Human Performance Laboratory between 7 and 9 am after a 10-hour overnight fast. The BOD POD and weight scale were calibrated 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 Siri equation:

$$\% \text{ Body Fat} = (495 / \text{Body Density}) - 450.$$

Waist to hip ratio (WHR) was determined by measuring the circumference of the narrowest part of the waist and dividing this by the measurement of the largest circumference of the hips. A Gulick measuring tape was utilized for all circumference measurements.

### IV 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 (Alere, Waltham, MA). Blood samples were obtained from a fingerstick and processed according to the manufacturer's instructions. Samples were analyzed using the Cholestech LDX® System (Alere, Waltham, MA).

### V Blood Sampling

Subjects reported to the Human Performance Laboratory in the morning after an overnight fast for the baseline blood sample. 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. 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°C. 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.

### VI Blood Analysis

For analysis, samples were thawed and centrifuged for 30 seconds at 1000 x g at 4°C 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, TGF-β1, E2, 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; E2, Thermo Fisher Scientific, Waltham, MA; FSH, Ray Biotech, Norcross, GA). The sensitivities of the assays were 0.11, 0.17, 15.4, 5.0, and 8.0 pg/mL for IL-6, IL-10, TGF-β1, E2, and FSH, respectively. The intra assay coefficients of variation (CV) for each kit were 5.8%, 8.8%, 3.0%, 5.3%, and 5.2% for IL-6, IL-10, TGF-β1, E2, and FSH, respectively. High sensitivity kits were used when available and appropriate.

### VII 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 test battery which consisted of the following exercises: bilateral leg press, seated chest press, knee extension, knee flexion, triceps extension, biceps curl and vertical pull down exercise. All exercises were performed on Icarian cable-based weight stack machines (Precor, Woodinville, WA). 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.

### VIII Resistance Exercise Protocol

On the day of testing, participants arrived after an overnight fast and sat quietly for 5 minutes. After the initial blood draw, subjects performed 5 minutes of sub-maximal 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, seated knee extension, prone knee flexion, seated triceps extension, biceps curl and vertical pull down exercise machines. Exercise intensity was set at 75% 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 subject continued until completion of 10 repetitions. Participants followed a 2/0/2 tempo pattern with approximately 60-second rest intervals between sets. Pre-menopausal subjects were tested on the 7th day (mid follicular phase; ± 1 day) of their menstrual cycle.

### IX Statistical Analysis

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. Associations between variables were determined using Pearson product-moment correlation coefficient. Associations for non-normal distributions were assessed using Spearman's rank-order correlation coefficient. Correlations are displayed as individual plots with linear regression and 95% confidence bands. Tables and figures are displayed as the mean ± standard error. GraphPad Prism (version 5.0) was used for all analyses with .05 used as the level of significance.

### Results

Subject characteristics are displayed in (Table 1). Results for IL-6, IL-10, and TGF-β are presented in (Figures 1, 2, & 3) respectively. Correlations are displayed in (Figure 4).

**Table 1:** Subject Characteristics.

| Characteristic | Pre-Menopausal | | Post-Menopausal | | p-value (2-tailed) |
|---|---|---|---|---|---|
| | M | SEM | M | SEM | |
| Age (yrs) | 27 | 1.0 | 59 | 0.8 | <0.0001* |
| Height (cm) | 164.4 | 2.3 | 164.1 | 1.7 | 0.90 |
| Body Mass (kg) | 60.5 | 2.9 | 60.1 | 2.7 | 0.93 |
| Fat Free Mass (kg) | 45.9 | 1.7 | 43.1 | 1.5 | 0.24 |
| Fat % | 23.7 | 1.5 | 28.3 | 1.9 | 0.04* |
| Waist to Hip Ratio | 0.73 | 0.0 | 0.80 | 0.0 | 0.005* |
| Blood Glucose (mg/dL) | 83.3 | 2.0 | 89.5 | 2.5 | 0.13 |
| Total Cholesterol (mg/dL) | 167.7 | 9.6 | 183.5 | 7.6 | 0.22 |
| LDL-C (mg/dL) | 100.8 | 9.5 | 97.0 | 5.6 | 0.73 |
| HDL-C (mg/dL) | 59.8 | 7.0 | 66.8 | 4.4 | 0.41 |
| Triglycerides (mg/dL) | 65.7 | 10.5 | 80.6 | 13.3 | 0.39 |
| FFM-Adjusted 1RM Index | 7.89 | 0.5 | 6.53 | 0.3 | 0.026* |
| FSH (mIU/mL) | 5.2 | 0.9 | 58.0 | 8.0 | <0.0001* |
| 17β-estradiol (pg/mL) | 109.8 | 13.5 | 17.7 | 4.9 | 0.001* |

Data are presented as mean ± standard error. LDL-C: low density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, FFM: fat free mass, 1RM: one repetition maximum, FSH: follicle stimulating hormone. FFM-Adjusted 1RM index was calculated by dividing an individual's raw total (kg) by their fat free mass (kg). *P<0.05 between groups.

There were significant differences in age (p<0.0001), waist to hip ratio (P<0.01), body fat (P<0.05), FFM-adjusted 1RM index (P<0.05), FSH (P<0.0001), and 17β-estradiol (P<0.001) (Table 1). The post-menopausal group was significantly older than the pre-menopausal group (range: pre-menopausal = 23-32, post-menopausal = 56-65 years), as well as exhibiting a significantly greater waist to hip ratio, body fat, FSH, and lower 17β-estradiol. The pre-menopausal group was significantly stronger than the post-menopausal group both in absolute and FFM-adjusted values (range: pre-menopausal = 6.21-11.65, post-

menopausal = 5.20-7.89 arbitrary units). There were no significant baseline differences in IL-6, IL-10, or TGF-β between groups.

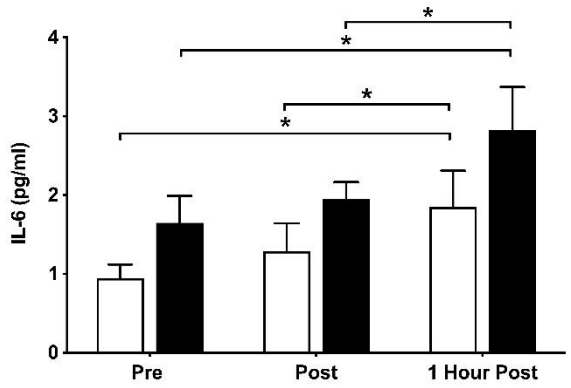


**Figure 1:** Plasma concentrations of IL-6 prior to, immediately after, and one hour after the cessation of resistance exercise in pre- (□white) and postmenopausal (■black) women. *(P<0.05) indicates significant effect of time.

There was a significant main effect for time (P<0.05), with both groups exhibiting increased plasma concentrations of IL-6 pre to post exercise (Figure 1). Post hoc analysis revealed a significant increase (P<0.05) in IL-6 pre to 1 hour post exercise, as well as immediately post to 1 hour post exercise in both groups. There was no significant group by time interaction. There was a significant main effect for time (P< 0.05) with the pre-menopausal group displaying increased plasma concentrations of IL-10 pre to post exercise followed by a reduction to near basal levels 1 hour post exercise (Figure 2). Post hoc analysis revealed a significant increase (P<0.05) in IL-10 pre to immediately post exercise. A significant, main interaction effect (P<0.05) was also observed. Post hoc analysis further identified a significant interaction for IL-10 immediately post exercise between groups, such that the pre-menopausal group significantly increased after exercise, while the post-menopausal group decreased but not significantly.

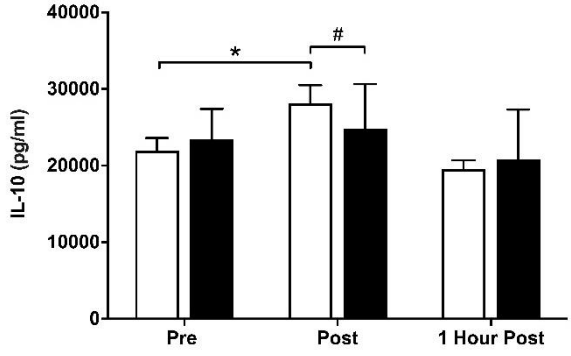


**Figure 2:** Plasma concentrations of IL-10 prior to, immediately after, and one hour after the cessation of resistance exercise in pre- (□white) and postmenopausal (■black) women. *(P<0.05) indicates significant effect of time. #(P<0.05) indicates significant time by group interaction.

TGF-β1 levels increased slightly, but not significantly, pre to post exercise in both groups. No significant effects for time or time by group interactions were observed for TGF-β1 (Figure 3). Bivariate analysis of study variables revealed a moderate, significant positive correlation (r2=0.33, P=<0.01) between E2 and WHR, as well as E2 and change in IL-10 (r2=0.45, P<0.001) from pre to immediately post exercise (Figure 4).

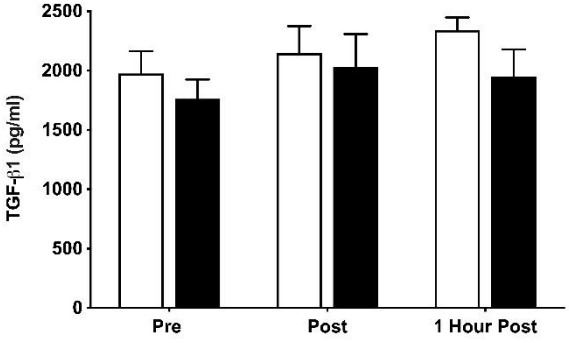


**Figure 3:** Plasma concentrations of TGF-β1 prior to, immediately after, and one hour after the cessation of resistance exercise in pre- (□white) and postmenopausal (■black) women.

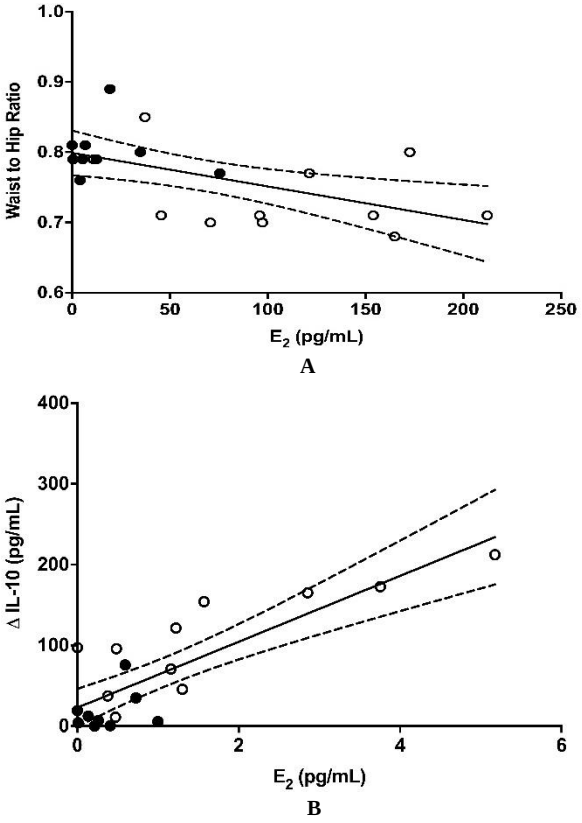


**Figure 4: A)** Significant correlation (P<0.01, $r^2$=0.33) between $E_2$ and waist to hip ratio in pre- (Owhite) and post-menopausal (●black) women. **B)** Significant correlation (P<0.001, $r^2$=0.687) between $E_2$ and change in IL-10 concentration from pre to immediately post exercise in pre- (Owhite) and postmenopausal (●black) women.

## Discussion

Menopause is characterized by a loss of E2 synthesis from reproductive tissues. Given the role of E2 in tissue repair, menopause associated reductions in E2 may result in loss of inflammatory recovery in post-menopausal women. We therefore examined 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, and TGFβ1 over time. It was hypothesized that post-menopausal women would display altered inflammatory recovery, characterized by either elevated IL-6 or decreased IL-10. It was also hypothesized that post-menopausal women would display a basal low-grade inflammation phenotype. Overall, we observed differences in the inflammatory response between pre- and post-menopausal women, supporting our first hypothesis. However, contrary to our second hypothesis, there were no baseline differences.

**I IL-6 Response to RE**

Significant elevations in IL-6 concentrations over time were observed in both pre and post-menopausal women. Skeletal muscle secretes IL-6 in response to exercise-induced stress and accounts for the majority of the circulatory protein. Under basal conditions, skeletal muscle IL-6 mRNA is hardly detectable. Upon onset of skeletal muscle contraction, IL-6 mRNA levels increase exponentially up to 12 hours post exercise [14]. Plasma IL-6 has been widely demonstrated to increase significantly upon an acute bout of aerobic exercise (AE) [15]. However, variable results have been reported for RE. Initial findings suggested no acute RE induced changes in IL-6 concentrations occurred in post-menopausal women [16]. However, several other studies described significant changes following RE, with an increase into the recovery period in both post-menopausal women and the general population [12, 17, 18]. The latter studies employed hypertrophy-oriented, high intensity RE protocols and revealed changes as described herein. It appears that factors such as repetition range, rest interval, contraction tempo and relative intensity may influence the magnitude of IL-6 induction.

**II IL-10 Response to RE**

Interleukin-10 is an anti-inflammatory cytokine that suppresses the induction of several pro-inflammatory cytokines [19]. Herein we reported that pre-menopausal women displayed significantly elevated plasma IL-10 concentrations immediately post exercise. Prolonged AE induces a marked increase in IL-10 concentration [20, 21]. However, multiple acute exercise studies have reported no significant changes in IL-10 activity [22-24]. In contrast, several other groups have reported significant changes after RE [25-27]. Hirose *et al.* (2014) reported significant increases in IL-10 one hour after performing moderate intensity RE. Izquierdo *et al.* (2009) found significantly increased post-exercise levels of IL-10 after a seven week RE intervention. Jajtner *et al.* (2015) found elevated plasma concentrations of IL-10 30 minutes after high volume lower body RE. Interestingly, amongst the studies reviewed there was a trend in elevated post-exercise IL-10 in trained individuals, providing a potential mechanism for training induced improvements to post-exercise inflammatory recovery and immunity.

The majority of studies assessing IL-10 responses to acute RE 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 RE, and fasting status appeared lacking. Overall, variable exercise protocols and lack of data with well-controlled cohorts limits generalizability and warrants further experimentation.

**III Menopausal Mechanisms for Exercise-Induced IL-10 Variation**

Significant differences in IL-10 concentrations immediately post-exercise were observed. Stress-induced impairments in post-menopausal IL-10 production may be attributed to altered transcription or post-transcriptional modifications in immune cells regulated by E2. Immune cells rapidly infiltrate skeletal muscle, connective tissue, and bone after RE [28]. Adequate response by immune cells ensures proper tissue healing and recovery, as well as musculoskeletal adaptation to exercise. E2 is ubiquitously expressed in immune cells [29]. Upon activation, E2 binds to its alpha, beta, or plasma membrane receptor (ERα, ERβ, or plasma membrane ER), leading to homo- or hetero-dimerization and subsequent phosphorylation. The E2 complex then translocates to the nucleus and regulates transcription. The specific complex formation of E2 is dependent upon receptor activation, binding domain and activity, and ligand involvement. E2 action is further augmented by a number of receptor mediated post-transcriptional modifications.

$E_2$ is a known regulator of IL-10 transcription in neutrophils, macrophages, dendritic, and type 2 T helper (Th2) cells [30]. Whole blood and isolated dendrites exposed to inflammatory stimuli exhibited enhanced IL-10 secretion in the presence of $E_2$ in a dose-dependent manner [31-33]. Skeletal muscle tissue from Sprague-Dawley rats displayed reduced neutrophil infiltration after exercise when treated with E2 [34]. More recently, macrophages treated with E2 exhibited enhanced immune recovery and were protected against endotoxins [35]. Cell and animal models in conjunction with our current data support the hypothesis that $E_2$ may be an essential regulator of inflammatory recovery, potentially through an IL-10 mediated pathway. Further research is needed to determine the direct or indirect mechanisms of E2 inflammatory regulation.

**IV Menopause Induced Visceral Adiposity**

Secondary analysis of study data revealed a significantly greater body fat % and WHR in post-menopausal women, despite similarities in total body mass. WHR also negatively correlated with $E_2$. Prior to menopause, females display greater subcutaneous & lower visceral fat accumulation than males [36]. The onset of menopause leads to a distinct phenotypic shift characterized by increased visceral adiposity. It is well established that estrogen deficiency from reproductive sites augments synthesis in peripheral tissues [37]. Extragonadal sites, such as adipose tissue, synthesize E2 via aromatization of androgens and activity of the enzyme aromatase [38, 39]. Aromatic activity in adipose tissue is regulated by a number of factors, including local cytokine production, promoter activity, and lipid mediators such as prostaglandin E2 (PGE2) [40]. Both IL-6 and TNF-α secretion from adipose tissue have been implicated in the activity of aromatic enzymes [41, 42]. Though no circulatory differences in IL-6 were observed, it remains possible that IL-6 from adipose depots is altered after menopause, driving visceral adiposity as a result of excess fat accumulation from reproductive estrogen deficiency.

In conclusion, an acute bout of 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 immediately post-exercise illustrated by increased IL-10 production. Though mechanistically unclear, recent developments in cytokine biology have elucidated a potential estrogen-dependent signaling pathway ultimately resulting in stress-induced IL-10 production. Thus, enhancing IL-10 secretion, via RE training and/or pharmacologic intervention, may provide clinical therapeutic value in estrogen deficient, post-menopausal women.

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**Abbreviation**

**1RM:** 1 repetition maximum
**AE:** aerobic exercise
**AMH:** anti-müllerian hormone
**ANOVA:** analysis of variance
**$E_2$:** 17β-estradiol
**ELISA:** enzyme-linked immunosorbent assay
**FFM:** fat free mass
**FSH:** follicle stimulating hormone
**HDL:** high density lipoprotein cholesterol
**IL-6:** interleukin-6
**IL-10:** interleukin-10
**IRB:** institutional review board
**LDL:** low density lipoprotein cholesterol
**RE:** resistance exercise
**TC:** total cholesterol
**TGF- β1:** transforming growth factor beta-1
**TRG:** triglycerides
**$VO_{2max}$:** maximal oxygen consumption
**WHR:** waist to hip ratio

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