Resistance Training at Eight-Repetition Maximum Reduces the Inflammatory Milieu in Elderly Women

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ABSTRACT


Introduction/Purpose: Inflammatory cytokines are associated with age- and inactivity-related diseases. We examined the influence of moderate- to high-intensity resistance trainings (RT) on inflammatory cytokines (interleukin 6 (IL-6) and IL-1β (IL-1β) and tumor necrosis factor α (TNF-α)) in circulation and lipopolysaccharide (LPS)-stimulated whole blood in elderly women. Method: Previously sedentary women (72 ± 1.1 yr) were grouped according to their hormone replacement regimens: traditional estrogen/progesterone (HRT, n = 12), selective estrogen receptor modulator (SER, n = 7), hormone replacement (NHR, n = 9), or no hormone replacement (CON, n = 7). Participants in the HRT, SER, and NHR groups trained (three sets, 10 exercises at an 8 repetition maximum (8RM)) 3 dwk⁻¹, whereas participants in the CON group maintained their "normal" activity for 10 wk. Participants performed a bout of resistance exercise (RE at 8RM; HRT, SER, and NHR groups) or sat quietly (CON) before (BT) and after (AT) RT to assess the influence of training on the acute responses to RE. Blood samples were obtained preexercise (PR), postexercise (PO), and 2 h postexercise (2H; same time points for resting CON). Results: Hormone status had no influence on dependent variables, so HRT, SER, and NHR groups were collapsed into one exercise group (EX, n = 28) and compared with CON. RT significantly reduced circulating TNF-α level by 37%. RT also reduced LPS-stimulated production of IL-6, IL-1β, and TNF-α at all time points (PR, PO, and 2H; per monocyte). Acute RE transiently increased plasma TNF-α, but blunted the circadian increase in LPS-stimulated inflammatory cytokines observed in CON. The blunting effect in EX was significantly greater AT compared with BT. RE also resulted in an increase in plasma IL-6, which was significantly reduced AT (BT: PR = 1.6 ± 0.5, PO = 2.8 ± 0.5; AT: PR = 1.8 ± 0.3, PO = 2.4 ± 0.3). Conclusions: We found that 10 wk of moderate- to high-intensity RT 1) reduced the systemic inflammatory milieu and 2) abrogated exercise-induced circulating IL-6 in previously sedentary elderly women. Key Words: IL-6, IL-1β, TNF-α, LIPOPOLYSACCHARIDE (LPS), EXERCISE, CIRCADIAN

Resistance exercise (RT) confers many health benefits and can be especially valuable for older individuals by helping them maintain independence. In elderly women, RT of sufficient intensity improves whole-body strength and peak oxygen consumption; conversely, aerobic training fails to improve upper extremity strength (8). RT has also been shown to ameliorate risk factors associated with aging and the metabolic syndrome by improving insulin resistance and glucose metabolism, resting metabolic rate, bone mineral density, hypertension, and percent body fat (9,38). Some benefits of RT in an elderly population have been well characterized, such as decreased risk of falls and increased muscle mass (9,12); however, the influence of habitual RT on the inflammatory milieu at rest and in response to acute resistance exercise (RE) in elderly persons has not been fully investigated.

Inflammatory cytokines such as interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) are involved in the initial development and progression of age- and inactivity-related diseases (13,17,27). Furthermore, a positive association exists between age and inflammatory-related cytokine expression or production (7,17,28). Several researchers have reported the positive effects of habitual exercise on inflammatory biomarkers. Smith et al. (30) observed a significant decline in spontaneous and mitogen-stimulated production of cytokines typically associated with
atherosclerosis (TNF-α, interferon γ, IL-1α) after 6 months of mixed aerobic and RE in persons at risk for cardiovascular disease (CVD). Recently, 12 wk of aerobic training decreased plasma IL-6 in sedentary lean and obese men without concurrent weight loss (4), whereas 12 wk of high-intensity but not moderate-intensity aerobic exercise reduced lipopolysaccharide (LPS)-stimulated TNF-α in young, healthy, sedentary volunteers (29). Monocytes are the chief responder cells to LPS stimulation, and their activation in vivo is directly linked to increased systemic inflammation. Given that monocytes and macrophages are key players in arterial plaque formation (27), LPS stimulation of whole blood may serve as an adequate ex vivo indicator of arterial or endothelial inflammatory status.

IL-6 exerts both inflammatory and anti-inflammatory actions. In fact, transient elevations in serum IL-6 consequent to muscle contraction during exercise exert positive metabolic actions. Contraction-induced IL-6 seems to act as a metabolic or substrate sensor and stimulates lipolysis, lipid oxidation, and glucose uptake in muscle (20). Exercise training may reduce the magnitude of IL-6 release from muscle (6) in athletes, but no difference was observed in postexercise circulating IL-6 after training in elderly persons (1). Bautmans et al. (1) did observe consistent acute exercise-induced elevations in circulating IL-6 in elderly men and women. It has been speculated that the anti-inflammatory effects of transient contraction-induced elevations in circulating IL-6 may protect against chronic systemic low-grade inflammation (18–20). Recent data from our laboratory support that theory (23).

We recently observed an apparent circadian increase in LPS-stimulated IL-1β (LPS–IL-1β) and TNF-α production (LPS–TNF-α), such that these measures were significantly elevated at approximately 1000 h compared with approximately 0700 h in resting elderly women (23). Nevertheless, a single bout of RE (three sets, 10 exercises, 80% one-repetition maximum (1RM)) abolished the circadian effect. Given the clearly defined anti-inflammatory properties of IL-6, we ascribed at least part of the “blunting effect” to the significant increase in serum IL-6 observed after exercise in the exercise group. It is unknown whether the observed “blunting” action of an acute bout of RE is conveyed to an overall anti-inflammatory effect when an individual engages in regular RT; therefore, we investigated the acute effects of RE after 10 wk of consistent RT.

Hormone replacement therapy (HRT) such as traditional conjugated estrogens or selective estrogen receptor (SER) modulators is frequently prescribed for postmenopausal women. Both estradiol and raloxifene (a SER) have been shown to inhibit cytokine production; therefore, hormone replacement status should be considered when investigating cytokine modulation (25,37). It is unknown if HRT or SER modulators influence the cytokine response to consistent RE.

Much of the existing data on cytokine profile in response to exercise training are resultant of aerobic exercise (4,29). Little information exists regarding the influence of RT on inflammatory cytokine production in sedentary elderly women. Given the potential diverse health-related benefits that RE may provide, the purpose of this investigation was to examine the influence of 10 wk of moderate- to high-intensity RT on inflammatory-related cytokines in serum and stimulated whole blood cultures at rest and in response to an acute bout of RE in elderly women. A second purpose was to determine whether traditional HRT or SER modulators influenced exercise-induced changes in the inflammatory milieu.

**METHODS**

**Subjects/prescreening.** Thirty-five apparently healthy postmenopausal women (65–85 yr) who had not exercised regularly for the previous 6 months volunteered to participate in the current study (Table 1). Participants completed a medical history, obtained written approval from their personal physician, and signed an informed consent form. The project was approved by the Committee on the Use of Human Research Subjects at Purdue University.

**Medical screening and exclusion criteria.** Potential subjects underwent a medical screening (physical examination and dementia screening) by our study physician and completed a submaximal treadmill and a resistive exercise stress test while blood pressure and ECG were monitored. The treadmill test began with a 3-min warm-up at 2 mph. The workload was increased by approximately 1 MET every 2 min until HR reached 85% of the age-predicted maximum. After a 15-min rest period, the proper lifting and breathing techniques were demonstrated for the leg press exercise, and subjects were given a chance to “practice” with light resistance. Each subject then performed an 8RM test with continuous monitoring of the ECG. During the test, the resistance was increased during each set until the subject could perform no more than eight consecutive repetitions (no more than three sets). Blood pressure was measured immediately on completion of each set and at 5 min of recovery. Any subject meeting the exclusionary criteria of the American College of Sports Medicine for exercise testing was not allowed to participate. Other exclusion criteria were severe arthritis, CNS or peripheral nervous system disorders, previous stroke, use of antidepressant medications, acute or chronic infection,
major affective disorder, human immunodeficiency virus infection or autoimmune disorders, metabolic disorders (type I or type II diabetes mellitus), smokers or smokeless tobacco users, regular aerobic or resistive exercise within the previous 6 months, oral steroid use other than estrogen/progesterone replacement, and alcohol intake greater than “moderate” (one drink per day). Participants who met the study criteria for participation were asked to maintain their “normal” diet regimen throughout the testing and training period and to consume no alcohol the day before the experimental trials. Subjects were verbally counseled each week to ensure no changes were made in the diet. In addition, users of nonsteroidal anti-inflammatory drugs were asked to refrain from taking their medication until after the experimental trials on test days.

**Group assignment.** Approved subjects were assigned to one of three exercise groups or to a nonexercise control group. Subjects were assigned to one of the exercise groups on the basis of their medical history of hormone therapy: HRT group (n = 12), a SER modulator group (n = 7), or a no-hormone replacement group (NHR, n = 9). In addition, seven subjects not taking hormone replacement were assigned to a nonexercise control group (CON, n = 7) to account for circadian changes during experimental trial days or seasonal changes between trials before and after 10 wk of exercise training or “normal” activity. Those in the HRT and SER groups had been taking uninterrupted estrogen/progesterone replacement (e.g., Prempro, Premarin, Provera, Estrace) or SER modulators (60 mg d−1; raloxifene) for at least 1 yr, respectively. One subject from each of the SER, NHR, and CON groups was taking alendronate. These subjects were initially excluded from all analyses because of the potential influence of bisphosphonates on cytokine profile (20). The exclusion of these data did not change the statistical outcome of any analysis except for preexercise LPS-stimulated IL-6 production (LPS–IL-6); therefore, data from these subjects were included in all other analyses (23).

**Pretesting and acclimation.** Body composition was estimated via the Jackson and Pollock seven-site skinfold procedure. All subjects were acclimated to the postresistive exercises (Cybex International, Owatonna, MN): chest press, lat pull-down, seated rows, shoulder press, leg abduction, leg adduction, chest fly, leg press, leg curl, and leg extension. They performed three acclimation sessions on nonconsecutive days. They performed three acclimation sessions on nonconsecutive days. 8RM was reassessed at the end of the 5th and 10th weeks. 8RM was assessed for each exercise on acclimation day 1. 1RM was estimated from the 8RM for each exercise. On acclimation day 2, subjects performed three sets (eight reps, eight reps, failure/fatigue) of each exercise at 50% estimated 1RM. 8RM were reassessed on acclimation day 3. To assess 8RM, participants performed each exercise using a weight estimated to allow a maximum of eight repetitions before “muscular failure” while using proper form. Resistance was adjusted on the basis of the participants’ ability to “lift” the initial resistance. If participants performed more or fewer repetitions than eight, the resistance was adjusted accordingly and the participant repeated the exercise. 8RM assessments were accomplished within one to three attempts allowing ample recovery time between sets.

**Experimental trials.** Experimental trials were conducted at least 1 wk after the last acclimation or exercise session. Between 0630 and 0730 h (overnight fast), subjects performed three sets (eight reps, eight reps, failure/fatigue) of the 10 exercises listed in the previous paragraph at their 8 RM with 2-min rest between sets (HRT, NHR, and SER) or rested quietly for 1 h (CON). After the exercise bout (HRT, NHR, and SER) or a 1-h rest (CON), subjects rested for 2 h and were allowed water ad libitum. Blood samples were obtained after a 15-min seated rest immediately preexercise (PR), within 4 min postexercise (PO), and 2 h postexercise (2H; same time points for CON). All participants underwent an experimental trial both before (BT) and after (AT) 10 wk of RT (or normal activity, CON). Muscle soreness was assessed by subjects rating their soreness on a scale (1–10 with 1 indicating no soreness and 10 indicating the most soreness/pain possible) when performing each of three movements. While standing, subjects abducted both arms until arms were perpendicular to the floor (arm raise), stood with arms at their side while flexing both forearms (bicep curl), or stood up from a seated position using their legs only (standing). Soreness was assessed before training began, 48 h after the first experimental trial, 1 wk after the last exercise training session, and 48 h after the second experimental trial.

**RT.** The experimental training period consisted of 10 wk of RT (HRT, NHR, and SER) or normal activity (CON). Exercise participants performed three sets of each exercise three times per week on nonconsecutive days. The first two sets consisted of eight repetitions with the last set being to muscular failure/fatigue. During the first week of training, they exercised at 70% of estimated 1RM. Intensity was increased to 80% of estimated 1RM at the beginning of the second week. Throughout the 10 wk, if subjects were able to complete 12 or more repetitions in the third set of an exercise on the last training day of each week, the resistance was increased the following week. The 8RM for each exercise was reassessed at the end of the 5th and 10th weeks.

**Blood sample treatment and estradiol.** Blood was drawn into prechilled serum separator tubes with clot activator (SST) or room-temperature tubes containing sodium heparin or EDTA. Serum from chilled SST was analyzed for estradiol concentration (double antibody 125I radioimmunoassay kit; Diagnostics Products Corporation, Los Angeles, CA). The interassay and intra-assay coefficients of variation for serum IL-6, IL-1β, and TNF-α concentrations were 7% and 5%, 16% and 16%, and 4% and 6%, respectively. EDTA plasma was analyzed for estradiol concentration (double antibody 125I radioimmunoassay kit; Diagnostics Products Corporation, Los Angeles, CA).

**Total and differential leukocyte count.** Total leucocyte number was measured in duplicate using a particle counter (Z-2 Particle Count & Size Analyzer; Beckman-Coulter, Hialeah, FL). Whole blood smears were stained...
with Wright-Giemsa stain (Sigma Diagnostics, St. Louis, MO), and a differential count was determined manually.

Stimulated cytokine production. LPS-stimulated cytokine production was assessed by diluting 200 μL of heparinized whole blood into 1800 μL of culture medium (RPMI (Sigma Diagnostics), cell culture medium (100 mL) supplemented with 2 mL of penicillin (100 U·mL⁻¹), 2 mL of streptomycin (100 μg·mL⁻¹), and 1 mL of glutamine (2 mM)). Two milliliters of diluted blood was placed in 24-well flat-bottomed plates and stimulated with LPS (from Salmonella enteriditis; final concentration = 25 μg·mL⁻¹; Sigma Diagnostics). After 24 h of incubation (37°C, 5% CO₂), culture supernatants were harvested and analyzed for IL-1β, IL-6, and TNF-α using ELISA (OptEIA; PharMingen, San Diego, CA). LPS-stimulated cytokine production was expressed as concentration in supernatant (pg·mL⁻¹) and per monocyte in each culture well (fg per monocyte). Two milliliters of 1:10 diluted whole blood was used for each sample in the LPS stimulation assay; therefore, total number of monocytes per well (MPW) was calculated as follows: absolute no. MPW = no. leukocytes per milliliter × percent monocytes × 1:10 dilution × 2 mL. The interassay and intra-assay coefficients of variation for LPS–IL-6, LPS–IL-1β, and LPS–TNF-α were 6% and 11%, 14% and 9%, and 2.6% and 7.2%, respectively.

Statistical analyses. To determine whether hormone status affects RE- or RT-induced changes in our dependent variables, initially four (group: HRT, NHR, SER, and CON) × three (exercise time point: PR, PO, and 2H) × two (training time point: BT and AT) repeated-measure ANOVA were conducted. The between-subjects factor was group, and the within-subjects factors were exercise time point and training time point. The results failed to reveal a significant effect of hormone status; therefore, the three exercise groups were collapsed to form one exercise group (EX, n = 28) to compare with the nonexercise control group (CON, n = 7). Statistical analyses were conducted using a two-factor (group × training time point) or three-factor (group × training time point × exercise time point) ANOVA with repeated measures on the within-subjects factors. The Mauchly test of sphericity was used. If the sphericity test confirmed that the covariance assumption was not satisfied, the Huynh–Feldt adjustment was used to correct degrees of freedom. When the ANOVA detected significant main effects, pairwise comparisons using the Bonferroni adjustment were used to determine where the differences were located. When the ANOVA identified significant interactions, independent samples t-tests were conducted to determine whether significant differences existed between groups, and one-way repeated-measures ANOVA were used to locate differences within the group across time points. We used the Bonferroni adjustment to adjust for multiple comparisons where appropriate. Post hoc power analyses were conducted using G*Power version 3.0.10. To determine the necessary sample size, a moderate effect size (0.35) was used, and power was set at 0.90 in the power calculations. The analysis indicated that a total sample size of 36 would be needed for four groups (HRT, SER, NHR, and CON) and 24 would be needed for two groups (EX, CON). Cohen’s d, a measure of effect size, is reported for the significant results where appropriate. Statistical analyses were performed using SPSS statistical analysis software (version 15.0; Chicago, IL). Descriptive information is expressed as mean ± SD, and dependent variables are presented as means ± SE. Threshold for significance was set at P < 0.05.

RESULTS

Subjects. Thirty-five elderly women (72.1 ± 6.1 yr) completed 10 wk of RT (EX, n = 28) or continued their normal daily activity (CON, n = 7). There were no significant changes in descriptive or anthropometric variables for either group (Table 1). Participants tolerated the moderate- to high-intensity RE well with no reports of significant fatigue. As expected, some muscle soreness occurred consequent to unaccustomed RE, but significant reductions in soreness were observed after training. There was a significant exercise effect for mean (BT and AT) arm raise soreness where 48-h PO soreness rating (1.7 ± 0.1) was slightly greater than PR (1.2 ± 0.6; P = 0.001). We observed a significant training × exercise interaction

![Figure 1: Percent change in 8RM strength after 10 wk of RT (EX, n = 28) or normal activity (CON, n = 7).](image)

TABLE 2. Strength (8RM).

<table>
<thead>
<tr>
<th>Exercise</th>
<th>EX (n = 28)</th>
<th>CON (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>AT</td>
</tr>
<tr>
<td>Chest press</td>
<td>17.3 ± 0.8</td>
<td>*23.4 ± 1.1</td>
</tr>
<tr>
<td>Lat pull-down</td>
<td>37.6 ± 1.1</td>
<td>*43.3 ± 1.5</td>
</tr>
<tr>
<td>Shoulder press</td>
<td>9.5 ± 0.5</td>
<td>*13.5 ± 0.6</td>
</tr>
<tr>
<td>Seated row</td>
<td>31.2 ± 0.9</td>
<td>*36.4 ± 1.0</td>
</tr>
<tr>
<td>Chest fly</td>
<td>7.4 ± 0.4</td>
<td>*11.5 ± 0.5</td>
</tr>
<tr>
<td>Leg abduction</td>
<td>32.1 ± 1.2</td>
<td>*37.7 ± 1.3</td>
</tr>
<tr>
<td>Leg adduction</td>
<td>30.9 ± 1.2</td>
<td>*41.5 ± 1.5</td>
</tr>
<tr>
<td>Leg press</td>
<td>70.0 ± 2.8</td>
<td>*84.9 ± 3.2</td>
</tr>
<tr>
<td>Leg extension</td>
<td>27.1 ± 1.0</td>
<td>*35.0 ± 1.2</td>
</tr>
<tr>
<td>Leg flexion</td>
<td>26.1 ± 0.9</td>
<td>*31.1 ± 0.9</td>
</tr>
</tbody>
</table>

8RM (kg) in EX and CON groups before (BT) and after (AT) 10 wk of RT or normal activity.

Values are mean ± SE.

* Different from BT (P < 0.001) and different from CON AT (P < 0.01).
† Different from BT in CON (P < 0.05).
(P = 0.03) for bicep curl soreness where soreness increased from PR (1 ± 0.05) to 48-h PO (1.2 ± 0.1) before training but did not change AT (PR: 1 ± 0.05; PO: 1 ± 0.04). We also found a training × exercise interaction for standing soreness (P = 0.026), which increased after the BT trial (PR: 1.07 ± 0.09; 48-h PO: 1.16 ± 0.09) but did not significantly change AT (PR: 1.1 ± 0.07; 48-h PO: 1.0 ± 0.05).

Resistance testing and training. The 8RM strength significantly improved for each exercise after 10 wk of RT in EX (Fig. 1 and Table 2). Mean 8RM strength for the 10 exercises improved by 31% (range, 18%–61%). Strength significantly declined for 10 wk for the lat pull-down (P = 0.047), seated row (P = 0.012), and leg flexion (P = 0.035) exercises in CON. Adherence to RT was good because the mean number of training sessions for exercise subjects during the 10-wk training period was 27.6 ± 1.7. Total weight lifted (TWL) during the experimental trials was calculated using the following equation: [(resistance (kg) × 8 repetitions × 2 sets) + (resistance (kg) × no. repetitions of third set)]. The average number of repetitions during set 3 of the experimental trials was 8 ± 3. After 10 wk of RT, mean TWL during the experimental trial was 18% greater than before training (BT: 3290 ± 112; AT: 3886 ± 131 kg; P < 0.001; Cohen’s d = −0.93).

Estradiol. As expected, baseline (PR/BT) plasma estradiol concentration was significantly greater in HRT compared with NHR, CON, and SER (data presented elsewhere [23]). Neither acute exercise (P = 0.072) nor RT (P = 0.338) had a significant effect on plasma estradiol concentration (data not shown).

Total leukocyte number. Acute RE increased circulating leukocyte number such that PO values were greater in EX than in CON (P < 0.001; Cohen’s d = 1.11). Leukocyte number at 2H remained elevated BT in EX but decreased AT, so that it was significantly different from the BT value (Cohen’s d = 0.31; Fig. 2).

Monocyte number per sample well. We observed a significant main effect of training and an exercise × group interaction (P = 0.010) for MPW. RE increased MPW at PO (Cohen’s d = 1.55) and 2H (Cohen’s d = 0.79) with the mean (BT and AT) being greater than the CON mean at the same time points (Fig. 2). After training, the grand mean for MPW with groups collapsed and was significantly greater than the mean BT (Fig. 2; Cohen’s d = −0.40).

Serum IL-6 concentration. We observed a significant exercise × group interaction in serum IL-6 concentration (P = 0.042). Research participants in EX experienced an increase in mean (BT and AT) serum IL-6 at PO (Cohen’s d = −0.67), which returned to baseline by 2H, whereas no change was observed in CON over time. To further evaluate the influence of chronic exercise training on exercise-induced IL-6 release, we calculated ΔIL-6 between the PR and PO time points both before and after 10 wk of RT. Plasma IL-6 increased 75% after RE when subjects were considered “untrained,” (BT) (23), whereas RE-induced IL-6 increased only 33% AT (P = 0.058; Fig. 3).
Serum IL-1β concentration. No changes in serum IL-1β were observed consequent to exercise time point or training in either EX or CON. BT Serum IL-1β concentrations for EX were 2.7 ± 0.8, 3.6 ± 1.0, and 3.1 ± 1.1 pg·mL⁻¹ for PR, PO, and 2H, respectively, whereas values for the same time points AT were 2.8 ± 0.8, 3.5 ± 1.2, and 3.0 ± 1.1 pg·mL⁻¹, respectively. CON serum IL-1β values for BT PR, PO, and 2H were 2.2 ± 1.4, 2.8 ± 1.8, and 2.2 ± 1.8 pg·mL⁻¹, respectively. Their AT values were 1.3 ± 1.2, 1.3 ± 1.9, and 1.3 ± 1.1 pg·mL⁻¹, for PR, PO, and 2H, respectively.

Serum TNF-α concentration. We observed an exercise × group interaction (P = 0.003), a training × exercise interaction (P = 0.02), and a tendency for a training × exercise × group interaction (P = 0.081). Similar to serum IL-6 results, EX experienced an increase in mean serum TNF-α at PO (Cohen’s d = −0.26), which then returned to baseline by 2H (Fig. 3). Post hoc analysis of the training × exercise interaction revealed that mean PR and PO values with groups collapsed were lower AT compared with BT. The training effect seemed to be driven by the EX group values; therefore, group data were analyzed separately. Our analyses revealed that a training × exercise interaction occurred in EX (P < 0.001), whereas no changes occurred in CON (Fig. 3). RT resulted in a decrease in serum TNF-α for EX at PR (Cohen’s d = 0.24) and PO (Cohen’s d = 0.26).

LPS–IL-6. We observed an exercise × group interaction, whereby mean PO LPS–IL-6 in EX was greater than in CON (Cohen’s d = 0.63; Fig. 4). Mean (BT and AT) LPS–IL-6 returned to preexercise levels and was not different from CON after 2 h of recovery. Although resting LPS–IL-6 was 20% lower at PR after training and mean LPS–IL-6 (PR, PO, and 2H) was 11% lower in EX, no significant training or training × group interaction occurred. When LPS–IL-6 was expressed per monocyte, we observed a training effect with groups collapsed (P = 0.044; Cohen’s d = 0.42) and a tendency for a training × group interaction (P = 0.055; Fig. 5). There was a group effect such that the grand mean for EX (308.7 ± 31.2 fg per monocyte) was...
significantly lower than that for CON ($550.2 \pm 62.4 \text{ fg per monocyte}$). There was also an exercise \times group interaction ($P = 0.006$), where mean LPS–IL-6 per monocyte was greater in CON at PO (Cohen’s $d = -1.61$) and 2H (Cohen’s $d = -1.19$) compared with EX (Fig. 5). The training effect seemed to be driven by the exercise group, so group data were analyzed separately. RT decreased mean (PR, PO, and 2H) LPS–IL-6 per monocyte by almost one-half in EX ($P < 0.001$; Cohen’s $d = 0.79$; Fig. 5), whereas no significant changes were observed in CON.

**LPS–IL-1β.** We observed a training \times exercise \times group interaction for LPS–IL-1β ($P = 0.011$). An apparent circadian increase was observed for LPS–IL-1β in CON, as it increased from PR to PO, becoming significantly higher than PR and PO by the 2H time point both BT (Cohen’s $d = -1.15$) and AT (Cohen’s $d = -0.91$; Fig. 4). LPS–IL-1β increased in EX immediately after exercise ($P < 0.001$; BT: Cohen’s $d = -0.55$; AT: Cohen’s $d = -0.94$) and remained elevated (BT: Cohen’s $d = -0.75$) (23) or continued to increase (AT: $P < 0.001$; Cohen’s $d = -1.46$) by 2H. RT reduced LPS–IL-1β per monocyte by 38% at PR ($P < 0.001$; Cohen’s $d = -0.69$) and 15% at PO (NS) in EX.

When expressed per monocyte, ANOVA identified a training \times exercise \times group interaction ($P = 0.011$) in LPS–IL-1β. RT decreased LPS–IL-1β per monocyte at all time points in EX such that AT values for EX (PR, PO, and 2H) were significantly lower than all other means at the same time points (Fig. 5). Cohen’s $d$ for EX for BT versus AT at PR, PO, and 2H was 0.90, 0.58, and 0.32, respectively. Cohen’s $d$ for EX versus CON at AT PR, PO, and 2H was $-1.45$, $-1.96$, and $-1.05$, respectively. There was a time effect for CON ($P = 0.049$), indicating a circadian increase in LPS–IL-1β per monocyte; however, multiple comparison did not identify specific differences between time points (Fig. 5). By 2H, LPS–IL-1β per monocyte was significantly greater than PR in EX (Cohen’s $d = -1.0$; Fig. 5).

**LPS–TNF-α.** We observed an exercise \times group interaction ($P = 0.032$), indicating that acute RE increased mean postexercise (BT and AT) LPS–TNF-α in EX (Cohen’s $d = -0.52$; Fig. 4). When expressed per monocyte, we found a training \times exercise \times group interaction for LPS–TNF-α ($P = 0.046$). RT significantly decreased LPS–TNF-α per monocyte at all time points in EX such that AT values for EX were significantly lower than all other values at each time point (Fig. 5). In addition, CON means (BT and AT) for PO and 2H were significantly greater than EX means at the same time points. Cohen’s $d$ for BT versus AT in EX at PR, PO, and 2H was 0.75, 0.39, and 0.39, respectively. Cohen’s $d$ for EX versus CON at BT PO and 2H was $-1.17$ and $-1.04$, respectively. Cohen’s $d$ for EX versus CON at AT PO and 2H was $-1.6$ and $-1.44$, respectively.

**DISCUSSION**

The key findings in this study were that 10 wk of RT at 8RM significantly reduced LPS–IL-6, LPS–IL-1β, and LPS–TNF-α and circulating concentrations of TNF-α. We recently reported the effects of acute RE on the inflammatory profile in untrained elderly women (23). These results, taken together with the current findings, demonstrate that both a single bout of moderate-intense RE and 10 wk of RT reduced inflammatory reactivity and overall inflammatory milieu in elderly women. These changes are generally associated with reduced risk of age- and inactivity-related diseases such as CVD.

There was a remarkably consistent pattern in LPS-stimulated cytokine production expressed per monocyte across exercise time (PR, PO, 2H) before and after RT, with the AT responses shifted downward, reflecting the training-induced decrease in inflammatory reactivity in EX across all exercise time points. In addition, the acute exercise-induced blunting of a circadian increase in cytokine production previously reported in untrained subjects (23) is supported here by the same suppressive response to acute RE after 10 wk of RT, though at an even greater magnitude. RT also attenuated the increase in acute contraction-induced serum IL-6 in our subjects by more than 50%, indicating potential metabolic training adaptations. Finally, RT significantly improved 8RM strength for each of the 10 exercises used in the RT regimen. The positive adaptations reported here occurred with subjects experiencing minimal soreness or fatigue overall. Given that RT confers improvements in strength and may lengthen the ability to live independently, these findings support the use of RT as a means not only to improve strength and functionality but also to reduce proinflammatory markers associated with components of the metabolic syndrome as well as overt diseases such as diabetes, atherosclerosis, CHD, and osteoporosis (9,13,17,27,38).

The absence of an influence of hormone status on inflammatory milieu in response to RT is not without precedence. We previously reported no interaction of hormone status with acute RE (23). In fact, whereas both estradiol and raloxifene (SER modulators) have been shown to decrease inflammatory cytokine production at rest or in vitro (25,37), we observed higher resting IL-6 concentrations in women taking raloxifene compared with those taking no hormone replacement whatsoever (23). The question of hormone replacement effects on inflammatory milieu both at rest and in response to exercise warrants further investigation.

Two- to threefold elevations in inflammatory cytokine concentrations indicate low-grade inflammation and accompany many age- and inactivity-related diseases. Habitual exercise has been reported to favorably influence the inflammatory cytokine profile in serum, muscle, or stimulated blood cultures (7,10,19,30). Our findings of reduced serum TNF-α after RT are similar to those of Kohut et al. (10), who reported that 10 months of flexibility and RE reduced circulating TNF-α but not IL-6 concentration. Kohut et al. (10) also reported that elderly men and women in an aerobic training group experienced decreases in both IL-6 and TNF-α. The authors acknowledged that the aerobic training was...
more rigorous than was the flexibility/RT; thus, their results may reflect a difference in physiological stress rather than differences in training mode. We used a shorter-duration (10 wk) full-body RT protocol that was significantly more rigorous than that described by Kohut et al. (10). We hypothesized that RT would decrease resting serum IL-6 along with IL-1β, in addition to TNF-α, similar to that reported in the aerobic training group, but we did not observe a significant change in serum IL-6 or IL-1β at rest.

Training duration or subject population may account for the lack of a training effect on resting serum IL-6 and IL-1β because our subjects trained for only 10 wk and were all apparently healthy females, whereas those in the study reported by Kohut et al. (10) had various disease states such as hypertension, type II diabetes, osteoporosis, thyroid dysfunction, and others. Training mode may also explain circulating inflammatory cytokine responses to chronic training because aerobic training resulted in a decrease in serum IL-6, but concurrent with our results, RT did not (10). However, the most likely explanation for the conflicting findings in circulating IL-6 response is the differences in both intensity and duration of training between the two studies. Participants with reduced circulating IL-6 in the study by Kohut et al. (10) trained aerobically for 10 months compared with just 10 wk of RT in the current study. In addition, the RT/flexibility used in the study by Kohut et al. (10) was considerably less strenuous than in the current study. We show not only that RT at 8RM elicits a reduction in inflammatory reactivity but also that this moderate- to high-intensity exercise is quite tolerable for an elderly population of women. It is important for future researchers to focus on the differences between training mode and the time course of positive changes to elucidate accurate exercise prescriptions for optimal health benefits. Most importantly, our observations indicate that significant beneficial changes in inflammatory milieu can be observed after a relatively short training period when using moderate- to high-intensity RT as the sole exercise mode.

In the present study, the 37% training-induced decrease in serum TNF-α at rest (PR) indicates a reduction in systemic, low-grade inflammation. TNF-α is the first of the inflammatory cytokines produced in the inflammatory cascade and directly impairs insulin signaling and, consequently, glucose uptake (24). The relationship between circulating levels of TNF-α and insulin resistance in elderly persons has been apparent for some time (17). In addition, TNF-α is related to the development of atherosclerosis, dementia, and osteopenia (2,7,11,13). Chronically elevated TNF-α has been associated with sarcopenia and muscle wasting in elderly persons (7,11). The reduction in circulating TNF-α observed in the current study may have contributed to strength gains consequent to RT. Three months of RT significantly decreased skeletal muscle TNF-α mRNA and protein levels in elderly individuals (7). In addition, muscle protein synthesis rate was inversely related to levels of TNF-α protein content. The data reported by Greiwe et al. (7) indicate that by reducing muscle TNF-α, the benefits of RT on skeletal muscle are augmented by improved protein synthesis. Although we did not measure muscle TNF-α expression or protein synthesis, our results support those of Greiwe et al. (7) by revealing a positive effect of RT on TNF-α, which is reported to be catabolic when chronically elevated. The finding of a training-induced reduction in circulating TNF-α, both at rest and immediately after an acute RE bout, after only 10 wk of moderate- to high-intensity RT without a change in body composition, underscores the significance of potential early, health-related, RT-induced benefits before observable changes in muscle or fat mass.

It has been established that skeletal muscle contraction stimulates IL-6 release, resulting in an increase in circulating IL-6 during and after exercise (32). Elevations in plasma IL-6 during and immediately after exercise are proportional to exercise duration, intensity, and active muscle mass. Exercise-induced increases in serum TNF-α and IL-1β are observed less frequently, and their biological roles during/after exercise are not as well understood. Exercise-induced IL-6 is thought to work as a “metabolic sensor,” signaling the need for available substrate for working muscles because it stimulates lipolysis, fat oxidation, and glucose uptake in muscle (20). We observed a 75% increase in serum IL-6 immediately after a single bout (~70 min) of RE (23). After 10 wk of RT, the postexercise IL-6 increase was only 34% despite an 18% increase in total work performed. The difference in ΔIL-6 did not reach statistical significance (P = 0.058); however, given that circulating levels were decreased by one-half, the difference may be biologically relevant and indicate a training adaptation. Regardless, consistent exercise training improves the capacity of skeletal muscle to oxidize fat and spare glycogen along with other adaptations such as increased metabolic enzyme activity, enhanced glycogen storage capacity, increased capillarization, and other positive adaptations. Although we did not measure the aforementioned training adaptations, it is reasonable to suspect that some may have occurred in our previously sedentary elderly subjects after 10 wk of moderate-intensity RT and thus, at least, partially account for the decrease in exercise-induced IL-6 after training. If these muscular training adaptations account for changes in IL-6 levels after exercise, this indicates that the majority of circulating IL-6 after exercise is due to muscle release as a metabolic sensor and is in concordance with previous reports where direct muscle IL-6 release was measured (32).

At present, there are few data on the influence of training on acute exercise-induced IL-6 levels. A recent cross-sectional study revealed that athletes experienced a lower magnitude of change in IL-6 after acute exercise compared with nonathletes (6). Another group found no difference in exercise-induced IL-6 after 6 wk of intensive RT in elderly subjects (1). The authors reported exercise-induced increases in IL-6 of ~20% both before and after training, which is substantially less than our observations. The disparity between our data and theirs may be consequent
to the duration of the training period, total number of exercises performed, total active muscle mass, or that our subjects were women, whereas they studied men and women. In the current study, we show that elderly women responded to a single RE bout with an increase in circulating IL-6 and that exercise-induced increases in IL-6 were attenuated by training.

Acute exercise-induced increases in serum TNF-α and IL-1β have been reported (15,36,39) but are not as consistently observed as those of IL-6 (1,6,16). We detected a significant main effect of exercise for TNF-α immediately after acute RE but no significant change in serum IL-1β. The source of these cytokines consequent to a single exercise bout remains unclear; however, the variation in observed responses may be influenced by multiple factors such as subject population, exercise duration, intensity, mode, or sampling time point. Exercise results in a mobilization of leukocytes with increased intracellular levels of TNF-α, IL-1α, and IL-6 as well as other pro-inflammatory and anti-inflammatory cytokines into circulation (39). It is possible that these cells may contribute to exercise-induced elevations in circulating cytokines (26). In addition, oxidative stress during exercise resulting in the production of reactive oxygen species (ROS) may also provide a stimulus for cytokine production from various tissues (36). Antioxidant supplementation abrogated (IL-6) or completely abolished (TNF-α, IL-1β) the exercise-induced increases in circulating cytokines after 45 min of cycling at 70% VO2max in untrained volunteers (36). Exercise training enhances the ability to withstand ROS by improving the natural antioxidant defense system (14). ROS may have played a role in the exercise-induced elevations we observed in serum IL-6 and TNF-α, whereas positive training effects on the antioxidant system may have contributed to the decreased change in IL-6 after exercise and reduction in TNF-α.

As expected, acute RE resulted in a significant leukocytosis both BT and AT. Circulating leukocyte number remained significantly elevated after 2 h of recovery BT (2H) (23); however, after 10 wk of RT, the total number of leukocytes was significantly lower at 2H compared with the same time point BT, indicating a more rapid return to baseline levels. Stress hormones such as the catecholamines and cortisol stimulate release of leukocytes from the marginal pool. Exercise training is known to blunt the stress hormone response to acute exercise. This blunting effect may explain the speedy return toward baseline we observed in leukocyte number after training. We found a significant main effect of training (groups collapsed) in MPW, which seems to be driven by the exercise group. Cellular adhesion molecules (CAM) are responsible for the attachment of monocytes to the endothelium, and exercise training has been shown to reduce the expression of inflammatory and atherogenic CAM (21). In addition, TNF-α induces adhesion of leukocytes to the endothelium (40). It is possible that RT may have resulted in reduced CAM, which might lead to higher circulating monocyte numbers; however, we cannot confirm this hypothesis in the present study. We did, however, observe a significant decrease in circulating TNF-α after RT in exercise subjects, which may support a link between a reduction in serum TNF-α and elevations in monocyte number because TNF-α has been shown to induce firm attachment of leukocytes to microvessel walls (40).

The cytokine response in LPS-stimulated whole blood cultures (pg·mL⁻¹) was significantly enhanced immediately after exercise both before and after 10 wk of RT. We expected greater cytokine production in whole blood cultures immediately after exercise because of the elevation in postexercise leukocytes due to exercise-induced leukocytosis. Mean MPW was greater in EX compared with CON at PO and 2H. After 2 h of recovery, stimulated production of IL-6 and TNF-α returned to preexercise levels, although leukocyte number remained elevated both BT and, to a lesser extent, AT. IL-1β production on the other hand continued to increase. In fact, after a total of 3 h of rest in CON, LPS–IL-1β became significantly different from PR after training, confirming the previously reported circadian effect before training (23).

When corrected for monocyte number in each culture well (fg per monocyte), circadian variation in cytokine production became apparent for all three cytokines. We previously reported that acute RE blunted an apparent diurnal/circadian response in LPS–IL-1β and LPS–TNF-α (fg per monocyte) in untrained elderly women (23). Here, we affirm our previous report with the observation of a similar response, although at an even greater magnitude after 10 wk of RT. Our current analysis also revealed that LPS–IL-6 per monocyte, in addition to IL-1β and TNF-α, was blunted in EX after exercise compared with resting CON. In fact, after training, the curves of cytokine production per monocyte were remarkably similar to those before training but shifted downward. The downward shift reflects the RT-induced decrease in inflammatory reactivity. As previously discussed (23), the acute RE-induced blunting of circadian elevations in cytokine production from whole blood cultures may be partially due to the significant postexercise increase in serum IL-6 observed in the exercise subjects. Our observations after 10 wk of training in the present study show that the acute exercise-induced inhibition of LPS-stimulated cytokine production is present in trained as well as in untrained subjects, despite a smaller increase in postexercise circulating IL-6. IL-6 has strong anti-inflammatory actions, which include inhibition of TNF-α and IL-1β and stimulation of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (18–20). Infusion of rHIL-6 to physiological levels inhibits the production of TNF-α elicited by low-grade Escherichia coli LPS endotoxemia in young healthy humans (31). In the same study, the authors reported that when subjects cycled...
for 3 h at 75% VO₂max, thereby increasing circulating IL-6 from approximately 2 to 25 pg·mL⁻¹. TNF-α production to endotoxemia was reduced in a similar manner to that of the IL-6 infusion (31). Compared with data presented by Starkie et al. (31), we saw modest increases in serum IL-6 yet still detected an inhibition of LPS-stimulated cytokine production after RE both before and after 10 wk of RT. Our data indicate that a RE bout that works all major muscle groups at 8RM (three sets per 10 exercises) is sufficient to elicit skeletal muscle release of IL-6 in elderly women, which in turn may act as a beneficial anti-inflammatory agent limiting low-level systemic inflammation in the short term and long term if the exercise is consistently performed.

Previous reports of training-induced decreases in ex vivo cytokine production have shown that long-term combination of aerobic and RE (6 months) (30) or high-intensity aerobic exercise for a shorter duration (12 wk) (29) is required to elicit beneficial effects. We detected a significant reduction in monocyte inflammatory reactivity after just 10 wk of moderate- to high-intensity RT. LPS–IL-1β, LPS–TNF-α, and LPS–IL-6 were significantly reduced at all exercise time points after correcting for monocyte number. The mean (PR, PO, 2H) percent change in IL-1β, TNF-α, and IL-6 was 45%, 42%, and 40%, respectively. In addition, absolute LPS–IL-1β at rest, before monocyte number correction, was also significantly reduced (32%) by RT, reflecting the robust influence of just 10 wk of moderate- to high-intensity RE in our subject population. Furthermore, there was an apparent downward shift in absolute production of IL-6 (17%) and TNF-α (14%) at rest in EX, although these changes were not statistically significant. Overall, production of the two proinflammatory cytokines, IL-1β and TNF-α, decreased to the greatest extent in response to habitual RE, which supports our hypothesis that consistent RE using all major muscle groups at moderate to high intensities will reduce the inflammatory milieu and markers associated with CVD, insulin resistance, osteoporosis, and other age- or inactivity-related disease states.

The training-induced reduction in LPS-stimulated cytokine production may be partially explained by the influence of exercise training on the toll-like receptor 4 (TLR4). Toll-like receptors recognize pathogen-associated molecular patterns and TLR4 is the primary signaling receptor for LPS, which is a component of gram-negative bacterial walls (3). We have previously observed a difference in whole blood mRNA expression of TLR4 in RT-trained versus untrained elderly women (5), with trained women having lower TLR4 than untrained women. In addition, when subjects were grouped into high versus low TLR4 expressers, the former had significantly higher LPS–IL-6, LPS–TNF-α, and LPS–IL-1β (5). Subsequently, Stewart et al. (33) reported that 12 wk of a combination of aerobic and RT reduced LPS–IL-6 concomitant with lower CD14⁺ cell-surface TLR4 expression. Consistent exercise seems to reduce both TLR4 mRNA and cell surface protein expression. RT seems to reduce cytokine production in whole blood, at least partially, by a reduction in TLR4, the primary signaling receptor for LPS.

Another potential mediator of exercise training-induced decreases in endotoxin-stimulated cytokine production may be improved parasympathetic (vagal) tone. Consistent exercise increases vagus nerve activity. Via the cholinergic anti-inflammatory pathway stimulation of the vagus nerve, acetylcholine and cholinergic agonists inhibit cytokine synthesis in macrophages and other cytokine-producing cells (35). The association between exercise-induced changes in vagal tone and its effects on low-level systemic inflammation in humans is an intriguing concept and warrants further investigation. In addition, the consistent but transient elevations in serum IL-6 due to muscle contraction during exercise may have a cumulative anti-inflammatory effect, helping to reduce systemic inflammation (19). Given the anti-inflammatory properties of IL-6, exercise-induced IL-6 release from muscle may have played a role in the reduction of serum TNF-α as well as decreased monocyte inflammatory reactivity observed in the current study.

The reduced responsiveness of monocytes to LPS stimulation after RT reflects a strong anti-inflammatory effect of habitual whole-body RE. Our observations suggest that RT may also induce production of monocytes with a different phenotype from circulating monocytes before RT. Indeed, Timmerman et al. (34) found that untrained elderly women had significantly more circulating inflammatory monocytes (CD14⁺CD16⁻; 13%) compared with trained women (7%). They reported that 12 wk of a combination of aerobic and RT reduced the inflammatory monocyte percentage in untrained women to approximately 5%. Given that CD14⁺CD16⁻ cells are responsible for the majority of the monocytic response to LPS, our findings of reduced monocyte reactivity to LPS stimulation after RT support their observations of a reduction in inflammatory monocytes in circulation.

When elderly individuals engage in consistent RE, they experience not only improvements in strength but also increases in peak oxygen consumption, increased independence, and decreased risk of falls (8,9,12). Age, as well as inactivity and disease, is accompanied by elevations in systemic inflammation and inflammatory biomarkers (7,9,11,13,17,28). Our data indicate that acute and habitual RE reduces inflammation and may create an anti-inflammatory environment conducive to improved health and a reduction in risk of disease. In summary, we show that moderate- to high-intensity (8RM) RE in elderly women has a positive and profound influence on low-level systemic inflammation and inflammatory reactivity, which often accompany CVD, insulin resistance, osteoporosis, dementia, muscle wasting, and other disease states. The positive adaptations to RT for elderly persons described above are especially significant in women who have less upper body muscle mass and strength compared with men. Our findings
add substantial knowledge on the positive effects of a relatively short-term RT period on the inflammatory milieu associated with age- and inactivity-related diseases in women aged 65–85 yr.

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