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Original article

Influence of sex on cytokines, heat shock protein and oxidative stress markers in response to an acute total body resistance exercise protocol

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Abstract

Background/Objective: This study evaluated the influence of sex on changes in cytokines, heat shock proteins (HSPs), and oxidative stress in response to a single bout of total body resistance exercise.
Methods: Sixteen healthy volunteers (8 men and 8 women), active and recreationally trained in resistance exercise, were subjected to a single bout of total body resistance exercise (3 × 8–10 repetition maximum, 10 exercises, rests periods of 90–120 seconds). Serum creatine kinase (CK), interleukin (IL)-6, IL-10, tumor necrosis factor-α, HSP60, HSP70, thiobarbituric acid reactive substance, and reduced glutathione were assessed at pre-protocol, and 1 hour, 4 hours, and 24 hours post-protocol.
Results: Both men and women had a similar increase in CK ($p < 0.05$) activity at 24 hours post-exercise. Significant sex differences were observed for IL-6. In the men, an increase from baseline was noted at 1 hour for IL-6. In women, an increase from baseline was noted at 4 hours only for IL-6. There was a significant correlation between peak IL-6 blood level and CK level at 24 hours only in the women. No significant changes were observed in IL-10, tumor necrosis factor-α, HSP60, HSP70, thiobarbituric acid reactive substance, and reduced glutathione.
Conclusion: Acute total body resistance exercise altered circulating levels of IL-6 and sex differences existed in the temporal pattern and magnitude of this response.

Keywords: Cytokines; Heat shock protein; Oxidative stress; Sex; Weight bearing exercise program

Introduction

A single bout of heavy resistance exercise increases plasma cytokines[1,2] that can influence metabolism[2,3] and tissue remodeling, especially in response to muscle damage.[3] Cytokines may be classified as pro- and anti-inflammatory. The proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, are considered to promote inflammatory responses followed by exercise-induced muscle damage. By contrast, anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1ra) inhibit inflammatory responses.[4] IL-6 can act as both a pro- and anti-inflammatory cytokine, depending on the situation.[4,5] Specifically, IL-6 may act as an essential regulator of satellite-cell-mediated hypertrophic muscle growth,[6] and may also play a role in tissue remodeling (muscle), especially in response to muscle damage.[3,7] In the metabolic context, IL-6 contributes to glucose homeostasis by inducing hepatic glucose output and mediating lipolysis.[2,3] Furthermore, exercise-induced cytokines may be

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associated with alterations in extracellular heat shock protein (HSP) levels and oxidative stress.[8]

Most HSP families are predominantly intracellular proteins that bind to nascent peptides and proteins and facilitate proper assembly and folding. Their concentrations are increased in response to a variety of stressful conditions to protect against protein denaturation and cell death. Under conditions of stress the 70-kDa HSP (HSP70) is released into the circulation where it induces a proinflammatory immune response.[9] It has been suggested that circulating HSP70 exerts an immunomodulatory effect following exercise.[8] Furthermore, thiobarbituric acid reactive substance (TBARS), a lipoperoxidation marker, reduced glutathione (GSH), and non-protein small thiols, have previously been demonstrated to be involved in oxidative stress and inflammatory responses following exercise.[8]

Previous studies have failed to demonstrate consistency in the temporal pattern, magnitude of response, and/or a significant change in systemic cytokines after a single bout of resistance exercise in men and women.[1,7] In general, after resistance exercise levels of IL-6 and IL-10 increased between one- and fivefold and peaked between 1 hour and 24 hours, while TNF-α levels remained unchanged or decreased.[1,7] Although these variations have been attributed to different variables used in the individual studies (physical fitness level, nutrition intake, and training intensity and volume)[1,3,10] it is still unclear to what extent sex difference influences cytokine responses to resistance exercise because these studies have focused predominantly on male populations or have not been directed to this purpose.[1,3,10] Recently, only two studies have observed resistance exercise effects on IL-6, IL-10, and TNF-α responses in women.[11,12] Although these studies were not directed to investigate sex difference on cytokines, no change was reported in IL-6, IL-10, and TNF-α concentrations after resistance exercise.

Significant sex dimorphisms in immune responses to non-exercising conditions have been well documented.[13] Men and women display sex differences in regulating substrate utilization,[14] muscle fatigue,[15] endocrine function,[10,16] oxidative stress,[17] muscle damage,[18] and inflammatory responses during exercise.[10,19,20] These exercise responses may result in sex-specific immunomodulatory effects.[2−4,8]

Thus, new insights into physiological adjustments may be gained by further elucidation of relationships between sex and temporal changes in cytokines, oxidative stress markers, and HSP during a single bout of resistance exercise. We hypothesized that changes in cytokines, HSP, and oxidative stress markers in response to an acute resistance exercise regimen would be attenuated in women.

Methods

Sample selection

Before the study, participants completed a physical activity questionnaire indicating their weekly physical training activities and how long they had been training. Participants involved with a resistance training program for a minimum of 1 year and at least 2 days per week were selected. Participants involved with competitive weight lifters, engaged in any specific training cycle outside of this investigation, any dietary or performance enhancing supplements, or any injuries or any pre-existing medical conditions that could affect safety and the protocol response were excluded.

Thus, 16 healthy recreationally resistance trained (8 men and 8 women), aged 20−30 years, and eutrophic, participated in the study (Table 1). All women were oral hormonal contraceptive users (combination of ethinyl estradiol and progestin). Four volunteers (2 male and 2 female) participated in some form of endurance exercise (running or treadmill exercise two or three times/week). Participation was voluntary and participants were advised of their right to cease participation at any time. Participants were informed about the possible risks and discomfort involved before their written consent was obtained. The research was approved by the Research Ethics Committee of the Institute of Health Sciences, Federal University of Triângulo Mineiro, Minas Gerais, Brazil, and was in accordance with the Declaration of Helsinki.

Experimental protocol

Before the beginning of the resistance exercise session, the participants were submitted to a two-session learning period to adapt themselves to the protocol (range 8−10 repetition maximum, RM). Then, each participant took part in two exercise sessions: (1) familiarization and (2) test. During the familiarization, after a warm-up pattern (5 minutes), the participants attempted one set of 8−10 repetitions (all exercises) with their self-selected 8−10 RM load. In case of under- or overestimation they rested for 3−5 minutes prior to the next attempt with a new 8−10-RM load. On average, this procedure was repeated 3−5 times until the individual 8−10 RM (real) was obtained. After an interval of 4−5 days, all participants were advised to abstain from exercise for 2 days prior to the acute testing visit (see Total body resistance exercise session).

One day before the resistance exercise session, a standard diet was provided (in writing) to all volunteers. On the

Table 1
Participants and training session characteristics.

Participant characteristics	Men ($n = 8$)	Women ($n = 8$)
Age (y)	27.1 ± 1.2	24.5 ± 0.8
Body mass (kg)	77.7 ± 2.2	57.2 ± 2.4*
Height (cm)	175.5 ± 1.2	158.8 ± 2.3*
Body fat (%)	11.3 ± 0.6	24.8 ± 1.8*
Lean body mass (kg)	68.8 ± 1.7	42.9 ± 1.7*
Total load (kg)	768.5 ± 24.2	449.2 ± 29.7*
Total load corrected for lean mass (total load/lean mass)	11.2 ± 0.5	10.5 ± 0.6
Total repetition number	290.5 ± 4.1	299.9 ± 1.9
Total volume (repetition × sets × load)	22,492.4 ± 862.0	13,666.8 ± 945.4*
Total volume corrected for lean mass (total volume/lean mass)	329.1 ± 17.5	319.4 ± 20.4

Data are presented as mean ± standard error.
*Significantly different between groups ($p < 0.05$; independent t test).

resistance exercise session day, the volunteers arrived at the laboratory at 6 AM for a body composition analysis after an overnight fast. Then, a standard meal (breakfast) was provided. The resistance exercise session was started 1 hour after feeding. Blood samples were collected at pre-protocol, and 1 hour, 4 hours, and 24 hours post-protocol. Following the single bout of resistance exercise protocol, the participants remained seated at the laboratory. Between 4 hours and 24 hours the participants remained in their normal routines without exercise/sports and were advised to remain with the standard diet (Fig. 1).

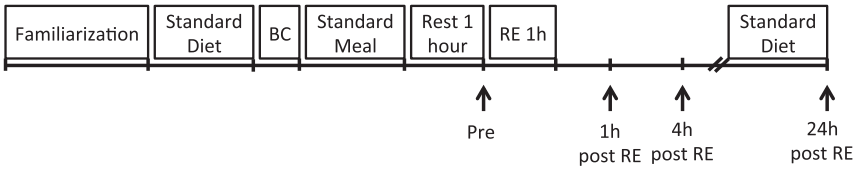

Fig. 1. Schematic representation of the experimental protocol. BC = body composition; Pre = before RE; Post = 1 hour, 4 hours, and 24 hours after RE; RE = resistance exercise session of 1 hour's duration; ↑ = blood sample.

Anthropometric measurements and diet monitoring

Height and weight were obtained using a stadiometer and a standard balance beam scale, respectively. Bioelectric impedance analysis was used to measure body composition (Model 450; Biodynamics, Shoreline, WA, USA). Before testing, all participants were instructed to avoid exercise, not to consume caffeine or alcohol within 1 day of the test, to fast for at least 10−12 hours prior to testing, and to drink 2 L of water and to empty their urinary bladder in order to reduce factors that might influence the results. Relative body fat (%) was calculated and lean mass (kg) was estimated by the equation proposed by Lukaski et al.[21]

One day before the resistance exercise session, a standard diet was provided to all volunteers containing about 30 kcal/kg and 60% carbohydrate, 23% protein, and 17% fat.[22] One hour before the resistance exercise session, a standard meal (breakfast) was provided to all volunteers containing about 8 kcal/kg and 60% carbohydrate, 23% protein, and 17% fat. The standard diet was elaborated by a qualified nutritionist.

Total body resistance exercise session

Previous to the testing visit, all participants were advised to abstain from exercise for 3 days prior to the resistance exercise session. The weight training was supervised by a qualified professional. The protocol consisted of dynamic exercises for the lower and upper limbs for a total of 1 hour. The training protocol consisted of the following: thighs (hack squat, leg extension, and leg curl), back (seated row and lat pull down), chest (bench press and pec deck), biceps (biceps curl), triceps (triceps pulley), and calf (calf raise). The training zone consisted of three series of 8−10 RM. A period of 90−120 seconds rest was established between series and exercises. During the training sessions, the participants were advised to perform eccentric actions for 1 second and concentric actions for 1 second. In order to obtain sex characteristics of the resistance training protocol, the volume and load of all participants were corrected for whole body lean mass.

Blood samples

Blood samples were collected from each participant after 12 hours of fasting. The samples (10 mL) were centrifuged (1500*g*, 4°C, 15 minutes) and the serum was aliquoted and stored at −80°C for subsequent analysis. TNF-α, IL-6, and IL-10 were assessed by enzyme-linked immunosorbent assay (Human TNF, Human IL-6, Human IL-10, Quantikines Kits; R&D Systems, Minneapolis, MN, USA), in a solid phase of 5-hours duration, according to the manufacturer's instructions. Intra- and inter-assay variation coefficients were lower than 7%. Analytical sensitivity, according to the manufacturers of the kits, was: TNF-α = 1.6 pg/mL, IL-6 = 0.70 pg/mL, and IL-10 = 3.9 pg/mL. Serum concentrations of HSP60 and HSP70 were assessed by enzyme-linked immunosorbent assay (Assay Designs, Stressgen, Ann Arbor, MI, USA) according to the manufacturer's recommendations. Analytical sensitivity was: HSP60 = 3.125 ng/mL and HSP70 = 0.090 ng/mL. Intra- and inter-assay variation coefficients were lower than 10% according to the manufacturer's instructions.

Creatine kinase (CK) activity was determined spectrophotometrically using a commercially available kit (Gold Analisa Diagnostica, Belo Horizonte, Brazil). The inter- and intra-assay coefficients of variation of the CK assay were 2.4% and 3.5%, respectively. Concentrations of reduced glutathione (GSH; in fact, non-protein small thiols) in whole blood were determined with Elman's reagent (dithionitrobenzoic acid), at a wavelength of 412 nm, in a spectrophotometer, as described previously.[23] Lipoperoxidation was assessed in the sera by measuring the levels of thiobarbituric acid reactive substance (TBARS) as described previously.[23] First, the serum was precipitated by the addition of 12% trichloroacetic acid (1 mL 12% trichloroacetic acid per 100 μL sample) followed by vigorous shaking for 5 seconds. Next, the sample was incubated for 1 hour at 100°C in the presence of 0.9 mL 60mM Tris−HCl buffer, pH 7.4 (0.1mM diethylenetriaminepentaacetic acid) and 1 mL 0.73% TBA. After incubation, the material was chilled for 30 minutes at

5°C, and later centrifuged. Samples were read at 535 nm in a spectrophotometer. The inter- and intra-assay coefficients of variation of the TBARS assay were 7.3% and 8.6%, respectively.

All evaluations were performed in duplicate by the same experienced researcher in a single experiment to minimize inter-assay variations.

Statistical analysis

A Kolmogorov–Smirnov test was conducted to verify a normal distribution. Data were analyzed using time (pre-protocol, and 1 hour, 4 hours, and 24 hours post-protocol) repeated-measures analysis of variance. When appropriate, the Fisher's *post hoc* test was performed to determine differences. Mauchly's test of sphericity was used to assess homogeneity of variance in the repeated-measures design. When significant, a Greenhouse–Geisser correction was used. Differences between groups (men vs. women) were determined by the independent *t* test. Correlation analysis was used to determine the association between the significant values obtained. Data are presented as mean ± standard error with the α level for significance set at $p < 0.05$. Statistical power (observed power) was used to report the probability of a type II error. The effect size (partial η, η^2) was used to report the strength of intervention.

Results

Significant sex effects were observed in anthropometric variables. Both sexes performed the protocol with the same RM number. Men performed a greater absolute amount of load and volume. However, the total load and volume corrected for lean mass showed no difference between men and women (Table 1). There was a significant ($p = 0.027$) increase in CK activity at 24 hours post-exercise compared to pre-exercise (Fig. 2). No significant difference was observed between sex or interaction between sexes by time.

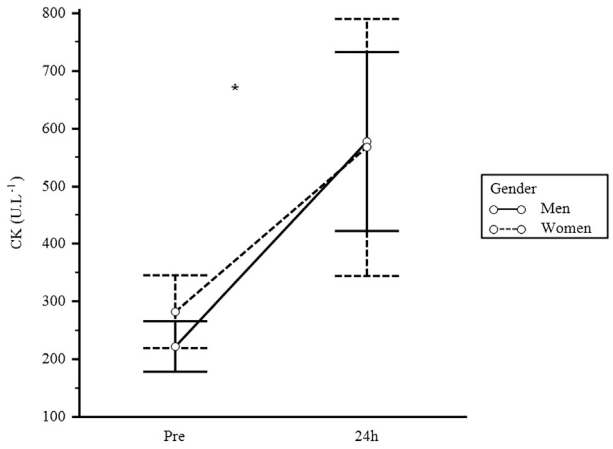

Fig. 2. Mean ± standard error serum creatine kinase (U/L) responses in men and women to an acute resistance exercise session. *$p \leq 0.05$ from corresponding pre-exercise values.

Significant time effects were observed for IL-6. In men, an increase from baseline was noted at 1 hour, returning to baseline levels at 4 hours and 24 hours. In women, an increase from baseline was noted at 4 hours, returning to baseline levels at 24 hours (Table 2). There was a significant correlation between the peak IL-6 blood level and the CK level at 24 hours only in women (Table 3).

No significant change was observed in IL-10, TNF-α, HSP60, HSP70, TBARS, and GSH (Table 2).

Discussion

The cytokine changes during exercise are proposed to play a role in metabolism, tissue remodeling, and inflammatory responses.[3] For this reason, many previous studies have investigated the kinetics of cytokine production with resistance exercise. However, these studies did not focus on the sex difference in cytokine response to resistance exercise.[1,3,10] Men and women display sex differences in physiological responses during exercise; these exercise responses may result in sex-specific immunomodulatory effects.[2–4,8] Thus, the purpose of this study was to evaluate the acute effects of a total body resistance exercise protocol on cytokines, HSP, and oxidative stress markers in recreationally resistance trained men and women. TNF-α, IL-10, HSP70, HSP60, TBARS, and GSH did not change following an acute total body resistance exercise protocol in women and men recreationally trained. However, the resistance exercise protocol was associated with modest increases in IL-6 and CK activity. Men and women had differences in the temporal pattern and magnitude of IL-6 responses. Women exhibited a delayed change in IL-6 levels and showed a correlation between peak serum IL-6 and CK response compared to men.

CK activity is used to indicate muscle damage following exercise. Values of 1000 IU/L may be used to separate participants with mild and severe muscle damage.[7] Our study showed that the resistance training protocol induced an increase of approximately 600 IU/L in the CK activity at 24 hours post-exercise in men and women. Resistance exercise typically causes mild muscle damage (<1000 IU/L).[7]

The physiological response to muscle damage is inflammation, which involves the production of cytokines.[7,10] In this context, IL-6 may act as an essential regulator of satellite-cell-mediated hypertrophic muscle growth,[6] and may also play a role in tissue remodeling (muscle), especially in response to muscle damage.[3,7] Exercise protocols that induce muscle damage (i.e., downhill running and eccentric actions of the leg or arm muscles) increase plasma IL-6 concentration for several hours after exercise.[7] By contrast, it has been suggested that the skeletal muscle *per se* (regardless of muscle damage) is the main source of IL-6 in the circulation in response to exercise and the peak plasma IL-6 concentration occurs at cessation of the exercise or shortly after, followed by a rapid decrease towards pre-exercise levels.[2,3,24] IL-6 values are markedly enhanced by exercise when glycogen levels are low.[2] IL-6 values observed in the present investigation show significant increases (~3-fold) from baseline at 1 hour in men, while in

Table 2
Cytokine, HSP and stress oxidative marker responses in men and women to an acute resistance exercise session.

		Pre	1 h	4 h	24 h	ANOVA p	Partial η^2	Power observed
IL-6 (pg/mL)	**Men**	1.5 ± 0.4	4.4 ± 0.6**	2.4 ± 0.3	1.1 ± 0.2	0.001	0.514	0.99
	Women	1.6 ± 0.3	2.1 ± 0.4	3.4 ± 0.5*	1.3 ± 0.1	<0.001	0.346	0.98
IL-10 (pg/mL)	**Men**	1.2 ± 0.1**	4.1 ± 1.8	2.3 ± 0.6**	0.7 ± 0.1**	0.105		
	Women	5.8 ± 2.1	3.6 ± 1.4	5.7 ± 1.4	5.6 ± 1.9	0.538		
TNF-α (pg/mL)	**Men**	2.8 ± 0.6	2.0 ± 0.4	2.5 ± 0.5	2.4 ± 0.6	0.055		
	Women	1.9 ± 0.3	2.1 ± 0.3	1.6 ± 0.2	1.2 ± 0.1	0.126		
HSP60 (ng/mL)	**Men**	2.7 ± 1.1	2.3 ± 1.1	2.3 ± 1.0	2.3 ± 0.9	0.417		
	Women	2.6 ± 1.1	3.1 ± 1.2	2.9 ± 1.1	2.4 ± 0.9	0.218		
HSP70 (ng/mL)	**Men**	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.434		
	Women	1.9 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.1 ± 0.1	0.139		
TBARS (mmol/L)	**Men**	63.8 ± 4.8	78.1 ± 7.4	86.2 ± 12.4	90.7 ± 16.6	0.065		
	Women	92.2 ± 4.2	94.3 ± 9.6	79.4 ± 2.8	85.0 ± 6.8	0.163		
GSH (mmol/L)	**Men**	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 0.4	1.8 ± 0.4	0.358		
	Women	1.7 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	0.303		

Date are presented as mean ± standard error.
* Significantly different from corresponding pre-exercise values ($p < 0.05$; Fisher's *post hoc* test).
** Significantly different between groups ($p < 0.05$; independent t test).
GSH = reduced glutathione; HSP = heat shock protein; IL = interleukin; Pre = pre-protocol; TBARS = thiobarbituric acid reactive substance; TNF = tumor necrosis factor.

women the IL-6 values increased (~2-fold) only at 4 hours. There was a positive correlation between the peak serum IL-6 concentration (4 hours) and CK activity (24 hours after exercise) only in the women (Table 3). Taken together, it seems likely that the late response of IL-6 in women was due to muscle damage but not in men. Men and women display sex differences (attributed to sex hormone) in regulating substrate utilization,[14] which translates into a proportionately lower carbohydrate and higher fat oxidations. All women in the present study were oral hormonal contraceptive users. The use of oral contraceptives may alter glucose metabolism and insulin sensitivity during exercise[25] and attenuate IL-6 response.[26] This would explain the lack of IL-6 response to resistance exercise in women at 1 hour after resistance exercise.

It has been consistently demonstrated that the serum concentration of IL-6 increases during muscular exercise.[2]

Table 3
Correlation between IL-6 (before, and 1 hour, 4 hours, and 24 hours after) and CK at 24 hours and ΔCK.

	Pre	1 h	4 h	24 h
Women CK−24 h	$r = 0.12$ $p = 0.972$	$r = 0.20$ $p = 0.631$	$r = 0.87$ $p = 0.005$	$r = 0.65$ $p = 0.082$
%Δ CK	$r = -0.07$ $p = 0.867$	$r = 0.01$ $p = 0.986$	$r = 0.86$ $p = 0.007$	$r = 0.68$ $p = 0.064$
Men CK−24 h	$r = 0.57$ $p = 0.140$	$r = 0.36$ $p = 0.378$	$r = 0.10$ $p = 0.809$	$r = 0.60$ $p = 0.115$
%Δ CK	$r = 0.09$ $p = 0.841$	$r = 0.59$ $p = 0.123$	$r = 0.05$ $p = 0.893$	$r = 0.10$ $p = 0.813$

CK = creatine kinase; IL = interleukin; Pre = pre-protocol.

Although the basal IL-6 concentration may increase up to 100-fold, less dramatic increases are more frequent, especially in resistance exercise.[1,2,27] Nieman et al[28] reported an increased IL-6 value immediately post-exercise and at 1 hour with values similar to ours in men. Other studies have reported IL-6 values ranging from 2.0 pg/mL to 7.5 pg/mL (1−4-fold) immediately after and 45−90 minutes after exercise in men.[27,29,30] In women, a study reported no change in serum IL-6 at 3 hours, 24 hours, or 2 days after the exercise session, but there was mRNA upregulation for IL-6 post-exercise.[12] However, few studies have been directed to this purpose in women, which makes it difficult to compare values and peak time point.[1]

Anti-inflammatory cytokines such as IL-10 inhibit inflammatory responses[4] and no difference in baseline IL-10 level between both men and women has been suggested.[31] However, the women showed a higher circulating IL-10 level at baseline than did the men. Our data suggest differences in immune responses between men and women in nonexercising conditions.[20] In men, a decreased T lymphocyte count as compared to that in women may play a role in the differences in immune responses between the sexes.[31,32] Furthermore, differences in *in vitro* stimulation of lymphocytes with phytohemagglutinin and sex differences in the T helper 2 cytokine profile (IL-4 and IL-10) have been previously demonstrated under nonexercising conditions. Women possess a greater T helper 2 cytokine release (IL-4 and IL-10) than men do.[33] It is known that estrogen and progesterone induce IL-10 production.[34,35] In this context, oral contraceptives may have influenced baseline IL-10 levels in women.

Infusion of recombinant human IL-6 drastically affects the levels of IL-10 1 hour after infusion.[36] Therefore, we expected IL6-induced increases in IL-10 in men. In the present investigation there were no significant increases in IL-10 in men and women (Table 2). Steensberg et al[36] showed that an increase of 36-fold in IL-6 affects the levels of IL-10 by fourfold 1 hour after infusion. Therefore, it is possible that exercise-induced IL-6 (2−3-fold) was not enough to stimulate IL-10 in men and women. Nevertheless, our data are different from others. Nieman et al[28] reported increased IL-10 immediately and 1 hour post-exercise in men. In women, few studies have been directed to this purpose, which makes it difficult to compare values.[1]

It has been suggested that exercise modifies redox status. The consequent oxidative modification induces HSP70 release by the liver. HSP70 has been shown to bind to Toll-like receptors 2 and 4, inducing an acute inflammatory state.[8] TNF-α, HSP70, HSP60, TBARS (lipoperoxidation marker), and GSH (non-protein small thiols) observed in the present investigation showed no significant change in men and women. Therefore, it is possible that the resistance exercise protocol used was not sufficient to modify redox status and induce an acute inflammatory state in men and women. Furthermore, it has been reported that the plasma concentrations of TNF-α does not change following exercise[1,7] by inhibitory effect of exercise-released IL-6.[5,37−39]

Given the small sample size, it is possible that the present studies were underpowered ($n = 16$; small sample size) to detect small differences in cytokines, HSPs, or lipoperoxidation marker. Another limitation of the present study was oral contraceptive use. Therefore, future work should examine whether oral contraceptives influence IL-6 release from contracting muscle during exercise.

In conclusion, TNF-α, IL-10, HSP70, HSP60, TBARS, and GSH do not change following an acute total body resistance exercise protocol in women and men recreationally trained in resistance exercise. However, acute total body resistance exercise results in increases in circulating IL-6 and CK. Men and women recreationally trained have significant differences in the temporal pattern and magnitude of IL-6 responses to a resistance exercise protocol. Women showed a delayed change in IL-6 levels compared to men. Thus, this study provides novel insight into the relationships between sex and temporal changes in IL-6 during a total body resistance exercise protocol.

Conflicts of interest

The authors declare no conflicts of interest.

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