Original Article

Differential inflammatory response of men and women subjected to an acute resistance exercise

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ABSTRACT

Background: The purpose of this study was to investigate the inflammatory response, lipid peroxidation and muscle damage in men and women athletes subjected to an acute resistance exercise.

Methods: Twenty college athletes (10 men and 10 women) performed a half-squat exercise consisting of five incremental intensities: 20%, 40%, 60%, 80% and 100% of the one-repetition maximum. Blood samples were collected at rest, 15 min and 24 h post-test. The concentration of lipid peroxidation markers and the activities of a skeletal muscle damage marker and a cardiac muscle damage marker were determined in serum. Serum α-actin was measured as a marker of sarcomere damage. Serum levels of interleukin-6, interleukin-10, and tumor necrosis factor alpha were determined to assess the inflammatory response.

Results: Interleukin-6 levels were higher at 24 h post-test than at rest and 15 min post-test in men ($p < 0.05$). Moreover, men showed significantly higher hydroperoxide levels in response to resistance exercise at 24 h post-test than at 15 min post-test ($p < 0.05$). No differences were found in muscle damage parameters regardless of sex or the time point of the test. No differences regarding the studied variables were found when comparing among different time points in women.

Conclusion: Our results show a larger influence of half-squat exercises on the release of IL6 and on lipid peroxidation in men than in women at equivalent workloads.

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At a glance of commentary

Scientific background on the subject

Males and females have the same muscle structure, but show differences in the hormonal regulation that influence the immune response. Indeed, estrogens have a protective effect on the inflammatory response. Nevertheless, there is no clear consensus on how sex could modulate the inflammatory response after an acute resistance training.

What this study adds to the field

In this study, greater pro-inflammatory response and lipid peroxidation took place in men after an acute resistance exercise compared with women. Therefore, women could have a better protective defense against muscle damage induced by resistance exercise than men.

Currently strength exercises are used not only to improve athletic performance, but also to achieve a better quality of life [1]. Strength training is critical in old people to counteract the loss of muscle mass due to aging [2]. In addition, strength training is applied in the field of competition with two key objectives, to improve athletic performance and to prevent future injuries [3]. Furthermore, resistance exercise could induce a particular immune response depending on several factors, such as the nature of the performed exercise, the exercise intensity, the recruited muscle mass volume (sets x repetitions) or the duration of rest periods (recovery between sets) [4]. Therefore, management of strength training is crucial for both athletic performance and health.

Physical exercise is known to induce an immune response in healthy subjects. Physical exercise produces a physiological stress acting as a major stimulus for efficient repair of muscles that require a well-coordinated and controlled inflammatory response [5]. Consequently, it induces a precise proliferation of immunological cells, such as natural killer cells, monocytes and neutrophils in the bloodstream [5]. In fact, an increase in the intensity and/or duration of the exercise tends to alter the cytokine release balance [4]. In fact, an excess of vigorous training can increase up to 6-fold the possibility of suffering some type of respiratory disease [6]. However, a moderate-intensity exercise reduces the possibility of suffering from any type of infection [6]. Therefore, it is vitally important to apply an adequate volume and intensity in resistance training programs to prevent the induction of a high immune response.

Men and women have the same muscle structure, which is able to generate strength. However, both sexes show differences in the metabolism of energy substrates in exercise and in fatigue, in addition, both sexes show differences in the immune response [7]. After resistance exercise, catecholamines and cortisol have been shown to respond differently in men compared to women [5]. Furthermore, it is known that estrogens have a protective effect in women on the integrity of cell membranes and on the inflammatory response after performing strength exercises [8]. Accordingly, women show lower concentrations of creatine kinase (CK) than men after eccentric leg press and leg extension exercises [9]. Nevertheless, inconsistent results on how sex could influence the inflammatory response after strength training has been found [10].

Strength training induces an improvement in the recruitment of muscle fibers during muscle contraction and a gain in muscle mass [11]. However, a breakdown of muscle fibers is necessary to achieve such improvement [12]. After an intense session of strength training, changes in the extracellular matrix, distortion of the contractile components of myofibrils and release of cellular proteins into the bloodstream can occur [13]. This damage can be generated by the mechanical action involved in strength training [14]. Immediately after the damage is produced, a local inflammatory response is generated [15]. Predominantly pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6), are released to initiate the breakdown of damaged muscle tissue [16]. However, anti-inflammatory cytokines, such as interleukin 10 (IL10), are released to prevent excessive secretion of pro-inflammatory interleukins, such as TNFα [17].

The data available on sex-based differences after an acute strength training are inconsistent. Therefore, we aimed to assess the inflammatory response, lipid peroxidation and muscle damage after a maximal strength test during half-squat exercises. This exercise, which is widely used to increase and evaluate muscular strength, involves a concentric phase and an eccentric phase. Thereof, the half-squat exercise can be used as a valid exercise for the management of strength training.

Methods

Participants

A total of 20 healthy university student volunteers (10 men and 10 women) were enrolled in this study. Table 1 shows the

Table 1 Characteristics of the participants (men and women).			
Parameter	Units	Men (n = 10)	Women (n = 10)
		Mean SEM	Mean SEM
Age	Years	22.76 ± 0.77	22.70 ± 0.42
Weight	kg	76.97 ± 1.11	60.01 ± 2.23*
Height	cm	180.00 ± 1.44	167.60 ± 2.15*
BMI	kg/m^2	23.79 ± 0.15	21.30 ± 0.13*
1 RM	kg	131.00 ± 1.24	73.45 ± 3.31*
Fat mass	kg	6.78 ± 0.48	11.35 ± 0.29*
Muscle mass	kg	38.86 ± 0.60	26.52 ± 0.92*
Leg - Muscle area	cm^2	195.01 ± 6.80	132.35 ± 6.67*
Thigh Perimeter	cm	53.30 ± 0.79	49.03 ± 0.79*
Leg Perimeter	cm	37.64 ± 0.48	35.56 ± 0.52*

p-values were obtained using Student's t test. Abbreviations: BMI: Body mass index; 1 RM: One repetition maximum. Results are expressed as mean $\pm$ standard error of the mean (SEM). *$p < 0.05$ men *vs*. women.

baseline features of these volunteers. All participants had recreational experience (1–3 years) in strength training, including half-squat exercise. Participants trained their lower body muscles 2–3 times per week by lifting loads of 60–90% of the one repetition maximum (1RM). We ensured that women participation in our study did not occur within one week either side of menstruation by using a self-report questionnaire. In addition, all women were not oral contraceptive users. Smoking, high intake of antioxidant-rich foods, ergogenic supplementation or drug use were considered exclusion criteria. All participants were fully informed of the purpose, nature, practical details and potential risks associated with the study before signing the informed consent. The study was approved by the Ethics Committee of the University of Granada (Spain) and it was in accordance with the Declaration of Helsinki.

Design

All the procedures were performed at an exercise physiology laboratory. Half-squat exercise was the chosen procedure because it is a common method used for the assessment of maximum strength in athletes [18]. All participants were assessed in the morning, in particular, two hours after breakfast, at a laboratory with controlled humidity and temperature according to the guidelines established by the National Strength and Conditioning Association [19]. Three days before the experiment, the participants received dietary recommendations by a nutritionist about the type of food they are allowed to eat. In addition, the dietary intake was recorded and subsequently assessed. The participants received a standard breakfast for athletes two hours before the experiment. Breakfast was prepared containing 57% carbohydrates, 18% proteins and 25% fats; thereby, breakfast met the general recommendations of international institutions [20].

All participants underwent an anthropometric assessment. Anthropometric data were obtained following the protocol developed by the International Society for the Advancement of Kinanthropometry (ISAK). Pre-test sessions were conducted 2 weeks prior to the experiment initiation. During the first week, participants became familiar with the experimental testing procedures. During the second week, participants took part in a control test to identify their 1RM [21]. Four days of testing over two consecutive weeks were required to evaluate the participants.

One repetition maximum strength testing sessions

All the participants underwent a progressive maximal test to determine their 1RM. Before the test, the participants performed a warm-up, which included 5 min on a cycle ergometer, dynamic stretching and 2 sets of 5 repetitions of half-squat with 20 kg and 30 kg each set. We used the 1RM test described by Fernandez-Gonzalo et al. for half-squat exercise [22]. The test was performed using a Smith machine (Life Fitness, Brunswick Corporation, USA). The load was increased by 10 kg when the participants succeeded, whereas the load was reduced by 5 kg when the participants failed the task. The test finished when the participants failed to perform two consecutive attempts. All the repetitions were interspersed by

a 3-minute rest. The 1RM was achieved between the third and sixth attempt.

Maximal incremental strength test

The participants were asked to refrain from exercise for at least 72 h prior to the experiment. The maximal incremental exercise test was performed between 08:30 and 15:00 h following the protocol described by Aragón-Vela et al. [23].

The test was performed in a random order. The participants performed a warm-up routine consisting of 5 min of jogging and leg stretching, as well as a familiarization set of 15 repetitions with a load at 40% of 1RM in the half-squat exercise [24]. The maximal incremental exercise test consisted of the performance of a half-squat exercise on a Smith Machine in which the barbell was attached at both ends with linear bearings on two vertical bars allowing only vertical movements. Shoulders were in contact with the bar, and the starting angle of the knee was 180°. Eccentric and concentric phases were performed consecutively. Knee flexion angle was maintained at 90°. To guide the displacement path, the position that the individual needed to take to initiate the half-squat was determined using a manual goniometer and marked using a rod on a tripod. The participant was then asked to do a half-squat in each repetition until touching the rod with the glutei. The accuracy error of the procedure was 1 cm [25]. Repetitions not covering the whole distance were not considered. Foot spacing on the half-squat was set at approximately the same width as, or slightly wider than, shoulder width while keeping toes pointing slightly outwards [26]. The torso was kept as straight as possible and a security belt was used by all participants [27].

Five incremental intensities derived from the individual's 1RM test were used: 20%, 40%, 60%, 80% and 100%. Five repetitions of 5 s each at 20%, 40% and 60% intensities were performed. Two repetitions were performed at 80% intensity without a time limit. Finally, only 1RM (100% intensity) was completed by each participant without any help for the last load. A 3-minute resting period was allowed after each of the first four intensities (20%, 40%, 60% and 80%), and a 5-minute resting period was allowed between 80% and 100% intensities.

Blood sampling

Blood samples were collected from the participants' antecubital vein at time 0, as well as 15 min post-test and 24 h post-test. Samples were centrifuged at 3000 rpm for 15 min at 4 °C to separate serum from cells.

Lipid peroxidation

Hydroperoxides (HPX) were measured in serum with the PeroxiDetect kit from Sigma (St. Louis, MO) following the manufacturer's instructions. Briefly, an hydrogen peroxide standard curve was plotted, and plasma samples (100 μL) were mixed with the working color reagent and incubated at room temperature for 30 min. Subsequently, absorbance changes were monitored spectrophotometrically at 560 nm.

Plasma thiobarbituric acid reactive substances (TBARS) concentration is an indirect method for estimating

malondialdehyde (MDA) and it was measured as described by Orrenius et al. [28]. Plasma samples were lysed and 100 μL of each one were mixed with 200 μL ice cold 10% trichloroacetic acid to precipitate proteins. After 15-minute incubation, 200 μL of thiobarbituric acid (TBA) were added to the supernatants and standards, and incubated for 10 min in a boiling water bath. Then, the samples were analyzed spectrophotometrically at 532 nm.

Serum creatine kinase MB isoform (CK-MB) and lactate dehydrogenase (LDH) activities

CK-MB analysis was performed using a commercial kit purchased from Spinreact, S.A. and following the manufacturer's instructions (Girona, Spain, ref: TK41255). Briefly, we mixed in a cuvette 1 mL of the working reagent and 40 μL of sample supernatant and incubated for 10 min at room temperature. Absorbance changes were monitored spectrophotometrically at 340 nm, reading the initial absorbance and the absorbance after 5 min.

LDH analysis was performed using a commercial kit purchased from Spinreact, S.A. (Girona, Spain, ref: 41223). A total of 3 mL of working reagent were mixed in a cuvette with 50 μL of sample and incubated for 1 min at constant temperature (37°). Absorbance changes were monitored spectrophotometrically each minute at 340 nm for 3 min in total.

Alpha-actin quantification

α-Actin concentration was determined as a marker of sarcomeric damage by Western blot as recently described by Aragón Vela J. et al. [4]. A serum sample (5 μL) from each subject was placed in sample buffer (62.76 mM Tris—HCl pH 6.8, 1% 2-mercaptoethanol, 1% SDS, 10% glycerol, and 0.01% bromophenol blue) at 1:3 ratio, boiled for 10 min, microfuged for 10 s and analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis in a Mini Protean transfer system (Bio-Rad, Hercules, California, USA) at 60 mA and room temperature for 90 min. Proteins were separated electrophoretically and transferred onto a 0.2 μm nitrocellulose membrane (Trans-Blot transfer pack, Bio-Rad) by applying a current of 25 V at room temperature for 7 min using a Trans-Blot Turbo Transfer system (Bio-Rad). The blots were treated with blocking solution (5% non-fat milk in TBST buffer: 20 mM Tris, 0.9% NaCl) for 1 h at room temperature. Then, the blots were incubated with the primary antibody (Actin (C-11): sc-1615, 1:1000 dilution, Santa Cruz Biotechnology, Dallas), overnight at 4 °C. Membranes were subjected to three 15-minute washes in 5% TBS-T, incubated with the secondary antibody (bovine anti-goat IgG-HRP: sc-2350, 1:5000 dilution, Santa Cruz Biotechnology, Dallas) for 1 h at room temperature, and again washed 3 times in TBS-T. Proteins were visualized by enhanced chemiluminescence (SuperSignal West Dura Chemiluminescent Substrate, 34075, Thermo Scientific).

Densitometric analysis was performed by scanning radiographic images of membranes. Image resolution was 100 points per inch. Adobe Photoshop 5.0 (Adobe Systems) was the image editing software used. Images were saved as TIFF files to allow them to be accessed by the quantification software (Quantity One 1-D, Bio-Rad). Alpha-actin was quantified in samples by comparison with a standard curve drawn with increasing concentrations of pure actin (Sigma—Aldrich Química, S. L., Madrid, Spain) and run in parallel.

Cytokine assay

Serum IL6 (# 88-7066-22), IL10 (# 88-7106-22), and TNFα (# 88-7346-22) levels were measured using commercially available ELISA kits according to the manufacturer's instructions (Invitrogen, Thermo Fisher Scientific, Canada). All samples and standards were analyzed in triplicate.

Briefly, the Corning™ Costar™ 9018 ELISA plates were coated with 100 μL/well of capture antibody in Coating Buffer. Then, the plates were sealed and incubated overnight at 4 °C. After aspiration and washing the wells, 200 μL of ELISA/ELISPOT Diluent (1X) were used for blocking non-specific binding in the wells, then plates were incubated at room temperature for 1 h. After washing, 100 μL/well of samples or standard were added to the appropriate wells and 100 μL of ELISA/ELISPOT Diluent (1X) were added and plates were incubated overnight at 4 °C. After aspiration and washing, 100 μL/well of diluted detection antibody were added to each well and incubated at room temperature for 1 h. After washing, plates were incubated with the enzyme and the substrate of the detection system. Finally, absorbance was measured at 450 nm in a microplate reader (Synergy NEO 2, BIOTEK, Izasa Scientific).

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The normality of distribution was assessed by using Shapiro—Wilk test. The homogeneity of variance was analyzed using Levene's test. Characteristics of the participants (age, weight, height, body mass index (BMI), 1 RM, fat mass and muscle mass, leg and thigh perimeter and total leg muscle areas) were compared by sex using a Student's t test. A two-way mixed analysis of variance was used; sex was the between-participant factor (men vs. women), whereas time was the within-participants factor (at rest, 15-min post-test and 24-h post-test). Multiple comparisons were performed through the Bonferroni post hoc test. Effect sizes were calculated using partial eta squared ($\eta^2 p$). The level of significance was set at $p < 0.05$. All statistical procedures were performed using SPSS/PC V. 22 (SPSS Inc., Chicago, IL, USA).

Results

Anthropometry

The features of the participants are described in Table 1. Men and women participants were of the same age. However, participants significantly differed in weight, height, BMI, 1RM, fat and muscle mass, leg muscle area, and perimeters of thigh and leg.

Lipid peroxidation

TBARS and HPX were used as biomarkers for oxidative stress [Fig. 1]. A significant interaction between time (F (1.16) = 4.530,
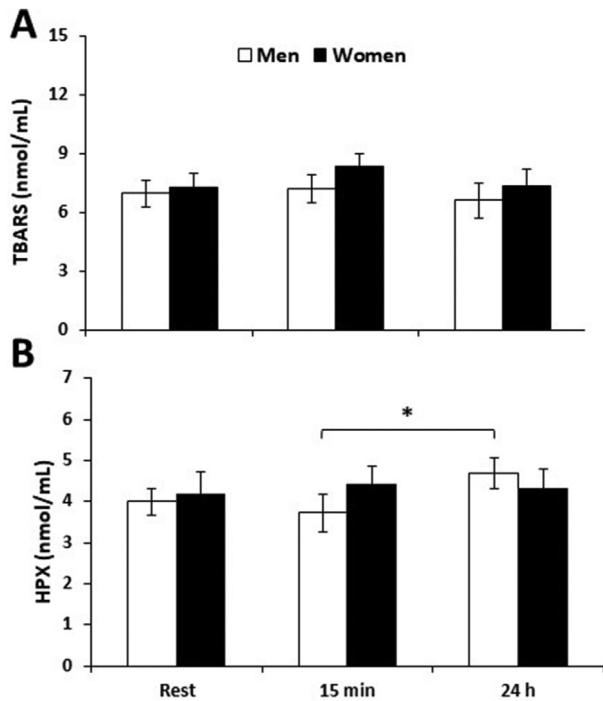
Fig. 1 Levels of lipid peroxidation markers in men (white bars) and women (black bars) in response to maximum incremental strength tests at different time points. (A) Thiobarbituric acid reactive substances (TBARs). (B) Hydroperoxides (HPX). *$p < 0.05$.

$p = 0.49$ $\eta^2 p = 0.220$) was observed for HPX in men. A significant increase 24 h post-test compared with 15 min post-test (0.971 ± 0.219; $p = 0.001$) was found in HPX values. However, no statistically significant differences between sexes were found in TBARS and HPX concentrations using the Bonferroni test [Fig. 1].

Cytokine assay

We found a statistically significant main effect of time on IL6 (F (1.16) = 7.342, p = 0.015 $\eta^2 p = 0.315$) in men. A significant increase in IL6 levels was found at 24 h (3.461 ± 1.283; $p = 0.048$) and 15 min post-test (3.20 ± 1.382; $p = 0.036$) compared with at rest in men [Fig. 2]. However, no significant differences were found in the levels of IL10 and TNFα, regardless the time point and sex.

Muscle damage

CK-MB and LDH activities along with α-actin concentration were used as markers of muscle damage. No significant differences between different time points nor between both sexes were found regarding these variables [Table 2].

Discussion

The purpose of this study was to analyze and compare sex differences in the inflammatory response, oxidative stress
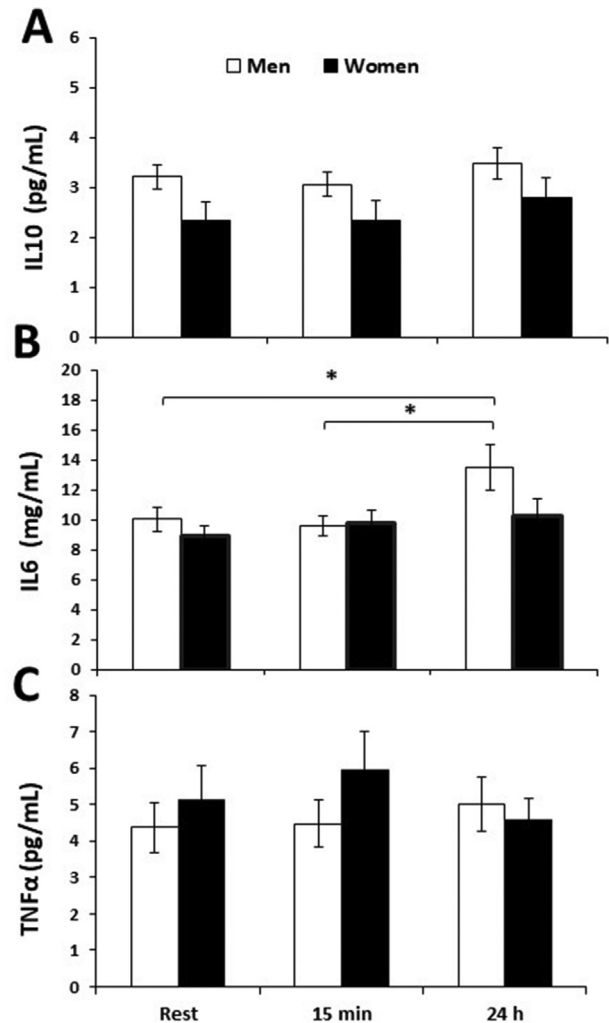

Fig. 2 Values of the inflammatory response in men (white bars) and women (black bars) in response to maximum incremental strength tests at different time points. (A) Interleukin 10 (IL10). (B) Interleukin 6 (IL6). (C) Tumor necrosis factor alpha (TNFα). *$p < 0.05$.

and muscle damage after an acute resistance exercise. Our main results showed a larger influence on the pro-inflammatory response (IL6) and on lipid peroxidation (HPX) after half-squat exercises in men than in women at equivalent workloads.

Acute sessions of resistance training have been shown to induce both pro-inflammatory and anti-inflammatory plasma cytokines in both sexes [29]. However, the physiological response induced by an acute strength training is still controversial. An increase in plasma levels of IL10, TNFα and IL6 is found immediately after a marathon [30], showing that there are both an acute anti-inflammatory effect (IL10) and an acute pro-inflammatory effect (IL6 and TNFα). Nevertheless, the response found after a 60-minute strength training session was only a pro-inflammatory response (IL6) [10]. Furthermore, according to our results, neither men nor women showed an increase in the levels of IL10. Several potential mechanisms could explain this result. First, it has been

Table 2 Levels of muscle damage markers.

Parameter	Unit	Men			Women		
		Rest	15 min	24 h	Rest	15 min	24 h
CK-MB	U/L	20.97 ± 2.44	19.07 ± 2.04	17.91 ± 3.43	15.99 ± 2.16	19.71 ± 2.38	16.80 ± 2.78
LDH	U/L	334.32 ± 19.32	306.27 ± 18.48	272.82 ± 39.62	281.85 ± 26.55	321.74 ± 30.25	286.98 ± 26.89
α-actin	%	100.00 ± 0.00	103.73 ± 3.81	102.42 ± 3.98	100.00 ± 0.00	109.15 ± 4.40	111.56 ± 8.74

Abbreviations: CK-MB: creatine kinase MB isoenzyme; LDH: lactate dehydrogenase. Results are expressed as mean ± standard error of the mean (SEM).

shown that 48 (i.e. 4 × 12) concentric repetitions at 100% of 1RM produce an increase in plasma levels of IL10 [31]. However, our protocol uses only 18 repetitions, therefore, a higher number of repetitions might be needed to release more IL10. Similarly, we found no changes in the levels of TNFα after an acute strength test. Therefore, the pro-inflammatory response could be insufficient to activate the anti-inflammatory response through the release of IL10. Secondly, the results described by Smith et al. showed an increase in IL10 at 48 h post exercise [31]. Therefore, an extension of our study for an additional 24 h would be interesting to corroborate if there are increases in the release of IL10 at 48 h post-test.

The main finding of the present study was that men showed significantly higher levels of IL6 after 24 h compared to the levels of IL6 at rest and at 15 min post-test. However, no significant differences were found in the levels of IL6 in women when comparing different time points. These results are consistent with those published by R. Benini et al. who showed that men expressed significantly higher levels of IL6 after a strength training session [32]. Overall, evidence about differences in the immune responses between sexes has been found. Even when the muscle damage is similar, women show a lower post-test inflammatory response than men [7]. These immunological differences could be due to the endocrine response characteristic of each sex. Indeed, estrogens could have a protective effect on the muscle during sports practice [33]. This effect could be explained by two reasons. First, because estrogens are liposoluble hormones, therefore, estrogens could be interspersed among the phospholipids in cell membranes, which would lead to greater stabilization of the membranes [34]. Secondly, this increase in estrogens in the membrane, being a lipophilic molecule, would alter the fluidity of the membrane, decreasing the exchange or release of substances through it [35]. Accordingly, the presence of estrogens in women could justify a lower inflammatory response.

In addition, another mechanism that can justify this pro-inflammatory response could be the oxidative stress generated by the strength exercise. One of the main physiological mechanisms associated with oxidative damage due to strength exercises is respiratory burst of neutrophils [36]. After acute resistance exercise, the damaged muscle cells release chemokines that recruit neutrophils, macrophages and natural killer cells to the damaged tissue [5]. These neutrophils aid macrophages in muscle repair and remove cellular debris through phagocytosis, inducing oxidative damage into muscle cell membranes [5,37], besides inducing a proinflammatory response by IL6 release. Furthermore, other studies have described that reactive oxygen species (ROS)

level is increased by IL6 release by immune cells. Thus, a cycle in which the release of IL6 and the production of ROS are connected in both directions could occur [38]. Ramel et al. (2004) showed an increase in plasma neutrophil count after a series of strength exercises with a duration of less than 20 min [39]. In this way, even during an activity with low oxygen consumption, there is the possibility of generating ROS. Therefore, this theory would justify that, in our study, men showed significantly higher levels of lipid peroxidation at 24 h post-test. Therefore, the post-test pro-inflammatory response (IL6) could be a result of neutrophil activation, responsible for the lipid peroxidation levels found in men. However, at equivalent loads, no significant differences in lipid peroxidation were found in women at different time points. In consistency with the mentioned above, the antioxidant capacity found in women could be due to the antioxidant characteristics of estrogens [40]. Basically, it is because estrogens have a hydroxyl group in their A ring, in the same position as tocopherol (vitamin E) [40]. Thereby, estrogens are able to donate hydrogen atoms from the phenolic hydroxyl group, neutralizing thus lipoperoxidation chain reactions [41]. Therefore, after strength exercises, women have a larger protective effect against lipid peroxidation than men.

Some limitations of the present study should be considered. A larger number of blood samples is recommended. Samples collected at additional time points (after few hours and also after 48 or 72 h) would be interesting to obtain more information about the muscle damage generated by our test. In contrast, the main strength of this study is that it is the first study evaluating and comparing the differences between men and women in the inflammatory response, oxidative stress and muscle damage after a strength exercise.

Conclusion

Overall, our data show that a larger muscle mass is involved and more significant net loads are achieved in men performing half-squat than in women, thereby, a larger increase in the pro-inflammatory profile is found in men than in women. Therefore, our results suggest that men should initially perform other workout routines that are less physiologically aggressive and that can minimize the pro-inflammatory impact of half squat exercise to prevent injuries. We suggest that women could have a protective factor, probably hormonal, inducing these results. However, further studies elucidating the molecular mechanisms involved in these sex-based differences after strength exercises are needed.

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