



Inflammatory response after session of resistance exercises in untrained volunteers

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ABSTRACT. The present study aimed to investigate the interaction between the blood cells, inflammatory markers, oxidative stress parameters and delayed onset muscle soreness (DOMS) after a session of resistance exercise (SRE). The sample consisted of sixteen untrained men (26.4 ± 5 years; 25.9 ± 3 kg m⁻²). The SRE was composed of 4 sets of 10 repetitions maximum (extensor bench, squat and leg press) for each exercise. Complete blood cell count, C-reactive protein (CRP), creatine kinase (CK), lipid peroxidation and antioxidant capacity against peroxy radicals were previously evaluated (baseline), and at 0, 30 and 120 min. after the SRE. DOMS was assessed 24 hours after the exercises. Immediately after the SRE, an increase of blood cell number was observed; returning to baseline after 30 min. However, after 120 min., neutrophils showed higher values than the baseline and 30 min. assessments. CK and CRP increased progressively throughout the experiment. LPO increased immediately and 120 min. after the SRE. Untrained volunteers presented an apparent biphasic inflammatory response after an acute SRE and the changes in oxidative stress, inflammatory markers and leukocytosis were best evidenced two hours after exercise.

Keywords: exercise tolerance, pain, inflammation, oxidative stress.

Resposta inflamatória após sessão de exercícios resistidos em voluntários destreinados

RESUMO. O presente estudo investigou a interação entre as células sanguíneas, marcadores inflamatórios, parâmetros de estresse oxidativo e dor muscular de início tardio (DMIT) após uma sessão de exercícios de resistência (SER). A amostra compreendeu dezesseis voluntários destreinados (26.4 ± 5 anos; 25.9 ± 3 kg m⁻²). A SER consistiu em 4 séries de 10 repetições máximas (extensão do joelho, agachamento e *leg press*) para cada exercício. Hemograma completo, proteína C-reativa (PCR), creatina quinase (CK), lipoperoxidação e capacidade antioxidante contra o radical peróxido foram avaliados previamente (basal), 0, 30 e 120 min. após a SER. A DMIT foi avaliada 24h após os exercícios. Imediatamente após a SER ocorreu um aumento no número de células sanguíneas, retornando aos valores basais após 30 min. Entretanto, após 120 min. os neutrófilos apresentaram valores mais elevados que as avaliações basais e 30 min. A CK e a PCR aumentaram progressivamente ao longo do experimento. A lipoperoxidação aumentou imediatamente e 120 min. após a SER. Voluntários destreinados apresentam uma aparente resposta inflamatória bifásica após SER e as alterações dos parâmetros de estresse oxidativo, marcadores inflamatórios e leucocitose são melhor evidenciadas duas horas após os exercícios.

Palavras-chave: tolerância aos exercícios, dor, inflamação, estresse oxidativo.

Introduction

The regular practice of physical exercises promotes anti-inflammatory effects, which reduces the mortality from all causes, especially by chronic inflammatory diseases, such as cardiovascular diseases and type 2 diabetes (PETERSEN; PEDERSEN, 2005). On the other hand, physical exercise induces acute inflammatory responses (CHEUNG et al., 2003; NAKAJIMA et al., 2010), which arise from mechanical, metabolic and/or

thermal changes (PEAKE; SUZUKI, 2004), leading to a transient oxidative stress that can cause damage to lipids, proteins and DNA, depending on its intensity and duration (FINAUD et al., 2006; POWERS; JACKSON, 2008).

Resistance exercises are widely used as a specific training for hypertrophy and/or strength (HUDSON et al., 2008), but can also fit any exercise program. However, the variables of the training program and/or exercise session, such as

load, volume, time interval between series and exercises, frequency of the sessions, exercise modes, repetition rate and the sequence of exercises, exert influence over the inflammatory response (SIMÃO et al., 2012). During these exercises, reactive oxygen species (ROS) are formed and, when its production exceeds the antioxidant enzymatic capacity (Superoxide dismutase, Catalase and Glutathione peroxidase) and non-enzymatic capacity (Vitamins A, C, E and uric acid) results in oxidation of cell constituents (FINAUD et al., 2006; RIETJENS et al., 2007).

Such oxidative stress status promotes the activation and mobilization of circulating white blood cells, the neutrophils (MENDHAM et al., 2011), which characterizes a transient leukocytosis observed during and just after the exercises with current activation of the vascular endothelium by ROS, and favors the migration of neutrophils to the injured muscle tissue (POWERS; JACKSON, 2008). This migration is stimulated by chemotactic factors, such as prostaglandins, tumor necrosis factor (TNF- α) and interleukins (IL)1 α , IL-1 β and IL-6 (PETERSEN; PEDERSEN, 2005). The infiltration of phagocytes results in increased ROS production and inflammation via Nuclear Factor kappa β (NF- $\kappa\beta$), which contributes to the secondary muscle damage (RIETJENS et al., 2007; VELLA et al., 2012).

These changes are clinically manifested by delayed onset muscle soreness (DOMS) (CHEUNG et al., 2003), since beginners in resistance exercises programs are more vulnerable to the effects of oxidative stress (RIETJENS et al., 2007) as they exhibit lower antioxidant enzymatic capacity (FINAUD et al., 2006), greater lipid peroxidation (RAMEL et al., 2004), higher levels of creatine kinase (CK) (FLANN et al., 2011) and present increased perception of pain after exercises (CHEUNG et al., 2003). The understanding of the mechanisms involved in this process allows the development of interventions targeted to alleviate the discomfort and minimize the abandonment of resistance exercises practice. The goal of the present study was to analyze the interaction between blood cells, inflammatory markers, oxidative stress parameters and delayed onset muscle soreness (DOMS) after a session of resistance exercise (SRE) in untrained adults.

Material and methods

Participants

The subjects were previously evaluated by an assistant physician who was invited to participate in

the study. Inclusion criteria were being male, clinically healthy, between 20 and 35 years old, with body mass index (BMI) lower than 30 kg m⁻², who had practiced bodybuilding, but currently not practicing physical and/or regular physical exercises (less than two times per week), not engaged in any diet programs and with no previous diagnosis of rheumatic, cardiovascular, metabolic, neurologic, oncologic, immunological and hematologic diseases. Smokers or individuals using any type of medication, diet, vitamin and/or ergogenic supplements were not included in the study. The participants were suggested to maintain the daily routine and eating habits. The consumption of fruit juice, alcoholic beverages and the practice of physical exercises were restricted to 72 hours before data collection. The study was approved by the Research Ethics Committee in the Health Area at the Universidade Federal do Rio Grande (FURG, protocol 2010-48) and all volunteers were informed about the study protocol and gave their informed written consent before starting their participation. Data were collected at the Body Building Room from the Sports Center of FURG.

Initially, seventeen volunteers were selected, received the information of the experimental protocol and were conducted familiarization with the exercises. The volunteers were fasted for 12 hour on data collection day, and those showing, in the laboratory evaluation, inflammatory response (CRP > 3 mg dL⁻¹), glycemia (> 100 mg dL⁻¹), leukocytosis (> 11.000 x 10³ mm⁻¹³), hyperthermia (> 38°C), changes in the systemic blood pressure (>140 90 mmHg⁻¹) and/or any pain or discomfort, were excluded from the study. Based on these criteria, one volunteer who presented CRP > 3 and leukocytosis was excluded. Thus, the sample consisted of sixteen volunteers which were subjected to muscular strength assessment and resistance exercise session with an interval of one week.

Muscle strength assessment

The test of 10 repetitions maximum (10RM) was adopted to conduct the training protocol with controlled overload (KRAEMER et al., 2002). The selected exercises were the extensor bench, the squat and the leg press. Previous to the strength testing, the volunteers were subjected to specific warm up for each exercise adopted (a series of 15 repetitions with 40% body weight to be used in the first attempt of each test, for each exercise respectively). They were performed in *Physicus*[®] equipment (Brazil). During the collection, a recovery time of five minutes between the exercises was adopted. The

workload values in the 10RM test were reached along three to five attempts, when the assessed volunteer presented a concentric fault outlook for the dynamic movement. There was addition of progressive increments of five kg, in each new attempt, with an interval of three to four minutes between each exercise series. Therefore, the last movement was validated as the maximum load.

Exercise session

The exercise session was based on previous studies (TEIXEIRA et al., 2012; TEIXEIRA et al., 2014). Before the exercise session, the volunteers were subjected to specific warm up for each exercise adopted (a series of 15 repetitions with 40% the load Max obtained in the 10RM test). The exercise sessions were comprised of four series of 10RM, with an interval of one minute between series and two minutes between exercises. The sequence of exercises (extensor bench, squat and leg press) was randomized by drawing sealed brown envelopes (CHRUSCH et al., 2001). Before the 10RM test and data collection, standard instructions were given concerning the experimental procedure and execution technique of the exercises. Verbal stimuli to the volunteer were given during the assessment and exercises. In the exercise of the extensor bench, the volunteers started the execution of the exercise with hips and knees flexed at 90 degrees and the movement was carried out to its full extent. For the implementation of the hack squat exercises, the volunteers were instructed to reach 90 degrees of knee flexion and subsequent trunk flexion. In the performance of the leg press exercise, the hip was positioned with 90 degrees and admitted to achieve around 120 degrees at the end of the movement. In this exercise, knees were positioned at the maximum extent and assuming that 90 degrees were achieved at the end of the movement. Standard instructions, verbal stimuli and execution technique of the exercises were conducted by the same researcher. After the exercise session, water intake was allowed ad libitum.

Data collection

Blood samples were taken before the exercises, immediately after the resistance exercises protocol (0 min.), 30 and 120 min. after the completion of the resistance exercise protocol. During the period between the basal collection and that immediately after the exercise, the volunteers were oriented to remain without hydration. Twenty-four hours after the exercise session, the delayed onset muscle soreness (DOMS) was evaluated by visual analog scale (NOSAKA; NEWTON, 2002).

Biochemical measurements

The levels of total cholesterol, triglycerides, high density lipoproteins (HDLc), glucose, uric acid and urea were assessed by using *LAB TEST* commercial kits (Lagoa Santa, Minas Gerais State, Brazil) and analyzed by the *LAB MAX 240*[®] device (Tokyo, Japan). Low density lipoproteins (LDLc) were calculated using Friedewald's formula. Fibrinogen was analyzed by the *START* device (Diagnóstica Stago, Asnieres, France) with *LAB TEST* commercial kits (Lagoa Santa, Minas Gerais State, Brazil). Concerning total plasma proteins, the Biuret colorimetric method was employed (Doles, Goiás State, Brazil). Lactate was evaluated through tapes (Roche Diagnostics GmbH, Mannheim, Germany) and was analyzed by the Accutrend PLUS device (Roche, Schweiz, Switzerland). Serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase were measured by the IFCC method (Hitachi 917[®] device, Roche Diagnostics, Florida, USA). Creatine kinase (CK) was measured using CK-NAC Liquiform reactive (Mindray, BS200, China). The ultrasensitive C-reactive protein (CRP) was determined by Nephelometry (Nephelometer Beckman Coulter, Immage model with laboratory reagents CCRP Immage, Fullerton, CA, USA). Red blood cell and differential white blood cell counts were made automatically (kits ABX, Horiba Diagnóstica, Curitiba, Paraná State, Brazil) and by microscopy. Regarding the measurement of hematologic variables, samples were counted twice and the values expressed by the average of the measurements. When a difference higher than 10% between the two results was observed, the procedure was repeated.

Oxidative stress

Lipid damage, superoxide dismutase (SOD) activity and antioxidant capacity against peroxy radicals ($RO_2\bullet$) were measured. Lipid peroxidation of plasma samples was determined by FOX method, according to Hermes-Lima et al. (HERMES-LIMA et al., 1995), based on the oxidation of Fe^{2+} by lipid hydroperoxides in acidic medium with Xylenol orange dye (SIGMA, São Paulo State, Brazil). The spectrophotometric determination was conducted in a microplate reader (550 nm). Cumene hydroperoxide (SIGMA, São Paulo State, Brazil) was adopted as standard. The results were expressed as η mol of cumene hydroperoxide mL^{-1} .

The SOD activity was spectrophotometrically determined (480 nm) by the method based on SOD inhibition of the autoxidation of adrenaline to adrenochrome in alkaline medium. SOD activity was expressed as U SOD mg^{-1} of protein at 30°C

and pH 8.0, where one unit is defined as the amount of enzyme that inhibits 50% of adrenaline autoxidation (MISRA; FRIDOVICH, 1972). Antioxidant capacity against peroxy radicals ($RO_2\bullet$) was measured following a protocol modified by Amado et al. (2009). Plasmatic protein concentration was determined by the Biuret method. Plasma samples were diluted with PBS to a final concentration of 3.3 mg mL^{-1} . The measurements were performed in triplicates. Ten microliters of diluted plasma were spiked into a fluorimetric plate. Subsequently, $127.5 \mu\text{L}$ of reaction buffer (30 mm HEPES, 200 mm KCl, 1 mm $MgCl_2$, pH 7.2) were added to the plates. The 2,20-azobis-2-methylpropionamide dihydrochloride (ABAP; 4 mm; Sigma-Aldrich, São Paulo State, Brazil) was employed as a $RO_2\bullet$ generator. The fluorophore used was 2,7-dichlorofluorescein-diacetate (2,7- H_2DCF -DA – Invitrogen, USA). Background and fluorescence intensity was measured in a microplate fluorometer (VICTOR 2, Perkin-Elmer, Turku, Finland). The wavelengths used for excitation and emission were 488 and 525, respectively. ABAP thermolysis generates $RO_2\bullet$, which reacts with H_2DCF -DA and generates fluorescence. The kinetics of fluorescence generation was measured in the presence and absence of ABAP during one hour. The results were calculated as the inverse fluorescence area min^{-1} in the presence and absence of ABAP relative area without ABAP (background) ($1/(\text{ROS area with ABAP} - \text{ROS area without ABAP})/\text{ROS area without background}$).

Statistical analysis

Data are expressed as mean and standard deviation (\pm SD). The Kolmogorov-Smirnov test was used to check data distribution. Student's paired t-test was used to compare the variables with two measurements. Variables with more than two measurements were analyzed by one-way ANOVA for repeated measurements or by Friedman's ANOVA followed by post hoc Least Significant Difference (LSD). Lactate variation magnitude (Δ) was calculated by the difference between 0 min. and basal measurements. Regarding the biochemical criteria, hematological variables, inflammatory markers and oxidative stress, the parameters were calculated by the difference between the first and the last measurement ($\Delta = 120 \text{ min.} - \text{basal}$). The correlations between variables were assessed by the Pearson (r) or Spearman (ρ) correlation coefficient, whenever appropriate. Statistical significance was accepted at $p \leq 0.05$.

Results

Mean values of absolute strength (10RM test) observed for leg extension, squat and leg press exercises were $53.8 \pm 13 \text{ kg}$, $73.8 \pm 19 \text{ kg}$ and $124.3 \pm 27 \text{ kg}$, respectively. Table 1 presents the characteristics of the sixteen participants who joined the study. The volunteers presented plasma levels of glucose, lipids, urea, uric acid, transaminases and systemic blood pressures as expected for the sample. No complications were reported by the volunteers during or immediately after the exercise session. The pain reported 24 hours after the exercise session reached 6.1 (± 2) points. The exercise session increased five times the plasma lactate levels (basal: 1.8 ± 0.5 vs. 0 min.: $9.3 \pm 0.9 \text{ mmol L}^{-1}$; $p < 0.001$).

Table 1. Clinical and fasting metabolic characteristics of the studied subjects ($n = 16$). Data are expressed as mean \pm SD.

Characteristic	Mean \pm SD
Age (y)	26.4 \pm 5
Body mass index (kg m^{-2})	25.9 \pm 3
Plasma glucose (mg dL^{-1})	88.0 \pm 9
Total cholesterol (mg dL^{-1})	148.9 \pm 29
Triglycerides (mg dL^{-1})	117.9 \pm 69
High-density lipoprotein cholesterol (mg dL^{-1})	34.1 \pm 6
Low-density lipoprotein cholesterol (mg dL^{-1})	92.3 \pm 27
Urea (mg dL^{-1})	29.3 \pm 7
Uric acid (mg dL^{-1})	5.0 \pm 1
Glutamic-oxaloacetic transaminase (U L^{-1})	31.1 \pm 11
Glutamic-pyruvic transaminase (U L^{-1})	32.4 \pm 11
Systolic Blood Pressure (mmHg)	117.5 \pm 5
Diastolic Blood Pressure (mmHg)	79.3 \pm 6

Data are mean \pm SD. DOMS: delayed onset muscle soreness.

Changes in blood cells due to exercise sessions were presented in Table 2. Hematocrit, erythrocytes and hemoglobin levels increased 5% ($p < 0.001$) immediately after the exercise (0 min.) and, for platelets, it was observed a 20% increase ($p < 0.001$). However, these variables returned to basal values in 30 and 120 min. after the exercises. Total leukocytes, segmented neutrophils and rods increased approximately 25% after the exercises, though, after 30 min. (except for segmented neutrophils) these values returned to normal plasma levels. Two hours after the end of the exercise session, these cells increased again compared to 30 min. after the exercises, when total leukocytes ($p < 0.001$), segmented neutrophils ($p = 0.015$) and rods ($p < 0.001$) were, respectively, 17, 35 and 70% higher than their normal levels. These values are similar to those found immediately after the end of the exercises, except for monocytes. The monocytes increased 60% ($p < 0.001$) after the exercises and returned to normal values in the other assessments. The number of lymphocytes increased immediately after the exercise session but, half an hour later, the level was lower than normal and returned to base levels after two hours ($p < 0.001$).

The plasma levels of oxidative stress and inflammatory markers parameters are presented in Figure 1. Lipid peroxidation (LPO) increased 35% immediately (0 min.) after the exercise session ($p < 0.001$) and returned to basal values after 30 min. On the other hand, after 120 min. from the end of the exercise session, LPO increased approximately 80% when compared to the basal levels (Figure 1A). Superoxide dismutase specific activity remained unchanged during analyzed period (Basal: 103.8 ± 42 vs. 0 min.: 101.3 ± 35 vs. 30 min.: 107.4 ± 43 vs. 120 min.: 98.8 ± 32 U SOD; $p = 0.627$). The antioxidant capacity against peroxy radicals (ACAP) did not change ($p = 0.539$) after the SRE (Figure 1B). The creatine kinase (CK) increased in all assessed times, in comparison to normal levels, and the values immediately after the exercises were similar to those found 30 min. after the end of the session. However, after 120 min., highest CK plasma levels were found and these values were 38% ($p < 0.001$) higher than the basal ones (Figure 1C). CRP increased after the exercise session ($p = 0.001$) and 2 hours later its plasma levels were the highest, representing approximately 70% from their basal levels (Figure 1D). Fibrinogen levels did not change during the study (Basal: 305.8 ± 68 vs. 0 min.: 297.3 ± 83 vs. 30 min.: 300.3 ± 85 vs. 120 min.: 298.1 ± 94 mg dL⁻¹; $p = 0.851$).

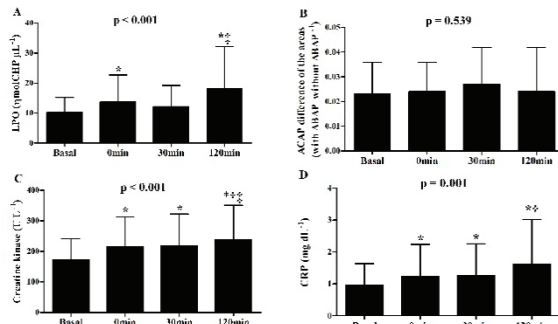


Figure 1. Changes in the oxidative stress parameters and inflammatory markers resulting from the resistance exercise session. CRP: C-reactive protein, CK: Creatine kinase, LPO: Lipid peroxidation; ACAP: Antioxidant Capacity Against Peroxyl radicals (RO₂*). Data are mean ± SD. * $p < 0.05$ vs. basal; † $p < 0.05$ vs. 0 min.; ‡ $p < 0.05$ vs. 30 min.

CK delta variations were directly correlated to segmented neutrophils (Figure 2A) and rods deltas (Figure 2B). Two hours after the SRE, the LPO presented a correlation with CRP (Figure 2C). The lactate evaluated immediately after the SRE was correlated with the CRP at that time (Figure 2D), as well as the variation deltas of these variables ($\rho = 0.570$; $p = 0.021$). The DOMS was directly correlated with the 2-hour CRP (Figure 2E) and with the CRP delta variation (Figure 2F). Also, the DOMS did not correlate with the blood cells, fibrinogen, CK and LPO as well as with the respective variation deltas of these variables.

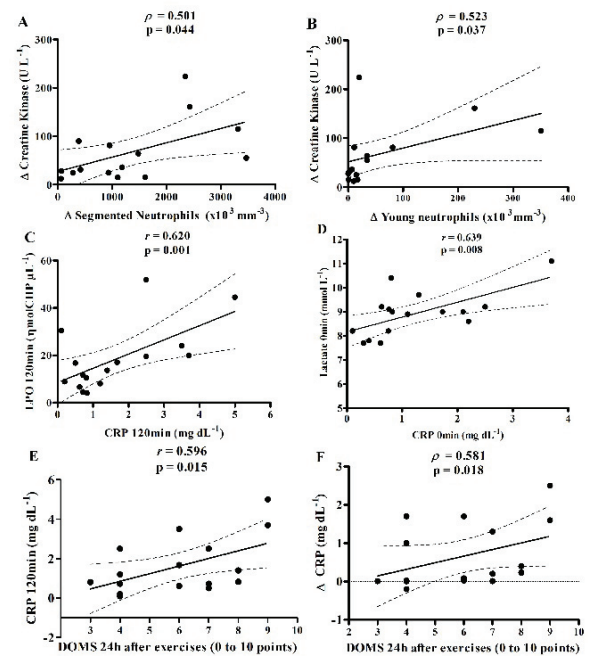


Figure 2. Correlation of hematological variables, oxidative stress parameters and inflammatory markers. LPO: lipid peroxidation; CRP: C-reactive protein, ACAP: antioxidant capacity against peroxy radicals (RO₂*). DOMS: delayed onset muscle soreness. r : Pearson correlation; ρ : Spearman correlation.

Table 2. Hematological changes resulting from the session of resistance exercise (n = 16). Data are expressed as mean ± SD.

Variables	Basal	0 min.	30 min.	120 min.	ANOVA p value
Hematocrit (mL % ⁻¹)	47.6 ± 2.8	49.9 ± 2.9 [†]	47.5 ± 2.3 [†]	47.7 ± 3.0 [†]	<0.001
Erythrocytes (x10 ³ mm ⁻³)	5.2 ± 0.3	5.5 ± 0.4 [*]	5.2 ± 0.4 [†]	5.2 ± 0.4 [†]	<0.001
Hemoglobin (g dL ⁻¹)	15.5 ± 0.8	16.2 ± 1.0 [*]	15.5 ± 1.0 [†]	15.4 ± 1.0 [†]	<0.001
Platelets (x10 ³ mm ⁻³)	245 ± 33	291 ± 57 [*]	245 ± 29 [†]	249 ± 31 [†]	<0.001
Leukocytes (x10 ³ mm ⁻³)	6875 ± 905	8569 ± 1392 [*]	6681 ± 835 [†]	8056 ± 1596 ^{††}	<0.001
Segmented neutrophils (x10 ³ mm ⁻³)	3739 ± 755	4776 ± 985 [*]	3982 ± 739 ^{††}	5049 ± 1622 ^{††}	<0.001
Young neutrophils (rods) (x10 ³ mm ⁻³)	72 ± 14	92 ± 32 [*]	67 ± 8 [†]	123 ± 102 ^{†*}	0.015
Monocytes (x10 ³ mm ⁻³)	289 ± 74	464 ± 193 [*]	278 ± 193 [†]	293 ± 110 [†]	<0.001
Lymphocytes (x10 ³ mm ⁻³)	2616 ± 500	3073 ± 754 [*]	2255 ± 497 ^{††}	2452 ± 456 ^{††}	<0.001
Eosinophils (x10 ³ mm ⁻³)	123 ± 73	113 ± 49	88 ± 37	127 ± 81	0.115

Data are mean ± SD. min.: min. * $p < 0.05$ vs basal; † $p < 0.05$ vs 0 min.; ‡ $p < 0.05$ vs 30 min.

Discussion

A session of resistance exercises caused lipid peroxidation, increase in inflammatory markers and leukocytosis. These exercises induced an increase of plasma leukocyte, erythrocyte and platelet concentrations and provoked an elevation of hematocrit immediately after the exercises. Besides, these changes (except the neutrophils) have disappeared within an hour after the end of the exercises. High intensity SRE causes disturbances in homeostasis by reorganizing the physiological responses of several tissues, including the hematopoietic, besides inducing changes in the concentration of circulating leukocytes immediately after its practice (AHMADIZAD; EL-SAYED, 2005; MENDHAM et al., 2011). However, the leukocytosis observed immediately after the SRE does not occur only due to mechanisms involved in the inflammatory response, but also to plasma volume reduction, and consequent viscosity increase, resulting in hemoconcentration (NAKAJIMA et al., 2010), which is reversed 30 min. after the end of exercises (AHMADIZAD; EL-SAYED, 2005). Our results are in accordance to previous studies (PEAKE et al., 2005; MENDHAM et al., 2011), which showed that leukocytosis occurs due to neutrophilia (segmented and rods) after the SRE. The activation of neutrophils during exercises may be related to many factors, such as muscular damage, hormonal factors and IL6 (PEAKE; SUZUKI, 2004). We emphasize that the period of 2 hours after SRE coincided with the peak concentration of inflammatory markers and the resulting neutrophilia (VELLA et al., 2012), once the hemoconcentration has already been reverted (AHMADIZAD; EL-SAYED, 2005).

Nikolaidis et al. (2008) suggested that the oxidative stress in blood and muscular tissues may manifest and persist for several days after the exercise. These changes were observed in our study, once the increases of lipid peroxidation were observed 2 hours after the SRE. The increase of ROS production during and after the SRE is due to different reasons: the activation of the mitochondrial electron transport chain, the lengthy process of ischemia/reperfusion, the increased synthesis of the enzymes xanthine and NADPH oxidase, the increase phospholipase A₂ activity, the catabolism of purines, tissue acidosis, catecholamines, the release of iron ions and the subsequent phagocytic activity, which characterizes the inflammation after exercises (PEAKE et al., 2005; FINAUD et al., 2006). Our data demonstrated lipid peroxidation immediately after the exercises, which is reverted half an hour

later but returned 2 hours after the end of the SRE. These changes are partially because of the hemoconcentration, once our data was not corrected according to the amount of lipids in the sample. The occurrence of lipid peroxidation just after the SRE was previously mentioned in the literature (RAMEL et al., 2004; RIETJENS et al., 2007). Hudson et al. (2008) used the same methodology of the present study and reported no immediate lipid peroxidation neither 1h after SRE. The differences from our results are due to a lower volume (only 4 sets *vs.* 12 sets), different body segments (upper members) and level of training of volunteers (HUDSON et al., 2008).

The antioxidant capacity against peroxy radical (ACAP) did not change after 2 hours from the SRE. This data agrees with other assessment methods of this variable (QUINDRY et al., 2011). Nevertheless, some studies have shown an increase in the antioxidant capacity after SRE (RAMEL et al., 2004; RIETJENS et al., 2007; HUDSON et al., 2008), which occurs due to the elevation of exogenous antioxidant plasma concentrations, especially of vitamins E (RAMEL et al., 2004; RIETJENS et al., 2007) and C (RIETJENS et al., 2007; HUDSON et al., 2008). These divergences are, somehow, due to the effects of hemoconcentration (AHMADIZAD; EL-SAYED, 2005; NAKAJIMA et al., 2010). In relation to the methodology applied in the present study, the plasma samples were diluted to protein concentrations (3.3 mg dL⁻¹) in saline solution (0.9%), in order to eliminate the effects of hemoconcentration. In addition, the studies differ regarding the methodologies applied to determine the antioxidant capacity. Considering these studies, despite the increase of antioxidant defense mobilization, during and after the exercises, such increase was not enough to prevent the oxidative damage.

Damage to the musculoskeletal tissue led to the inflammatory response, observed in our study by the increase in CK and CRP. CK is considered the best marker for muscular damage (NOSAKA; NEWTON, 2002) and an indirect marker of oxidative stress due to lipid peroxidation of membranes (FINAUD et al., 2006). This enzyme may present increases immediately after the SRE (NAKAJIMA et al., 2010), although these changes do not always occur (MENDHAM et al., 2011), and the highest levels are usually found between 24 and 72 hours after the SRE (MENDHAM et al., 2011; QUINDRY et al., 2011). Our results suggest that the changes in plasma levels of CK and circulating neutrophils (segmented and rods) are correlated, reinforcing that this muscle damage marker

reverberates in the systemic neutrophilia, although no correlations were found between CK and leukocyte infiltration in the muscular tissue, after the SRE (RAASTAD et al., 2003).

Increasing plasma levels of CRP, immediately after the SRE, are in accordance with the results found by Nakajima et al. (2010). Risøy et al. (2003) have already demonstrated these changes in CRP 3 hours after exhausting run, only in untrained volunteers, and these results were also observed after SRE (KRÜGER et al., 2011). However, the changes in CRP were not detected after 3 (MENDHAM et al., 2011) and 4 hours (MANSOURI et al., 2011) from the SRE, which are normally observed 24 hours after long lasting and/or high intensity exercises (PETERSEN; PEDERSEN, 2005) regardless the type of exercise (MENDHAM et al., 2011). Changes in CRP immediately after the exercises found here were also reported in other studies (RISØY et al., 2003; NAKAJIMA et al., 2010) with similar training level of the volunteers and intensity of the exercises.

CRP is an acute phase inflammatory marker, produced by the hepatocytes in response to IL-6 (JOHNSON et al., 2011). Usually, increases in CRP are associated with increased TNF- α and IL-1 β , which stimulate the production of IL-6 in the adipose tissue and in mononuclear blood cells (PETERSEN; PEDERSEN, 2005). Nakajima et al. (2010) suggested that the oxidation and degranulation of circulating neutrophils would be responsible for the increase in CRP immediately after the SRE. Another source of CRP release could be the musculoskeletal cell stress, during and after high intensity SRE, by increasing the expression of NF- κ B and, at the same time, decreasing its inhibitor (I κ B α), which regulates the release of several cytokines, including the IL-6, independently from the TNF- α (VELLA et al., 2012), which cause plasma elevation of the IL-6 during the exercises (RISØY et al., 2003; PETERSEN; PEDERSEN, 2005; MENDHAM et al., 2011) and would increase the CRP. Our results indicate that the lactate and the plasma lipid peroxidation are correlated to the CRP. This interaction reinforces that the intensity of the exercise is determinant to plasma alterations of CRP found in our study and in others (RISØY et al., 2003; NAKAJIMA et al., 2010).

These changes suggest an apparent biphasic inflammatory response. The increase in leukocytes, inflammatory markers and oxidative stress immediately after the SRE are due to hemoconcentration (RAMEL et al., 2004; AHMADIZAD, EL-SAYED, 2005; TEIXEIRA et al., 2014), and the interpretation of results should be adjusted for changes in plasma volume

(TEIXEIRA et al., 2014), which can vary from 2 to 10% (RAMEL et al., 2004; AHMADIZAD, EL-SAYED, 2005). The return to the baseline values 30 min. after the SRE is related the replenishment of the plasma volume (TEIXEIRA et al., 2014) especially after post-exercise hydration. The increase in the parameters two hours after exercise is more evident and derives from the systemic inflammatory response and we believe that these results best reflect musculoskeletal inflammation, since hemoconcentration has been reversed (AHMADIZAD; EL-SAYED, 2005; TEIXEIRA et al., 2014).

Changes in oxidative stress, inflammatory markers and blood cells, clinically reflected in the DOMS, observed here and in other studies, 24 hours after the SRE (NOSAKA; NEWTON, 2002; HOFFMAN et al., 2010). Muscle damage interferes with the production of strength, and promotes pain sensation (VELLA et al., 2012). These changes may persist for several days after the exercises (NIKOLAIDIS et al., 2008). CRP is an unspecific systemic inflammatory marker (JOHNSON et al., 2011) and the changes resulting from the SRE are correlated with the DOMS that, despite the influence of psychological aspects, is considered an indirect measure of muscle damage (CHEUNG et al., 2003). The lack of evaluation parameters for the enzymatic antioxidant capacity and the uric acid, which could occur simultaneously with the biochemical collection, as well as the evaluation of these variables with the DOMS, are limitations of the present study.

Conclusion

Resistance exercises in untrained volunteers caused a state of oxidative stress, increasing inflammatory markers and leukocytosis. These alterations were more evident two hours after the end of the exercises. Changes in these variables interact directly and indirectly with the DOMS in untrained volunteers. These changes suggest an apparent biphasic response, as neutrophils (mature and young) and lipid peroxidation increased immediately after exercise, and returned to baseline in 30 min. and increased again two hours after SRE. The understanding of these mechanisms that occur immediately after the session of resistance exercises favors the adoption of clinical and prophylactic measures to minimize muscle damage.

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