TITLE PAGE

ACUTE EFFECT OF ANAEROBIC AND INTERMITTENT EXERCISES ON BLOOD CELLS COUNT AND OXIDATIVE STRESS IN MIDDLE AGED WOMEN

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ABSTRACT

The aim of this study was to analyze the effect of an intermittent exercise session and an anaerobic exercise session on blood cell counts and oxidative stress in middle-aged women. A number of 26 middle aged women were divided in three groups: RE group (which was submitted to a 60 min of resistance exercises), Spinning Group (which was submitted to a 60 min of Spinning) and control group (which remained at rest). Sample collection was carried out at rest, immediately after, and 1 hour after exercise sessions. The blood count was performed by differential count of leukocytes. Oxidative stress was assessed by determining the lipid peroxidation and protein carbonylation as well as the measurement of enzymatic and non-enzymatic antioxidant body defenses. In both groups lymphocytes and monocytes had a decrease after 1 h recuperation when compared to after exercise. TBARS levels showed an increase in lipid peroxidation after exercise. Protein carbonyl had a significant increase after exercise. SOD and CAT activities had a decrease in both groups immediately after exercise. In conclusion, we found that an acute bout of intermittent and anaerobic exercises induces immune suppression and increases the production of reactive oxygen species, causing oxidative stress in middle aged women.

INTRODUCTION

For many women middle age is a difficult transitional period of life associated with decreasing well-being and body dissatisfaction as well as a number of other changes due to the aging process. [15] To attenuate this process by women, currently, there is a great demand for strenuous exercise, which promotes more evident physiological changes in a short term. Intense physical exercise is characterized by having values above 75% of maximal oxygen (VO_2) and heart rate (HR).[2]

In addition to the cardiovascular parameters, the number of blood cells also changes during an acute bout of exercise. High-intensity exercises are associated with a biphasic change of circulating leukocytes. In the immediate post-exercise, an increase of the total number of leukocytes is observed, which is mainly at the expense of lymphocytes, neutrophils and a lesser proportion of monocytes.[23] After a recovery period, a decrease in the lymphocyte number, which lasts for three to six hours, has been reported.[23,33,35] These alterations compromise the body's defense against infection and oncogenic agents as well as allergic processes and auto-immunity. [33,35]

Thus, intensity, volume, and frequency of exercise play a key role in determining the immune responses to an effort and may increase or reduce the immune function.[37] Regular exercise and/or physical training of moderate intensity improve the defense systems, while intense training causes immune suppression with increased susceptibility to infections.[18]

One explanation for the immune suppression in response to intense physical exercise load may be an increased use of functions of the body with exaggerated production of reactive oxygen species (ROS) and increased oxidative stress in tissues.[3]

In the last time, it is recognized that free radicals, besides being one of the factors that causes damage to physical exercise, also influence the immune system and essential metabolic functions.[41] Oxidative stress occurs when the action of free radicals exceeds the activity of antioxidants.[17] Indirect assessment of oxidative stress involves the measurement of the more stable molecular products formed via the reaction of ROS with certain biomolecules. Some of common molecular products include concentrations of oxidation target products, including lipid peroxidation end products, like malondialdehyde and thiobarbituric acid reactive substances (TBARS) and oxidized proteins (protein carbonyls). Additionally, oxidative stress can be measured by observing alterations in the body's antioxidant defense system. This is typically done by measuring the redox changes in the glutathione. Moreover, the activity of certain

antioxidant enzymes (superoxide dismutase and catalase) can be assessed as indicators of the oxidative stress imposed on the tissue. One of the main consequences of oxidative stress is lipid peroxidation and a possible damage to proteins and DNA (deoxyribonucleic acid) altering thus cell function. [17]

Moreover, in situations which exercise periodization is bad, the ROS can induce to a condition known as overreaching, which is a disturbance of the metabolic and recuperative systems.[49] The decreased activity of the antioxidant defense system only becomes worrisome if after a period of recovery the body does not restore the redox balance. Overreaching can be easily reversed in a few days or weeks. This condition of decline in performance happens before a much more serious and harmful situation to the body, the overtraining, which in addition to specific signs of metabolic fatigue, induces severe neuroendocrine disorders.[3,49]

Currently, it is clear that both acute aerobic and anaerobic exercise has the potential to result in increased free radical production, which may or may not result in acute oxidative stress.[10] However, only a few single-study reports have so far compared the effects of anaerobic exercise and aerobic exercise on oxidative stress[7,43] and no studies were found about intermittent exercise as well as comparing intermittent and anaerobic exercises protocols.

It is fairly well accepted that ROS production and subsequent tissue damage resulting from aerobic exercise is mainly due to an increased flux in electron transport leading to an increased leakage of superoxide radicals, while the generation of ROS during and following anaerobic exercise may be mediated through a variety of other pathways.[21] These include xanthine and NADPH oxidase production, iscbemia reperfusion, prostanoid metabolism, phagocytic respiratory bursts, disruption of iron containing proteins, and excessive calcium accumulation, often resulting from the performance of muscle-damaging isotonic or eccentric biased muscle actions, which commonly produce muscle injury.[21]

Besides, it is important to note that with the aging process, especially in women after menopause, there is a natural increase of ROS, suggesting a link between oxidative stress and diseases.[40] Thus, with the increased fragility of the immune system and increased susceptibility to infections, health professionals are required a good understanding of the processes occurring in a woman's body during this period of life as well as in situations where physical abilities are required, such as in the case of an

exercise session. This way, health professionals play a key role in helping to avoid overtraining.

As previously mentioned, an acute bout of strenuous exercise produces cardiovascular changes and increased production of ROS, causing oxidative stress and changes in the immune system activity. Therefore, the study of biochemical variables involved during a session of intense exercise in middle-aged women is extremely important. Thus, the aim of this study was to analyze the effect of an intermittent exercise session and an anaerobic exercise session on blood cell counts and oxidative stress in middle-aged women.

Materials and methods

Experimental Group

Twenty-six healthy women aged between 45 and 55 years were selected for this study. They presented normal blood pressure and were free from diabetes mellitus, obesity, alcoholism, cigarette smoking, and chronic diseases. Moreover, they had not been submitted to any pharmacological therapy during the month before the study. The women were divided into three groups:

- Resistance Exercise (RE) Group: Eleven women engaged in resistance exercises for at least two years in fitness centers in Santa Maria/RS.
- Spinning Group: nine women engaged in spinning activity for at least 1 year in fitness centers in Santa Maria / RS.
- Control Group: six sedentary women.

The women were informed of the purpose, nature, and possible side effects involved in the study, and gave their written informed consent. The protocol was approved by the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria, protocol number 0288.0.243.000-09, Brazil. Moreover, in this research were considered the ethical issues of the Declaration of Helsinki (1986) from the World Medical Association.

Procedures

All participants underwent a physical assessment before the day of collection, which included body composition assessment, measurement of heart rate (HR) and blood pressure (BP) at rest, measurement of body mass index (BMI) and waist-hip ratio

(WHR) and one-repetition maximal (1RM) test to estimate a load of approximately 75% of maximum strength for the RE group.

Before the experiment, all subjects had a medical certificate proving they were able to perform physical tests. Experiments began at the same time of the day to avoid circadian and circaseptan effects. Data collection was performed in three days, one day for each group.

On the first day, procedures were carried out with the RE group. The session required a completion of a standard circuit of resistance exercises in which three sets of 10 repetitions at 75 to 80% of 1-RM force were completed at each of the ten stations: leg press, knee station, hamstring curl, buttocks, hip adduction, hip abduction, bench press, high pull, biceps curl, and triceps curl. In this protocol, exercises sets were usually performed up to muscular failure.

On the second day, procedures were carried out with the Spinning group. Subjects performed 50 min of intermittent aerobic exercise on a special cycle-ergometry and were completed from 70 to 85% of the individual's personal HR.

In both experiments, subjects remained seated for a 1-hour recovery period following the exercises. The heart rate was monitored continuously throughout each of the sessions, using a POLAR® frequency counter, whose results are expressed in beats per minute (bpm). The rating of perceived exertion (RPE) was monitored continuously using a scale of Borg[11] and BP was monitored before, immediately after, and 1 hour after the exercise sessions by the auscultatory technique. Sample collection was carried out at rest, immediately after, and 1 hour after the exercise sessions.

On the third day, procedures were carried out with the control group. The participants were not submitted to the effects of the exercise. They remained in rest for 2 h and the same procedures were performed only once.

Anthropometric Measurements

Body mass was measured with an anthropometric scale ARJA® brand with a resolution of 100 g and body height with a stadiometer SANNY®. BMI was calculated as weight divided by the square of the height. WHR were calculated as waist divided by hip. For BMI and WHR was used the standard cut points recommended by the World Health Organization.[48]

The estimation of body fat (% BF) was performed using the method of skin folds, which are measured with a scientific adipometer CESCORF® with a resolution of 1mm. The % BF was determined by Jackson *et al.*[20] equation.

Sample Collection

The blood was collected in tubes with and without anticoagulant system. The serum was obtained for centrifugation at 1800 x g for 10 min and the precipitate was discarded. Then, the serum was used to determine biochemistry parameters: glucose levels, lipid profile, substances reactive to thiobarbituric acid (TBARS) and protein carbonyl. For non-protein thiols, the blood was collected using EDTA as anticoagulant. The sample was then centrifuged (1800 x g for 10 min) and the plasma was used to determine plasmatic thiols. CAT and SOD activities were determined using whole blood collected in citrated tubes and diluted in a 1:10 in saline solution. The samples were kept at -80ºC until analysis. For hematological determinations the total blood with EDTA was used and the counting was made immediately afterwards.

Evaluation of Glucose levels and Lipid Profile

Glucose levels, serum total cholesterol, and triglyceride concentrations were measured through standard enzymatic methods using Ortho-Clinical Diagnostics® reagents on a fully automated analyzer (Vitros 950® dry chemistry system; Johnson & Johnson, Rochester, NY, USA). High-density lipoprotein (HDL) cholesterol was measured in the supernatant plasma after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride.[4]

Hematological determinations

Quantitative determinations of white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), lymphocyte percentage (LYM), monocyte percentage (MON), and percentage of granulocytes (GRA) obtained by venipuncture were performed using a Coulter-STKS analyzer (Miami, USA).

Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring TBARS in serum samples according to a modified method of Jentzsch *et al.*[22] Briefly, 0.2 ml of serum was added to the reaction mixture containing 1 ml of 1% ortho-phosphoric acid, 0.25 ml alkaline

solution of thiobarbituric acid -TBA (final volume 2.0 ml) followed by 45 min heating at 95ºC. After cooling, samples and standards of malondialdehyde were read at 532 nm against the blank of the standard curve. The results were expressed as nanomole MDA per milliliter of plasma.

Carbonylation of serum protein

The carbonylation of serum proteins was determined by a modified Levine *et al.*[26] method. Firstly, from 1 ml of serum, the proteins were precipitated using 0.5 ml of 10% trichloracetic acid (TCA) and centrifuged at 1800 x g for 5 min discarding the supernatant. One half milliliter of 10 mmol/l 2.4-dinitrophenylhydrazine (DNPH) in 2 mol/l HCl was added to this precipitate protein and incubated at room temperature for 30 min. During incubation, the samples were mixed vigorously every 15 min. After incubation, 0.5 ml of 10% TCA was added to the protein precipitate and centrifuged at 1800 x g for 5 min. After discarding the supernatant, the precipitate was washed twice with 1 ml of ethanol/ethylacetate (1:1), centrifuging out the supernatant in order to remove the free DNPH. The precipitate was dissolved in 1.5 ml of protein dissolving solution (2 g SDS and 50 mg EDTA in 100 ml 80 mmol/l phosphate buffer, ph 8.0) and incubated at 37ºC for 10 min. The color intensity of the supernatant was measured using a spectrophotometer at 370 nm against 2 mol/l HCl. Carbonyl content was calculated by using the molar extinction coefficient ($21x10^3$ 1/mol cm) and results were expressed as nanomoles per milligram protein.

Determination of non protein thiols

Non-protein thiols were assayed in plasma by the method of Ellman.[16] Aliquots (0.1 ml) of plasma were added to a phosphate buffer 0.3 mol/l (0.85 ml), pH 7.4 and the reaction was read at 412 nm after the addition of 10 mM 5-5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (0.05 ml). Results were expressed as μmol/ml of plasma.

Catalase (CAT) and Superoxide dismutase (SOD) activities

The determination of CAT activity was carried out in accordance with a modified method of Nelson & Kiesow.[32] This assay involves the change in absorbance at 240 nm due to CAT dependent decomposition of hydrogen peroxide. An aliquot (0.02 ml) of blood was homogenized in potassium phosphate buffer, pH 7.0. The spectrophotometric determination was initiated by the addition of 0.07 ml in an aqueous

solution of hydrogen peroxide 0.3 mol/l. The change in absorbance at 240 nm was measured for 2 min. CAT activity was calculated using the molar extinction coefficient (0.0436 cm^2/μmole) and the results were expressed as picomoles per milligram protein.

SOD activity measurement is based on the inhibition of the radical superoxide reaction with adrenalin as described by Mc Cord & Fridovich.[28] In this method, SOD present in the sample competes with the detection system for radical superoxide. A unit of SOD is defined as the amount of enzyme that inhibits by 50 % the speed of oxidation of adrenalin. The oxidation of adrenalin leads to the formation of the colored product, adrenochrome, which is detected by spectrophotometer. SOD activity is determined by measuring the speed of adrenochrome formation, observed at 480 nm, in a reaction medium containing glicine-NaOH (50 mM, pH 10) and adrenalin (1 mM).

Statistical Analysis

Data were analyzed statistically using SAS program, version 9.1. A descriptive analysis of data was carried out. For comparison between the same group Wilcoxon test was used and for comparison between groups mann-whitney test was used. The significance level was 0.05.

RESULTS

Table 1 shows the characteristics of the variables age, body mass, stature, BMI, WHR, fat percentage, fat mass, and lean mass for the three groups included in this study. According to these data, we could observe that the groups are homogeneous and all participants have good levels of fat percentage, BMI classified as normal,[48] and WHR indicating low risk for the development of heart problems.[48]

Table 2 shows cardiovascular and biochemical variables at rest, after exercise, and after recuperation of the RE, Spinning, and control groups. We observed that there is a significant difference between the RE group and Spinning Group when compared to the control group regarding HR variable. At rest and after recuperation, the RE and Spinning groups presented HR lower than the control group. The RE and the Spinning groups after exercise had a major increase of HR compared with the moments at rest in the same groups and the control group.

No significant difference between groups in blood pressure as a result of the exercises was observed (data not shown). The rating of perceived exertion (RPE) did not present difference between groups when participants were at rest. However, a

significant raise (p<0.0001) was observed after exercise, when participants felt an extreme physical exertion verified from mean 17.81 of Borg scale[11] for the RE group and 16.66 for the Spinning group.

The glucose level of the control group was significantly higher (p<0.044) than the RE and Spinning groups at rest. However, no difference in this variable was observed for the groups regarding exercise.

Both exercises tested did not exert significant changes on cholesterol, cholesterol HDL and triglycerides. On the other hand, we observed that RE group and Spinning group had significant better values when compared to control group showing lower values from cholesterol (p<0.0203 and p<0.046, respectively), triglycerides (p<0.0207 and p<0.041, respectively), and higher values from cholesterol HDL (p<0.0203 and P < 0.044, respectively) at rest.

With respect to blood cell count, no difference was found from white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT), monocyte percentage (MON), and percentage of granulocytes (GRA) between the groups at rest (some data not shown). Lymphocytes percentage (figure 1) had a major decrease after recuperation in the RE group and Spinning group when compared to after exercise (p<0.047 and p<0.030, respectively). Monocytes (table 2) also had a decrease after recuperation when compared to after exercise (p< 0.048 and p<0.049, respectively). Differently from the RE group, the Spinning group presented granulocytes significantly increased after recuperation when compared to after exercise (p<0.047).

Figures 2, 3, 4, 5 and 6 shows oxidative stress biomarkers of the RE and Spinning groups at rest, after exercise and after recuperation. In both exercised groups, the TBARS levels (figure 2) showed an increase in lipid peroxidation after exercise when compared to rest (p<0.0008 for RE group and p<0.003 for Spinning group) and a decrease after recuperation (p<0.006 for RE group and p<0.008 for Spinning group). When compared to control group, no differences were found in this variable.

The protein oxidation, determined by protein carbonyl content in serum (figure 3), of the RE and Spinning groups had an increase after exercise (p<0.0007 and p<0.002, respectively) that remained after recuperation (p<0.001 and p<0.003) when compared to rest. No differences were found when compared to control group.

The non-protein thiols (figure 4), in the RE and Spinning groups, had a higher increase immediately after exercise (p<0.002 and p<0.01, respectively) and only in the

RE group a decrease was observed after one hour of recuperation ($p<0.01$), approaching baseline levels. There was no difference between groups at rest.

The SOD activity (figure 5) was significantly decreased in the RE group after exercise and remained after recuperation when compared to rest ($p<0.001$ and $p<0.007$, respectively). The same occurred with the CAT activity (figure 6). There was a decrease in the CAT activity after exercise ($p<0.001$), which remained after recuperation ($p<0.001$) when compared to the rest. It is interesting to note that women who practice resistance exercise had higher levels in the CAT activity when compared to the control group ($p<0.0009$), formed by sedentary women.

SOD and CAT activities had a significant decrease in the Spinning group after exercise ($p<0.02$ and $p<0.003$, respectively) and remained after recuperation ($p<0.04$ and $p<0.004$, respectively) when compared to rest. Moreover, the activity of these two enzymes are higher in women who practice spinning (at rest) when compared with sedentary ones ($p< 0.004$ to SOD and $p<0.001$ to CAT).

Statistical comparison between the RE and the Spinning groups (data not shown) shows significant differences only in HR, where the Spinning group had higher values after exercise ($p<0.02$) and lower values after recuperation ($p<0.04$) when compared to the RE group. In most of the variables, differences were not observed.

DISCUSSION

Considerable uncertainty still remains in the population studied (middle-aged women) about the relationship between exercise and the oxidative stress that may occur owing to increased oxygen circulation during exercises. Few studies, until now, have compared aerobic exercise and anaerobic exercise in terms of the oxidative stress that they may induce.[1,7,43] Until now, there are no studies that propose to assess changes in blood cells compared with the production of ROS comparing intermittent and anaerobic exercises. Thus, this is the first study witch aimed to analyze the effects of intermittent exercise and anaerobic exercise on cardiovascular and biochemical parameters in middle-aged womenin. Furthermore, in this study we have assembled several variables that may change with exercise and decided to test this population for being in the aging process.

Heart rate has been measured in many sports and physical activities to examine exercise intensity and can be used to examine fitness exercise effectiveness.[2] According to our results, even the women having a good level of physical training, we could

observe that both types of exercise were very intense including the resistance exercises, which do not possess the characteristic of high elevation in HR. However, intermittent exercise showed more elevated HR than the resistance type. Such result was expected, since this is a characteristic of the exercises which have aerobic components.[2,40]

The high-intensity physical activities proposed here can be observed through the perception of effort, which in both exercises showed women to feel extreme effort when performing activities with the same high intensity, according to the Borg scale.[11] Although the exercises were of high intensity, no significant changes in the blood pressure were detected. This is probably due to the time it takes to put the cuff on the person up to finish of the measurement. According to Baum *et al.*[6] the relaxation interval of 3 seconds is sufficient to permit the prompt recovery of BP.

Glucose was analyzed on postprandial state, explaining high levels of this variable at rest in all groups. Indeed, levels from the control group were significantly higher than the other groups, perhaps due to the sedentary lifestyle of the subjects. Mondazzi & Arcelli[30] say that after an acute bout of physical activity, it is expected a decline on blood glucose levels. However, our results showed no significant decrease probably because they are trained women and their metabolism is already adapted.

Cholesterol and triglycerides in the trained woman were lower than in the sedentary ones. On the other hand, we observed that cholesterol HDL was higher in trained woman than in sedentary ones. The better values in woman engaged in physical training may be explained by the increased demand of the working muscle for fatty acids as an energy-yielding substrate as well as the replenishment of fatty acid containing stores for the regeneration of damaged muscle fibers.[19,38] High HDL levels can also be explained through the biochemistry of exercises because there is an increased activity of lipoprotein lipase, which augments the degradation of triglycerides from very low-density lipoproteins and causes the lipoprotein particles to shrink.[19,38] These adaptations, which the body is subjected, explain the good lipid profile of trained women. However, there were no acute changes in lipid profile in both types of exercises, which highlighted that these changes may be long term.

Differently from several other authors[31,46] we did not observe a significant increase in total leukocyte, neutrophil, monocyte, and lymphocyte counts immediately following the two types of exercise. Indeed, the cited studies were conducted with young populations, and perhaps the fact that we have not found this increase is because the women of our study are in aging process and their cell defenses may not be

responding as they should to a so aggressive stimulus. According to Olsen,[36] low levels of estrogen, observed in middle aged women, have shown to attenuate the immune response and predispose the organism to microbial invasion and infection. This gap in the defense system coming from middle age may explain the fact that there is not an increase in the amount of blood cells immediately after exercise.

However, our results corroborate with Nieman & Nehlsen-cannarella[33] who found intense exercise induced strong lymphocytopenia 30 min after exercise. In our study, the last sample collection was carried out one hour after exercise, and we found a major decrease on lymphocyte and monocyte percentage in both exercises. This result shows that intense physical exercise could induce an immune suppression, which has already been well documented by other authors[18,33,37] as well, however, not in the population of our study. Our results also are in accordance to Nieman *et a*l.[34] with respect to different exercises. Nieman *et al*.[34] found that leg squat exercise induced strong leukocytosis, lymphocytosis and lymphocytopenia, similar in magnitude to the changes reported after endurance exercise. Although protocols differences, we can observe that intense exercises show same responses in terms of immune system. These responses are highly dependent on the ability of leukocytes to migrate from the blood into peripheral tissues at sites of inflammation,[17] in this case, provoked by exercises.

Moreover, this immune suppression seems to be linked to oxidative stress induced by exercise because there is an increased use of the functions of the organism with exaggerated production of ROS and increase of oxidative stress in the tissues.[3,41]

The most common method utilized to indicate exercise induced oxidative damage has been the assessment of lipid peroxidation with TBARS.[10] Our results showed an increase in the lipid peroxidation through TBARS levels in both exercises. Similar findings have reported an increase in TBARS following intense exercise in young humans, with values typically returning to baseline within one hour post exercise[44,45] which was also observed in our study.

Proteins are major targets for ROS because of their high overall abundance in biological systems. Furthermore, it has been estimated that proteins can scavenge the majority of ROS generated.[14] Oxidative damage to proteins can occur directly by the interaction of the protein with ROS or indirectly by the interaction of the protein with a secondary product (resulting from the interaction of the radical with lipid or sugar molecule.[14] The formation and accumulation of protein carbonyls has been one of the most commonly used methods for assessing overall protein oxidation in relation to

exercise.[10] Increased protein oxidation, evident by the accumulation of protein carbonyl, has been reported by several authors.[9,29] It has been shown to increase in a duration dependent fashion[9] as well as remains elevated for several hours post aerobic exercise.[29] In our study, the protein oxidation also had an increase after exercise that remained after one hour recuperation, being consistent with the reported studies. No differences were found between exercises protocols, suggesting similar effects in protein oxidation.

In addition to lipid peroxidation and protein oxidation, the measurement of redox changes in glutathione (the major non-enzymatic endogenous antioxidant), measured by non-protein thiols, has also been routinely performed as a representation of exercise inducing oxidative stress.[10] Typically, a decrease in non-protein thiols has been reported following a variety of exercise protocols like an indicative of oxidative stress.[25,29,44] However, in our study we observed an increase in non-protein thiols in both groups studied after exercises. The higher non-protein thiol levels in our study could be a result from a depressed enzymatic activity of glutathione peroxidase due to the rise in the oxidative stress.[12]

Free radical-scavenging enzymes such as SOD and CAT are the first line of cellular defense against oxidative injury, decomposing $O_2^{\bullet -}$ and H_2O_2 before interacting to form the more reactive hydroxyl radical ($^{\bullet}OH$).[41] Our study showed high leves of these enzymes in trained woman when compared with the sedentary ones at rest. It can be explained by participants training level, which may have provided to women trained, an improvement of endogenous enzymatic antioxidant defenses, as seen through the high levels of these two enzymes.[10]

As a consequence of the exercise which the participants underwent, our study showed a decrease in these antioxidant body capacities in both exercises that remained after one hour recuperation. A decrease in the SOD and CAT activities immediately after exercise corroborate to Watson *et al.*[47] and Steinberg *et al.*[44,45] who found similar results. However, these studies showed an increase above basal conditions of these enzymes during the recovery period, which differs from our study. According to Bloomer & Fisher-Wellman,[10] in response to conditions of strenuous physical work, the body's antioxidant capacity may be temporarily decreased as its components are used to quench the harmful radicals produced, and this decreased time depends on the intensity of the exercise. In our study, the high intensity of both exercises can explain this decrease in enzymes activities. Furthermore we can observe the synergism between the activity of these two enzymes, well described in literature.[10]

Our findings are in accordance with Cuevas *et al.*[13] and Bloomer *et al*.[7], who support the contention that more ROS are generated in response to high-intensity exercise, although less oxygen is consumed during anaerobic exercise. Shi *et al*.[43] found similar results, but they suggest that similar workloads of anaerobic exercise and aerobic exercise induce ROS differently: aerobic exercise seems to initially generate more ROS, whereas anaerobic exercise may induce prolonged ROS generation. These findings are not equal to ours, but we have to take into account the differences between protocols exercises and population.

Bloomer *et al*.,[7] in according with us, did not found differences between protocols exercises, but they found only an indicative of oxidative stress, witch was not significant, justified by trained subjects. In our study, we found greater evidences of oxidative stress even women being trained. In a study conducted by Larrea *et al*.,[27] the female sex hormone estrogen was shown to exhibit antioxidant properties in vitro. This study was conducted with women in middle age, witch sex hormone functions are in the decline process. Thus, it is reasonable to assume that high ROS generation after workouts in this study are also relates to the aging process.

Indeed, though the term anaerobic means "without oxygen", resistance training does result in increased oxygen consumption both during and following acute exercise. Nevertheless, the magnitude of this increase in VO_2 is far less than what is observed following acute intermittent or aerobic exercise.[7,8] Despite the comparatively low increase in VO_2, it has been shown that acute anaerobic exercise serves as a sufficient stimulus to elicit an increase in ROS formation[5] and oxygen consumption per se may not be the major cause of exercise-induced oxidative damage.[43] It was corroborated by our study.

Furthermore, unlike intermittent and aerobic exercise, where increased mitochondrial respiration is thought to be the primary target of increased ROS, it has been suggested that the increased radical production and subsequent oxidative stress observed during and following resistance exercise may be meditated to a large degree by the activities of certain radical generating enzymes (xanthine and NADPH oxidase), prostanoid metabolism, phagocytic respiratory burst, disruption of iron containing proteins, as well as altered calcium homeostasis.[8] Brief periods of ischemia followed by reperfusion, resulting from intense muscular contraction as well as mechanical stress and/or muscle damage, are thought to be the mechanisms underlying the increase in ROS triggering the activity of radical generating enzymes and initiating the migration of

inflammatory cells to the affected area.[21] This way, this is the possible explanation on the formation of ROS through anaerobic activity and it could be the link between oxidative stress and immune suppression verified in our study.

Thus, our findings indicate that similarly to aerobic exercises, although the mechanisms are not fully understood, anaerobic and intermittent exercises, when performed at high intensity, clearly possess the ability to result in acute oxidative stress and immune suppression. These metabolic changes, mainly in immunity, may become clinically relevant after repeated exercise bouts with insufficient recovery.

Therefore in order to prevent metabolic disorders such as overreaching and/or overtraining, recuperative intervals between exercise sessions should be carefully observed for the body to recover its antioxidant systems and not suffer greater consequences arising from the exacerbated practice of physical exercises.

REFERENCES

1. Alessio HM, Hagerman AE, Fulkerson BK, Ambrose J, Rice RE, Wiley RL. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med Sci Sports Exerc* 2000; **32:**1576-81.

2. American College of Sports Medicine (ACSM). The recommended quality and quantity of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in adults. *Med Sci Sports Exerc*1998; **30:** 975–991.

3. Angeli A, MInetto M, Dovio A, Paccotti P. The overtraining syndrome in athletes: a stress-related disorder. *J Endocrinol Invest*. 2004; **27**: 603-12.

4. Bachorik PS, Albers JJ. Precipitation methods for quantification of lipoproteins. *Methods Enzymol* 1986; **129**: 78–100.

5. Bailey DM, Lawrenson L, McEneny J, Young IS, James PE, Jackson SK, Henry RR, Mathieu-Costello O, McCord JM, Richardson RS. Electron paramagnetic spectroscopic evidence of exercise induced free radical accumulation in human skeletal muscle. *Free Radic Res* 2007; **41**: 182-190.

6. Baum K, Rüther T, Essfeld, D. Reduction of blood pressure response during strength training through intermittent muscle relaxations. *Int J Sports Med*. 2003; **24:** 441-5.

7. Bloomer RJ, Goldfarb AH, Wideman L, McKenzie MJ, Consitt LA. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J Strength Cond Res* 2005; **19**: 276–285.

8. Bloomer RJ. Energy cost of moderate-duration resistance and aerobic exercise. *J Strength Cond Res* 2005; **19:** 878-882.

9. Bloomer RJ, Davis PG, Consitt LA, Wideman L. Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *Int J Sports Med* 2007; **28:** 21-25.

10. Bloomer RJ, Fisher-Wellman k. Acute exercise and oxidative stress: a 30 year history. *Dynamic Medicine* 2009; **8**: 1-25.

11. Borg, G. Borg´s perceived exertion and pain scales. Champaign: *Human Kinetics*, 1998.

12. Campolo J, De Maria R, Caruso R. Blood gluthatione as independent marker of lipid peroxidation in heart failure. *Int J Cardiol* 2007; **117**: 45-50.

13. Cuevas MJ, Almar M, Garcia-Glez JC, Garcia-Lopez D, De Paz JA, Alvear-Ordenes I. Changes in oxidative stress markers and NF-kappaB activation induced by sprint exercise. *Free Radic Res* 2005; **39**: 431–439.

14. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; **52**: 601-623.

15. Debus G, Gugger B, Gruchalla B, Hahlweb-Widmoser B, Teschner B. Peri- and postmenopausal changes--body awareness, sexuality, and self- image of the middle-aged and older woman. *Zentralbl Gynakol* 2003; **125**: 202-8.

16. Ellman GL. Tissue sulphydryl groups, *Arch. Biochim. Biophys* **82,**1959.

17. Finaud J, Lac G, Filaire E. Oxidative stress. Relationship with exercise and training. *Sports Med* 2006; **36**: 327-58.

18. Friman G, Ilbäck NG. Acute infection: Metabolic responses, effects on performance, interaction with exercise, and myocarditis. *Int J Sports Med* 1998; **19**: 172-82.

19. Herzberg GR. Aerobic exercise, lipoproteins, and cardiovascular disease: benefits and possible risks. *Can J Appl Physiol* 2004; **29**: 800–807.

20. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. *Med Sci Sports Exerc* 1980; **12**: 175-81.

21. Jackson MJ. Exercise and oxygen radical production by muscle. In Handbook of oxidants and antioxidants in exercise Edited by: Sen CK, Packer L, Hanninen O. Amsterdam: *Elsevier Science* 2000; **12:** 57-68.

22. Jentzsch AM, Bachmann H, Furst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. *Free Radic Biol Med* 1996; **20**: 251–256.

23. Kendall A, Hoffman-goetz L, Houston M. Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects. *J Appl Physiol* 1990; **69**: 251-60.

24. Knight, J.A. Free Radicals, Antioxidants, Aging, and Disease. Wasbington, DC: American Association for Clinical Chemistry, 1999.

25. Laaksonem DE, Atalay M, Niskanem L, Uusitupa M, Hanninem O, Sen CK. Blood glutathione homeostasis as a determinant of resting and exercise-induced oxidative stress in young men. *Redox Rep* 1999; **4**: 53-59.

26. Levini RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990; **186**: 464–478.

27. Larrea MB, Garrido MJ, Lacort M. Estradiol-induced effects on glutathione metabolism in rat hepatocytes. *J Biochem* 1993; **113:** 563-567.

28. Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969; **244:** 6049-6055.

29. Michailidis Y, Jamurtas AZ, Nikolaidis MG, Fatouros IG, Koutedakis Y, Papassotiriou I, Kouretas D. Sampling time is crucial for measurement of aerobic exercise-induced oxidative stress. *Med Sci Sports Exerc* 2007; **39**: 1107-1113.

30. Mondazzi L; Arcelli E. Glycemic index in sport nutrition. *J Am Coll Nutr* 2009; ***18:*** 30-45.

31. Natale VM, Brenner IK, Moldoveanu AI, Vasiliou P, Shek P, Shephar RJ. Effects of three different types of exercise on blood leukocyte count during and following exercise. *Rev Paul Med* 2003; **121:** 9-14.

32. Nelson DP, Kiesow LA. Enthalpy of decomposition of hydrogen peroxide by catalase at 25 -C (with molar extinction coefficients of H_2O_2 solutions in the UV). *Anal Biochem* 1972; **49:** 474–478.

33. Nieman DC, Nehlsen-cannarella SL. The immune response to exercise. *Semin Hematol* 1994; **31**: 166-79.

34. Nieman DC, Henson DA, Sampson CS. The acute immune response to exhaustive resistance exercise. *Int J Sports Med* 1995; **16**: 322-8.

35. Nobrega AC. The subacute effects of exercise: concept, characteristics, and clinical implications. *Exerc Sport Sci Rev* 2005; **33:** 84-7.

36. Olsen NJ, Kovacs WJ. Gonadal steroids and immunity. *Endocr* 1996; **17**: 369-84.

37. Ortega E. Neuroendocrine mediators in the modulation of phagocytosis by exercise: physiological implications. *Exerc Immunol Rev* 2003; **9**: 70-93.

38. Paschalis V, Nikolaidis M G, Giakas G, Theodorou A A, Sakellariou G K, Fatouros I G, Koutedakis Y, Jamurtas A Z. Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. *Scand J Med Sci Sports* 2010; **20**: 103–111.

39. Pedersen BK, Hoffman-goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000; **80**: 1055-81.

40. Pialoux V, Brown AD, Leigh R, Friedenreich CM, Poulin MJ. Effect of cardiorespiratory fitness on vascular regulation and oxidative stress in postmenopausal women. *Hypertension* 2009; **54**: 1014-20.

41. Powers SK, Lennon S. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proc Nutr Soc* 1999; **58:** 1025-34.

42. Radak Z, Nakamujia A, Nakamotcj H, Akano K, Ohno H, Goto DS. A period of anaerobic exercise increases the accumulation of reactive carbonyl derivatives in the lungs of rats. *Pftugers Arc. Eur J Physiol* 1998; **435**: 439-41.

43. Shi M, Wang X, Yamanaka T, Ogita F, Nakatani K, Takeuchi T. Effects of Anaerobic Exercise and Aerobic Exercise on Biomarkers of Oxidative Stress. *Env Health Prev Medicine* 2007; **12**: 202–208.

44. Steinberg JG, Ba A, Bregeon F, Delliaux S, Jammes Y. Cytokine and oxidative responses to maximal cycling exercise in sedentary subjects. *Med Sci Sports Exerc* 2007; **39**: 964-968.

45. Steinberg JG, Delliaux S, Jammes Y. Reliability of different blood indices to explore the oxidative stress in response to maximal cycling and static exercises. *Clin Physiol Funct Imaging* 2006; **26**: 106-112.

46. Tvede N, Pedersen BK, Hansen FR. Effect of physical exercise on blood mononuclear cell subpopulations and *in vitro* proliferative responses. *Scand J Immunol* 1989; **29:** 383-9.

47. Watson TA, Callister R, Taylor RD, Sibbritt DW, MacDonald-Wicks LK, Garg ML. Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. *Med Sci Sports Exerc* 2005; **37**: 63-71.

48. World Health Organization (WHO). Obesity: Preventing and Managing the Global Epidemic. WHO; Geneva: Program of Nutrition, Family and Reproductive Health. Technical Report Series 1998; **894.**

49. Zoppi CC, Hohl R, Silva FOC, Lazarim FL, Antunes NJMF, Stancanelli M. Vitamin C and E supplementation effects in professional soccer players under regular training. *J Int Soc Sports Nutr* 2006; **3**: 37-44.