OXIDATIVE STATUS OF THE MYOCARDIUM IN RESPONSE TO DIFFERENT INTENSITIES OF PHYSICAL TRAINING

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Short Title: **MYOCARDIAL RESPONSES TO DIFFERENT TRAINING INTENSITIES**

Summary

The intensity of exercise determines the metabolic pathway and the energetic substrate that is spent. Our study sought to identify the effects of different intensities of swimming on myocardial oxidative status and the blood lipid profile. Eighty Wistar rats (male and female) submitted to different intensities of a swimming regimen (low, LS; moderate, MS; or high, HS) for 16 weeks. Samples of blood and myocardium from the left ventricle were collected to determine lipid profiles and oxidative status. Reactive oxygen species (ROS) and antioxidant capacity against peroxyl radicals (ACAP), lipid profiles and lipid peroxidation was analyzed. ROS levels and ACAP were higher in male rats than in female rats overall ($p<0.05$). However, ACAP in the myocardium was significantly elevated in LS female rats compared to the MS and HS female rats, which had a significantly lower ACAP compared to all other groups. LS and MS training in both sexes and HS training (in females) led to significant decreases in the heart's lipid peroxidation. Amelioration of the lipid profile and reduction in oxidative damage contributed to a physiological state that benefits cardiovascular function in exercised animals. The results show that low and moderate intensity exercise promotes beneficial adaptations.

Key Words: Cardiovascular; Exercise: Lipid Profile: Swimming Intensity; Oxidative Stress;

Introduction

Exercise training has been shown to improve cardiovascular capacity and is associated with lower resting and submaximal heart rates, increased ventricular weight, and an improved lipid profile. It also increases exercise capacity and skeletal muscle strength (Thompson 2003; Trejo-Gutierrez and Fletcher 2007; Sugawara *et al*. 2012; Perrino *et al*. 2011). Exercise training represents one of the most severe, yet physiological, stresses to the cardiovascular system and determines which metabolic substrates are used (Perrino *et al.* 2011).

The intensity of exercise influences the metabolic pathway and the substrate spent as a source of energy, affecting the levels of energy stores and the lipid profile (Hernandez-Torres *et al*. 2009). Despite numerous investigations that emphasize the benefits of exercise training on the blood lipid profile, some uncertainty remains regarding the intensity of exercise that is needed to induce this and whether an optimum exercise intensity can be determined (Duncan *et al*. 2005; Ensign *et al*. 2002). Physiological adaptations to exercise are dependent on program length and session duration, frequency, and intensity. These factors make the prescription of physical exercise, which is intended to simulate situations similar to those applied to humans in experimental models, more complex (Araujo *et al*. 2012). The transposition of experimental data and physical training protocols to experimental physiology frequently becomes inadequate, leading to misunderstood data before publishing (Booth *et al*. 2010). Studies concerning the determination of aerobic and anaerobic metabolism have been developed, with the intent of improving the prescription of physical training in rats (Pilis *et al*. 1993; Gobatto *et al*. 2001; Carvalho *et al*. 2005; Manchado *et al*. 2006; Contarteze *et al*. 2008). The control of exercise intensity can be based on the production, accumulation, and removal of lactate in the blood stream. The anaerobic threshold (AT) is defined as the workload at which the blood lactate starts to accumulate excessively during progressive

exercises. During physical exercise, the AT indicates the workload equivalent of the maximal lactate steady state (MLSS), which represents the highest concentrations of lactate in a determined load of work, which can be maintained over time (Manchado *et al*. 2006; Araujo *et al*. 2012; Ferreira *et al*. 2007; Gobatto *et al*. 2001). The MLSS intensity in rats can be identified during continuous exercise, considering an exercise intensity equivalent to a concentration of 4 mmol/L^{-1} of lactate in humans (Benek *et al*. 2011). The lactate concentrations at MLSS in rats have been studied (Carvalho *et al*. 2005; Gobatto *et al*. 2001; Manchado *et al*. 2006). During high-intensity physical activity, lactate blood levels higher than MLSS indicate that anaerobic metabolism predominates over aerobic (Manchado *et al*. 2006; Gobatto *et al*. 2001).

Some studies have shown that the cardiovascular adaptations of females differ from those of males; this includes the responses of morphological and physiological systems after exercise (Lindqvist *et al*. 2012; Aune *et al*. 2009; Dalen *et al*. 2010). These differences may stem from existing distinctions between males and females in types of muscle fuel stores, fuel utilization during exercise, and hormonal makeup (Steffensen *et al*. 2002). Following this line of logic, we hypothesized that exercise training at different intensities promotes distinct changes in the cardiovascular system and that these changes are dependent on gender.

Regular physical exercise has been shown to improve the lipid profile and increase mean life span (Kraus *et al*. 2002; Radak *et al*. 2008). An inevitable consequence of exercise is that the increased oxygen consumption induced by exercise creates favorable conditions for increased generation of reactive oxygen/nitrogen species (RONS), an increased cardiac oxidative metabolic rate, leading to alterations in the cellular redox status and activating pathways of cell death (Ascensão *et al*. 2007).

In addition to RONS generation, the products of lipid peroxidation may cause alterations in the cell membrane, as well as damage to proteins and DNA (Radak *et al*. 1999).

Together, lipid oxidation and inflammation of vessels play a fundamental role in the development of atherosclerosis (Wang *et al*. 2006). Exercise training in diverse experimental protocols is capable of augmenting peroxidation in humans and murine models (Groussard *et al*. 2003; Chirico *et al*. 2012). Oxidative conditions in the heart have been associated with cardiovascular diseases, mainly myocardial infarction (Shiomi *et al*. 2004). Scientific evidence indicates that physical exercise may improve antioxidant capacity and reduce damage to cardiac tissue caused by oxidative stress (Pinho *et al*. 2012; Powers *et al*. 2008). During and after exercise, various mechanisms are activated in different organs and systems to maintain or restore cell homeostasis and exercise intensity can affect the cardiovascular system. Few studies have focused on the chronic effects of aerobic and anaerobic exercise on oxidative status and lipid profile. The aim of this study was to investigate the effects of different intensities of exercise training (low, moderate, and high) on the oxidative status of the myocardium and lipid profile in male and female rats.

Methods

Experimental model

Sixty-day-old Wistar rats (*Rattus norvegicus*), forty males and forty females, were obtained from the Central Animal House of the Universidade Federal do Rio Grande (FURG), Brazil. Mean weight was 312±10 g (male rats) and 214±4 g (female rats). Animals were housed in plastic cages (five animals per cage) and maintained at 24°C±1°C in a 12-hour light-dark cycle, and they received commercial rodent food and water *ad libitum*. Rats were weighed once a week, and food consumption was monitored daily by weighing leftover food. Training sessions were performed during the dark cycle. The experiments were performed according to Guide for the Care and Use of Laboratory Animals and Use of Vertebrate Animals in Research and Training and official Brazilian governmental guidelines,

in compliance with the Federation of Brazilian Societies of Experimental Biology. The study was approved by the Ethics Committee on Animal Use, Universidade Federal do Rio Grande (CEUA-FURG).

Training protocol

Training protocol followed Guerreiro *et al.* (2015). Rats were randomly distributed into four groups (n = 10): sedentary control (SC); low-intensity swimming training (LS) and moderate-intensity swimming training (MS), both of which induced aerobic metabolism; and high-intensity swimming training (HS), which induced anaerobic metabolism. The untrained SC group was placed in the swimming apparatus for 1 minute one time per day, 5 days per week, to mimic the water stress associated with the experimental protocol and handling.

The continuous exercise swimming system of low and moderate intensity consisted of a rectangular tank (90 cm width × 100 cm length × 80 cm height) filled with water up to 50 cm. Ten PVC pipes of 20 cm width and 50 cm length were placed inside the tanks. The water temperature was kept close to 32ºC. Therefore, the animals were able to realize the exercise individually, each individual in a different PVC pipe. The high-intensity intermittent swimming system was realized using a pulley system coupled to a cylinder of 60 cm width with 10 PVC pipes. The cylinder was immersed in the swimming tank described above for 15 seconds, forcing the animals to swim vigorously. Then the cylinder was lifted by the pulley system, allowing the animals to recover for 15 seconds out of the water (Guerreiro *et al*. 2015).

Rats performed a training program one time per day, 5 days per week, during a 16-week period divided into two periods. An initial period of acclimation was 8 weeks long; during this period, the training load and duration of swimming progressively increased (20 minutes in the first week, up to 60 minutes at the eighth week; Figure 1). The second training period was characterized by the maintenance of higher loads of swimming training and duration of

exercise (60 minutes and a workload of 5% [MS] or 15% [HS]) for 8 more weeks. The load was adjusted weekly according to body weight (Table 1). The protocols were as follows:

The LS experimental group performed low-intensity aerobic swimming without overload, during 60 minutes, 5 times a week, for 8 weeks. This methodology intended to simulate an exercise in which the intensity is not controlled by overload. This protocol aimed to simulate a common physical activity that is performed by humans, such as walking or jogging at controlled frequency and duration. The MS experimental group engaged in moderate swimming for 60 minutes, 5 times a day, with progressive overload during acclimatization periods (increased from 1% to 5% of body weight). This overload was created by attaching pieces of lead to the back of the animal, and was equivalent to the MLSS. The HS experimental group performed high-intensity intermittent anaerobic swimming during 60 minutes, 5 times a week, for 16 weeks. Each 60-minute session consisted of alternate periods of vigorous swimming for 15 seconds, followed by a recovery resting period for 15 seconds, with overload increased from 8% to 15% of body weight equivalent at intensities above the MLSS (Figure 1) (Guerreiro *et al*. 2015).

Determination of blood lactate levels

The lactate levels were determined during the exercising procedure. The lactate was collected at three different time points. First, to determine the loads of swimming, a test of initial lactate was conducted. Second, another collection was made after 8 weeks of acclimation training. The third collection of lactate was made after 12 weeks of training. During the exercise, the rats were removed removed from the water at 10, 20, 30 and 40 min and dried, and blood samples (25 μL) were collected by cutting off the tip of the tail (Voltarelli *et al*. 2002). Lactate concentrations were determined in mmol/L with a lactate analyzer (Accutrend).

Tissue samples

At the end of the acclimation period of swimming training (eighth week), the rats were sedated and blood samples were collected from the retro-orbital sinus. Seventy-two hours after the end of the training period (16 weeks), the rats were euthanized by beheading. The period of 72 hours before euthanasia was chosen because it is known that some effects of physical exercise on the lipid profile and oxidative stress can last up to 48 hours (Hughes *et al*. 1990; Abernethy and Azarnoff 1990). Their blood was collected in tubes and centrifuged to obtain plasma for analyses of the blood lipid profiles. The hearts were rapidly removed by dissection, weighed, and stored at –80°C.

Lipid Profile

Levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined by colorimetry kits (Labtest). Plasma samples were mixed and incubated with the specific reagent for 10 minutes at 37°C, and the absorbance (510, 520, and 505 nm, respectively) was read by spectrophotometer. The difference between TC and HDL-C was recorded as the low-density lipoprotein cholesterol (LDL-C) concentration. All levels were expressed as mg/dL.

Generation of reactive oxygen species

Left ventricle tissue (approximately 100 mg) was homogenized in potassium chloride (KCl) 1.15% and phenylmethanesulfonyl fluoride (PMSF) (10 mmol/L) for 30 seconds, followed by 3000 ×g centrifugation for 10 minutes at 4°C (Jacob *et al*. 2006). Total protein content was determined by colorimetric assay (Biuret method, Labtest) in triplicate using a microplate reader (BioTek LX 800) at 550 nm. Each sample was diluted to 2 mg of protein/mL in homogenization buffer. Reactive oxygen species (ROS) generation was assayed by 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) indicator (Invitrogen). The non-fluorescent compound H_2DCF-DA is first de-acetylated and then oxidized by ROS

to the fluorescent compound dichlorofluorescein (DCF), which is detected at 488 and 525 nm for excitation and emission, respectively, into a fluorescence microplate reader (Victor 2, Perkin Elmer). Readings were performed every 5 minutes during a 30-minute period. Fluorescence data were adjusted to a second-order polynomial function and integrated to calculate the area that expressed ROS concentration (Amado *et al*. 2009).

Antioxidant capacity against peroxyl radicals

Total antioxidant capacity against peroxyl radicals (ACAP) is a new method described by Amado et al. (2009) that determines the tissue's capacity to confront peroxyl radicals. According to these authors, peroxyl radicals are produced by thermal decomposition of the generator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP) 4 mM (Aldrich) at 35°C. The assays were run at 37°C because this temperature is indicated for mammalian models. This methodology is conducted as an ROS assay, but in this case DCF production is increased by ABAP-peroxyl radical generation. ROS concentration in the presence of ABAP is also expressed by the area calculated from the second-order polynomial function, resulting from the adjustment of fluorescence units during the measurement time. Total antioxidant competence was expressed as relative area calculated by the rate of the difference between ROS area, with ABAP and without ABAP, divided by ROS area without ABAP, as a standardization factor of ROS background production.

Lipid peroxidation

Myocardium samples (50 mg) were homogenized with KCl 1.15% plus 35 mM of butylated hydroxytoluene according to Oakes and Van Der Kraak (2003). This method, previously described, involves the reaction of malondialdehyde (MDA), a degradation product of lipid peroxidation (LPO), with 2-thiobarbituric acid (TBA) under conditions of high temperature and acidity to generate a fluorescent adduct that was measured by spectrofluorometry (Victor 2, Perkin Elmer). After excitation at 515 nm and emission of 553

nm, the concentration of TBA-reacting substances (TBARS) was calculated and expressed in nmol/mg of proteins.

Statistical analysis

Data were expressed as means ± standard deviations (SDs) and analyzed by one-way analysis of variance (ANOVA) or Kruskal-Wallis ANOVA when the assumption of normality or homoscedasticity was not reached, followed by *posteriori* Tukey Highly Significant Difference test. Sex differences were compared by an independent Student's T-test. Statistical significance was set at p-values ≤ 0.05.

Results

Lactate levels during swimming training behaved according to expectations for each workload intensity. LS training did not change the rats' lactate levels versus the SC group, confirming the low workload. Lactate rose significantly ($p<0.05$) in HS rats (female rats: 9.88±1.77 mmol; male rats: 9.27±3.4 mmol) and MS rats (female rats: 5.26±0.56 mmol; male rats: 5.68±0.84 mmol) compared to SC rats (female rats: 2.4±0.3 mmol; male rats: 3.04±0.2 mmol) (Figure 2), demonstrating that both loads resulted in high and moderate efforts, respectively. Gender was not statistically related to changes in lactate levels ($p>0.05$).

Male rats had significantly ($p<0.05$) higher weights than female rats throughout the experiment. A significant reduction in body weight of male rats in the MS and HS groups (-11% and -12%, respectively) was observed at the end of the adaptation period ($p<0.05$; Table 1). In female rats, body weight reduction became apparent only in the 12th week of the experiment (Table 1). At that time, male rats in the MS (-10%) and HS (-16%) groups and female rats in the LS (-5%), MS (-6%), and HS (-6%) groups presented a significant ($p<0.05$) lower weight than the SC group rats (Table 1).

Food intake might explain the observed reductions in body weight. Rats of both sexes in the MS and HS groups consumed a significantly smaller quantity of food (in grams) than

the SC and LS rats (Table 1). Following the tendency to observe the highest body weight in male rats, food intake was also significantly higher in male rats than in female rats (Table 1).

After the adaptation period, female rats did not show any change in lipid profile between treatments (Figure 3), while male rats showed a significant reduction ($p<0.05$, MS -28% and HS -26%) in TC (Figure 3 A). LDL-C and HDL-C also decreased in male rats in the HS group ($p<0.05$). TG levels in male rats did not differ significantly between intensities, and they were higher than in female rats during this period ($p<0.05$, Figure 3 B).

By the end of the training period, female rats (LS -27%, MS -50%, HS -52%) and male rats (MS -28% and HS -27%) presented a significantly ($p<0.05$) lower TC content compared to SC rats (Figure 4 A). Also, LDL-C showed the same reduction pattern (female rats LS: -44%; MS: -60%, HS: -74%; male rats MS: -48% and HS: -29%) compared to the respective SC group (Figure 3 D). Swimming training, regardless of intensity, decreased TG in male rats (LS -28%, MS -28%, HS -27%) compared to SC. Male rats continued to present higher levels of TG than female rats in the SC and MS groups ($p<0.05$, Figure 4 B). There were no changes in the TG and HDL-C levels in female rats. HDL-C in male rats increased in the MS group and decreased in the HS group relative to SC ($p<0.05$, Figure 4 C). Female rats showed higher levels of HDL-C in SC, LS, and HS groups compared to the respective groups of male rats.

Cardiac ROS generation at 72 hours after the end of the experiment did not show any difference between training and SC groups ($p>0.05$). However, sex-related differences were observed, with male rats presenting higher ROS generation than female rats ($p<0.05$, Figure5 A). Myocardium ACAP was significantly increased only in LS female rats compared to the MS and HS groups (Figure 5 B). The MS and HS female rats showed a significant reduction in ACAP compared to all groups (Figure5 B). ACAP levels in male rats were not altered by swimming training. LPO in myocardium was significantly diminished in female rats (LS,

MS, and HS groups) and in male rats (LS and MS groups only) compared to SC rats ($p<0.05$, Figure 6). Male rats had higher LPO than female rats ($p<0.05$, Figure 6).

Discussion

Studies in experimental physiology need to acclimate animals to the intended loads and activities to be performed. Araujo *et al*.(2013), who studied rats using a monotonous training regimen without progressive habituation to load/time volume, observed a reduction in the amount of training (less time spent engaged in physical activity), increased muscle damage and reduced anaerobic capacity. To guarantee the execution of an exercise program completely, it is important to progressively acclimate subjects to load/time volume, although this is commonly missing from studies of experimental exercise physiology (Booth *et al*. 2010; de Araujo *et al*. 2013). To this purpose, was included here an acclimation period, with gradual increment of time and load until reach 60 minutes of exercise 5 times per week and to achieve a maximum load at 5% for MS and 15% for HS. Such protocol was effective for inducing aerobic and anaerobic metabolism, as observed by the expected blood lactate values, similar to those already reported (Voltarelli *et al*. 2002). Chronic exercise can have negative effect on mitochondrial glycerol phosphate dehydrogenase, reducing lactate generation (Casimiro-Lopes *et al*. 2012). In our experiment, it was not seen, because the physical training loads are incremental over time, allowing an increased effort during swimming keeping lactate levels adequate for the intended intensity.

The relationship between swimming intensity and reduced body weight detected herein could be associated with the reduction in food intake. Fatty acid oxidation increases in response to prolonged low- and moderate-intensity exercise for the purpose of skeletal muscle maintenance (Trejo-Gutierrez and Fletcher 2007; Ensign *et al*. 2002). Our swimming protocol could be promoting fatty acid oxidation, not exclusively in LS or MS, but also in HS training. This overall weight reduction is one of the changes stimulated by exercise that may

ameliorate cardiovascular risk factors (Durstine and Haskell 1994). Herein, males ate more food than females but lost weight faster; A study of obesity (Ropelle *et al*. 2006) observed that physical exercise suppressed hyperphagia, which was to linked to central action of insulin and leptin in the hypothalamus of mice. Our swimming protocols at all intensities and in both genders could be promoting weight loss by: (1) increasing the amount of fat burned and/or (2) changing the central mechanism that signals satiety.

In both sexes, exercise led to reduced TC and LDL-C concentrations compared to SC animals. In addition, all swimming intensities promoted a significant reduction in TG in male rats. These findings support that exercise induces fatty acid lipolysis, resulting in the improved blood lipid profile (Tunstall *et al*. 2002). However, HDL-C in female rats was not altered by any swimming training, although its levels were decreased in HS male rats. Such a decrease in HDL-C in male rats could also be a consequence of exacerbated fatty acid oxidation, including cholesterol, promoted by the high-intensity protocol.

The increase in HDL-C seen in MS male rats could be mediated by some key enzymes, such as lecithin cholesterol acyltransferase and lipoprotein lipase (Hamilton *et al*. 1998; Wittrup *et al*. 1997), as has already been verified in Sprague-Dawley rats exercised with voluntary running wheels. This augmented HDL-C profile imparts a benefit to cardiovascular health and could be considered a good physiological adaptation to exercise that is achieved with moderate-intensity activity. LS or HS training may be too mild or too intense, respectively, to promote this benefit. Female rats, for instance, already had a higher HDL-C than male rats, so exercise could not result in higher values.

An interesting finding of this work was that the HS training protocol was able to reduce TC, TG, and LDL-C. Another study, which examined 22-month-old rats submitted to swimming training for 60 minutes/day for 4 months in a high-intensity (5% load) situation, did not observe significant changes in lipid profile (Ravi *et al*. 2006). The reason for this

difference could be that 5% for older rats may be too intense to promote an alteration in lipid profiles. Another study, in which rats utilized a resistance exercise protocol for 3 days a week for 8 weeks, did not observe any reduction in lipid profile (Yang *et al*. 2006).

Our results suggest that low-intensity (in female rats only), moderate-intensity, and high-intensity swimming training may increase fatty acid oxidation. These findings point to the possibility that high-intensity training could be beneficial in elevating fatty acid oxidation and reducing TC, LDL-C, and TG, as was commonly observed in LS and MS rats.

The varying patterns of body weight reduction and lipid profile amelioration observed in male rats and female rats could be caused by different adaptive enzyme responses and/or influenced by sex hormones. Some authors (Campbell and Febbraio 2001), who studied the effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation of female rats, observed that ovarian sex steroids participated in the control of the maximal activity of several key enzymes of lipid oxidation.

We observed that even low-intensity swimming might reduce female rats' body weight, food intake, and TC and LDL-C levels, while these benefits were observed only in MS and HS male rats. This could be a result of a dimorphic interaction between exercise and sex hormones. In contrast, neither TG nor HDL-C changed in trained female rats, regardless of exercise intensity, attesting to the hypothesis that exercise does not have a similar influence on lipid metabolism in the different genders. Perhaps as a means of hormonal protection, female rats tend to have a higher HDL-C concentration than male rats. Another sex difference observed was the lapse in time response to exercise: whereas male rats presented significant differences after the 8-week adaptation period, female rats did not show differences until after the 16-week training period. The present protocol was effective in promoting reductions in body weight and blood lipids, although these reductions were the

slowest in female rats on the low-intensity regimen. In spite of these differences, both sexes benefited from the swimming protocols.

An inevitable consequence of the increased oxygen consumption induced by exercise is a favorable condition for increased generation of RONS and, apparently, an increase in oxidative stress (Powers *et al*. 2008). Previously, Storey (1996) showed that ascorbic free radical signals were increased in the rat heart after several consecutive days of exercise. Using DCF, another study (Kumar *et al*. 1992) demonstrated that the ROS production rate was significantly increased in the muscle of rats that were exhaustively exercised. Ramos *et al*. (2013) presented data that the oxidative stress biomarkers measured in the plasma immediately after a single bout of swimming exercise were generated primarily in the liver, not in muscle. Our data showed that long-term training (16 weeks) via a low-, moderate-, or high-intensity swimming protocol did not alter basal ROS generation in myocardium at 72 hours after the last training session in resting rats. But do not rule out the hypothesis that there may be oxidative modifications in other organs in response to physical training. Several works have verified elevation of ROS following exercise, and this is probably a result of very close analysis of an exercise session. In this situation, higher ROS levels could reflect an acute response to exercise, rather than a chronic effect of it. In addition, overtraining could be responsible for the increase in ROS, indicating a maladaptive effect of exhaustive exercise. With respect to generation of ROS, males showed significantly greater production of ROS than females. Previous studies demonstrate that estrogen confer favorable effects on cardiovascular system, diminishing generation of reactive species of oxygen elicited by regulation of ROS-generating enzymes or by promoting ROS eliminating (Colom *et al*. 2007, Arias-Loza *et al*. 2013). Protection of females against ROS can be explained by sexual dimorphism in the transcriptional levels of genes associated with fatty acid metabolism,

pyruvate dehydrogenase complex, and oxidative phosphorylation in hearts, highering resistance to oxidative stress (Vijay *et al*. 2015)

Our data also point to a beneficial effect of exercise in myocardial reduction of LPO in female rats (all swimming groups) and male rats (LS and MS groups). These results agree with other studies that observed a LPO reduction in skeletal muscle of mice exercised with a low-intensity treadmill (Kaczor *et al*. 2007). Another study (Ravi *et al*. 2006) observed that swimming training (low, moderate, and high) decreases myocardial MDA content compared to sedentary controls. We also found significantly lower levels of MDA in females than in males, which is in agreement with previous studies (Bloomer and Fisher-Wellman 2008; Goldfarb *et al*. 2007). In contrast, other research found increased MDA in the hearts of rats submitted to chronic (8 weeks) and acute exhaustive treadmill exercise (Liu *et al*. 2000), but this was probably an effect related to overtraining. However, the apparently unexpected uncoupled response of LPO, which diminished in all swimming groups, and ACAP, which rose only in LS female rats, could be explained by the differing influences of exercise intensities on the antioxidant systems. This was already detected in one study; different combinations of exercise intensity (low, moderate and high) and duration (30, 60, and 90 min/day) of swimming protocols were observed, and they produced different regulations of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase (GPX) in the left ventricle of trained rats (Powers *et al*. 2008). In Sprague-Dawley rats submitted to endurance and exhaustive swimming training, during exercise of increased intensity, glutathione and glutathione reductase enzyme activity decreased, whereas an increase was observed in GPX activity (Aydin *et al*. 2007).

The adaptive responses of the antioxidant system, and the expression of the various indirect markers of oxidative tissue damage, would be specific to either the type of tissue or the different defense mechanism affected (Ascensão *et al*. 2007). It would explain why only

LS showed an elevated antioxidant capacity against peroxyls, indicating an exercise intensity–specific response. However, MS and HS rats did not show increases in antioxidant capacity, which does not exclude the possibility that other defense systems are being affected by these workloads. Radicals may seem beneficial, as they act as signals to enhance defenses, rather than as deleterious, as they are when cells are exposed to high levels of them (Jones 2006; Powers *et al*. 2008). Following this point of view, the ROS generation that is habitually observed during exercise could be a sign of an adaptive antioxidant response. Our swimming training program caused a reduction in lipid peroxidation, no alteration in ROS during rest, and, under a low-intensity regimen, enhanced antioxidant systems.

In humans, genetic endowment and nutritional habits can lead to increased lipid profile, oxidative stress, inflammatory process, hypertension, myocardial infarction, atherosclerosis, collectively known the cardiovascular diseases (CVDs), contribute greatly to the mortality, morbidity (Buttar *et al*. 2005). This study, in rats we observed that intensity of exercise can modulate blood lipid profile and myocardial oxidative status, indicated that all levels of intensity of exercise training promoted amelioration of lipid profiles, especially reducing TC and LDL-C, which could improve health and diminish the risk of cardiovascular disease in both sexes. This study suggested that female rats showed improvement in lipid profiles following low-intensity exercise, while male rats required moderate- and high-intensity exercise to achieve this same benefit. Male rats and female rats responded similarly to the exercise intensities, but some sex-specific patterns were distinguished, as male rats responded earlier to exercise, whereas female rats responded later but after lower-intensity exercise than male rats. Over a chronic (16-week) training period, the exercise intensities that helped females were LS and MS. In male rats, MS was the best intensity for cardiac adaptation, considering adaptation patterns observed for TC, LDL-C, antioxidant capacity, and LPO.

Future investigations in this model will unveil novel molecular, cellular, and oxidative stress and integrative mechanisms of adaptation to different intensities of swimming training in comparing genders. Understanding the mechanisms of training-induced amelioration of cardiovascular function may help identify molecular targets for the beneficial adaptation of the cardiovascular system induced by exercise.

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Disclosures

The authors have no conflicts of interest to disclose.

Table 1. Body weight and food intake of groups submitted to different intensities of swimming training.

	SC		LS		MS		HS	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
BW initial (g)	225±12	320±18*	217±13	313±24*	222±17	295±33*	227±23	295±25*
BW (8 week)(g)	250±16	385±16[a]*	238±14	381±32[a]*	243±15	350±41[b]*	244±22	343±31[b]*
BW (12week)(g)	269±20[a]	450±16[a]*	257±16[b]	447±35[a]*	257±22[b]	419±48[b]*	256±26[b]	396±46[c]*
BW end (g)	285±22[a]	467±23[a]*	268±18[b]	460±35[ab]*	268±23[b]	440±45[b]*	271±24[b]	403±43[c]*
Food intake (g/day)	19.59±0.40[a]	27.44±0.72[a]*	19.01±0.90[ab]	26.25±0.85[a]*	18.35±0.35[bc]	23.58±1.47[b]*	17.61±0.88[c]	22.55±1.43[b]*

BW initial: body weight before initiation of exercise training. **BW (8 week)**: body weight after eight weeks of exercise training. **BW (12 weeks)**: body after twelve weeks of exercise. **BW end**: body weight after the end of the experiment (16 weeks) **Food intake**: mean of food consumption through of the experiment. Treatments: SC (sedentary control); LS (low-intensity); MS (moderate-intensity); HS (high-intensity). Data were expressed as mean ± S.D. Different letters mean significant differences between treatments and (*) means statistical differences between sexes at the significant level of 5%.

CAPTIONS

Fig. 1 Training program during a 16-week period divide into two periods: Acclimation (8 weeks) and maintenance (8 weeks) LS: low-intensity swimming, without workload. MS: moderate-intensity swimming with load equal to 5% of body weight. HS: high-intensity swimming with load equal to 15% of body weight.

Fig. 2 Lactate Test in acclimation period (8 weeks)**.** SC: sedentary control. LS: low-intensity swimming, without workload. MS: moderate-intensity swimming. HS: high-intensity swimming. Data are expressed as mean ± SDs (mg/dL). Different letters indicate significant differences between groups at significance level of 5%.

Fig. 3 Lipid profile at the end of the acclimation period (8 weeks) of *Rattus norvegicus* submitted to different intensities of swimming training. (A) TC; (B) TG; (C) HDL-C; (D) LDL-C. Data are expressed as means ± SDs (mg/dL); white bars, female rats; black bars, male rats. Different letters indicate significant differences between groups; *statistically significant differences between sexes at the 5% significance level.

Fig. 4 Lipid profile at the end of the experiment (16 weeks) of *Rattus norvegicus* submitted to different intensities of swimming training. (A) TC; (B) TG; (C) HDL-C; (D) LDL-C. Data are expressed as means ± SDs (mg/dL); white bars, female rats; black bars, male rats. Different letters indicate significant differences between groups; *statistically significant differences between sexes at the 5% significance level.

Fig. 5 (A) Generation of ROS in the myocardium; (B) ACAP in the myocardium after different intensities of swimming training. Data are expressed as means ± SDs (fluorescence area); white bars, female rats; black bars, male rats; *statistically significant differences between sexes at the 5% significance level.

Fig. 6 Lipid peroxidation determined by MDA levels in the myocardium of *Rattus norvegicus* submitted to different intensities of swimming training. Data are expressed as means ± SDs (mg/protein); white bars, female rats; black bars, male rats. Different letters indicate significant differences between groups; *statistically significant differences between sexes at the significant level of 5%.

References

ABERNETHY DR, AZARNOFF DL: Pharmacokinetic investigations in elderly patients. Clinical and ethical considerations. *Clin Pharmacokinet* **19:** 89-93, 1990.

AMADO LL, GARCIA ML, RAMOS PB, FREITAS RF, ZAFALON B, FERREIRA JL, YUNES JS, MONSERRAT LM. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organism: application to evaluate microcystins toxicity. *Sci Total Environ* **407**: 15-213, 2009.

ARAUJO GG, PAPOTI M, DOS REIS IG, DE MELLO MA, GOBATTO CA. Physiological responses during linear periodized training in rats. *Eur J Appl Physiol* **112**: 839-852, 2012.

ARAUJO GG, PAPOTI M, MANCHADO-GOBATTO FDE B, DE MELLO MA, GOBATTO CA. Monitoring chronic physical stress using biomarkers, performance protocols and mathematical functions to identify physiological adaptations in rats. *Lab Anim* **47**: 36-42, 2013.

ARIAS-LOZA PA, MUEHLFEDLDER M, PELZER T. Estrogen and estrogen receptors in cardiovascular oxidative stress. *Pflugers Arch* **465:** 739-746, 2013.

ASCENSÃO A, FERREIRA R, MAGALHARES J. Exercise induced cardioprotection biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. *Int J Cardiol* **117**: 16-30, 2007.

AUNE E, BAEKKEVAR M, ROISLIEN J, RODEVAND O, OTTERSTAD JE. Normal reference ranges for left and right atrial volume indexes and ejection fractions obtained with real-time thee-dimensional echocardiography. *Eur J Echocardiogr* **10**: 738-744, 2009.

AYDIN C, INCE E, KOPARAN S, CANGUL IT, NAZIROGLU M AKF. Protective effects of long term dietary restriction on swimming exercise induced oxidative stress in the liver, heart and kidney of rat. *Cell Bochem Funct* **25**: 19-137, 2007.

BENEK R, LEITHAUSER RM, OCHENTEL O. Blood lactate diagnostic in exercise testing and training. *In tJ Sports Physiol Perform* **6**: 8-24, 2011.

BLOOMER RJ, FISHER-WELLMAN KH. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gend Med* **5**: 218-228, 2008.

BOOTH FW, LAYE MJ, SPANGENBURG EE. Gold standards for scientists who are conducting animal-based exercise studies. *J Appl Physiol* **108**: 219-221, 2010.

BUTTAR HS, LI T, RAVI N. Prevention of Cardiovascular diseases: Role of exercise, dietary interventions, obesity and smoking cessation. *Exp Clin Cardiol* **10**: 229-249, 2005.

CAMPBELL SE, FEBBRAIO MA. Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol Endocrinol Metab* **281**: 803-808, 2001.

CARVALHO JF, MASUDA MO, POMPEU FA. Method for diagnosis and control of aerobic training in rats based on lactate threshold. *Comp Biochem Physiol A Mol Integr Physiol* **140**: 409-413, 2005.

CASIMIRO-LOPES G, RAMOS D, SORENSON MM, SALERNO VP. Redox balance and mitochondrial glycerol phosphate dehydrogenase activity in trained rats. *Eur J Appl Physiol*. **112**: 3839-3846, 2012

CHIRICO EN, MARTIN C, FAES C, FEASSON L, OYONO-ENGUELLE S, AUFRADET E, DUBOUCHAUD H, FRANCINA A, CANET-SOULAS E, THIRIET P, MESSONNIER L, PIALOUX V .Exercise training blunts oxidative stress in sickle cell trait carriers. *J Appl Physiol* **112**: 1445-1453, 2012.

COLOM B, OLIVER J, ROCA P, GARCIA-PALMER FJ. Caloric restriction and gender modulate cardiac muscle mitochondrial H_2O_2 production and oxidative damage. *Cardiovasc Res*. **74:** 456-465, 2007.

CONTARTEZE RV, MANCHADO FDE B, GOBATTO CA, DE MELLO MA .Stress biomarkers in rats submitted to swimming and treadmill running exercises. *Comp Biochem Physiol A Mol Integr Physiol* **151**: 415-422, 2005.

DALEN H, THORSTENSEN A, VATTEN LJ, AASE SA, STOYLEN A. Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ Cardiovasc Imaging* **3**: 614-622, 2010.

DUNCAN GE, ANTON SD, SYDEMAN S, NEWTON RL, CORSICA JA, DURNING PE, KETTERSON TU, MARTIN AD, LIMACHER MC, PERRI MG. Prescribing exercise at varied levels of intensity and frequency - A randomized trial. *Archives of Internal Medicine* **165**: 2362-2369, 2015.

DURSTINE JL, HASKELL WL. Effects of exercise training on plasma lipids and lipoproteins. *Exerc Sport Sci Rev* **22**: 477-521, 1994.

ENSIGN WY, MCNAMARA DJ, FERNANDEZ ML. Exercise improves plasma lipid profiles and modifies lipoprotein composition in guinea pigs. *J Nutr Biochem* **13**: 747-753, 2012.

FERREIRA JC, ROLIM NP, BARTHOLOMEU JB, GOBATTO CA, KOKUBUN E, BRUM PC. Maximal lactate steady state in running mice: effect of exercise training. *Clin Exp Pharmacol Physiol* **34**: 760-765, 2007.

GOBATTO CA, DE MELLO MA, SIBUYA CY, DE AZEVEDO JR, DOS SANTOS LA, KOKUBUN E. Maximal lactate steady state in rats submitted to swimming exercise. *Comp Biochem Physiol A Mol Integr Physiol* **130**: 21-27, 2001.

GOLDFARB AH, MCKENZIE MJ, BLOOMER RJ. Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. *Appl Physiol Nutr Metab* **32**: 1124-1131, 2007.

GROUSSARD C, RANNOU-BEKONO F, MACHEFER G, CHEVANNE M, VINCENT S, SERGENT O, CILLARD J, GRATAS-DELAMARCHE A. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. *Eur J Appl Physiol* **89**: 14-20, 2003.

GUERREIRO LF, PEREIRA AA, MARTINS CA, WALLY C, GONÇALVES CAN. Swimming physical training in rats: Cardiovascular adaptation to exercise training protocols at different intensities. *J Exerc Physiol online* **18**: 1-11, 2015.

HAMILTON MT, ETIENNE J, MCCLURE WC, PAVEY BS, HOLLOWAY AK. Role of local contractile activity and muscle fiber type on LPL regulation during exercise. *Am J Physiol* **275**: 1016-1022, 1998.

HERNANDEZ-TORRES RP, RAMOS-JIMENEZ A, TORRES-DURAN PV, ROMERO-GONZALEZ J, MASCHER D, POSADAS-ROMERO C, JUAREZ-OROPEZA MA. Effects of single sessions of low-intensity continuous and moderate-intensity intermittent exercise on blood lipids in the same endurance runners. *J Sci Med Sport* **12**: 323-331, 2009.

HUGHES RA, THORLAND WG, EYFORD T, HOOD T. The acute effects of exercise duration on serum lipoprotein metabolism. *J Sports Med Phys Fitness* **30**: 37-44, 1990.

JACOB MH, PONTES MR, ARAUJO AS, BARP J, IRIGOYEN MC, LLESUY SF, RIBEIRO MF, BELLO-KLEIN A. Aortic-banding induces myocardial oxidative stress and changes in concentration and activity of antioxidants in male Wistar rats. Life Sci **79**: 2187-2193, 2006.

JONES DP. Redefining oxidative stress. *Antioxid Redox Signal* **8**: 1865-1879, 2006.

KACZOR JJ, HALL JE, PAYNE E, TARNOPOLSKY MA. Low intensity training decreases markers of oxidative stress in skeletal muscle of mdx mice. *Free Radic Biol Med* **43**: 145-154, 2007.

KRAUS WE, HOUMARD JA, DUSCHA BD, KNETZGER KJ, WHARTON MB, MCCARTNEY JS, BALES CW, HENES S, SAMSA GP, OTVOS JD, KULKARNI KR, SLENTZ CA. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med* **347**: 1483-1492, 2002.

KUMAR CT, REDDY VK, PRASAD M, THYAGARAJU K, REDDANNA P. Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress. *Mol Cell Biochem* **111**: 109-115, 1992.

LINDQVIST P, MORNER S, HENEIN MY. Cardiac mechanisms underlying normal exercise tolerance: gender impact. *Eur J Appl Physiol* **112**: 451-459, 2012.

LIU J, YEO HC, OVERVIK-DOUKI E, HAGEN T, DONIGER SJ, CHYU DW, BROOKS GA, AMES BN. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol* **89**: 21-28, 2002.

MANCHADO FDE B, GOBATTO CA, VOLTARELLI FA, ROSTOM DE MELLO MA . Non-exhaustive test for aerobic capacity determination in swimming rats. *Appl Physiol Nutr Metab* **31**: 731-736, 2006.

OAKES KD, VAN DER KRAAK GJ. Utility of the TBARS assay in detecting oxidative stress in white sucker (Catostomus commersoni) populations exposed to pulp mill effluent. *Aquat Toxicol* **63**: 447-463, 2003.

PERRINO C, GARGIULO G, PIRONTI G, FRANZONE A, SCUDIERO L, DE LAURENTIS M, MAGLIULO F, ILARDI F, CAROTENUTO G, SCHIATTARELLA GG, ESPOSITO G. Cardiovascular effects of treadmill exercise in physiological and pathological preclinical settings. *Am J Physiol Heart Circ Physiol* **300**: 1983-1989, 2011.

Pilis W, Zarzeczny R, Langfort J, Kaciuba-Uscilko H, Nazar K, Wojtyna J. Anaerobic threshold in rats. *Comp Biochem Physiol Comp Physiol* **106**: 285-289, 1993.

PINHO CA, TROMM CB, TAVARES AM, SILVA LA, SILVEIRA PC, SOUZA CT, BENETTI M, PINHO RA. Effects of different physical training protocols on ventricular oxidative stress parameters in infarction-induced rats. *Life Sci* **90**: 553-559, 2012.

POWERS SK, QUINDRY JC, KAVAZIS AN. Exercise-induced cardioprotection against myocardial ischemia-reperfusion injury. *Free Radic Biol Med* **44**: 193-201, 2008.

RADAK Z, CHUNG HY, GOTO S. Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic Biol Med* **44** (2):153-159, 2008.

RADAK Z, KANEKO T, TAHARA S, NAKAMOTO H, OHNO H, SASVARI M, NYAKAS C, GOTO S. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radic Biol Med* **27**: 69-74, 1999.

RAMOS D, MARTINS EG, VIANA-GOMES D, CASIMIRO-LOPES G, DALERNO VP. Biomarkers of oxidative stress and tissue damage release by muscle and liver after a single bout of swimming exercise. *Appl Physiol Nutr Metab* **38**: 507-511, 2013.

RAVI KT, SUBRAMANYAM MV, PRATHIMA S, ASHA DEVI S. Blood lipid profile and myocardial superoxide dismutase in swim-trained young and middle-aged rats: comparison between left and right ventricular adaptations to oxidative stress. *J Comp Physiol B* **176**: 749-762, 2006.

ROPELLE ER, FLORES MB, CINTRA DE, ROCHA GZ, PAULI JR, MORARI J, DE SOUZA CT, MORAES JC, PRADA PO, GUADAGNINI D, MARIN RM, OLIVEIRA AG, AUGUSTO TM, CARVALHO HF, VELLOSO LA, SAAD MJ, CARVALHEIRA JB. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol **8**, 2006.

SHIOMI T, TSUTSUI H, MATSUSAKA H, MURAKAMI K, HAYASHIDANI S, IKEUCHI M, WEN J, KUBOTA T, UTSUMI H, TAKESHITA A. Overexpression of

glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* **109**: 544-549, 2004.

STEFFENSEN CH, ROEPSTORFF C, MADSEN M, KIENS B. Myocellular triacylglycerol breakdown in females but not in males during exercise. *Am J Physiol Endocrinol Metab* **282**: 634-642, 2002.

STOREY KB. Oxidative stress: animal adaptations in nature. *Braz J Med Biol Res* **29**: 1715-1733, 1996.

SUGAWARA J, KOMINE H, MIYAZAWA T, IMAI T, FISHER JP, OGOH S. Impact of chronic exercise training on the blood pressure response to orthostatic stimulation. *J Appl Physiol* **112**: 1891-1896, 2012.

THOMPSON PD. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. *Arterioscler Thromb Vasc Biol* **23**: 1319-1321, 2003.

TREJO-GUTIERREZ JF, FLETCHER G. Impact of exercise on blood lipids and lipoproteins. *J Clin Lipidol* **1**: 175-181, 2007.

TUNSTALL RJ, MEHAN KA, WADLEY GD, COLLIER GR, BONEN A, HARGREAVES M, CAMERON-SMITH D. Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* **283**: 66-72, 2002.

VIJAY V, HAN T, MOLAND CL, KWEKEL JC, FUSCOE JC, DESAI VG. Sexual dimorphism in the expression of mitochondria-related genes in rat heart at different ages. *PLoS One* **10**: 117-147, 2015.

WANG JS, LEE T, CHOW SE. Role of exercise intensities in oxidized low-density lipoprotein-mediated redox status of monocyte in men. *J Appl Physiol* **101**:740-744, 2006.

WITTRUP HH, TYBJAERGHANSEN A, ABILDGAARD S, STEFFENSEN R, SCHNOHR P, NORDESTGAARD BG. A common substitution (Asn291Ser) in lipoprotein lipase is

associated with increased risk of ischemic heart disease. *Journal of Clinical Investigation* **99**:1606-1613, 1997.

VOLTARELLI FA, GOBATTO CA, DE MELLO MAR. Determination of anaerobic threshold in rats using the lactate minimum test. *Brazilian Journal of Medical and Biological Research* **35**: 1389-1394, 2002.

YANG JY, NAM JH, PARK H, CHA YS. Effects of resistance exercise and growth hormone administration at low doses on lipid metabolism in middle-aged female rats. European Journal of Pharmacology **539**:99-107, 2006.

Figure 1

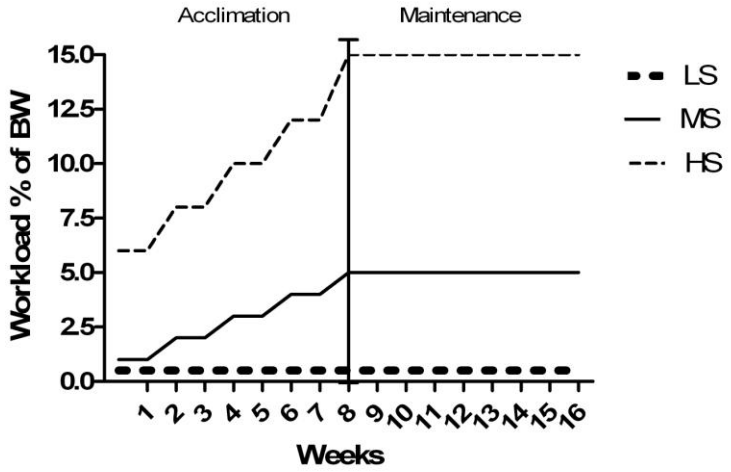

Figure 2

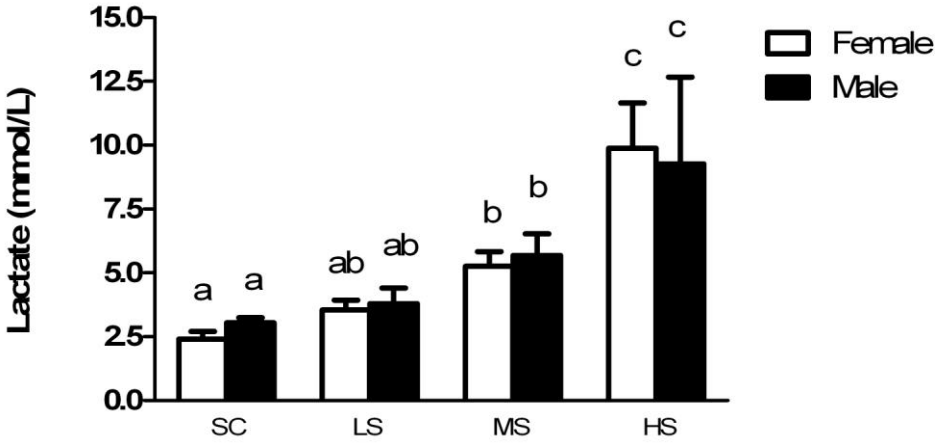

Figure 3

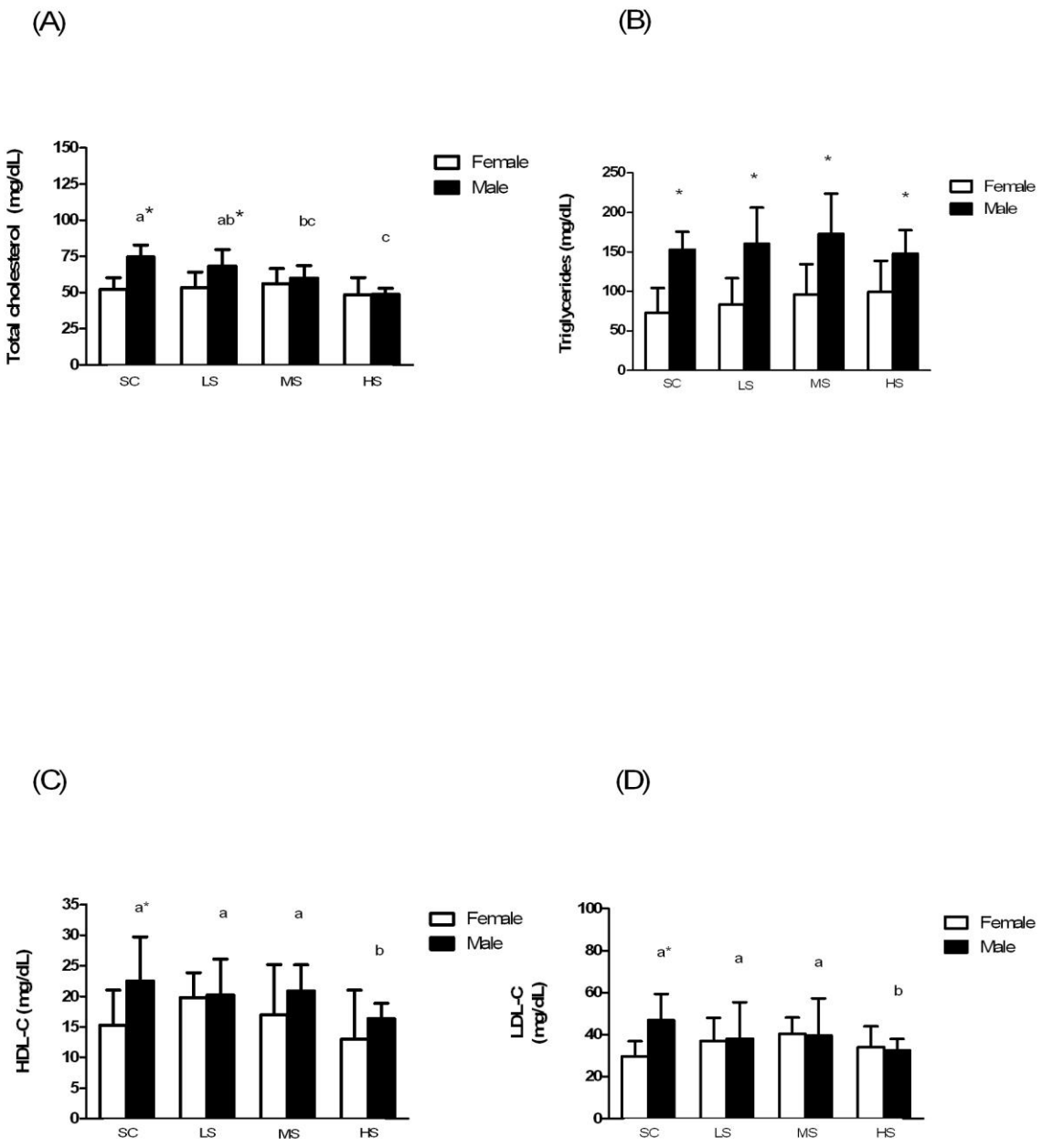
(A)
(B)
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
Female
Male
a*
ab*
bc
c
*
SC
LS
MS
HS
(C)
(D)
HDL-C (mg/dL)
LDL-C (mg/dL)
a*
a
a
b

Figure 4

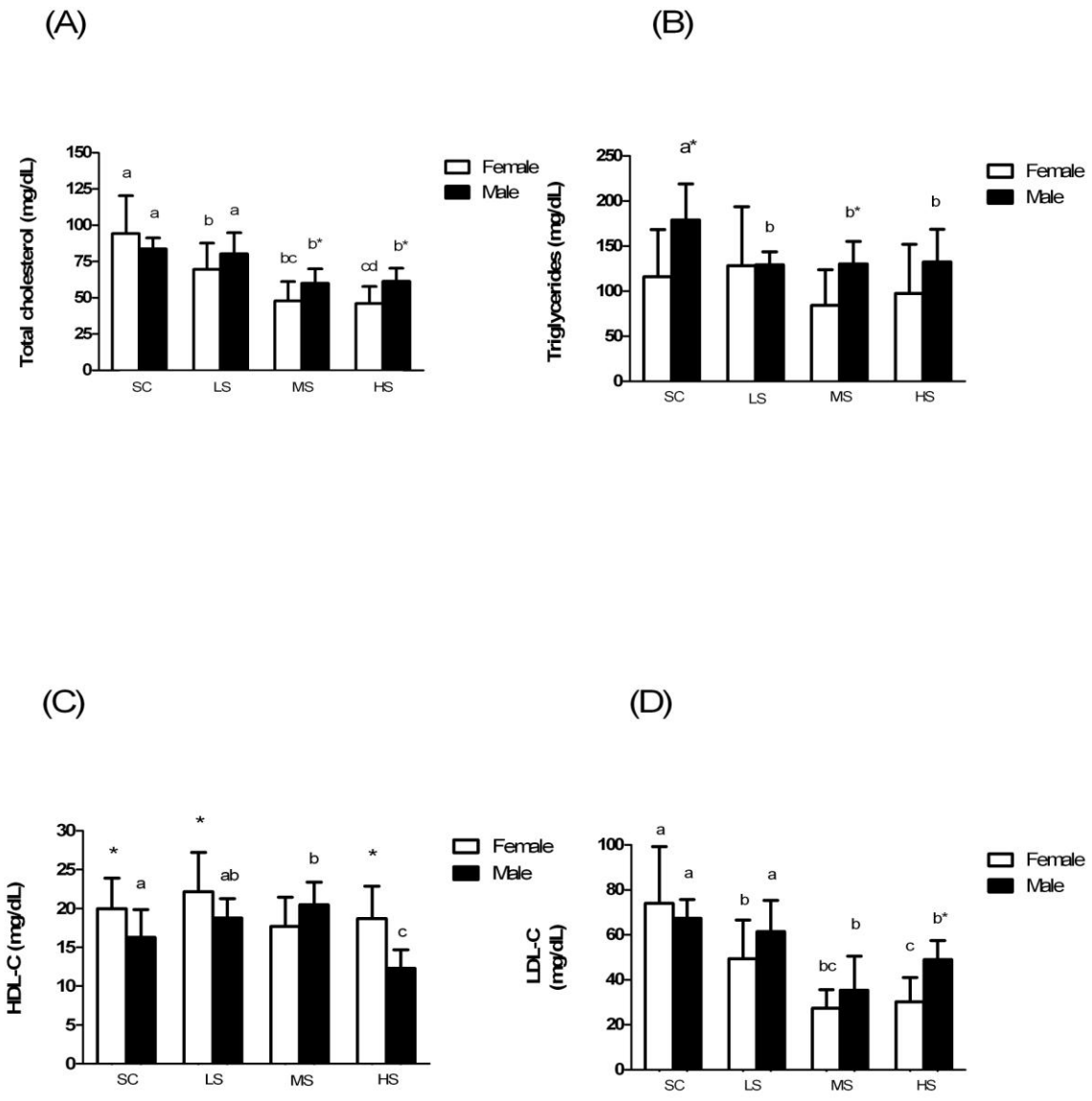
(A)
(B)
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
Female
Male
SC
LS
MS
HS
(C)
(D)
HDL-C (mg/dL)
LDL-C (mg/dL)

Figure 5

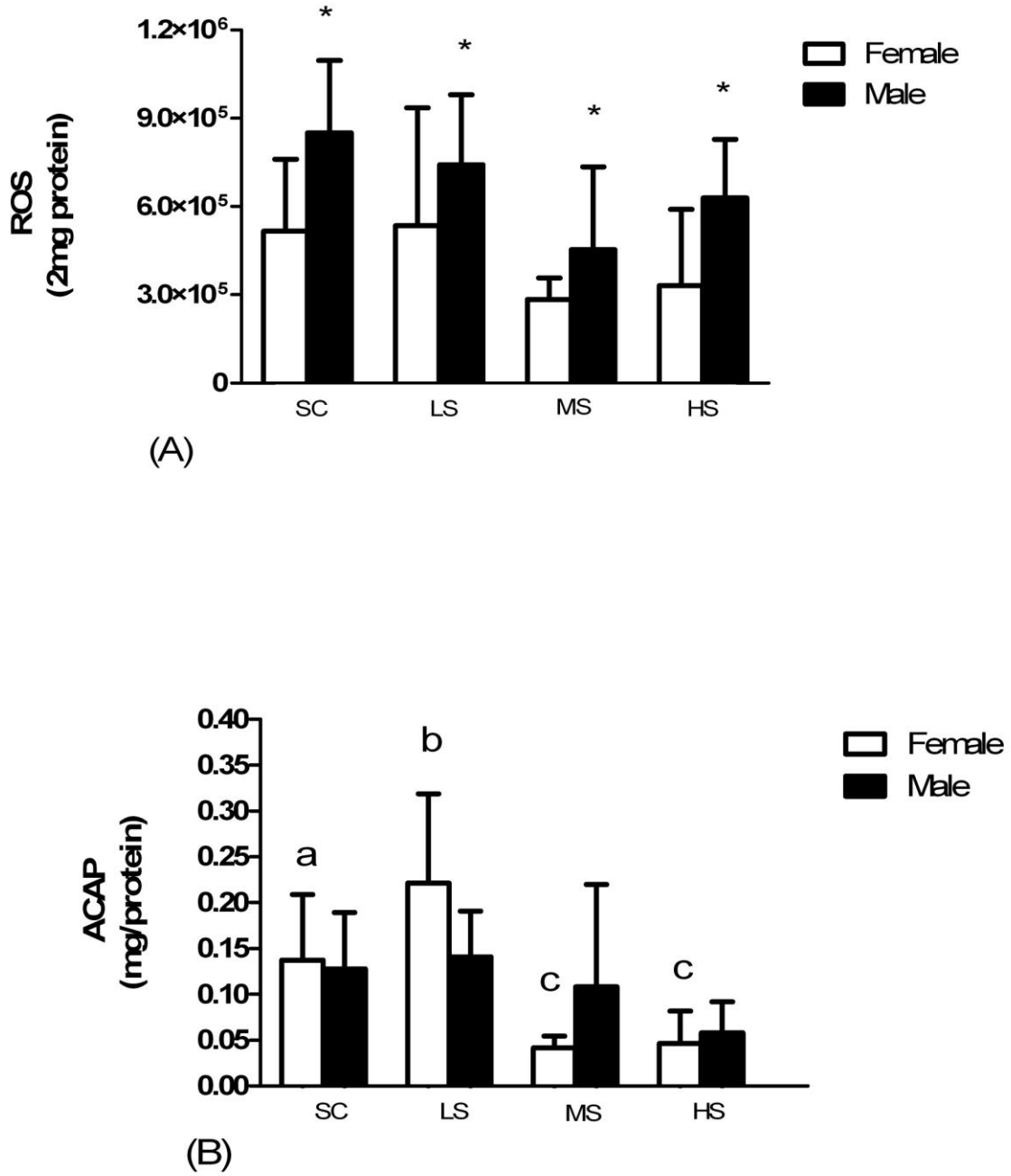
ROS
(2mg protein)
1.2×10⁶
9.0×10⁵
6.0×10⁵
3.0×10⁵
0
SC
LS
MS
HS
*
Female
Male
(A)
ACAP
(mg/protein)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
b
c
c
(B)

Figure 6