Effect of Aerobic Training of Moderate and Low Volume on Electron Transport Chain Activity and Oxidative Stress Markers in Skeletal Muscle

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ABSTRACT

Silva LA, Scheffer DL, Alves A, Pereira LT, Moneretto DB, Tromm C, Streck EI, Silveira PCL, Pinho RA. Effect of Aerobic Training of Moderate and Low Volume on Electron Transport Chain Activity and Oxidative Stress Markers in Skeletal Muscle. JEPonline 2015;18(6):81-93. The purpose of this study was to compare the adaptations induced by moderate and low volume aerobic exercise programs on mitochondrial enzyme activity oxidative stress markers in skeletal muscle of mice. Eighteen male mice (CF1) weighing 30 to 35 g were randomly distributed into three groups (n = 6): (a) untrained (UT); (b) moderate training volume (MTV); and (c) low training volume (LTV). Animals were submitted to an 8-wk training program. Forty-eight hours after the last training session the animals were killed by decapitation and the quadriceps muscles were removed and stored at –70°C. Succinate dehydrogenase (SDH), mitochondrial respiratory chain enzyme activities (complex I-II), thiobarbituric acid reactive species (TBARS), protein carbonyls (PC), superoxide dismutase (SOD), and catalase (CAT) were measured. Results indicate that MTV program increase SDH (19%) activity, complex I (56%) and II (67%) more than the LTV,
when compared to untrained mice. However, the LTV program decreased oxidative damage (TBARS by 32% and CP by 22%) more than MTV, against UT. Antioxidant enzyme activity (SOD 71% / CAT 73%) increased similarly in both programs. In conclusion, MTV caused greater increases in mitochondrial respiratory chain enzyme activity as compared to LTV, while LTV caused a sharper decrease in oxidative stress in comparison to MTV.

**Key Words**: Mitochondrial enzyme activity, Oxidative stress, Physical exercise, Skeletal muscle

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**INTRODUCTION**

Regular physical exercise has many health benefits, including a lower risk of all-cause mortality along with a reduced risk of cardiovascular disease, cancer, and diabetes (5,9). Paradoxically, it is also clear that the contraction of skeletal muscles generates free radicals and that exercise can result in oxidative stress to skeletal muscle and thus affect health negatively (2,35).

Reactive oxygen species (ROS) levels during aerobic exercise have been attributed to high oxygen consumption, and may go up to 10 to 20 times the systemic levels and as much as 100 to 200 times the skeletal muscle levels, resulting in substantially increased mitochondrial electron flux (16). However, the oxidative stress observed after aerobic physical training depends on the intensity and duration of the exercise session.

It is generally accepted that oxidative stress after exercise might be reduced by augmenting activities of antioxidant enzymes and facilitating electron transport from mitochondria (45), which can be achieved by regular exercise (3,6,36). Antioxidant enzymes can be defined as substances that help reduce the severity of oxidative stress either by forming a less active radical or by quenching the damaging ROS chain reaction on substrates such as proteins, lipids, and DNA (10). In our laboratory we demonstrated that higher antioxidant enzyme activity in liver (44) and increased mitochondrial respiratory chain enzyme activities in muscle (45) occurred after 8 wks of running training, in association with a drop in oxidative damage.

However, studies comparing the effect of the volume of the exercise sessions on these markers are scarce. We hypothesized that different exercise volumes (low to moderate) can promote different responses with regard to mitochondrial activity and oxidative stress markers in the skeletal muscle of trained animals. Nevertheless, it was investigated whether moderate training is more effective than low training volume to increase mitochondrial respiratory chain and antioxidant enzyme activities and to decrease oxidative stress. Thus, the purpose of the present study was to compare the effects of moderate and low training moderate volume on mitochondrial enzyme activity and oxidative stress on skeletal muscle.

**METHODS**

**Animals**

The study protocol was reviewed and approved by the Ethics Committee of the Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil, according to the Guidelines for Animal Care and Experimentation (32). A total of 18 male mice (CF1), aged 3 months, weighing 30 to
35 g, were housed in cages containing six animals with water and food ad libitum. The animals were kept on a 12-hr light and 12-hr dark cycle and were maintained at 23°C.

**Exercise**
The animals were divided into the following groups (n = 6): (a) untrained (UT); (b) moderate training volume (MTV); and (c) low training volume (LTV). The exercise training group was subjected to running on a motor driven treadmill according to a progressive exercise training regimen. All animals were accustomed to treadmill running for 1 wk (10 m·min⁻¹ without inclination, for 10 min·d⁻¹). After a 1-wk adaptation period, the trained groups were submitted to an 8-wk training program with a 1% treadmill inclination.

**Moderate Training Volume (MTV)**
The MTV program was performed in the night (between 6 and 8 p.m.) and consisted of a 45 min·d⁻¹ session, 5 d·wk⁻¹ for 8 wks. The velocity of the treadmill was 13 m·min⁻¹ during the first 4 wks and 16 m·min⁻¹ during the subsequent weeks. This training velocity corresponded to moderate intensities of approximately 50% and 60% VO₂ max (7).

**Low Training Volume (LTV)**
The LTV program consisted of a three session of 15 min·d⁻¹, 5 d·wk⁻¹ for 8 wks. The exercise sessions were carried out at 9:00 am, 3:00 pm, and 8:00 pm. During all training programs the velocity was the same as THL (Table 1).

**Sacrifice of Animals**
Forty-eight hours after the last training session, the animals were killed by decapitation. The quadriceps muscles (red portion) were surgically removed and the samples were immediately stored at -70°C for subsequent analysis. Mitochondrial respiratory chain enzyme activities (SDH, complex I and II), oxidative damage (thiobarbituric acid reactive species and protein carbonyl), and antioxidant enzyme activities (superoxide dismutase and catalase) were analyzed.

**Biochemical Analyses**
**Homogenate Preparation**
The quadriceps muscles were homogenized (1:10, w/v) in SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/mL heparin, pH 7.4). The homogenates were centrifuged at 800 g for 10 min and the supernatants were kept at −70°C until use in the determination of succinate dehydrogenase (SDH) and mitochondrial respiratory chain enzyme activities (complexes I and II). The maximal period between homogenate preparation and enzyme analysis was always less than 5 d.

**Mitochondrial Respiratory Chain Enzyme Activities**
**Succinate Dehydrogenase (SDH)**
Phenazine oxidoreductase (soluble succinate dehydrogenase - SDH) was measured in terms of the decrease in absorbance due to the reduction in 2,6-dichloroindophenol (DCIP) at 600 nm as reference wavelength (ε = 19.1 mM⁻¹·cm⁻¹) in the presence of phenazine methosulphate (PMS). The reaction mixture consisted of 40 mM potassium phosphate, pH 7.4, 16 mM succinate and 8 mM DCIP. DCIP preincubated with 40 to 80 mg homogenate protein at 30°C for 20 min. Subsequently, 4 mM sodium azide, 7 mM rotenone and 40 mM
DCIP were added and the reaction was initiated by adding 1 mM PMS, and was monitored
for 5 min (13).

Complex I and II
On the day assays were carried out, the samples were frozen and defrosted in hypotonic
assay buffer three times to fully expose the enzymes to substrates and achieve maximal
activities. NADH dehydrogenase (complex I) was evaluated, according to the method
described by Cassina and Radi (8) by measuring the rate of NADH-dependent ferricyanide
reduction at 420 nm. The activities of succinate: DCIP oxidoreductase (complex II) were
determined according to the method developed by (13). Complex II activity was measured by
following the decrease in absorbance due to the reduction in 2,6-DCIP at 600 nm. The
activities of the mitochondrial respiratory chain complexes were expressed as nmol·min⁻¹ mg
protein.

Oxidative Damage
Thiobarbituric Acid Reactive Species (TBARS)
The development of TBARS during the acid-heating reaction was used as an index of lipid
peroxidation (11). Briefly, the samples were mixed with 1 ml 10% trichloroacetic acid and 1 ml
0.67% thiobarbituric acid, and then heated in boiling water for 30 min. TBARS were
determined by the absorbance at 535 nm using 1,1,3,3-tetramethoxypropane (Sigma
Chemical) as an external standard. The results were expressed as malondialdehyde (MDA)
equivalents per milligram of protein.

Protein Carboxyls
The oxidative damage to protein was measured by the determination of carbonyl groups
based on the reaction with dinitrophenylhydrazine (DNPH) (26). Proteins were precipitated by
adding 20% trichloroacetic acid and reacted with DNPH. The samples were then redissolved
in 6 M guanidine hydrochloride and carbonyl contents were determined by measuring
absorbance at 370 nm using a molar absorption coefficient of 22 0000 M⁻¹.

Antioxidant Enzymes
Superoxide Dismutase total (SOD) And Catalase (CAT) Activities
In order to determine CAT activity, tissue portions were sonicated in 50 mM phosphate buffer
and the resulting suspension was centrifuged at 3000 g for 10 min. The supernatant was
used for enzyme assay. CAT activity was measured based on the rate of decrease in
hydrogen peroxide (10 mM) absorbance at 240 nm (1). SOD activity was determined by
measuring the inhibition of adrenaline self-oxidation absorbance at 480 nm (4).

Protein Determination
The amount of protein in SDH, complex I and II enzyme activities, TBARS, PC, CAT, and
SOD activities was determined using the Lowry technique (27).

Statistical Analysis
Data were expressed as mean and standard error mean (mean ± SEM), and analyzed
statistically by the one-way analysis of variance (ANOVA), followed by the post hoc Tukey’s
HSD test. The level of significance was set at 95% (P<0.05). The Statistical Package for the
Social Sciences (SPSS) version 16.0 for Windows was used to analyzed the data.
RESULTS

Mitochondrial Respiratory Chain Enzymes Activities

**Succinate Dehydrogenase (SDH)**

MTV (8.0 ± 0.6 nmol·min⁻¹·mg⁻¹ protein) caused a greater increase in SDH (19%) than LTV (7.2 ± 0.3 nmol·min⁻¹·mg⁻¹ protein), when compared to the UT group (4.2 ± 0.3 nmol·min⁻¹·mg⁻¹ protein, P<0.05), as shown in Figure 1A.

![Figure 1A](image1.png)

**I Complex Activity**

Data show that MTV (159 ± 13 nmol·min⁻¹·mg⁻¹ protein) caused a greater increase (56%) in I complex activity than LTV (118 ± 9 nmol·min⁻¹ protein, P<0.05), when compared with the UT group (73.1± 6 nmol·min⁻¹·mg⁻¹) (Figure 1B).

![Figure 1B](image2.png)
**II Complex Activity**

Results show that MTV (1.4 ± 0.02 nmol·min⁻¹·protein) led to a greater increase in II complex activity (67%) than LTV (1.0 ± 0.06 nmol·min⁻¹·protein), against UT (0.6 ± 0.04 nmol·min⁻¹·protein, P<0.05) (Figure 1C).

**Figure 1C**

**Figure 1 (A-C):** Mitochondrial Respiratory Chain Enzyme Activities (SDH, Complex I and II) were Determined in the Skeletal Muscle 48 h after the Last Training Session, According to Materials and Methods. The animals were distributed into four groups (n = 6): untrained (UT); moderate training volume (MTV); low training volume (LTV). Data are presented as Mean ± SEM and results expressed in nmol·min⁻¹·mg⁻¹·protein. Difference in relation to the untrained group (*) and MTV (#) was significant at P<0.05.

**Oxidative Damage**

**Thiobarbituric Acid Reactive Species (TBARS)**

Data demonstrate (Figure 2A) that LTV (0.13 ± 0.03 nmol/mg protein) caused a shaper decrease in MDA levels (32%) as compared to MTV (0.25 ± 0.06 nmol/mg protein), against UT (0.37 ± 0.06 nmol/mg protein, p<0.05).

**Figure 2 (A):** Malondialdehyde (MDA) Levels were Determined in the Skeletal Muscle (Red-Quadriceps) 48 h after the Last Training Session, According to Materials and Methods. The animals were distributed into four groups (n = 6) untrained (UT); moderate training volume (MTV); low training volume (LTV). The values are presented as Mean ± SEM and results expressed in nmol of MDA/mg of proteins. Difference in relation to the untrained group (*) and MTV (#) was significant at P<0.05.
**Protein Carbonyls**
As shown in Figure 2B, LTV (0.23 ± 0.01 nmol·mg⁻¹ protein), caused a sharper decrease in protein carbonyls (22%) than training MTV (0.32 ± 0.30 nmol·mg⁻¹ protein, P<0.05), against UT (0.42 ± 0.04 nmol·mg⁻¹ protein).

![Protein Carbonyls Graph](image1)

**Figure 2 (B): Carbonylation Levels were Determined in the Skeletal Muscle (Red-Quadriceps) 48 hrs after the Last Training Session, According to Materials and Methods.** The animals were distributed into four groups (n = 6): untrained (UT); moderate training volume (MTV); low training volume (LTV). Data are presented as Mean ± SEM and results expressed in nmol·mg⁻¹ of proteins. Difference in relation to the untrained group (*) and MTV (#) was significant at P<0.05.

**Antioxidant Enzyme**
**Superoxide Dismutase (SOD)**
Figure 3A demonstrates increases in SOD (71%) activities after the different physical training programs (MTV: 1.65 ± 0.21 U·mg⁻¹ protein; LTV: 1.61 ± 0.15 U·mg⁻¹ protein, P<0.05), when compared with UT (0.95 ± 0.12 U·mg⁻¹ protein).

![SOD Graph](image2)

**Figure 3 (A): Superoxide Dismutase Activities were Determined 48 hrs after the Last Training Session, according to Materials and Methods.** The animals were distributed into four groups (n = 6): untrained (UT); moderate training volume (MTV); low training volume (LTV). Data are presented as Mean ± SEM and results expressed in U/mg of proteins. Difference in relation to the untrained group (*) and MTV (#) was significant at P<0.05.
**Catalase (CAT)**

Results show a significant increase in catalase (73%) activity in the MTV group (1.1 ± 0.05 U·mg\(^{-1}\) protein) and LTV group (1.05 ± 0.10 U·mg\(^{-1}\) protein), in comparison with UT (0.62 ± 0.07 U·mg\(^{-1}\) protein) (Figure 3B).

![Catalase Activities](image.png)

**Figure 3 (B): Catalase Activities were Determined 48 hrs after the Last Training Session, According to Materials and Methods.** The animals were distributed into four groups (n = 6): untrained (UT); moderate training volume (MTV); low training volume (LTV). Data are presented as Mean ± SEM and results expressed U/mg of proteins. Difference in relation to the untrained group (*) and MTV (#) was significant at P<0.05.

**DISCUSSION**

This study investigated the effects of physical exercise on mitochondrial respiratory chain activity, oxidative damage, and antioxidant enzyme activity in terms of training volume. The data demonstrate that the MTV program increases muscle oxidative capacity (SDH, complex I and II) more than the LTV program, when compared to untrained mice. However, the LTV was more effective in decreasing oxidative damage than the MTV (i.e., in comparison to the UT). Similar increases in antioxidant enzyme activity (SOD/CAT) were caused by both programs.

It has been reported that endurance exercise training results in physiological adaptations and increased aerobic energy metabolism (18,29). The metabolic response of the muscle to endurance training has been estimated by measuring markers of oxidative capacity with an increase in the activities of enzymes involved in mitochondrial metabolism (14,19,45). This suggests that the maximal capacity of mitochondria, in particular, may be increased by exercise training. Our results demonstrate that the MTV program increased muscle oxidative capacity more than the LTV program (Figures 1A, 1B, and 1C). Aerobic exercise is accompanied by increased VO\(_2\), which may increase mitochondrial enzymes activity. It is well known that muscle adaptation to regular exercise involves mitochondrial biogenesis and synthesis of new components of the respiratory chain to match increased energy demands...
It is possible that increased mitochondrial enzyme activity is a consequence of the higher energy demand induced by training in the mitochondria. Results published in the literature also demonstrate that the adaptive response to aerobic exercise can be explained by increases in mtDNA (20,21,29). These data indicate that the MTV exercise of 45 min per exercise training session is better than the LTV exercise of 15 min per exercise training session in terms of the increase in mitochondrial respiratory chain enzymes activities in skeletal muscle.

It is well known that intense exercise increases the formation of reactive oxygen species (ROS) (39), causing lipid peroxidation and protein oxidation. Lipid peroxidation is the end result of a damaging radical chain reaction that usually begins with a single hydrogen abstraction from an unsaturated fatty acid (42). Oxidation of proteins is the result of damaging ROS, which induce the oxidation of arginine, lysine, threonine, or proline amino acid residues and generate protein carbonyls (38). It is also clear that moderate regular exercise is likely to cause adaptations to antioxidant and oxidative damage repair systems. Previous studies have shown the decrease in oxidative damage following physical training (39-41,44,45). However, the volume of physical training has not been investigated.

Our results demonstrated that the LTV decreases oxidative damage more than the MTV (Figures 2A and 2B). It is possible that the shorter the exercise programs, the lower the production of ROS and, consequently, the lower the oxidative damage. The decrease in oxidative damage induced by exercise can be explained by several mechanisms. First, exercise training increases the activity of the proteasome complex, the induction of repair mechanism (38). Second, chronic exercise enhances heat shock protein accumulation within skeletal muscle and may prevent oxidative damage (43). Third, exercise causes the activation of NF-κB and, consequently, increases the expression of important enzymes associated with defense against ROS (15).

Exercise training has been reported to increase antioxidant defenses under chronic conditions (30,31,33,34). Superoxide dismutase (SOD) is the major defense upon superoxide radicals, and is the first defense line against oxidative stress. SOD represents a group of enzymes that catalyze the dismutation of O$_2^\bullet-$ and the formation of H$_2$O$_2$. Catalase (CAT) is second defense line against oxidative stress. CAT converts H$_2$O$_2$ into water and oxygen (12). The results of the present investigation demonstrate that SOD/CAT activity increased similarly in both programs (LTV and MTV). Chronic contractile activity also appears to influence the ability of muscle to detoxify superoxide and peroxide hydrogen, with increases in skeletal muscle total activity SOD/CAT (28,33). Depending on the characteristics of the training program, it is possible that alterations in SOD/CAT activity occur due to an increase in mRNA levels (25).

ROS may potentially act as signaling molecules to cause adaptations in muscle antioxidant capacity. For example, increased ROS production (mainly H$_2$O$_2$) induced by training can cause a transient activation of major signaling pathways such as MAPK and NF-κB, increasing active antioxidant enzyme (23). It has been established that SOD/CAT promoter contains NF-κB and AP-1 binding sites, which are sensitive to ROS stimulation (17,23). In the present study, both training programs (MTV and LTV) and training duration did not influence enzyme activity. Ji (22) suggests that contractile activity upregulates antioxidant defense systems and that ROS are essential in this adaptation in skeletal muscle. In fact, an increase
in catalase activity can control H$_2$O$_2$ concentration; whereas, upregulation of superoxide dismutase (SOD) reduces the possibility of forming hydroxyl radical through Haber–Weise reaction.

CONCLUSIONS

In the present study, we showed that the MTV increases mitochondrial enzyme activity more than the LTV, although the LTV decreased oxidative damage more than the MTV. Antioxidant enzyme activity (SOD/CAT) increased similarly in both programs.

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REFERENCES


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