Regular physical exercises decrease the oxidant pulmonary stress in rats after acute exposure to mineral coal

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ABSTRACT

Several studies have pointed the regular low to moderate intensity physical exercise an important agent to combat the oxidant stress. The purpose of this study was to investigate the effect of the physical exercise in the pulmonary oxidant response after inhaling mineral coal dust. Twenty-four male Wistar rats (200-250 g) were randomly divided in two groups with their respective controls (trained, n = 6; non-trained, n = 6). All animals received mineral coal dust via tracheal instillation (saline 3 mg/0.5 ml, 3 days/week for 3 weeks), or 0.5 ml of 0.9% saline solution. Forty-eight hours after the last instillation, the trained group was submitted to a progressive exercise program on treadmill for twelve weeks (up to 17 $m.min^{-1}$, 50 min/day^{-1}, 10% inclination). Forty-eight hours after the last training session, every animal was killed by decapitation, and their lungs and soleus were surgically removed for later biochemical analysis. The citrate synthase activity was determined in the soleus muscle, and the damages on the lipids and proteins were assessed into the lungs by means of the TBARS concentration and by determining the carbonyl groups, respectively. The results show that the regular practice of physical exercises reduces significantly the present TBARS levels in trained rats, decreasing the oxidation levels in proteins of both groups compared to their respective controls. The results lead us to suggest that the regular physical exercise on treadmill is an agent capable to soften the pulmonary oxidant damages caused by inhaling mineral coal particles.

Keywords: Physical training. Oxidant damages. Lungs. Free radicals.

INTRODUCTION

Inhaling occupational and atmospheric pollutants, such as mineral coal, has contributed significantly to the appearance and progression of several respiratory disorders, such as infection, inflammations, pneumoconiosis, chronic bronchitis, among others.

Recent studies[1-4] have shown that the pulmonary change after inhaling industrial particles is mediated mainly by the formation of reactive Oxygen specimens (ROS). According to Tao and co-workers[5], such biochemical response leads to the macrophage activation and to the recruitment of polymorphonuclear cells that induce to the increase in the inflammatory mediators.

The ROS are normally produced along the cellular metabolism, mainly at the mitochondrial level, and they are fundamental to several cellular processes, such as the cellular defense and signalization. However, the surplus in their production can cause damage to the cellular constituent, as it is involved in several physiological and pathological processes[6,7]. These substances can raise the unhealthy effects of the disease. But it seems quite possible that the long-term physical exercise can improve the defense system against the ROS action[8,9].

The routine therapeutic interventions, especially the pharmacological actions, have significantly contributed to decrease the problem severity, but it presents a few limitations. It is believed that the regular physical exercise exert a positive effect on the cardio respiratory capability and on the pulmonary biochemical response.

Several studies have described the metabolic ways in the ROS production while exercising[9-12], but little is yet known about the influence of the regular physical exercises on the ROS releasing after inhaling mineral particles. The information contained in the literature is only presuppositions that demand further scientific investigations.

Thus, the purpose of this paper was to investigate the effect of the regular physical exercise in the pulmonary oxidant response after inhaling mineral coal dust. It is believed that the disclosure of this aspect is extremely relevant in order to achieve a better understanding on the biochemical phenomenon of the pulmonary oxidant damages as well as its relationship to the physical exercise.

MATERIALS AND METHODS

Every procedure was performed according to the "Guiding Principles in the Care and uses of Animals"[13], and it was duly approved by the Ethics Committee of the Extremo Sul Catarinense University.

Coal preparation: 1 kg of rough mineral coal collected at the Carbonifera Cooperminas located in the city of Criciúma, Santa Catarina State, Brazil. A 300 g sampling was grinded in a sphere-crusher for 3 hours at a 25 Hz frequency. The coal was analyzed at the Ground and Fertilizer Analysis Laboratory of the Extremo Sul Catarinense University, Criciúma, Santa Catarina State, Brazil, and it presented the following mineralogical characteristics: cupper (0.003%), iron (2.5%), zinc (0.003%), and silica (27.3%). The coal particles used in this trial had diameter of up to 15 µm.

Coal instillation: Twenty-four male Wistar rats (200-250 g) were used in the trial protocol. The animals were gathered in specific cages at controlled 22ºC room temperature, 12:12 clare-obscure cycle, and with free access to food and water. To the coal instillation, the animals were ketamine (80 mg/kg of the body mass, i.d.), and zylazine (20 mg/kg of the body mass, i.d.) anesthetized, and they received direct administration by tracheal instillation of 3 mg/0.5 ml of saline or 0.5 ml of 0.9% saline solution once every three days for nine days, in a procedure adapted from the model described by Pinho and co-workers[4].

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The animals were randomly divided in two groups with their respective controls: Trained (TR, n = 6), and Non-trained (NTR, n = 6).

Training protocol: Forty-eight hours after the last coal instillation, every animal was adapted on the fitted treadmill (10 m/min^{-1}, no inclination, 10 min/day^{-1}). The trained groups (coal and saline) were submitted to a progressive training program for 12 weeks with velocity up to 17 m/min^{-1}, time of up to 50 min/day^{-1}, and up to 10% inclination gradually increased. Forty-eight hours after the last training session, every animal was killed by decapitation, and their lungs and soleum were surgically removed and immediately stored at –80ºC for later analysis.

Citrate synthase (CS): The citrate synthase activity was determined from the HS-group using 5. 5' dithiobis (2-nitrobenzoic acid – DTNB)[14]. 100 mg of muscle (soleum) were PGS-homogenized and centrifuged for 10 minutes, 1000Xg, 4ºC. To the reaction, 100 µl of 1 mM-DTNB were used dissolved in 10 ml of 1 M-Tris/HCl (8.1 pH), 30 µl of 10 mM-Acetyl-CoA dissolved in Milli Q water, 20 µl of the sampling, 800 µl of the solution (1 mM-EDTA/Triton X100 0.05%, pH 7.4, 25ºC). The enzymatic activity was measured at 412 nm absorbance for 3 minutes, with 7 minutes incubation. The process was repeated, and the CS activity initiated when 50 µl oxaloacetate dissolved was added to 0.1 M-Tris/HCL. The 13.600 M absorption coefficient was used.

Reactive specimens to the thiobarbiturate (TBARS): As peroxidation index of the lipids, the reactive substances formation was verified upon the warming of the thiobarbiturate acid spectrophotometrically measured (532 nm), and expressed as the malondialdehyde (MDA) (nmol/mg protein)[15].

Protein carbonylation: The oxidizing damages of proteins were measured through the determination of the carbonyl grouping based on the reaction with the dinitrophenylhydrazine[16].

The carbonyl content was spectrophotometrically determined in 370 nm using a 22000 M coefficient.

Protein determination: The total quantity of protein was determined using the Lowry technique[17].

Statistical analysis: The data was expressed in mean and standard deviation, and they were statistically analyzed using the *t* Student test to analyze the citrate synthase (CS) activity, as well as the one-way variance analysis (ANOVA) followed by the Tukey's post hoc test to the oxidizing damage assays. The significance level set for the statistical tests was $p < 0.05$. The SPSS (Statistical Package for the Social Sciences) version 10.0 was used as statistical package.

Reagents: Thiobarbituric acid, DTNB, acetyl-CoA, oxaloactate, dinitrophenylhydrazine, adrenaline, and Hydrogen peroxide were acquired from Sigma Chemical (St. Louis, MO).

RESULTS

Citrate synthase (CS): The activity of the CS was used as indicator for the activities of the physical exercises. The results (table 1) indicate that the training program performed on the treadmill was sufficient to increase the oxidant capability of the skeletal muscle (TR = 0.58 ± 0.01 U/mg protein, NTR = 0.32 ± 0.04 U/mg protein, $p < 0.05$).

TABLE 1
Citrate synthase activity in skeletal muscle (soleum) in trained and non-trained rats

Group	Mean	EPM
Trained (TR)	0.589 U/mg protein*	0.010
Non-trained (NTR)	0.327 U/mg protein	0.042

* $p < 0.05$

Lipoperoxidation: As the level of the oxidant damage in the membrane's lipids, the TBARS formation was assessed after exposure to mineral coal. As it is seen in figure 1, the results indicate a significant increase in the lipoperoxidation in rats exposed to the coal (coal = 0.123 ± 0.025 nmol/mg protein; Control = 0.082 ± 0.014 nmol/mg proteins, $p < 0.05$).

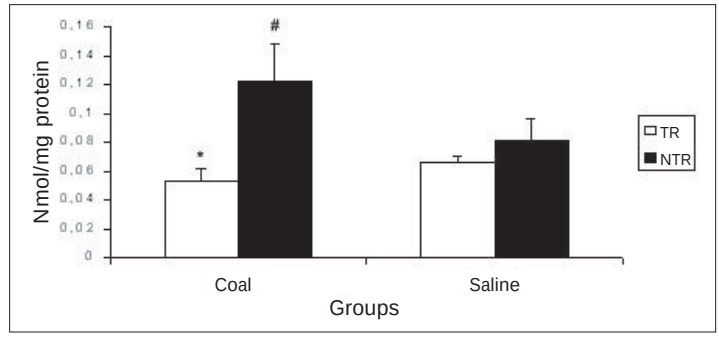

***Figure 1** – Lipoperoxidation in lungs of trained (TR) and non-trained rats (NTR) after exposure to mineral coal. The values are presented in Mean ± EPM, and the results were expressed in nmol of TBARS/mg proteins. The significant differences between the control* and non-trained# groups was $p < 0.05$.*

The results also show that the regular practice of physical exercises reduces significantly the TBARS formation in trained rats compared to non-trained rats (TR = 0.053 ± 0.009 nmol/mg protein, and NTR = 0.123 ± 0.025 nmol/mg protein, $p < 0.05$).

Protein carbonylation: In order to check the oxidant damages to proteins, the carbonyl groups were assessed based on the reaction to the dinitrophenylhydrazine. According to figure 2, the results do not show any significant difference in the protein damage in rats after exposure to mineral coal compared to the Control group (coal = 0.407 ± 0.075 nmol/mg protein, and Control = 0.249 ± 0.027 nmol/mg protein). Also, it was verified that the regular physical exercise decreases the oxidant response caused by the exposition to the mineral coal (TR = 0.136 ± 0.010 nmol/mg protein, and NTR = 0.407 ± 0.075 nmol/mg protein, $p < 0.05$).

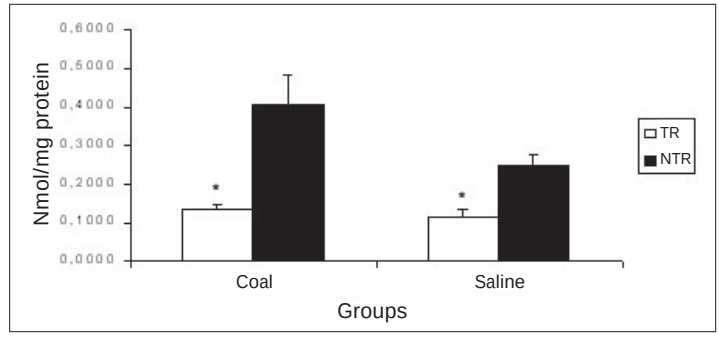

***Figure 2** – Protein carbonylation in lungs of trained (TR) and non-trained rats (NTR) after exposure to mineral coal. The values are presented in Mean ± EPM, and the results were expressed in nmol of carbonis/mg proteins. The significant difference between the control* and the non-trained# groups was $p < 0.05$.*

DISCUSSION

The citrate synthase is a regulatory enzyme in the production of the aerobic power that catalyzes the oxaloacetate and CoA acetyl condensation for the citrate in the tricarboxylic acid cycle[18]. Several studies have been using the mitochondrial enzyme activity to confirm or not the influence of the physical exercise in the oxidant adaptation of the skeletal muscle in rats[10,19,19]. But few studies have demonstrated the CS response after the endurance training on treadmill.

It is observed on table 1 a significant increase in the CS activity in the soleum muscle of rats after a training program. Likewise, Alessio and Goldfarb[20] have shown that the training on treadmill in young rats increases the CS activity in the gastrocnemius twice more than in the control group.

In another study, Rádak and co-workers[12] have shown an important increase in the CS activity in different skeletal muscles in young and middle age rats after a nine week training in water. Although our study confirms these results, other studies mentioned by Pereira and co-workers[21] has presented opposite responses. Probably such difference between these studies is due to the different types of muscular fibers used or the different training protocols or in the methods used to determine the enzymatic activity or even to the differentiated molecular adaptations (translational and post-translational modifications) in the CS adaptation during and post-physical training[22].

Recent studies[4,5,23,24] show that the pulmonary response by occupational and atmospheric pollutants such as the coal, causes a inflammatory chain-response mediated by the macrophage activation, and the recruitment of polymorphonuclear cells, cytosine, chymosin, and reactive Oxygen specimens (ROS). After inhaling these pollutants, the ROS production can be significantly increased, causing damages in the cellular constituents[2].

The oxidant response to the physical exercise is determined by the type, frequency, duration and intensity, that by one side can generate the ROS formation which is able to cause cellular damages and inflammation[25], and on the other hand, the regular physical endurance exercise can be the most efficient antioxidant defense system, establishing a balance between the damages induced by the ROS and the antioxidant repairing systems[26].

Therefore, due to both end points observed in the oxidant response to the physical exercise, the results attained through the physical training are not yet conclusive. In this study, we raised the hypothesis that the regular and continuing physical aerobic exercises can be an agent capable to soften the protein and lipid damage in the membrane caused by the exposure to mineral coal.

The data on the lipids initiated from their oxidation result from free radical reaction with polyunsaturated fatty acids present in the lipoproteins of the membranes. The changes in the lipoperoxidation markers are more frequent evidences observed into the pulmonary tissue after exposure to atmospheric pollutants[4], and in the response to the physical exercise[26].

The results attained in our study show that the regular physical exercise presented a significant decrease in the pulmonary lipoperoxidation levels in rats exposed to the mineral coal after physical training (figure 1). It is possible that these results are due to the levels of the antioxidant enzymes. Nevertheless, although such results are important, Oh-Ishi and co-workers[11] suggest that the effect of the physical training on the reduction of the lipoperoxidation levels is not as significant as the improvement in the ability of the tissue in resisting to the lipoperoxidation effects induced by the own physical exercise.

The ROS can change the amino acid by chain reactions through the protein aggregates susceptible to proteolytic degradations. Along the process, some amino acids are converted in carbonyl derivates[7].

The exposure to mineral coal stimulates the production of the carbonyl groups[4], and in this specific case, it can be softened by the regular practice of physical exercise, according to results showed in this study.

According to figure 2, it is observed a significant reduction in the protein carbonylation after the exercise program. Although without using atmospheric pollutants to induce pulmonary damages, Radák and co-workers[12] showed opposite results. The endurance training induces a significant rising of the protein oxidation in the rats' lungs. It is possible that these differences between studies can be related to four aspects: first, the use or not of damage-stimulating agents may present different responses induced by the physical exercise; second, the methodology used to determine the protean oxidation levels; third, the type, duration, and intensity of the exercise used; fourth, it is possible that these differences are related to the reduction in the rate of the protein turnover. It is well established in the literature that the modified or oxidized proteins are less degraded by proteosomes. This hypothesis is reinforced by the increasing proteosome activity as a secondary fitting process to the protein oxidation after the exercise[27]. Although it is yet a hypothesis, it may be the main explanation to the differences found in several studies.

Concluding, the results presented in this study show evidences that the regular physical exercise on treadmill can be an agent capable of soften the pulmonary oxidant damages induced by the inhalation of mineral coal particles.

All the authors declared there is not any potential conflict of interests regarding this article.

REFERENCES

1. Dörger M, Allmeling AM, Kiefmann R, Münzing S, Mesmer K, Krombach F. Early inflammatory response to asbestos exposure in rat and hamster lung: role of inducible nitric oxide synthase. Toxicol Appl Pharmacol 2002;181:93-105.
2. Castranova V, Porter D, Millecchia L, Ma JY, Hubbs AF, Teass A. Effect of inhaled crystalline silica in a rat model: time course of pulmonary reactions. Mol Cell Biochem 2002;234:177-84.
3. Mossman BT. Introduction to serial reviews on the role of reactive oxygen species (ROS/RNS) in lung injury and diseases. Free Radic Biol Med 2003;34:115-6.
4. Pinho RA, Bonatto F, Andrades M, Frota ML Jr, Ritter C, Klamt FF, et al. Lung oxidative response after acute coal dust exposure. Environ Res 2004;96:290-7.
5. Tao F, Gonzalez-Flecha B, Kobzik L. Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med 2003;35:327-40.
6. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-22.
7. Repine JE, Bast B, Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1997;156:341-57.
8. Leaf DA, Kleinman MT, Hamilton M, Deitrick R. The exercise-induced oxidative stress paradox: the effects of physical exercise training. Am J Med Sci 1999; 317:295-300.
9. Powers SK, Ji LL, Leeuwenburgh C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med Sci Sports Exerc 1999;31: 987-97.
10. Ji LL, Stratman FW, Lardy HA. Enzymatic down regulation with exercise in rat skeletal muscle. Arch Biochem Biophys 1988;263:173-9.
11. Oh-Ishi S, Kizaki T, Ookawara T, Sakurai T, Izawa T, Nagata N, et al. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 1997;156:1579-85.
12. Radák Z, Kaneko T, Tahara S, Nakamoto H, Ohno H, Sasvári M, et al. 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 1999;27:69-74.
13. Olert ED, Cross BM, McWillians AA. Guide to care and use of experimental animals. 2nd ed. Ottawa: Canadian Council on Animal, 1993.
14. Alp PR, Newsholme EA, Zammit VA. Activities of citrate synthase and NAD+-linked and NADP+-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. Biochem J 1976;154:689-700.
15. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421-31.
16. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186:464-78.
17. Lowry OH, Rosebough NG, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
18. Siu PM, Donley DA, Bryner RW, Alway SE. Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. J Appl Physiol 2003;94:555-60.
19. Lawler JM, Powers SK, Van Dijk H, Visser T, Kordus MJ, Ji LL. Metabolic and antioxidant enzyme activities in the diaphragm: effect of acute exercise. Respir Physiol 1994;96:139-49.
20. Alessio HM, Goldfarb AH. Lipid peroxidation and scavenger enzymes during exercise: adaptative response to training. J Appl Physiol 1988;64:1333-6.

21. Pereira B, Costa Rosa LFB, Safi DA, Medeiros MHG, Curi R, Bechara EJH. Superoxide dismutase, catalase, and glutathione peroxidase activities in muscle and lymphoid organs of sedentary and exercise-trained rats. Physiol Behav 1994; 56:1095-9.

22. Leek BT, Mudaliar SR, Henry R, Mathieu-Costello O, Richardson RS. Effect of acute exercise on citrate synthase activity in untrained and trained human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 2001;280:R441-7.

23. Vallyathan V, Goins M, Lapp LN, Pack D, Leonard S, Shi X, et al. Changes in bronchoalveolar lavage indices associated with radiographic classification in coal miners. Am J Respir Crit Care Med 2000;162:958-65.

24. Zhang Q, Huang X. Induction of ferritin and lipid peroxidation by coal samples with different prevalence of coal workers' pneumoconiosis: role of iron in the coals. Am J Ind Med 2002;42:171-9.

25. Liu J, Yeo HC, Övervik-Douki E, Hagen T, Doniger SJ, Chu DW, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 2000;89:21-8.

26. Carmeli E, Laviam G, Reznick AZ. The role of antioxidant nutrition in exercise and aging. In: Radák Z, editor. Free radicals in exercise and aging. Champaign: Human Kinetics, 2000;73-115.

27. Grune T, Reinheckel T, Davies KJ. Degradation of oxidized proteins in mammalian cells. FASEB J 1997;11:526-34.