

Impact of the Training Program on Lipid Profile and Cardiac Health

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ABSTRACT

The aim of this study was to investigate the effects of training programs on serum lipid profile and myocardial oxidative stress. Male *Wistar* rats (2 mo-old) were divided into three groups (n=8): sedentary (S), loadless trained (T) and trained-overload 2% body weight (TL). T and TL were trained through swimming for 9 weeks. T and TL rats had increased myocardial lipoperoxide (TBA) and lipid hydroperoxide (HP), whereas HP was higher in TL than in T animals. Superoxide dismutase (SOD) activities were lowest in TL. Myocardial glutathione peroxidase (GSH-Px) was lower in TL than in T and S rats. TL decreased HDL-cholesterol and increased LDL-cholesterol. The serum lactate dehydrogenase and TBA were increased, while SOD and GSH-Px activities were decreased in TL rats. Loadless training was able to improve HDL-cholesterol and to reduce LDL-cholesterol. In conclusion, the loadless training program induced beneficial effects on lipid profile, while overload training induced dyslipidemic profile that was associated with serum oxidative stress. The overload training program was deleterious relative to loadless training program, increasing myocardial oxidative stress.

Key terms: Training, lipid profile, oxidative stress, heart, serum

INTRODUCTION

Exercise has been recommended as a therapeutic measure with a significant role in preventive medicine and cardiac health (15, 24, 33). However, it is questionable whether all training programs are really safe with respect to cardiac function. Regular aerobic training appears to enhance myocardial performance (30): Isolated exercise sessions may elicit acute transient cardiovascular responses (34), and exhaustive exercise may contribute to alterations in heart processes, resulting in further injury (17, 19).

Reactive oxygen species (ROS) and free radicals are mediators of several forms of tissue damage, such as ischemic injuries (1) and cardiac damage (7, 8, 9, 12, 29). Antioxidant defense cell systems protect against or minimize oxidative damage induced by ROS, but there is conflicting information on the effects of exercise and training in these antioxidant defenses (17, 18, 19, 28, 35).

Although usually ascribed to dyslipidemic profile and atherosclerosis (1), myocardial dysfunction has been reported in the absence of increased serum lipids (5, 10, 20). Because there is a

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relationship between oxidative stress and myocardial disease with or without dyslipidemic profile, studies on the effects of different types of training programs on lipid profile and myocardial oxidative stress are of particular interest.

Thus, the aim of this study was to investigate the effects of the training program on lipid profile and myocardial oxidative stress.

MATERIALS AND METHODS

The Ethical Committee for Conduction of Animal Studies at the Faculty of Medicine, University of São Paulo State (UNESP), approved the experimental protocol and all animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care, as outlined in the "Guide to the Care and Use of Experimental Animals." Male *Wistar* rats (24 animals, 2 months old) were fed a Purina rodent chow (3074 SIF, Purina Ltd., Campinas, S.P., Brazil) and water *ad libitum*. They were housed in groups of four animals, in polypropylene cages in an environmentally-controlled clean-air room, with a temperature of $22 \pm 3^\circ\text{C}$, 12 h light/dark cycle and a relative humidity of $60 \pm 5\%$. The rats were randomly divided into three groups (n=8): sedentary-control group (S), loadless trained (T), and trained overload 2% body weight (TL). Swimming was performed in two 100 cm high plastic containers, and the water temperature was $32 \pm 1^\circ\text{C}$.

Swimming was selected as a model for exercise performance because it appears to belong to the natural behavior of rodents and humans. It is less stressful and can avoid foot injury as in the case of a treadmill, which may generate ROS unrelated to exercise (28). The trained groups swam in the morning, four animals each group, five days/week, for nine weeks in progressive depths of 10-50 cm during the first week. The training time was increased by 15 min each week (35). At the fourth week the trained animals swam for 60 min/day and were divided into T and TL groups until the end of the study. The load

was attached to the thorax and updated once a week. Loading 2% body weight was used to increase exercise intensity (28). At the end of the 9-week experimental period, all animals were sacrificed by decapitation (35). The sedentary-control group was sacrificed at rest at the same time as the others animals.

The blood was set into a centrifuge tube and allowed to clot to obtain the serum. The serum was used for total protein (23), triacylglycerol (26), total cholesterol (26), high-density lipoprotein (HDL-cholesterol) (26), low-density lipoprotein (LDL-cholesterol) (13) and lactate dehydrogenase (LDH, E.C.1.1.27.) (26) determinations.

The heart was removed, and the cardiac adipose tissue was discarded. The heart was weighed, and the left ventricle (LV) was separated and weighed. In this experiment the biochemical determinations were carried out on the LV (myocytes) free of external adipose tissue. Samples of 200 mg of LV were homogenized in 5 ml of a cold phosphate buffer (0.1M, pH 7.4). Tissue homogenates were prepared in a motor-driven Teflon glass Potter Elvehjem, tissue homogenizer (1 min, 100 rpm) immersed in iced water. The homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant was used for protein, lipoperoxide (TBA), lipid hydroperoxide (HP), glutathione peroxidase (GSH-Px, E.C.1.11.1.9.) and superoxide dismutase (SOD, E.C.1.15.1.1.) determinations.

Lipoperoxide (TBA) was measured by the thiobarbituric acid reactive substances (32). Lipid hydroperoxide (HP) was determined with mixtures containing 0.25 mM FeSO_4 , 0.1 mM xylenol orange and 4 mM butylated hydroxytoluene (16). GSH-Px was assayed using phosphate buffer 0.15 M, pH 7.0, containing 5mM EDTA (ethylenediaminetetracetic acid), 0.1 ml of 0.0084 M NADPH, 0.005 ml GSSG-reductase (Sigma), 0.01 ml of 1.125 M NaN_3 (sodium aside) and 0.1 ml 0.15 M GSH (glutathione reduced form) (27). SOD activity was determined based on the ability of the enzyme to inhibit the reduction of nitro blue tetrazolium (NBT), which was generated by hydroxylamine 37.5 mM (Carlo Erba, Milan, Italy) in alkaline solution (6). The

chemicals were from Sigma Chemical Co. (St. Louis, MO, USA). Spectrophotometric determinations were performed in a Pharmacia Biotech Spectrophotometer (Cambridge, England, UK).

Results are presented as means \pm standard deviation (SD). Significance of difference was tested by analysis of variance (ANOVA) and Tukey test to compare the treatment groups with each other, not just the control. The probability of 0.05 was chosen as the significant level (25).

RESULTS

TL decreased the body weight gain and the final body weight (bw), but no significant alterations were observed in heart weight (hw) and hw/bw ratio of TL in relation to S and T animals (Table I). No significant changes were observed in body weight gain, final body weight, hw and hw/bw ratio of T group from S. Table II shows that training induced a significant increase in TBA and HP myocardial levels, whereas

TABLE I

Characteristics of sedentary (S), loadless trained (T) and trained overload 2% body weight (TL) rats.

Parameters	S	T	TL
Final body weight (bw) (g)	474.0 \pm 13.0 ^c	447.0 \pm 46.0 ^c	438.0 \pm 22.0 ^{ab}
Body weight gain (g)	79.3 \pm 10.8 ^c	67.1 \pm 1.3 ^c	50.4 \pm 3.6 ^{ab}
Heart weight (hw) (g)	1.32 \pm 0.11	1.38 \pm 0.12	1.30 \pm 0.05
Hw /bw (g/ kg)	2.78 \pm 0.3	3.09 \pm 0.12	2.97 \pm 0.15

Data are means \pm SD.

^a Significantly different from S group, $p < 0.05$.

^b Significantly different from T group, $p < 0.05$.

^c Significantly different from TL group, $p < 0.05$.

TABLE II

Lipid hydroperoxide (HP), lipoperoxide (TBA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in cardiac tissue of sedentary (S), loadless trained (T) and trained overload 2% body weight (TL) rats.

Biochemical determinations	S	T	TL
HP (nmol/g tissue)	44.70 \pm 5.3 ^{bc}	57.47 \pm 5.13 ^{ac}	73.92 \pm 10.61 ^{ab}
TBA (nmol/g tissue)	15.22 \pm 1.82 ^{bc}	24.48 \pm 4.31 ^a	21.26 \pm 3.83 ^a
SOD (U/mg protein)	15.49 \pm 2.17 ^{bc}	9.26 \pm 1.40 ^{ac}	6.79 \pm 0.83 ^{ab}
GSH-Px (U/g tissue)	32.61 \pm 3.8 ^c	33.24 \pm 5.44 ^c	21.69 \pm 6.07 ^{ab}

Data are means \pm SD.

^a Significantly different from S group, $p < 0.05$.

^b Significantly different from T group, $p < 0.05$.

^c Significantly different from TL group, $p < 0.05$.

HP concentration was higher in TL than in T animals. The SOD activities were significantly lower in T than in S. TL rats had the lowest myocardial SOD activity. No alterations were observed in myocardial GSH-Px activities in T from the S group, whereas GSH-Px was lower in TL than in S and T-groups.

No significant alterations were observed in total protein, triacylglycerol and cholesterol serum concentrations. Loadless training decreased LDL-cholesterol and increased HDL-cholesterol. TL animals showed increased LDL-cholesterol and decreased HDL-cholesterol serum concentrations. The LDH activities were higher in the TL group. No alterations were observed in HP serum concentrations. The TBA serum concentrations were increased in TL, while SOD and GSH-Px activities were

decreased in these animals. No alterations were observed in HP, TBA, SOD and GSH-Px activities of T animals in relation to S group (Table III).

DISCUSSION

The general population frequently performs regular physical activity, with different types of training programs to maintain good cardiovascular health. Therefore, the effects of the training programs need to be evaluated. Many studies support the notion that regular exercise training has a protective cardiac effect (2, 21, 35), but in most of these studies the exercise effects have been analyzed independently of the intensity. The present study differs from previous research, in that it was designed to

TABLE III

Total protein, triacylglycerol, cholesterol, LDL-cholesterol (LDL), HDL-cholesterol (HDL), lactate dehydrogenase (LDH), lipoperoxide (TBA), lipid hydroperoxide (HP), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in serum of sedentary (S), loadless trained (T) and trained overload 2% body weight (TL) rats.

Biochemical determinations	S	T	TL
Total protein (mg/dl)	7.10± 1.27	7.35 ± 0.24	6.98 ± 0.62
Triacylglycerol (mg/ dl)	68.15 ± 6.32	71.56 ± 6.88	67.3 ± 3.54
Cholesterol (mg/dl)	81.4 ± 3.57	82.54 ± 1.89	77.3 ± 5.49
LDL (mg/dl)	34.64 ± 4.19 ^{bc}	27.22 ± 2.98 ^{ac}	40.72 ± 2.23 ^{ab}
HDL (mg /dl)	33.13 ± 1.36 ^{bc}	41.11 ± 2.27 ^{ac}	23.12 ± 2.51 ^{ab}
LDH (U)	138.42 ± 5.07 ^c	145.76±1.43 ^c	212.89±5.81 ^{ab}
TBA (nmol/dl)	12.23 ± 1.96 ^c	11.53 ± 0.16 ^c	17.09 ± 1.92 ^{ab}
HP (nmol/dl)	6.30 ± 0.75	5.62 ± 0.16	5.68 ± 0.37
SOD (U/mg protein)	0.71 ± 0.24 ^c	0.82 ± 0.18 ^c	0.43 ± 0.13 ^{ab}
GSH-Px (U/ml)	93.5± 5.80 ^c	81.01±6.92 ^c	36.17 ± 1.16 ^{ab}

Data are means ± SD.

^aSignificantly different from S group, p<0.05.

^bSignificantly different from T group, p<0.05.

^cSignificantly different from TL group, p<0.05.

mimic a free-living situation, where physical activity is performed with different types of training programs. In this study, although no significant changes were observed in body weight, body weight gain, hw and hw/bw index between sedentary and trained rats, the TL animals showed decreased body weight gain and final body weight (Table I). Serum LDH activities were significantly increased in TL animals, indicating cellular damage and reflecting the stress on endothelial cells (Table III).

In several organs, cell membrane damage is followed by the release of a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basis for clinical diagnosis (14). Enzymes are liberated from tissues as soon as a physical-chemical alteration has occurred. Since LDH activity is used for myocardial disease diagnosis, we can assume that the training program of TL group was sufficiently elevated to induce myocardial alterations, suggesting that the animals of this group were over-trained.

In recent years, a considerable interest has been shown in the deleterious action of oxygen radicals on the heart (7, 8, 9, 12, 29). This concept may be regarded as a paradox because myocardial function is strictly dependent upon oxygen delivery and use. However, in some situations, oxygen is converted to ROS, which can elicit widespread damage to cells such as lipoperoxidation (22). In this study, two indices of lipoperoxidation were measured. This involves the abstraction of hydrogen from fatty acid side chains by ROS, forming conjugated dienes that oxidize to lipid hydroperoxide (HP), and subsequently undergo cleavage to aldehydes, some of which react with TBA (1,11). TBA and HP concentrations were significantly increased in cardiac tissue of both trained groups. It is evident that both training programs had a higher workload than normal, which leads to an imbalance of the oxidant/antioxidant systems. These observations were demonstrated with antioxidant enzymes (Table II). The SOD activity was significantly decreased in response to the training (T group). Nevertheless, within the same group no changes were observed in

GSH-Px activities. It is interesting to note that when rats trained with load (TL) they showed the lowest SOD and GSH-Px activities. SOD removes superoxide radical by reducing them to hydrogen peroxide. GSH-Px catalyses the conversion of hydrogen peroxide to water (1). Therefore, our results support the idea that the alterations in antioxidant enzymes were not sufficient to inhibit the oxidative stress and the lipoperoxidation in cardiac tissue of both trained rats (T and TL).

On the other hand, Table II shows similar TBA values in T and TL animals, while HP concentration was markedly lower in T than in the TL group. These observations indicated that over-training, as occurred in the TL group, accelerated the breakdown of HP and increased aldehyde production. These observations are substantially important in explaining the adverse alterations on serum lipids (1, 4). Lipid hydroperoxides give rise to a large variety of products, many of which react with TBA and with serum lipids (1). Oxidation of LDL from hydroperoxide products may have substantial importance on dyslipidemic profile. Zadeh et al., (36) showed that LDL-cholesterol is the major carrier of lipid hydroperoxide in plasma. Oxidative modification of LDL is the key step in the sequence of events leading to atherosclerosis (4). Since serum TBA and LDL-cholesterol concentrations were increased in TL animals, we can assume that increased lipoperoxidation was associated to LDL-cholesterol. The HDL-cholesterol is extensively degraded due oxidative processes (3). The reduced SOD and GSH-Px activities in the serum were the result of load training. While no alterations were observed in HP serum concentrations, the increased TBA in the serum of TL animals indicated that they were largely affected by training intensity and suggested that substances that react with thiobarbituric acid were liberated to the blood from the tissues. These observations explained why the T rats had decreased myocardial HP levels and no differences were observed in myocardial TBA levels between T and TL animals.

The most significant risk indicators for cardiovascular alterations, which are

considered to be parameters of oxidative stress (1), are increased serum cholesterol, triacylglycerol, LDL-cholesterol (36) and decreased HDL-cholesterol (31). Various theories have suggested that cardiovascular damage was the result of an oxidative stress process (31, 36). Loadless training had beneficial effects on serum lipids (Table III). The beneficial effects of loadless training were demonstrated in relation to LDL and HDL-cholesterol. The T animals showed decreased LDL and increased HDL-cholesterol. These effects were not observed when the training was made with load. Note that the TL animals showed decreased HDL and increased LDL-cholesterol (Table III).

In conclusion, the effects of exercise on lipid profile and markers of oxidative stress were dependent on the training program performed. Loadless training programs induced beneficial effects on the serum lipid profile, while load training induced dyslipidemic profiles that were associated with serum oxidative stress. The training program with overload 2% body weight was deleterious relative to loadless training program, increasing myocardial oxidative stress.

REFERENCES

- ABUJA PM, ALBERTINI RA (2001) Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clin Chim Acta* 306: 1-17
- ATALAY M, SEN CK (1999) Physical exercise and antioxidant defenses in the heart. *Ann N Y Acad Sci* 874: 169-177
- BRADAMANTE S, BARENGHI L, GIUDICI GA (1992) Free radicals promote modifications in plasma high-density lipoprotein. *Free Radic Biol Med* 12: 193-202
- BRIZZI P, TONOLO G, CARUSILLO F, MALAGUARNERA M, MAIOLO M, MUSUMECI S (2003) Plasma lipid composition and LDL oxidation. *Clin Chem Lab Med* 41: 56-60
- CECONI C, BORASO A, CARGNONI A, FERRARI R (2003) Oxidative stress in cardiovascular disease: myth or fact? *Arch Biochem Biophys* 420: 217-221
- CROUCH R, GANDY SE, KINSEY G (1981) The inhibition of islet superoxide dismutase by diabetogenic drugs. *Diabetes* 30: 235-241
- DINIZ YS, CICOGNA AC, PADOVANI CR, FAINE LA, GALHARDI CM, RODRIGUES HG, NOVELLI ELB (2003) Dietary restriction and fiber supplementation: oxidative stress and metabolic shifting for cardiac health. *Can J Physiol Pharmacol* 81: 1042-1048
- DINIZ YS, CICOGNA AC, PADOVANI CR, SANTANA LS, FAINE LA, NOVELLI ELB (2004). Diets rich in saturated and polyunsaturated fatty acids: metabolic shifting and cardiac health. *Nutrition* 20: 230-234
- DINIZ YS, FAINE LA, ALMEIDA JA, SILVA MDP, RIBAS BO, NOVELLI ELB (2002) Toxicity of dietary restriction of fat enriched diets on cardiac tissue. *Food Chem Toxicol* 40: 1893-1899
- DOI K, SAWADA F, TODA G, YAMACHIKA S (2001) Alteration of antioxidants during progression of heart disease. *Free Radic Res* 34: 251-261
- ESPOSITO LA, MELOV S, PANOVA A, WALLACE B (1999) Mitochondrial disease in mouse results in increased oxidative stress. *Proceedings National Academy Sci* 96: 4820-4825
- FAINE LA, DINIZ YS, ALMEIDA JA, NOVELLI ELB, RIBAS BO (2002) Toxicity of *ad libitum* overfeeding: effects on cardiac tissue. *Food Chem Toxicol* 40: 663-668
- FRIEDWALD WT, LEVY RI, FREDRICKSON DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma. *Clin Chem* 18: 494-502
- GAW A, COWAN RA, O'REILLY DSJ, STEWART MJ, SHEPHERD J (2001) *Bioquímica Clínica*. 2nd ed. Rio de Janeiro: Guanabara Koogan
- GRANDJEAN PW, STEPHEN FC, ROHACK JJ (2000) Influence of cholesterol status on blood lipid and lipoprotein enzyme responses to aerobic exercise. *J Appl Physiol* 89: 472-480
- HERMES-LIMA M, WILLMORE WG, STOREY KB. (1995) Quantification of lipid peroxidation in tissue extracts based on Fe (III) xylenol orange complex formation. *Free Radic Biol Med* 19: 271-280
- JÓZKO AF, FAFF J, SIERADZAN-GABELSKA B (1996) Changes in concentrations of tissue free radical marker and serum creatine kinase during the post-exercise period in rats. *Eur J Appl Physiol* 74: 470-474
- KIM JD, YU BP, LEE SY, HERLIHY JT (1996) Exercise and diet modulate cardiac lipid peroxidation and antioxidant defenses. *Free Radic Biol Med* 20: 83-88
- KUMAR TC, REDDY VK, PRASAD M, THVAGARAJU K, REDDANNA M (1992) Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress. *Mol Cell Biochem* 111: 109-115
- LEFER DJ, GRANGER N (2000) Oxidative stress and cardiac disease. *Am J Med* 109: 315-323
- LEICHTWEIS SB, LEEWENBURG C, PARMELEE DJ, FIEBIG R, JI LL (1997) Rigorous swim training impairs mitochondrial function in post-ischaemic rat heart. *Acta Physiol Scand* 160: 139-148
- LIU J, YEO HC, OVERVIK-DOURI E, HAGEN T, DONIGER SJ, CHU DW (2000) Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol* 89: 21-28
- LOWRY OH, ROSENBROUGH NJ, FARR AL (1951) Protein measurement with folin-phenol reagent. *J Biol Chem* 193: 265-275
- MEILHAC O, RAMACHANDRAN S, CHIANG K, SANTANAM N, PARTHASARATHY S (2001) Role of arterial wall antioxidant defense in beneficial effects of exercise on atherosclerosis in mice. *Arteriosclerosis Thrombosis Vasc Biol* 21: 1681-1688
- MONTGOMERY DC (1991) Design and analysis of experiments. 3rd ed., New York: John Wiley
- MOURA RA (1982) *Técnicas de laboratório*. 2nd ed., São Paulo: Atheneu
- NAKAMURA W, HOJODA S, HAYASHI K (1974) Purification and properties of rat liver glutathione peroxidase. *Biochem Biophys Acta* 358: 251-61

28. NAKAO C, OOKAWARA T, KIZAKI T, OH-ISHI S, MIYAZAKI H, HAGA S, SATO Y, JI LL, OHNO H (2000) Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues. *J Appl Physiol* 88: 649-654
29. NOVELLI ELB, FERNANDES AAH, CAMPOS KE, DINIZ YS (2002) The adverse effect of a high energy dense diet on cardiovascular tissue. *J Nutr Environ Med* 12: 287-294; 2002
30. PALMER BM, THAYER AM, SNYDER S, MOORE RL (1998) Shortening and [Ca²⁺] dynamics of left ventricular myocytes isolated from exercise-trained rats. *J Appl Physiol* 85: 2159-2168
31. RAVEH O, PINCHUK I, FAINARU M (2001) Kinetics of lipid peroxidation in mixtures of HDL and LDL mutual effects. *Free Radic Biol Med* 31: 1486-1497
32. SATOH K (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 90: 37-43
33. TAYLOR RP, CICCOLO JT, STARNES JW (2003) Effect of exercise training on the ability of the rat heart to tolerate hydrogen peroxide. *Cardiovasc Research* 58: 575-581
34. THOMPSON PD, CROUSE SF, GOODPASTER B, KELLEY D (2001) The acute versus chronic response to exercise. *Med Sci Sports Med* 33: 438-445
35. VENDITTI P, DI MEO PL (1996) Antioxidants, tissue damage, and endurance in trained and untrained young male rats. *Arch Biochem Biophys* 331: 63-68
36. ZADEH JN, SARMADI JT, LINH KLD (1996) Low-density lipoprotein is the major carrier of lipid hydroperoxides in plasma. *Biochem J* 313: 781-786