Aerobical Physical Activities Improve Quality of Cardiac Health in an Animal Model: Parameters of Calorimetry, Profile Lipids and Oxidative Stress

Pedro OB*, Klinsmann CS, Camila B and Ana Angélica HF
São Paulo State University, São Paulo, Brazil

Abstract
Occidental life styles, including physical inactivity, are related to risk of developing heart diseases. To prevent or even mitigate these effects, the frequent recommendation has been the practice of physical exercise as a measure of therapeutic interaction in preventive medicine and rehabilitation. Thus, our study was carried out to evidence the effects of aerobic physical exercise regularly practiced, through swimming, calorimetry, lipid profile, and oxidative stress in myocardium. Thirty-two Winstar rats, males, 75 days old were used. Animals were divided into two groups: C (n=8) as the control, which received food and water ad libitum and TR (n=8) as the trained group received the same treatment as C and animals in this group were submitted to swim. Swimming was used as a model of exercise with moderate intensity training for eight weeks. The experimental design was completely randomized with 16 treatments and eight replications, with a significance level of 5% probability. Calorimetry shows lower carbohydrate oxidation and increased lipid oxidation in C when compared with TR. The trained group had higher glucose, higher HDL-cholesterol and lower accumulation of triglycerides, and verified the protective effect in cardiac tissue. Thus, we can conclude that the swimming protocol was effective in controlling deleterious effects caused by poor habits on the myocardium.

Keywords: Myocardium; Oxidative stress; Physical exercise

Introduction
Worldwide, cardiovascular diseases are the main cause of death in the occidental lifestyle [1]. In the countries of America, 75% of deaths are associated with heart diseases [2].

The risk of developing heart diseases can be explained by side-reactions of the metabolism, which has been shown to promote oxidative stress through the elevated reactive oxygen species (ROS) production and/or reduced antioxidant defense that provokes the decrease of health and well-being of biological systems and their functions [3-6].

According to biochemical theories, lipotoxicity may be an important target for the deleterious action of free radicals and trigger oxidative stress in the cardiac tissue [7,8], due to the imbalance between lipid entry in cardiac tissue and its ability to oxidize them [9].

In addition, it was possible to evidence that, in young people, risk factors for the development of diseases were the high serum concentration of total cholesterol and low density lipoprotein C (LDL-C) and low high-density lipoprotein C (HDL-C) [10]. Salvaro and Avila Jr [11] analyzed profile lipids of women and verified an association between sedentariness and high level of LDL-C, triglycerides (TG) and total cholesterol on the blood, beside the low level of HDL-C.

A non-pharmacological treatment is physical exercise. The positive effects obtained by practicing physical activity upon health, in general, are unquestionable. Among implications for the cardiovascular system, there may be the reduction of total cholesterol, LDL-C, TG and HDL-C, besides the contribution to the glycemic control, for the physiological responses related to the increase of the metabolic demand and to the oxygen supply associated with muscular expenses [12].

The beneficial effects of exercise practiced regularly on metabolic disorders and directly on the heart tissue [13,14] decreases the chances of dying from cardiovascular disease by the reduction of risk factors, such as hypertension and hyperlipidemia, and by practicing physical activities; it also enhances longevity by mechanisms independent of these risk factors [15,16]. In addition, the protocol of the moderate intensity of exercise can promote improvement in diastolic function in rats [17] and generates a physiological molecular pattern, different from that in pathological cardiac hypertrophy [18].

The prescription of the exercise involves bouts between 60 and 100% VO2 max heart rate [19], training volumes and rest periods to lead to benefits of physical activity [19,20].

However, inspite of the consensus regarding direct and indirect beneficial effects provided by the practice of physical activity, concerning the cardiovascular parameters, our study was carried out to evidence the effects of aerobic physical exercise regularly practiced, through swimming, calorimetry, lipid profile and the oxidative stress in myocardium.

Material and Methods
Experimental animals
Male Winstar rats [200-250 g, bw] aging 75 days were maintained in polypropylene cages under constant temperature [24 ± 1°C], 12 h light/dark cycle and relative humidity of 55 ± 5%. They, received standard chow [Purina Labina, Campinas/SP, Brazil, containing by weight: 22.0% protein, 3.8% fat, 44.5% carbohydrate and 3.81 kcal/g of metabolizable energy] and water ad libitum. Experimental procedures were approved
[protocol no. 421] by the Ethical Committee for Conduction of Animal Studies at the Institute of Biosciences of Botucatu, Universidade Estadual Paulista [UNESP], in accordance with guidelines of the Canadian Council on Animal Care as outlined in “Guide to the Care and Use of Experimental Animals”. The animals were randomly divided into two groups [n=8]: sedentary control [C] and group submitted to swimming sessions, considered trained [TR]. Food and water intake was daily measured [9:00-10:00 a.m.] and body weight was weekly measured.

Training protocol

The rats were submitted to swim in a rectangular polyethylene tank with dimensions 80 cm deep, 80 cm wide and 100 cm length, containing water at 31 ± 1°C. The adaptation period was initiated at a depth of 10 cm of water for 10 minutes, which gradually increased 10 cm/day and 10 min/day, for six consecutive days. After this period, the animals initiated swimming training sessions at 60 cm deep for 60 min daily [always between 18:00 and 19:00 p.m.], five times per week during eight weeks [experimental period]. The exercise intensity was obtained by placing small weights in the thoracic region of the animal, corresponding 5% of body weight. This load was previously established by determining the anaerobic threshold into progressive test swimming [21], ensuring therefore the physical exercise of moderate intensity.

Parameters calorimetrics

The parameters calorimetrics were obtained in rats, fasted overnight (for 12-12 h) confined in metabolic cages (air flow=1.01/min) coupled to calorimetric (CWE, Inc, St. Paul, USA), after experimental period of 56 days. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were registered by respiratory-based software program (MMX, CWE, Inc., USA) every 5 min for 20 min. The resting metabolic rate (RMR), respiratory quotient (RQ) and oxidation of carbohydrates were calculated from VO2 and VCO2 according to Strohl et al. [22].

Morfometric and nutritional parameters

After obtaining the calorimetric measurements, the animals were anesthetized (0.1 mL/100 g body mass of 2:1 solution of 10% ketamine chloride and 2% xylazine) for measurement body length, which was used to determine Lee index (LI) [g/cm]=cube root of body weight/length. Total energy intake (EI, kcal/day=mean food consumption per day [g] × metabolizable energy of the ration [kcal/g]) and feed efficiency (FE, g/kcal=weight gain [g]/EI) were calculated according to Kawahara et al. [23] and Santos et al. [24].

Obtaining serum samples and cardiac tissue samples

After corporal measurements, animals were anesthetized with 10% of cetamin and 2% xilazin and euthanized by decapitation. Firstly, after obtaining the calorimetric measurements, the animals were anaesthetized (0.1 mL/100 g body mass of 2:1 solution of 10% ketamine chloride and 2% xylazine) for measurement body length, which was used to determine Lee index (LI) [g/cm]=cube root of body weight/length. Total energy intake (EI, kcal/day=mean food consumption per day [g] × metabolizable energy of the ration [kcal/g]) and feed efficiency (FE, g/kcal=weight gain [g]/EI) were calculated according to Kawahara et al. [23] and Santos et al. [24].

Estimation of oxidative stress markers

Lipid hydroperoxide (HP) was estimated through the oxidation of ferrous ion, which in the presence of xilenol orange led to the formation of a Fe²⁺-xilenol orange complex, then measured at 560 nm [25]. Superoxide dismutase [SOD, EC 1.15.1.1] activity was assayed as described elsewhere [26], through the inhibition of the reduction of nitro blue tetrazolium [NBT] in the presence of reduced nicotinamide adenine dinucleotide (NADH) and phenazine. The amount of enzyme that gave 50% inhibition of NBT reduction/mg protein was taken as one unit of enzyme activity. Glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was indirectly determined by measuring the consumption of NADPH during the reduction of oxidized glutathione (GSSG) in a reaction catalyzed by glutathione reductase, one unit of enzyme was defined as the amount required to oxidize 1 umole GSH/min, which corresponded to 0.5 amol NADPH oxidized/min [27].

Statistical analysis

Data normality was confirmed using the Shapiro-Wilk test. Results are presented as the mean ± standard deviation. Comparisons between groups were performed by analysis of variance (t-student test). For all the analyses, the level of significance was set at p<0.05 (Sigma Plot 12.0, USA).

Results

According to Table 1, the trained group (TR) gained less weight than the control group [C], but it is not enough to evidence a statistically significant value (p>0.05). The TR group showed for the Lee index, feed intake, energy intake and energy efficiency of this study higher absolute values when compared with the C group, but it is not effectively and statistically different (p>0.05). Changes in calorimetric parameters are illustrated in Figure 1. Firstly, the TR group showed extremely increase in the parameters VO2 and RQ, which evidenced statistically difference between TR and C groups (p<0.01). Secondly, the C group showed lower values for the parameters VCO2, RMR and oxidation of carbohydrates, which resulted in statistical significance (p<0.05) and thirdly the parameter oxidation of lipids showed homogeneity between TR and C groups, which means that there is not a statistical significance (p>0.05).

On the blood, this study analyzed Glucose, Total Cholesterol, LDL-C, VLDL-C, TG and HDL-C, as shown in Table 2. The TR group have shown statistical significance to the Glucose when compared with C group (p<0.05). Total Cholesterol and LDL-C statistically differed C group (p<0.01) from TR group. Serum levels of VLDL-C and TG showed statistical significance between the groups of this study (p<0.05). TR group increased the plasmatic level of HDL-C (p<0.001) when compared with C group. The trained group (TR), lipids hydroperoxide (HP) and glutathione peroxidase (GSH-Px) activity decreased significantly when compared with control group (C), while the activity of superoxide dismutase (SOD) and catalase (CAT) were not effect to promote a statistical significance (p>0.05) when comparing C and TR groups (Table 3).

Discussion

In fact, the benefits of physical exercise are already well known in the literature regarding the prevention and control of risk factors. They need, however, a more comprehensive view and a specific detail of their relation with the pathophysiology of diseases. Thus, it is essential

![Table 1: Body weight (Wt), Lee index (LI), feed intake (FI), energy intake (EI) and energy efficiency (EE), observed between different experimental groups.](image-url)
Significant difference between control (C) and trained group (TR)
*: p<0.05; ** p<0.01

**Figure 1:** Consumption of $O_2$ (VO$_2$) (A) Production of CO$_2$ (VCO$_2$) (B) Respiratory quotient (RQ) (C) Metabolic rate basal (TMB) (D) Oxidation of carbohydrates (E) Oxidation of lipids (F) Observed between different experimental groups.

<table>
<thead>
<tr>
<th>Glucose mg/dL</th>
<th>Total cholesterol mg/dL</th>
<th>LDL-C mg/dL</th>
<th>VLDL-C mg/dL</th>
<th>TG mg/dL</th>
<th>HDL-C mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 75.98 ± 7.70</td>
<td>151.10 ± 13.76**</td>
<td>87.88 ± 9.20**</td>
<td>31.78 ± 7.23*</td>
<td>158.8 ± 36.14*</td>
<td>30.06 ± 3.82</td>
</tr>
<tr>
<td>TR 85.27 ± 8.64*</td>
<td>84.13 ± 9.80</td>
<td>21.14 ± 6.10</td>
<td>22.01 ± 6.03</td>
<td>110.06 ± 30.16</td>
<td>40.98 ± 5.24**</td>
</tr>
</tbody>
</table>

Significant difference between control (C) and trained group (TR)
*: p<0.05; ** p<0.01

**Table 2:** Plasmatic levels of glucose, total cholesterol, LDL-C, VLDL-C, TG, HDL-C, for the experimental groups.

<table>
<thead>
<tr>
<th>HP nmol/g</th>
<th>SOD nmol/mg</th>
<th>CAT µmol/g</th>
<th>GSH-Px nmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 238.69 ± 16.66</td>
<td>7.62 ± 1.13</td>
<td>96.09 ± 17.72</td>
<td>46.43 ± 5.70b</td>
</tr>
<tr>
<td>TR 205.63 ± 18.43*</td>
<td>7.32 ± 0.95</td>
<td>108.66 ± 28.66</td>
<td>59.82 ± 8.59*</td>
</tr>
</tbody>
</table>

Significant difference between control (C) and trained group (TR)
*: p<0.05

**Table 3:** Cardiac tissues concentration of hidroperoxide of lipids, activities in the cardiac tissue of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px).
to identify the factors and to know the effects of physical exercise on modulating cardiovascular risks, with the predominant purpose of medical conduct and the treatment of cardiopathy individuals [5,15,16].

Physical exercise can cause deviation in the homeostasis of an organism leading to reorganization of responses in different organ systems [21,28,29]. Intensity, duration and frequency of exercise have a key role in determining the metabolic responses to the effort, and may increase it or reduce it. It is recommended the intensity of physical exercise between mild to moderate, regularly performed, for the prevention of cardiovascular diseases [24,30]. Studies by Delmondes et al. [31] and Figueira et al. [32] also did not perform direct measurement of lactate and considered intense exercise as used when an overload of 5% of body weight, considering as reference the study by Gobatto et al. [21] and sorting the intense term as intense aerobic, in other words, corresponding to maximum steady state of blood lactate, which is the upper limit of heavy exercise domain.

Understanding factors related to morphometry is essential in the prevention of cardiovascular diseases because it allows better knowledge of the complications associated with overweight and diets [33]. The body weight has homogeneity between C and TR groups. Given this, it may be suggested that the influence of exercise on body weight loss is dependent on the feeding behavior, as exercised animals fed ad libitum diet showed no decrease in body weight. These results are consistent with McArdle et al. [34]. It is possible to observe that the Lee index also showed statistical difference between the groups. This result is closely related to the study by Rutledge and Adeli [35] regarding that the weight gain may be viewed not just as a result of a positive energy balance, but also as the mechanism by which the energy from energy substrates are metabolized and stored and consequently causes differences between groups.

Although it is known that the regular practice of physical exercise causes greater energy expenditure, in this study it is interesting to observe that the exercise program had no effect on feed and energy intake. However, feed efficiency has not changed between the groups. Thus, it is suggested that the intensity of moderate exercise did not affect the feeding behavior of groups.

Indirect calorimetry is a way to determine the use of energy substrates in the presence of O₂ and release of CO₂ by the analysis of air inhaled and exhaled by the lungs [36]. The amount of carbon dioxide produced, compared with the oxygen consumed during the oxidative processes, ranges according to the oxygen content present in oxidizable substrate. The catabolism of triglycerides in adipose tissue provides fatty acids into the blood stream and thus to oxidize them for energy tissues. Hence, the fatty acids are relatively small molecules (CH₃[CH₂]ₙ-COOH) compared with carbohydrates (C₆H₁₂O₆) and tissues require more oxygen to oxidize lipids. Moreover, complete oxidation of fatty acids for oxidative phosphorylation generates large amount of ATP, it contributes to further increase and the need for oxygen and reduce the ratio phosphate/oxygen (P/O), which reflects the number of ATP molecules produced by low oxygen atom in the mitochondrial electron transport chain [37].

The physical activity protocol used in this study normalized lipid oxidation rate due to less difference between VO₂ and VCO₂ observed in the TR group compared with the C group and the difference was smaller. Experimental studies have reported that aerobic physical activity increased oxygen consumption rate in patients with metabolic syndrome [38]. It is suggested that physical exercise was efficient to increase carbohydrate oxidation in TR tissues, which differ statistically from the C group.

Although lipolysis is a metabolic process stimulated by catecholamines during physical activity and therefore higher amounts of fatty acids available to be oxidized [39-41], animals of the trained group increased carbohydrate oxidation compared with the control group, suggesting greater balance in the oxidation of energy substrates. Melanson et al. [42] showed that the oxidation of lipids in individuals submitted to aerobic activity did not differ statistically from those physically inactive. The authors pointed out that such evidence could lead to the conclusion that the increased oxidation of lipids reported by some research may not represent the actual increase in the use of lipids as energy substrate. On the other hand, Bordenave et al. [43] reported that physical exercise of moderate intensity improved the ability of the organism to oxidize lipids, which was associated with the greatest oxidative capacity in skeletal muscle of patients.

The relation VCO₂/VO₂, was increased in trained animals, which showed rapid increase in RQ, indicating change in lipid metabolism and carbohydrate. Although the preference for oxidize carbohydrates or lipids depends on the intensity and duration of exercise, consensus seems to increase the RQ (VCO₂/VO₂) in the conditions proposed in this study [44]. Accordingly, Wisloff et al. [45], Wisloff et al. [46] and Kemi et al. [47] found an increase in RQ in rats submitted to moderate intensity training program.

The practice of aerobic training in animals of the TR group was not effective in reducing blood glucose to serum obtained for the C group. This result contrasts the study by Luciano et al. [48] and Pauli et al. [49], which report that exercises normalized glucose homeostasis by increasing the response of cells to insulin action such as muscle and fat. The molecular mechanism that causes the cellular internalization of glucose-mediated insulin associated with exercise training may be particularly related to the expression and activity of key regulatory proteins known as the glucose metabolism, resulting in improvement in insulin sensitivity.

Vedala et al. [50] and Vougari et al. [39] propose that regular exercise has been adopted as a strategy to reduce the harmful effects caused by poor life style and heart attacks, both associated with metabolic disorders. The basis for this strategy is evidenced mainly by the reduction of dyslipidemia, such as reduced levels of triglycerides, total cholesterol and low density lipoproteins (LDL-cholesterol) and increase high density lipoprotein (HDL-cholesterol) cholesterol [50,51]. Thus, changes in lifestyle are of particular interesting because the physical inactivity predisposes individuals to cardiovascular adverse events, most myocardial infarction index [52].

Physiological mechanisms based on exercise induce a positive lipid profile resulting from complex interactions involving hormones, enzymes and receptors. Studies suggest increase in lipoprotein lipase activity in skeletal muscle and adipose tissue while conducting moderate-to-intense physical efforts, and some post-exercise time, associated with the possible decrease in the hepatic synthesis of TG, can generate metabolic adjustments that favor lower concentrations of plasma lipids among the most physically active subjects [10,11,53].

Exercise was efficient in causing beneficial changes in lipid profile, which can also be demonstrated in human being [54].

The cholesterol serum concentration is dependent on exogenous source, from the diet, such as endogenous (synthesis) and this occurs when there is availability of acetyl-CoA molecules, a decrease in total cholesterol levels in animals if the TR group compared with the C group, which can be attributed to periods of systematic training that the animals of TR group were submitted. Such conditions also reflected in
some way at low levels of LDL-cholesterol. The training protocol used in this study was able to reduce the level of total cholesterol and LDL-cholesterol in serum, regardless of feeding behavior, to less than those obtained for the control group. These findings are confirmed by the study of Ishikawa et al. [55] who observed decrease in the concentration of both total cholesterol and LDL-cholesterol during aerobic exercise. In addition, the literature reports that exercise decreases the likelihood of present values for these parameters considered normal according to what was previously mentioned [56].

The concentration of triglycerides and VLDL-cholesterol in C group showed a significant change compared with TR group, suggesting that hepatic synthesis of triacylglycerols and consequently of VLDL-cholesterol lipoprotein, has been changed due to regular practice of physical exercise the TR animals were submitted. Kraus and Slentz reported that the triglyceride level was lower in individuals subjected to the intensity of exercise.

Approximately 90% of the triglycerides are synthesized in the liver and secreted incorporated into VLDL lipoprotein cholesterol. Beside this, Kraus and Slentz [56] reported that high levels of triglycerides and VLDL-cholesterol in the bloodstream indicates elevated hepatic lipogenesis (re-esterification of fatty acids), which can be related to training of exhaustive exercises, which can lead to excessive lipolysis. By comparison, the training protocol used in this study may explain the differences between the results obtained by these authors.

According to Otsuka et al. [6] and Ogasawara et al. [40], it is possible to relate heart disease and heart failure with triglyceride accumulation, due to the high uptake of fatty acids by the heart tissue at the expense of oxidation [56]. While the swim protocol used in this study attenuated deposit triglycerides in animals treated with diet ad libitum [TR]. These results are consistent with the literature. Bruce et al. [57] reported that exercise training is associated with fatty acid oxidation in peripheral tissues, limiting the abnormal accumulation in tissues. The triglyceride content in skeletal muscle and gastrocnemius medium in subcutaneous adipose tissue decreased in trained subjects for eight weeks compared with sedentary group [57,58].

The concentration of HDL cholesterol increased in trained rats [TR], which may indicate that moderate aerobic exercise protocol caused benefit in elevation of HDL-cholesterol. These results are consistent with Kraus and Slentz [56] and Rocco et al. [59] to report that exercise increased the HDL - cholesterol fraction and prevented the development of atherosclerosis.

Lipid accumulation in cardiac tissue may be a target of reactive oxygen species (ROS) during the lipid peroxidation process and formation of lipid hydroperoxide (HP), which has been an important biomarker of oxidative stress [53]. The result of this study evidenced a lower concentration of HP in the myocardium of the exercised animals. Considering that oxidative phosphorylation associated with mitochondrial electron transport is an important source of ROS, which may induce lipoperoxidation, the improvement in production of HP by the exercised animals may be due to a greater control in the oxidation of fatty acids and, therefore, the probability of producing EROs in the electron transport chain.

For the removal of EROs the cells depend on endogenous enzymatic antioxidant systems, represented by superoxide dismutase, which is the first line of defense against the action of ROS and catalyzes the conversion of O$_2^-$ (the first free radical formed from the partial reduction of oxygen) into H$_2$O$_2$, which is reduced to H$_2$O by glutathione peroxidase and catalase [60].

While in the TR group the activity of SOD was normalized, the activities of GSH-Px and CAT were increased in comparison with the C group. These results suggest lower H$_2$O$_2$ production and simultaneously higher conversion of these hydrogen peroxides into H$_2$O, indicating stabilization in the lipoperoxidation process, as verified by the values obtained for HP in these animals. Some authors related the beneficial effect of the daily aerobic training exercise, increasing the endogenous antioxidant defenses and myocardial resistance to lipoperoxidation and greater protection of the cardiac tissue against oxidative stress [53,61].

Considering that the flow of electrons increases in the mitochondria of the myocytes due to the demand of the redistribution of blood during the muscle contraction period, there is evidence that the benefit of physical activity against the oxidative stress depends basically on the intensity of the training [19,20].

**Conclusion**

It is important to avoid sedentariness as a life style. Based on these results, the regular practice of aerobic physical activity may promote beneficial changes in the calorimetric parameters and the lipid profile, which reduces the appearance of factors related to cardiac diseases from sedentary lifestyle.

**References**


