Interval and Continuous Exercise Training Produce Similar Increases in Skeletal Muscle and Left Ventricle Microvascular Density in Rats

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1. Introduction

Regular practice of moderate aerobic exercise is widely recognized to reduce cardiovascular risk [1]. Since acute exercise is the most important stimulus for increasing myocardial demand, it is not surprising that major structural and functional adaptations occur in the heart consequent to chronic regular exercise training. These adaptations include higher efficiency in myocardial substrate utilization and energetic [2] and enhanced antioxidative stress capacity [3], both potentially involved with the protection against ischemia/reperfusion myocardial damage [3] and infarction [4] developed after exercise training.

Along with metabolic and biochemical myocardial adaptations, major vascular growth and remodeling occur in coronary circulation after exercise training. Enhanced angiogenesis, that is, capillary growth, and vascularization along with altered regulation of coronary blood flow are well-known features of physiological adaptations to chronic exercise and have been extensively revised [5–7]. The balance between metabolic demand and oxygen delivery is maintained along the weeks of training by a dynamic and stepwise combination of early angiogenesis followed by an increase in small and larger arterioles, in part as a consequence of capillaries developing into small arterioles [8].

The increased capillary network and coronary blood flow play an important role in improving aerobic capacity and facilitating oxygen transport, conductance, and muscle extraction, therefore contributing to increase of maximal oxygen uptake and physical performance [3, 9, 10]. Capillary growth in skeletal and cardiac muscle is a complex process mediated by several metabolic alterations occurring during muscle activity and factors linked to increased shear stress induced by blood flow and passive stretch of the vascular smooth muscle tissue [5, 11, 12].
Increasing the intensity of exercise, blood flow also increases [2, 13]. Thus it would be expected that exercise at higher intensities provides a greater stimulus for capillary growth than that of moderate exercise. Surprisingly, the chronic effects of intense or extraneous exercise on myocardial vasculature have been sparsely studied [14, 15] and yet no specific impact of exercise intensity can be derived from those, since the total exercise volume was not controlled. In other words, if an exercise training regime is employed leading to an increased total training volume, a resulting greater physiological adaptation could be attributed to the higher total amount of work and not to the specific impact of the intensity itself. On the other hand, a series of well-planned and well-conducted studies have matched the total amount of work in order to investigate the influence of exercise intensity itself on a variety of different myocardial measures such as substrate utilization, oxygen consumption [2], and ventricular oxidative stress [4] in rats.

Therefore, despite the physiological and clinical relevance of myocardial vasculature and the unknown effects of higher exercise intensity on this variable, no studies have so far provided evidence on the comparisons between the effects of training regimes of different intensities but the same volume. Thus, the aim of the present study was to assess the effects of two different aerobic exercise programs on myocardial capillary density where exercise intensity was different, but total duration and amount of work were carefully matched. To accomplish this purpose, we submitted a group of Wistar rats to interval training (IT), consisting of alternated periods of high and low intensity exercise and compared the adaptations in skeletal muscle and myocardial microvasculature to those observed in rats submitted to a moderate intensity and continuous exercise protocol (CT) and a control sedentary group.

2. Methods

2.1. Animals. The experiments were performed with 12- to 14-week-old male WKY rats (Wistar Kyoto, Oswaldo Cruz Foundation animal facilities, Brazil). The animals were housed with controlled light (12:12 h light-dark cycle) and temperature (22 ± 1°C) with free access to water and standard rat chow until the day of the experiment. All of the procedures were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (protocol no. P 0034-08) and are consistent with the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised 1996).

2.2. Experimental Protocols. Thirty WKY rats were randomly assigned to an interval training (IT), continuous training (CT), or sedentary group (SED). The exercise performance tests were conducted immediately before experiment initiation, at six weeks after initiation of the experiment, and at the end of the training program. The intensity of the training program was adjusted after six weeks of training. Twenty-four hours after the last session of exercise training, the rats were anesthetized for intravital microscopy procedures; afterwards, the animals were deeply anesthetized and sacrificed, and the heart and gracilis muscle were immediately removed for subsequent analysis.

2.3. Training Program. The exercise training was performed on a low-speed motorized treadmill (Universidade de São Carlos, São Paulo, Brazil) and consisted of a 13-week period of running 30 min per day at no incline (0%), 4 times a week. The training program was preceded by a 10-day adaptation period to the aerobic exercise, during which the running time and speed of the treadmill were gradually increased from 10 min at 15 m/min to the training schedule described below. The exercise performance tests consisted of graded exercise on the treadmill at no incline, starting at 10 m/min with increments of 1 m/min every 1 min up to the maximal running speed attained by each rat (exhaustion). In the CT group, the treadmill speed was incrementally increased to attain 70% of maximal exercise capacity. In the IT group, the animals ran 1 min intervals at 90% of maximal exercise capacity, followed by 1 min intervals at 50% of maximal exercise capacity [16]. Therefore, the total volume of exercise (intensity × duration) of the anesthetic and fluorescent dye. The central temperature was monitored from several random microscopic fields over a total magnification of 100x at the video monitor. After intravenous injection of 0.15 mL of 5% fluorescein-isothiocyanate- (FITC-) labeled dextran (molecular weight 150,000), microscopic images of the right jugular vein was catheterized to permit injection of the anesthetic agents and fluorescent dye. The central temperature was monitored with a rectal probe, and the body temperature was kept at 38 ± 0.5°C with a homeothermic blanket system (Harvard Apparatus, Boston, USA).

The ear skin was scraped, and the animals were placed on their back on a Plexiglas pad. The gracilis muscle was exposed through an incision in the right thigh and was covered with an oxygen-impermeable plastic wrap. The animals were then placed under an upright, fixed-stage intravital microscope (Olympus BX51, WI, USA) coupled to a CCD digital video camera system (Optronics, Goleta, USA). A 10x objective magnification was used, resulting in a total magnification of 100x at the video monitor. After intravenous injection of 0.15 mL of 5% fluorescein-isothiocyanate- (FITC-) labeled dextran (molecular weight 150,000), microscopic images of the muscle and the skin were successively collected, and the capillaries were quantified with Saisam software (Microvision, Evry, France). The functional capillary density, which was defined as the total number of spontaneously perfused-capillaries per square millimeter of surface area (1 mm²), was determined from several random microscopic fields over a period of 4 minutes.

2.5. Histochemical Analysis

2.5.1. Gracilis Muscle. The tissue samples were dehydrated in a graded series of ethanol (70%, 95%, and 100%) and
Table 1: Exercise performance test results and body weight of sedentary (SED), continuous-trained (CT), and interval-trained (IT) rats.

|                | SED     | CT       | IT       | Body weight (g)       |
|----------------|---------|----------|----------|-----------------------|
|                | 0 weeks | 6 weeks  | 13 weeks | 0 weeks | 6 weeks | 13 weeks |
| SED Velocity   | 24.6 ± 1.3 | 19.4 ± 1.1 | 14.6 ± 1.8 | 276 ± 5 | 371 ± 10 | 436 ± 12 |
| CT             | 24.8 ± 1.5 | 28.0 ± 1.0* | 32.3 ± 1.3* | 270 ± 6 | 356 ± 9 | 396 ± 10* |
| IT             | 24.7 ± 1.4 | 28.8 ± 3.3* | 32.8 ± 1.3* | 272 ± 5 | 350 ± 5 | 381 ± 8* |

The values are the mean ± SEM; n = 10 for all groups. *P < 0.05 compared to the sedentary rats (SED).

2.6. Citrate Synthase Activity. Tissue samples from the gracilis muscle were obtained to biochemically analyze citrate synthase activity to determine the effectiveness of the exercise training. The muscle samples were immediately frozen in liquid nitrogen and stored at −80°C until processing. Citrate synthase activity was measured from whole muscle homogenate using the spectrophotometric method published by Srere [19].

2.6.1. Drugs. The following drugs were used: sodium pentobarbital, pancuronium bromide, FITC-labeled dextran, and FITC-conjugated G. simplicifolia I lectin (Sigma Chemical Co., St. Louis, USA).

2.6.2. Statistical Analysis. The results are expressed as the mean ± SD for each group, and comparisons between the different groups were made with one-way analysis of variance (ANOVA). If a significant difference was detected by ANOVA, the Bonferroni test was used to identify the statistically significant differences. Differences with a P value less than 0.05 were considered significant. All calculations were made by computer-assisted analyses with a commercially available statistical package (GraphPad Instat 5.0, GraphPad software).

3. Results

3.1. Energy Expenditure during IT and CT. Total running distance (IT: 496 ± 37 m; CT: 499 ± 60 m; P > 0.05) and total oxygen consumption during the exercise session (IT: 1,075 ± 184 mL; CT: 1,036 ± 299 mL; P > 0.05) were similar between training protocols.

3.2. Exercise Training Efficacy, Body Weight, and Citrate Synthase Activity. After six and 13 weeks of exercise, both exercise methods (CT and IT) induced several changes indicative of a trained state in rats (Table 1 and Figure 1). The maximal velocity achieved in the treadmill endurance test was significantly higher (P < 0.05) in the trained animals than in the SED animals (Table 1); the observed increase in velocity was similar for the CT and IT groups at both six and 13 weeks of training. Additionally, body weight was lower in the CT and IT groups than in the SED group at the end of the training period. Moreover, gracilis muscle homogenate citrate synthase activity was significantly higher in both the CT and IT rats (321±69 and 342±61 nmol/mg/min, resp.; P < 0.001) than in the SED animals (189 ± 100 nmol/mg/min). The percentage increase in citrate synthase activity was of 69.0 ± 38.3 and 81.2 ± 32.9 for the CT and IT groups, respectively (Figure 1). These results indicate that the exercise
programs enhanced the skeletal muscle oxidative capacity of the exercise groups relative to the control group.

3.3. Functional Capillary Density in Skeletal Muscle and Skin. Both the CT and IT groups had a significant increase in the functional capillary density of their skeletal muscle (282 ± 7 and 283 ± 8 capillaries/mm², resp.; P < 0.001), compared with that of the sedentary animals (SED: 216 ± 7 capillaries/mm²). The percentage increase in muscle functional capillary density was of 30.6 ± 11.7 and 28.7 ± 11.9 for the CT and IT groups, respectively (Figure 2). There was no significant difference in the functional capillary density of the skin for any of the groups. The percentage increase in skin functional capillary density was of 17.3 ± 19.1 and 16.3 ± 16.9 for the CT and IT groups, respectively (Figure 2).

3.4. Structural Capillary Density in Skeletal Muscle and the Left Ventricle. Compared to the control group, both exercise training regimens similarly increased the capillary-to-fiber ratio in skeletal muscle (CT: 1.66 ± 0.06 and IT: 1.80 ± 0.09 versus SED: 1.28 ± 0.06; P < 0.001). The percentage increase in muscle structural capillary density was of 28.7 ± 14.4 and 40.1 ± 17.2 for the CT and IT groups, respectively (Figure 3). For the left ventricle, the structural capillary density was evaluated in sections that were obtained with the “orientator” method [17], which allow for the visualization of unbiased and uniformly isotropic structures in anisotropic tissues, such as the heart. The capillary volume density-to-fiber volume density ratio (V̄V[cap]/V̄V[fib]) in the left ventricle of both exercise groups was increased to a similar magnitude, when compared to that in the control group (CT: 0.60 ± 0.19 and IT: 0.56 ± 0.11 versus SED: 0.41 ± 0.14; P < 0.05). The percentage increase in left ventricle structural capillary density was of 57.3 ± 53.1 and 54.3 ± 40.5 for the CT and IT groups, respectively (Figure 3).

4. Discussion

The most important novel findings of the present study are as follows: (1) CT and IT increased exercise endurance and muscle oxidative capacity and attenuated body weight gain to the same extent; (2) CT and IT increased similarly functional and structural alterations in the microcirculation of locomotor skeletal muscle and of the myocardium of rats. These results suggest that IT mode is equivalent to CT in increasing exercise capacity and in inducing microvascular adaptations in both skeletal and cardiac muscles, when total training volume is matched supporting the concept that total energy expenditure, and not exercise intensity per se, is the major physiological stimulus, at least for chronic changes in skeletal and myocardium microcirculation.

The large increase in oxidative enzyme citrate synthase, performance, and capillary density produced by both IT and CT in the present study is consistent with numerous studies of endurance trained athletes and animals [2, 16, 20, 21]. Many cross-sectional studies have demonstrated that trained endurance athletes present skeletal muscle oxidative enzyme activities that are much greater than those of their sedentary counterparts [10, 12, 22]. These increases in oxidative capacity are associated with increases in skeletal muscle mitochondrial content via mitochondrial biogenesis [23] and are consistent with the previously shown relationship between capillarity and mitochondrial content [9, 24, 25].
The present study reveals that in normal rats, IT induces significant changes in the structure and function of the gracilis muscle. These changes are similar to those induced by CT. The structural and functional adaptations within our rats gracilis muscle are consistent with those in earlier reports [21, 37, 41, 42]. For example, the observed values for capillary-to-fiber ratio of IT and CT are in line with previously published reports [2, 16, 20, 35]. Such changes within the muscles explain, at least in part, the substantially increased capacity for aerobic work observed after training.
The present results revealed a marked increase in the number of spontaneously perfused capillaries in locomotor skeletal muscle in both the CT and IT groups as assessed by intravital video microscopy. In contrast, there was no change in the functional capillary density of the ear skin. Thus, these results suggest that both CT and IT increase perfusion only in tissues that are actively participating in exercise and are subjected to repeated increases in metabolic demand, blood flow, and vascular shear stress.

The mechanisms underlying exercise-induced angiogenesis and vasculogenesis in skeletal muscle are well defined [43, 44]. By contrast, angiogenesis in the adult heart is a controversial issue. In general, studies have reported an increase in myocardial capillary density in young animals subjected to endurance training but no change or a decrease in adult animals, even though the training consisted of weeks or months [45]. In a comprehensive study that evaluated the time course of capillary growth during training, the authors reported that cardiac vascular synthesis and remodeling occurred in young animals subjected to endurance training but no change or a decrease in adult animals, even though the training consisted of weeks or months [45]. In a comprehensive study that evaluated the time course of capillary growth during training, the authors reported that cardiac vascular synthesis and remodeling occurred in young animals subjected to endurance training within the first 3 weeks of exercise [8]; thus capillary growth may be an early and transient feature of training that may have been missed by other long-term studies with a single end point. In the present study, even after a long period of either CT or IT, there is still a significant increase in the structural capillary density in the myocardium of exercised rats, compared to that in the sedentary control animals.

Finally, it is worth mentioning that the vascular adaptations, as well as their underlying mechanisms, induced by exercise training, are not consistently distributed along the arterial tree [46]. Moreover, the improvement of vascular endothelial function induced by exercise training is not limited to the active muscle vascular beds such as the skeletal, respiratory, and cardiac muscles [47]. Actually, these adaptations in the vasculature of nonworking skeletal muscle, brain, viscera, and skin can also differ substantially [48]. Thus, the results of our study are limited to active muscle vascular beds including the skeletal and cardiac muscles.

In conclusion, we found that when total exercise volume is matched, interval training that included periods of high intensity exercise produces similar adaptations to moderate and constant exercise with regard to effects on exercise capacity, skeletal muscle citrate synthase effects, and microcirculatory beds of skeletal muscle and myocardium.

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