

Association Between Anabolic Steroids and Aerobic Physical Training Leads to Cardiac Morphological Alterations and Loss of Ventricular Function in Rats



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ABSTRACT

Introduction: Anabolic-androgen steroids (AAS) associated with physical training induce changes from physiological cardiac hypertrophy (CH) to pathological hypertrophy. However, these studies were performed with strength athletes, and the AAS effects associated with aerobic training are still poorly understood. Thus, the aim of this study was to evaluate the effects of aerobic training and AAS on the cardiac structure and function. **Methods:** 28 Wistar rats divided in 4 groups were used: sedentary control (SC), sedentary anabolic (SA), trained control (TC) and trained anabolic (TA). The AAS was administered twice a week (10mg/Kg/week). The swimming training was conducted 5 sessions per week during 10 weeks. We evaluated blood pressure and heart rate by tail plethysmography, ventricular function by echocardiography, cardiomyocyte diameter and collagen volumetric fraction by histological methods. **Results:** There were no differences in BP. TC group showed reduction in rest heart rate after the experimental period, which did not occur in TA group. CH of 38% in SA group; 52% in TC group and 64% in TA group compared to SC group was observed. TA group presented decrease in diastolic function in relation to other groups. The trained groups showed significant increases in cardiomyocytes diameter. SA and TA groups showed increase in collagen volumetric fraction in relation to SC and TC groups. **Conclusion:** The results show that AAS treatment associated to swimming training induces CH, mainly by the increase in interstitial collagen, which can lead to loss of diastolic function.

Keywords: physical training, steroids, cardiac hypertrophy, collagen.

INTRODUCTION

Physical training can induce to several adaptive responses on the cardiovascular system, among these, cardiac hypertrophy (CH), which symmetrically occurs being considered a benefic adaptation to the cardiovascular system⁽¹⁾. CH induced by physical training may occur by different stimuli, depending on the exercise performed, in which exercises with more isometric characteristics end up generating pressoric overload over the heart, which results in hypertrophy of concentric pattern^(2,3). On the other hand, dynamic exercise, performed at moderate intensity in a cyclic manner, leads the heart to stress by increase of volume resulting in eccentric hypertrophy^(1,4).

However, when strength physical training is associated with use of anabolic-androgen steroids (AAS) alteration in the physiological CH has been observed, induced by exercise, to pathological, characterized by cardiac morphological, structural and functional alterations^(5,6).

The cardiac effects induced by the use of AAS has been observed both in animal models^(7,8) and athletes^(5,6). In a study which analyzed the cardiac alterations by echocardiogram, increase of the ventricular mass index and thickness of the intraventricular septum was observed in users compared to non-users, being also observed harm to the diastolic function associated to reduction in the velocity peak during the initial phase of diastolic filling⁽⁹⁾. Further studies^(10,11), observed harm of the diastolic function in weight lifters who used AAS compared to the ones who did not use them. Investigations with ex-users observed that their effects on the mass of the left ventricle and ventricular function persisted even after one year with the drug abstinence⁽¹²⁾.

However, the great majority of the investigations which study the cardiac effects of the AAS are carried out with strength or Power athletes, but athletes from aerobic modalities also make use of AAS⁽¹³⁾ trying to minimize the catabolic actions induced by training, increase the protein synthesis and increase disposition to training due to actions on the central nervous system⁽¹⁴⁾. Nevertheless,

the effects of the AE administration associated with aerobic physical training on the cardiovascular system are still little known. Therefore, the aim of the present study is to evaluate the effects of the AAS and their association with swimming aerobic physical training in rats on the cardiac structure and function.

METHODS

Sample: 28 male Wistar rats with initial weight between 180-250g were used. The animals were randomly divided in four groups: sedentary control (SC, n = 7), sedentary with anabolic steroids (SA, n = 7), trained control (TC, n = 7) and trained treated with anabolic steroids (TA, n = 7). The animals were kept in cages, separated by groups, in the animal facility of the Laboratory of Biochemistry of Motor Activity of EEF/USP with room temperature between 22-24°C and with light controlled at 12-hour cycle (light dark). Water and food were administered ad libitum and the animals were weekly weighted. All procedures were approved by the Ethics Committee of the Physical Education and Sport School of USP according to the guidelines of the Brazilian College of Animal Experimentation.

Treatment: subcutaneous injections of vehicle or nadrolone decanoate (decadurabolin; organon, roseland, NJ) two times a week were administered, totalizing 10mg/kg/week. Doses equivalent to the generally used by athletes (600mg/week)⁽¹⁵⁾.

Swimming training: It was performed according to protocol adapted by Medeiros *et al.*⁽¹⁶⁾, in a swimming system with warm water between 30-32°C, during 10 weeks, with five weekly sessions, with gradual increase of time of the session until it reaches 60 minutes, and of the work overload (weight on the animal's tail) until 5% of body weight is reached. Such protocol was characterized as aerobic training of low intensity and long duration⁽¹⁶⁾.

Blood pressure and heart rate evaluation: BP and HR were weekly checked by non-invasive method of plethysmography of tail artery (indirect BP record). The system AT/CODAS (DataQ Instruments, Inc., Ohio, USA), with sampling frequency of 1,000Hz was used.

Ventricular function evaluation: the ventricular function evaluation was performed by echocardiogram. The echocardiography measurements followed the guidelines by the Mode M Standardization Committee of the American Society of Echocardiography^(17,18). The exams were performed after the experimental period in all the studied groups and they were performed by a single evaluator blind for the animal groups. The exam was performed with the animals intraperitoneally anesthetized with xylazine (10mg/kg) and ketamine mixture (90mg/kg). The Sequóia 512 equipment (ACUSON Corporation, Mountain View, CA), with 15MHz transducer was used.

Systolic fraction was determined by the shortening fraction (SF) and ejection fraction (EF). The images obtained by Doppler were used for determination of the diastolic function (velocity peak of the E wave, velocity peak of the A wave, E/A ratio and IVRT). Moreover, the left ventricle mass was calculated (LVM) according to guidelines from the American Society of Echocardiography^(17,18), which estimates the LVM by using the following mathematic formula: $LVM = [(DDVE + SIV + PP)^3 - (DDVE)^3] \times 1.047$, where 1.047 (mg/mm³) corresponds to the myocardial density.

Morphometric analyses: After the experimental protocol (24 hours after the last training session), the animals were decapitated; the heart was removed from the thoracic cavity and dissected for separating the right ventricle from left ventricle (free wall and septum).

Cardiomyocytes diameter: histological cuts of 5µm thick which were stained with hematoxylin and eosin (HE) for visualization of the cellular structures. Three LV cuts for each animal were randomly selected for visualization on light microscope (Leica Q500 iw and Leica DMLS, *Leica Imaging Systems, Ltda.*, Cambridge, UK) using immersion objective with increase of 40x. Approximately 20 visual fields were determined for each histological cut. The cardiomyocytes selected were those which presented very visible nucleus and intact cellular membrane. The cardiomyocyte image was obtained on the computer screen and its transversal diameter hand drawn, where the traced line crossed the center of the nucleus. The calculation was done by a commercial program (Quantimet 500, Cambridge Instruments).

Cardiac collage volume fraction: histological cuts of 5µm thick were stained with picrosirius red. The cuts were evaluated in the image computer system (Leica Q500 iw and Leica DMLS, *Leica Imaging Systems, Ltda.*, Cambridge, UK), with increase of 20x. Cardiac collage was quantified by the fraction of the collage volume (FCV) calculated by the percentage ratio of the area of the myocardic tissue positively stained for the collagen fibers (absolute quantity of collagen) by the total area of the myocardic tissue. 20 visual fields were examined for each sample.

Statistical analysis: Data were presented as mean ± standard deviation. The statistical analysis was performed with the use of the Statistic Basic software by two-way analysis of variance ANOVA. Pre and post-experimental treatment blood pressure was analyzed by ANOVA for repeated measurements. When significant F was applied, Duncan post-hoc test of multiple comparisons was applied. Pre and post heart rate frequency was analyzed with the paired Student's t test. Significant values accepted were with $p \leq 0.05$.

RESULTS

Significant difference has not been observed in the BP between groups in the pre and post experimental treatment periods (figure 1A). Concerning the HR, differences between groups were not observed between groups in the pre-treatment period. However, the group which performed physical training (TC) presented rest HR reduction after the experimental period. However, when physical training was associated with AAS administration (TA) this decrease was not observed (figure 1B).

The results presented in figure 2 show increase of cardiac mass analyzed by echocardiogram in groups SA, TC and TA compared to the control group, and this increase was of 38% in the SA group, 52% in the TC group and 64% in the TA group. Although the cardiac mass in TA group is not significantly different in groups SA and TC, the AAS association with swimming physical training led to increase even more remarkable of myocardial mass.

Both groups which performed physical training, TC and TA, presented significant increase in the cardiomyocytes diameter

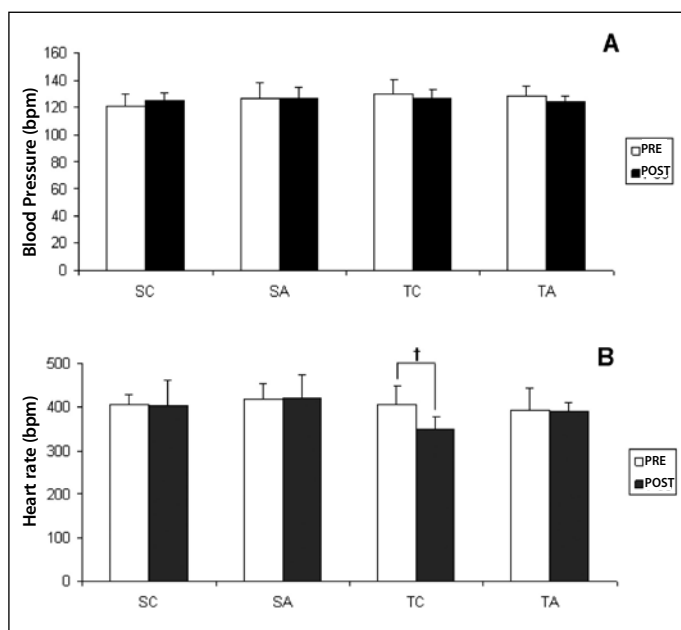


Figure 1. Pre and post-experimental protocol blood pressure (A) and heart rate (B). Sedentary control (SC), sedentary anabolic steroid (SA), trained control (TC), trained anabolic steroid (TA). Data are presented as mean \pm SD. † Significant difference concerning the pre and post-experimental treatment periods, $p < 0.05$.

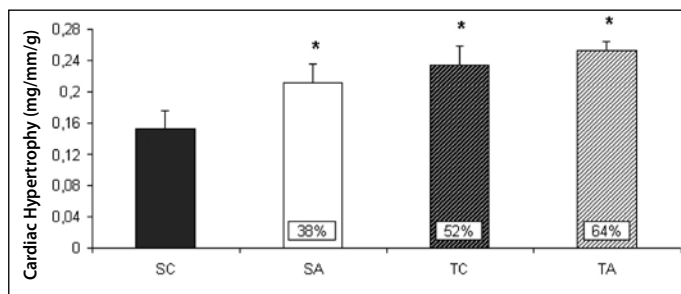


Figure 2. Cardiac mass analyzed by echocardiogram and increase percentage concerning the sedentary control group. Sedentary control (SC), sedentary anabolic steroid (SA), trained control (TC), trained anabolic steroid (TA). Data are represented as mean \pm SD. * Significant difference concerning group SC, $p < 0.05$.

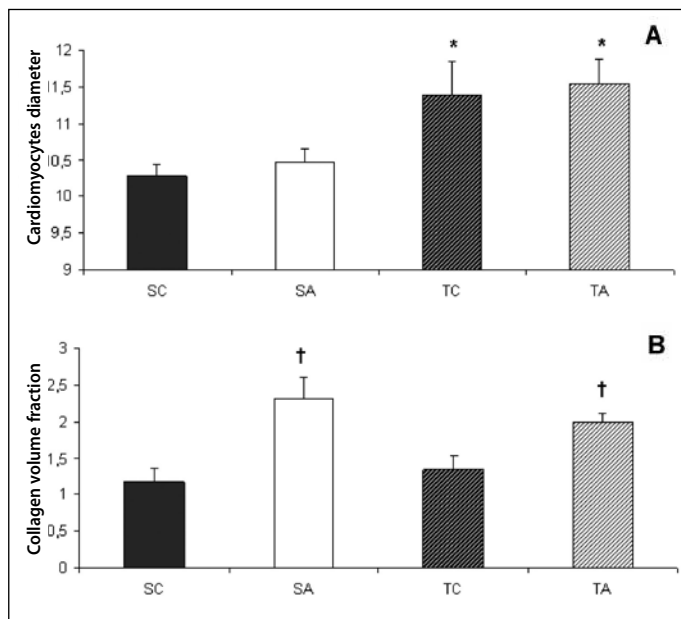


Figure 3. Cardiomyocytes Diameter (A) and collagen volume fraction (B). Sedentary control (SC), sedentary anabolic steroid (SA), trained control (TC), trained anabolic steroid (TA). Data are represented as mean \pm SE. * Significant difference concerning groups SC and CA, $p < 0.05$. † Significant difference concerning groups SC and TC, $p < 0.05$.

when compared to the sedentary groups, SC and SA (figure 3A). Nevertheless, when the collagen volume fraction is analyzed the groups which received treatment EA, SA and TA, presented significant increase compared to the SC and TC groups (figure 3B).

Significant difference has not been observed in the systolic function among the studied groups. However, the group which received AAS associated with swimming training (TA) presented decrease of diastolic function compared to the remaining groups, characterized by reduction in the peak of the E wave and decrease of the E/A ratio (table 1).

Tabela 1: Systolic and diastolic ventricular function indices obtained by echocardiography.

Measurement	SC	SA	TC	TA
Systolic function				
EjF	81.05 \pm 4.38	79.03 \pm 5.17	78.55 \pm 2.95	79.02 \pm 3.76
SF	44.30 \pm 3.44	42.39 \pm 4.09	40.23 \pm 2.74	40.77 \pm 3.78
Diastolic function				
E Peak	0.61 \pm 0.07	0.56 \pm 0.06+	0.62 \pm 0.06	0.42 \pm 0.10*
A Peak	0.43 \pm 0.08	0.40 \pm 0.05	0.40 \pm 0.09	0.38 \pm 0.04
E/A Ratio	1.53 \pm 0.08	1.34 \pm 0.12	1.68 \pm 0.23	1.07 \pm 0.25*
IVRT	25.2 \pm 1.7	24.2 \pm 2.5	30.6 \pm 2.8	23.5 \pm 2.3

Ejection fraction (EjF), shortening fraction (SF), isovolumetric relaxing time (IVRT). Sedentary Control (SC), sedentary anabolic steroid (SA), trained control (TC), trained anabolic steroid (TA). Mean \pm SD. * $p < 0.05$ vs SC, SA and TC.

DISCUSSION

The main results found in the present study show that the AAS administration associated with swimming physical training induces to pathological CH, characterized by increase of interstitial collagen and consequently decrease of diastolic function.

Alterations in the BP of the pre and post-experimental period animals have not been observed, showing that neither swimming physical training nor AAS administration influence on the BP control in this study. In normotensive animals, physical training has been well-demonstrated since it does not induce responses on the BP⁽¹⁹⁾; however, the AAS effects are still very controversial. Research shows that the AAS administration has been associated with BP increase in humans⁽²⁰⁾ and in rats^(7,22). Nevertheless, other researchers did not observe the same behavior in athletes who received supraphysiological doses of AAS for 16 weeks or in rats trained by swimming and treated with AAS⁽²¹⁾, which corroborates the data of the present study; BP difference was not observed with AAS administration either.

Rest HR decrease, or rest bradycardia, is one of the main adaptations promoted by training and can be induced by different mechanisms. According to Negrão *et al.*⁽²²⁾, rats trained on treadmill presented rest bradycardia especially due to alterations of the bypass cells, which reduced intrinsic BP. On the other hand, Medeiros *et al.*⁽¹⁶⁾ showed that rats trained by swimming presented reduction in rest HR, especially by increase of parasympathetic tonus. Our results show that the group which performed swimming training presented lower rest HR after the experimental protocol, which may have occurred especially by the increase of the parasympathetic component, showing that the physical training applied was able to promote aerobic adaptations on the cardiovascular system. Moreover, the rest HR reduction is considered an important marker of the aerobic physical training^(16,22). However, when physical training was associated with AAS administration, reduction in rest HR was not observed. These results may have occurred by a possible disorder in the autonomic control induced by the AAS in this group, since the AAS may increase the tachycardic responses and decrease the bradycardic ones⁽⁷⁾. AAS have shown to increase the central nervous system⁽²⁴⁾, harming hence the cardiac parasympathetic modulation⁽⁸⁾.

As expected, the group which performed swimming training (TC) presented increase of 52% in cardiac mass compared to the SC group. The increase of cardiac mass in this group was followed by increase in the cardiomyocytes diameter with no alteration of interstitial collagen or of ventricular function, which suggests a physiological CH. The effects of the physical exercise on the CH have been already well-described in the literature, in which physical exercise causes benefic and adaptative responses to the cardiovascular system⁽¹⁾. Aerobic exercise, as well as the one used in the present study, induces to CH predominately by volumetric overload, besides increasing length, it also increased the myocytes diameter, which occurs by the increase in the content of contractile proteins, with not great alterations of the structural matrix or of cardiac function⁽⁴⁾.

AAS administration per se led to increase of cardiac mass of 37% in comparison to the SC group. The increase of the cardiac mass in this group may be mainly associated with the interstitial collagen, since there was significant increase of the collagen fraction in this group, showing the important role of the AAS on the regulation of the structural matrix.

However, when the AAS administration was associated with physical training, increase of cardiac mass was even more remarkable, and increase of 64% was found when compared to the control group. Higher CH in this group may have occurred by a sum effect of the physical training and AAS, in which increase in the cardiomyocytes diameter, induced by physical training was observed, as well as increase of the collagen volume fraction induced by AAS.

Studies have shown increase of cardiac mass in AAS users⁽⁹⁾ and in animal models^(7,21), and this increase many times associated with greater cardiac fibrosis^(12,21). The CH induced by the AAS may occur by its interaction with nuclear receptors, directly acting in the DNA increasing the protein synthesis or, by affecting specific enzymes, ion flow and structural matrix in the myocardium as well⁽²⁵⁾. However, the exact mediators of these effects are diverse

and vary from mechanical stimuli to humoral circulating factors released by the heart and peripheral organs⁽²⁶⁾; however, the real mechanisms through which the AAS causes CH and increase of interstitial collagen are not well-known until the present moment.

Association of physical training and AAS has also been reported to lead to ventricular disorder, especially by decrease of the myocardial complacence, which may be attributed to fibrosis^(6,27), as observed in our results.

In order to better understand the functional importance of the results observed on the CH and increase of interstitial collagen, we analyzed the systolic and diastolic function of these animals by echocardiogram. Significant differences have not been observed concerning the results of systolic function among groups, which corroborates previous studies of our group in which ventricular function alterations have not been observed in the animals treated with AAS⁽²¹⁾. Similar data have also been found in humans, where increase of cardiac mass was observed by echocardiogram in AAS users and alterations of systolic function have not been noticed⁽⁹⁾.

The velocity peak of E wave, velocity peak of A wave, the E/A ratio as well as the isovolumetric relaxing time (IVRT) were used for analysis of the diastolic function. The E peak corresponds to the ventricle fast filling phase and the A peak corresponds to the slow filling or atrial contraction. In a normal echocardiogram the E peak is higher than the A peak, but in situations of diastolic dysfunction these values revert. In some pathological situations, the increase of A peak is followed by increase of IVRT indicating higher time needed for the ventricular relaxing⁽²⁸⁾. In the present study, we observed that trained animals treated with AAS presented decrease PF E peak, followed by reduction in E/A ratio when compared to the other groups, which indicates a possible diastolic dysfunction. On the other hand, no alteration of diastolic function was observed in any of the other experimental groups, including in the sedentary animals which received AAS, which suggests that diastolic dysfunction is only observed when the AAS use is associated with aerobic physical training.

Many investigations have shown the AAS effects on the diastolic function⁽⁹⁻¹¹⁾, where decrease in the velocity peak has been observed during the initial phase of diastolic filling and harm in the diastolic function in weight lifters who used AAS compared to the ones who did not use it, being these effects associated with the increase of the interstitial collagen^(10,11).

Thus, our results obtained with the association of AAS with aerobic physical training corroborate the data already observed in the literature with resistance training, which promoted adaptations to the cardiovascular system, such as the pathological CH, characterized by increase of interstitial collagen and decrease of diastolic function.

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