Effect of exercise training and carvedilol treatment on cardiac function and structure in mice with sympathetic hyperactivity-induced heart failure

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The present investigation was undertaken to study the effect of β-blockers and exercise training on cardiac structure and function, respectively, as well as overall functional capacity in a genetic model of sympathetic hyperactivity-induced heart failure in mice (α_{2A}/α_{2C}ArKO). α_{2A}/α_{2C}ArKO and their wild-type controls were studied for 2 months, from 3 to 5 months of age. Mice were randomly assigned to control (N = 45), carvedilol-treated (N = 29) or exercise-trained (N = 33) groups. Eight weeks of carvedilol treatment (38 mg/kg per day by gavage) or exercise training (swimming sessions of 60 min, 5 days/week) were performed. Exercise capacity was estimated using a graded treadmill protocol and HR was measured by tail cuff. Fractional shortening was evaluated by echocardiography. Cardiac structure and gastrocnemius capillary density were evaluated by light microscopy. At 3 months of age, no significant difference in fractional shortening or exercise capacity was observed between wild-type and α_{2A}/α_{2C}ArKO mice. At 5 months of age, all α_{2A}/α_{2C}ArKO mice displayed exercise intolerance and baseline tachycardia associated with reduced fractional shortening and gastrocnemius capillary rarefaction. In addition, α_{2A}/α_{2C}ArKO mice presented cardiac myocyte hypertrophy and ventricular fibrosis. Exercise training and carvedilol similarly improved fractional shortening in α_{2A}/α_{2C}ArKO mice. The effect of exercise training was mainly associated with improved exercise tolerance and increased gastrocnemius capillary density while β-blocker therapy reduced cardiac myocyte dimension and ventricular collagen to wild-type control levels. Taken together, these data provide direct evidence for the respective beneficial effects of exercise training and carvedilol in α_{2A}/α_{2C}ArKO mice preventing cardiac dysfunction. The different mechanisms associated with beneficial effects of exercise training and carvedilol suggest future studies associating both therapies.

Key words: Heart failure; Exercise training; Carvedilol treatment; Ventricular function; Cardiac remodeling; α_{2A}/α_{2C}ArKO mice

Introduction

Heart failure (HF) is a common endpoint for many forms of cardiovascular disease and a significant cause of morbidity and mortality (1). The development of end-stage HF often involves an initial insult to the myocardium that reduces cardiac output and leads to a compensatory increase in sympathetic nervous system activity, which ultimately leads to cardiac dysfunction and remodeling (2,3). In fact, cardiac hypertrophy in HF is associated with poor prognosis and constitutes an important independent risk factor for death (4). Thus, therapies that counteract the

deleterious effect of sympathetic hyperactivity on cardiac function and structure in HF are promising for the treatment of HF.

Pharmacological therapy of HF involves the use of β-blockers that recognizably protect the myocardium from excessive adrenergic stimulation (5). In fact, we have previously observed that carvedilol treatment of HF patients decreases sympathetic activity measured directly by microneurography performed on the peroneal nerve (6). Although β-blockers are known for their positive impact on cardiac function and their ability to reverse remodeling (7), they fail to improve forearm blood flow and overall functional capacity of HF patients (6). However, when β-blocker therapy is associated with exercise training there is an increase in forearm blood flow and exercise capacity (8).

Exercise training is considered to be an adjunctive therapeutic strategy for HF (9), since it improves exercise tolerance, endothelial function, and biochemical and structural parameters of skeletal muscles (10-12). Indeed, exercise training is effective in reducing a number of cardiovascular risk factors (13,14). However, the effects of exercise training on left ventricular structure and function are not completely understood. In fact, several studies in humans and animals have reported contradictory effects (15-19).

In the present study, we used a genetic model of sympathetic hyperactivity-induced HF in mice to determine the isolated effect of β-blockers and exercise training on cardiac structure and function, and overall functional capacity. It is important to point out that exercise training and β-blocker treatment were started before mice presented signs of HF in order to observe the potential preventive role of both therapies. We hypothesized that both exercise training and β-blocker treatment would improve cardiac function in HF mice. However, exercise training benefits would be related to an improved exercise tolerance and skeletal muscle capillarity density while β-blocker therapy benefits would be associated with reduced ventricular fibrosis and hypertrophy.

Material and Methods

Animal care

Male wild-type (WT) and congenic α_{2A}/α_{2C}-adrenoceptor knockout (α_{2A}/α_{2C}ArKO) mice with C57Bl6/J genetic background, 3 to 5 months of age, were studied. At 3 months of age, α_{2A}/α_{2C}ArKO mice display preserved cardiac function with no signs of lung water retention. In contrast, at 5 months of age, cardiomyopathy is associated with a modest but significantly increased water retention in lungs (20), consistent with pulmonary edema. Genotypes were determined by polymerase chain reaction on genomic DNA obtained from tail biopsies using primers to detect the intact and disrupted genes.

Mice were maintained in a light- (12-h light cycle) and temperature- (22°C) controlled environment and were fed a pellet rodent diet (Nuvital Nutrientes S/A, Brazil) *ad libitum* and had free access to water. WT and α_{2A}/α_{2C}ArKO mice were randomly assigned to control (N = 45), exercise-trained (N = 33) and carvedilol-treated (N = 29) groups. This study was carried out in accordance with Ethical Principles of animal research adopted by the Brazilian College of Animal Experimentation (www.cobea.org.br). In addition, this study was approved by the University of São Paulo Ethics Committee (CEP #004).

Procedures and measurements

Drug treatment and exercise training protocol. Drug treatment consisted of 8 weeks of carvedilol by gavage (38 mg/kg per day; Baldacci S.A., Brazil). The dose of carvedilol was optimized to achieve comparable heart rate (HR) levels as observed in the WT control group.

Exercise training consisted of swimming sessions 5 days/week with gradually increased duration up to 60 min, for 8 weeks in a warmed water (30-32°C) swimming apparatus adapted for mice (21). The training sessions were performed during the dark part of light cycle of the mice. This swimming protocol has been characterized as low to moderate intensity and associated with improved muscle oxidative capacity and resting bradycardia (21).

Graded treadmill exercise test. Exercise capacity, estimated by total distance run, was evaluated using a graded treadmill exercise protocol for mice. After being adapted to treadmill exercises for 1 week (10-min exercise session), mice were placed in the exercise lane and allowed to acclimatize for at least 30 min. Exercise began at 6 m/min with no grade and increased by 3 m/min every 3 min thereafter until exhaustion. The graded treadmill exercise test was performed with both WT and α_{2A}/α_{2C}ArKO mice before and after the experimental period.

Cardiovascular measurements. HR was determined non-invasively using a computerized tail-cuff system (BP 2000 Visitech Systems, USA) described elsewhere (22). Mice were acclimatized to the apparatus during daily sessions for 6 days, one week before starting the experimental period. HR measurements were obtained serially in WT and α_{2A}/α_{2C}ArKO mice once a week throughout the 8-week experiment.

Non-invasive cardiac function was assessed by two-dimensional guided M-mode echocardiography in halothane-anesthetized WT and α_{2A}/α_{2C}ArKO mice, before and after the experimental period. Briefly, mice were positioned in the supine position with front paws wide open,

and an ultrasound transmission gel was applied to the precordium. Transthoracic echocardiography was performed using an Acuson Sequoia model 512 echocardiographer (Siemens, USA) equipped with a 14-MHz linear transducer. Left ventricle systolic function was estimated by fractional shortening as follows: Fractional shortening (%) = [(LVEDD-LVESD)/LVEDD] x 100 where, LVEDD = left ventricular end-diastolic dimension, and LVESD = left ventricular end-systolic dimension.

Structural analysis

Twenty-four hours after the last drug administration or exercise-training session, all mice were killed and their tissues harvested. The heart was stopped at diastole (KCl, 14 mM) and dissected to obtain the left ventricle, which corresponds to the remaining organ upon removal of both atria and free wall of the right ventricle. For morphometric analysis, left ventricle samples obtained from the free wall, at the level of papillary muscle, were fixed in 4% buffered formalin and embedded in paraffin, cut in 4-µm sections and subsequently stained with hematoxylin and eosin. Two randomly selected sections from each animal were visualized by light microscopy using an objective with a calibrated magnification (400X). Myocytes with visible nucleus and intact cellular membranes were chosen for diameter determination. The width of an individually isolated cardiomyocyte displayed on a viewing screen was manually traced across the middle of the nuclei with a digitizing pad and determined by a computer-assisted image analysis system (Quantimet 520; Cambridge Instruments, UK). For each section, approximately 15 visual fields were analyzed.

Quantification of left ventricular fibrosis was achieved by Sirius red staining. Two randomly selected sections from each animal were visualized by light microscopy using an objective with a calibrated magnification of 200X. The interstitial collagen area was quantified by a computer-assisted image analysis system (Quantimet 520; Cambridge Instruments, USA). Approximately 5 visual fields were analyzed for each animal.

Capillary-to-fiber ratio was quantified in gastrocnemius muscle after periodic acid-Schiff staining. Two randomly selected sections from each animal were visualized by light microscopy using an objective with a calibrated magnification of 400X. The number of capillaries per fiber was analyzed in a computer-assisted morphometric system (Leica Quantimet 520, Cambridge Instruments). Approximately 15 visual fields were analyzed for each animal.

Statistical analysis

Data are reported as means ± SEM. Two-way ANOVA for repeated measurements with *post hoc* testing by Tukey (Statistica software, StatSoft, Inc., USA) was used to compare the effect of treatment (control, exercise-trained and carvedilol-treated) and genotype (WT and α_{2A}/α_{2C}ArKO) on HR measurements throughout the experimental period. Two-way ANOVA with *post hoc* testing by Tukey was used to compare the effect of treatment (control, exercise-trained and carvedilol-treated) and genotype (WT and α_{2A}/α_{2C}ArKO) on distance run, capillary density, fractional shortening and cardiac structure analysis. Statistical significance was set at P < 0.05.

Results

Effect of exercise training and carvedilol treatment on exercise tolerance, heart rate and capillary density

Before starting the experiment (at 3 months of age), α_{2A}/α_{2C}ArKO mice presented exercise capacity similar to WT mice (428 ± 99 *vs* 425 ± 107 m, respectively). However, after 8 weeks of experiment (at 5 months of age), α_{2A}/α_{2C}ArKO mice displayed exercise intolerance compared with age-matched WT mice (Figure 1A). Exercise training prevented exercise intolerance in α_{2A}/α_{2C}ArKO mice, and increased exercise tolerance in WT mice (Figure 1A). In contrast, carvedilol treatment had no impact on exercise tolerance in either WT or α_{2A}/α_{2C}ArKO mice. α_{2A}/α_{2C}ArKO mice displayed baseline tachycardia when compared with age-matched WT mice (Figure 1B). Both exercise training and carvedilol treatment significantly decreased baseline HR in α_{2A}/α_{2C}ArKO mice reaching WT control levels from the 5th week of experiment onward (Figure 1B). The magnitude of HR reduction was similar in both trained and carvedilol-treated α_{2A}/α_{2C}ArKO groups. As expected, exercise training and carvedilol treatment also reduced baseline HR in WT mice.

α_{2A}/α_{2C}ArKO mice displayed capillary rarefaction when compared with age-matched WT mice (Figure 1C). Exercise training significantly prevented capillary rarefaction in gastrocnemius muscle of α_{2A}/α_{2C}ArKO mice, and significantly increased the capillary-to-fiber ratio in WT mice. Carvedilol treatment had no effect on capillary density in either WT or α_{2A}/α_{2C}ArKO mice (Figure 1C).
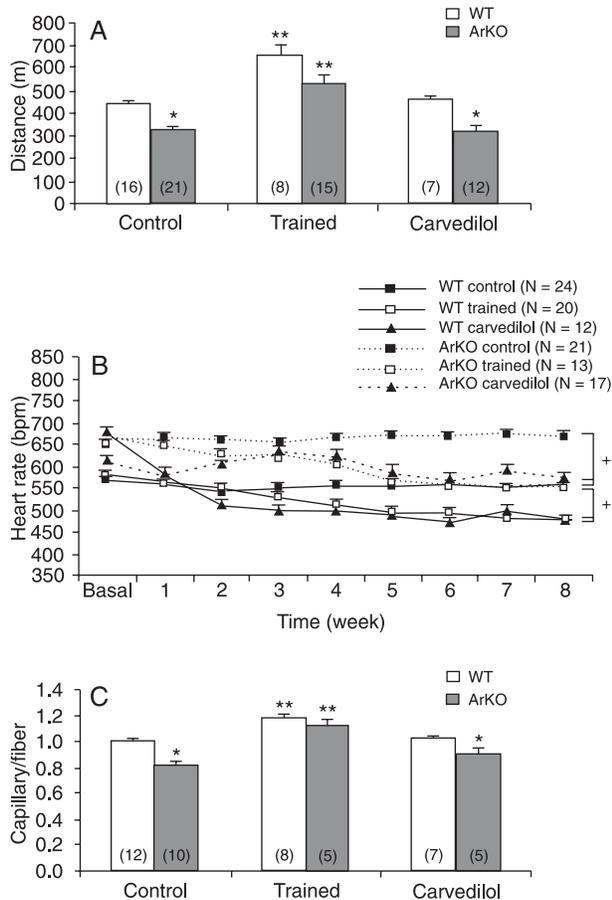
Effect of exercise training and carvedilol treatment on fractional shortening and cardiac structure

At the beginning of the experiment (at 3 months of age), there was no difference between WT and α_{2A}/α_{2C}ArKO mice in fractional shortening (20.9 ± 1.4 *vs* 18.0 ± 0.5%), left ventricle diastolic diameter (3.72 ± 0.1 *vs* 3.60 ± 0.1 mm), and left ventricle posterior wall thickness (0.60 ± 0.001 *vs* 0.60 ± 0.001 mm). However, after 8 weeks (at 5

months of age), α_{2A}/α_{2C}ArKO mice displayed systolic dysfunction when compared with age-matched WT mice (Figure 2A) and a trend toward increased left ventricle diastolic diameter (3.80 ± 0.07 *vs* 3.92 ± 0.03 mm, P = 0.08). Both exercise training and carvedilol treatment prevented the systolic dysfunction in α_{2A}/α_{2C}ArKO mice by increasing

fractional shortening towards WT mice values (Figure 2A). In addition, while exercise training significantly increased fractional shortening in WT mice, carvedilol treatment had no impact on cardiac contractility (Figure 2A).

Quantitative morphometric analysis showed that cardiac myocyte cross-sectional diameter was significantly

Figure 1. Effect of exercise training and carvedilol administration on exercise capacity measured as maximal distance run (A), heart rate (B) and capillary-to-fiber ratio in gastrocnemius muscle (C) evaluated in wild-type (open columns, WT) and α_{2A}/α_{2C}ArKO (filled columns, ArKO) mice. The exercise training significantly improved distance run and capillary-to-fiber ratio while it decreased heart rate in α_{2A}/α_{2C}ArKO mice. In contrast, carvedilol treatment had no effect on exercise capacity and capillary density, but reduced baseline heart rate in α_{2A}/α_{2C}ArKO mice. As expected, trained WT mice displayed resting bradycardia and increased exercise capacity and capillary density. The number in parentheses indicate the number of mice in each group. Data are reported as means ± SEM. *P < 0.05 compared to WT control group; **P < 0.05 compared to respective control group; +P < 0.05 compared to respective control group from the 4th week to the end of experimental period.
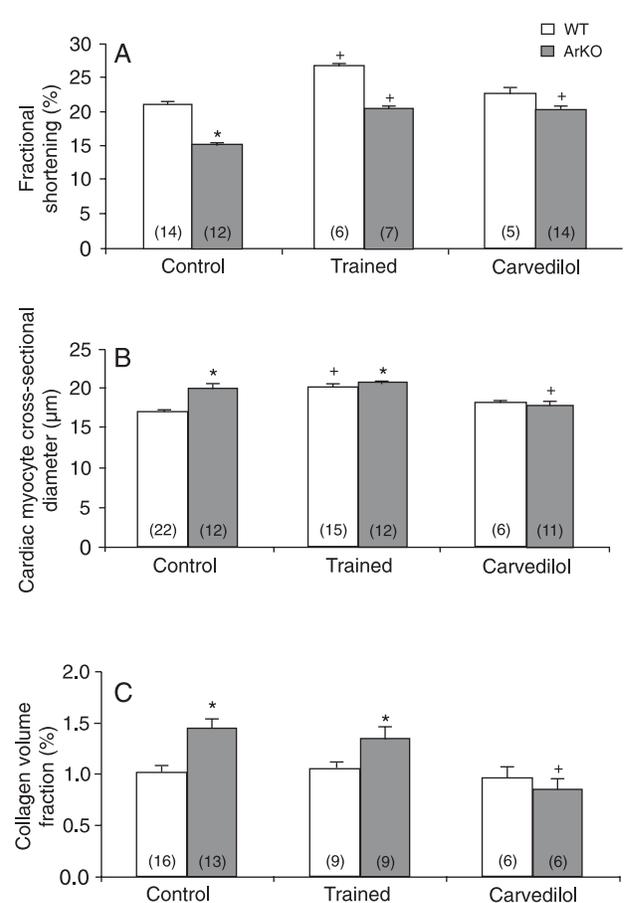
Figure 2. Effect of exercise training and carvedilol administration on fractional shortening used as an index of systolic function (A), cardiac myocyte cross-sectional diameter (B) and cardiac collagen volume fraction (C) evaluated in wild-type (open columns, WT) and α_{2A}/α_{2C}ArKO (filled columns, ArKO) mice. The exercise training and carvedilol treatment significantly increased fractional shortening in α_{2A}/α_{2C}ArKO mice. However, only carvedilol treatment prevented cardiac remodeling by reducing cardiac myocyte cross-sectional diameter and collagen volume fraction in α_{2A}/α_{2C}ArKO mice. The number in parentheses indicate the number of mice in each group. Data are reported as means ± SEM. *P < 0.05 compared to WT control group; +P < 0.05 compared to respective control group.

greater in α_{2A}/α_{2C}ArKO mice compared with WT control mice (Figure 2B). Exercise training had no effect on cardiac myocyte cross-sectional diameter in α_{2A}/α_{2C}ArKO mice but increased it in WT mice. Carvedilol significantly reduced cardiac myocyte width in α_{2A}/α_{2C}ArKO mice. The reduction of cardiac myocyte cross-sectional diameter in carvedilol-treated α_{2A}/α_{2C}ArKO mice was so remarkable that it reached WT control mice levels (Figure 2B).

Increased cardiac myocyte cross-sectional diameter in α_{2A}/α_{2C}ArKO mice was paralleled by ventricular fibrosis, represented by an increase of 45% in cardiac collagen volume fraction of α_{2A}/α_{2C}ArKO mice compared with WT control mice (Figure 2C). Exercise training did not change cardiac collagen volume fraction in either WT or α_{2A}/α_{2C}ArKO mice. In contrast, carvedilol treatment significantly reduced ventricular fibrosis in α_{2A}/α_{2C}ArKO mice, but cardiac collagen volume fraction of carvedilol-treated WT mice remained unchanged (Figure 2C).

Discussion

The main findings of the present study were that exercise training and β-blocker therapy improved ventricular function of HF mice similarly. However, the effect of exercise training was mainly associated with improved exercise tolerance and skeletal muscle capillary density while β-blocker therapy had a greater impact on cardiac structure.

Exercise training is emerging as an adjuvant non-pharmacological therapy for HF (9) and the benefits of exercise training on HF are related to improved function of both cardiac and skeletal muscles (10-12,23). In the present investigation, we observed improved fractional shortening paralleled by an increased gastrocnemius capillary density in exercise-trained α_{2A}/α_{2C}ArKO mice, which ultimately led to improved exercise tolerance. The importance of these results is striking because exercise intolerance is associated with a poor prognosis and reduced quality of life in HF patients (24). Indeed, an unacceptable burden of disability and unrelieved symptoms still remain even in optimally medically treated HF patients (25,26).

The mechanisms underlying the improved cardiac function and gastrocnemius capillary density in exercise-trained α_{2A}/α_{2C}ArKO mice were not investigated in this study, but we have previously demonstrated an improved cardiac myocyte Ca^{2+} handling after exercise training in α_{2A}/α_{2C}ArKO mice (27,28). Indeed, increased expression of vascular growth factors seems to be involved in angiogenesis induced by exercise training (29).

The effects of exercise training on left ventricular remodeling in HF are under investigation (15). Exercise training had no detectable impact on left ventricular fibrosis of α_{2A}/α_{2C}ArKO mice. In fact, previous studies demonstrated little effect of exercise training in total cardiac collagen fraction (30). Likewise, exercise training did not change the increased cardiac myocyte width of α_{2A}/α_{2C}ArKO mice. However, we cannot exclude the possibility that swimming training might preclude pathological hypertrophy, since it induces physiological remodeling that is associated with improved cardiac function. In fact, as we observed in WT mice, swimming exercise training led to a physiological hypertrophy, which we showed previously to be associated with beneficial cardiac effects (21).

In contrast to exercise training, carvedilol treatment had no effect on gastrocnemius capillary density and exercise tolerance in α_{2A}/α_{2C}ArKO mice. This response was somehow expected, since carvedilol is a non-selective β-blocker with transient α_1-adrenoceptor blockade, which could reduce skeletal muscle vasodilatation and preclude improvement in exercise tolerance.

The benefits of carvedilol were mainly due to its effect on cardiac function and structure. In fact β-blockers yield impressive benefits on cardiac contractility of patients with chronic heart failure (17). In the present study, we observed an increased fractional shortening in carvedilol-treated α_{2A}/α_{2C}ArKO mice associated with reduced left ventricular fibrosis and cardiac myocyte hypertrophy. These observations suggest that carvedilol prevents cardiac remodeling in α_{2A}/α_{2C}ArKO mice. The mechanisms involved in reduced cardiac fibrosis and hypertrophy of carvedilol-treated α_{2A}/α_{2C}ArKO mice were not examined in the present investigation; however, reduced cardiac angiotensin II levels might be involved. In fact, we have recently demonstrated that α_{2A}/α_{2C}ArKO mice display increased cardiac angiotensin II levels associated with ventricular fibrosis reversed partially by losartan treatment (20).

The present results demonstrate that both exercise training and carvedilol therapy improve, to the same extent, the ventricular function in a genetic model of sympathetic hyper-activity-induced heart failure in mice. However, while the benefits of exercise training are mainly associated with increased aerobic capacity and capillary density of skeletal muscle, carvedilol benefits were mainly due to its effect on cardiac structure. These results indicate that studies are needed to determine the efficacy of exercise training associated with β-blockers for treatment of heart failure therapy.

Acknowledgments

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