Sympathetic hyperactivity differentially affects skeletal muscle mass in developing heart failure: role of exercise training

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Sympathetic hyperactivity differentially affects skeletal muscle mass in developing heart failure: role of exercise training. J Appl Physiol 106: 1631–1640, 2009. First published January 29, 2009; doi:10.1152/japplphysiol.91067.2008.—Sympathetic hyperactivity (SH) is a hallmark of heart failure (HF), and several lines of evidence suggest that SH contributes to HF-induced skeletal myopathy. However, little is known about the influence of SH on skeletal muscle morphology and metabolism in a setting of developing HF, taking into consideration muscles with different fiber compositions. The contribution of SH on exercise tolerance and skeletal muscle morphology and biochemistry was investigated in 3- and 7-mo-old mice lacking both α2A- and α2C-adrenergic receptor subtypes (α2A/α2CARKO mice) that present SH with evidence of HF by 7 mo. To verify whether exercise training (ET) would prevent skeletal muscle myopathy in advanced-stage HF, α2A/α2CARKO mice were exercised from 5 to 7 mo of age. At 3 mo, α2A/α2CARKO mice showed no signs of HF and preserved exercise tolerance and muscular norepinephrine with no changes in soleus morphology. In contrast, plantaris muscle of α2A/α2CARKO mice displayed hypertrophy and fiber type shift (IIA/IIX) paralleled by capillary rarefaction, increased hexokinase activity, and oxidative stress. At 7 mo, α2A/α2CARKO mice displayed exercise intolerance and increased muscular norepinephrine, muscular atrophy, capillary rarefaction, and increased oxidative stress. ET reestablished α2A/α2CARKO mouse exercise tolerance to 7-mo-old wild-type levels and prevented muscular atrophy and capillary rarefaction associated with reduced oxidative stress. Collectively, these data provide direct evidence that SH is a major factor contributing to skeletal muscle morphological changes in a setting of developing HF. ET prevented skeletal muscle myopathy in α2A/α2CARKO mice, which highlights its importance as a therapeutic tool for HF.

HEART FAILURE (HF) is a clinical syndrome with poor prognosis characterized by exercise intolerance, early fatigue, and skeletal muscle myopathy associated with atrophy and shift toward fast-twitch fibers (27, 28, 49). The development of end-stage HF often involves a myocardial insult that reduces cardiac output, which leads to a compensatory increase in sympathetic nervous activity (4, 5). Although beneficial acutely, chronic increase of sympathetic activity leads to further pathological changes in the heart with a progressive deterioration of cardiac function (7, 24, 38, 39), which is closely related to increased cardiac oxidative stress (52).

Several lines of evidence suggest that sympathetic hyperactivity also contributes to the skeletal myopathy of HF, since it leads to chronic vasoconstriction in HF patients (22, 35, 44) associated with skeletal muscle oxidative stress (34, 46, 53) and increased concentrations of proinflammatory cytokines (12, 23). However, little is known about the influence of sympathetic hyperactivity on skeletal muscle morphology, redox balance, metabolism, and function in a setting of developing HF taking into consideration muscles with different fiber compositions.

Exercise training has been established as an adjuvant therapy for HF, since it counteracts exercise intolerance and improves quality of life (13, 18, 26). One of the most striking results achieved by exercise training in HF is a decrease in sympathetic nervous activity (9, 43) associated with decreased oxidative stress, proinflammatory cytokines, and inducible nitric oxide synthase (iNOS) expression (36). However, the influence of reduced sympathetic activity by exercise training on skeletal muscle mass, phenotype, and redox status requires further investigation.

Our group (2, 42) previously reported that mice lacking α2A- and α2C-adrenoceptors (α2A/α2CARKO) develop sympathetic hyperactivity-induced HF related to exercise intolerance and cardiac dysfunction with clinical signs of HF such as lung edema associated with increased mortality at 7 mo of age. In contrast, at 3 mo of age, α2A/α2CARKO mice display preserved cardiac function and exercise tolerance (14, 30). Therefore, these mice represent a good system for better understanding the influence of sympathetic activity on skeletal muscle morphology, metabolism, and function in different stages of cardiac disease and the potential beneficial effects of exercise training on skeletal myopathy.

The present investigation was undertaken to investigate the relative contribution of sympathetic hyperactivity to disease stage-related changes in skeletal muscles with different fiber compositions. In addition, we studied whether exercise training would prevent skeletal muscle myopathy in advanced-stage cardiomyopathy. We tested three hypotheses: 1) that sympathetic hyperactivity in α2A/α2CARKO mice with preserved cardiac function can lead to skeletal muscle hypertrophy and fiber type change toward a more glycolytic phenotype but preserved exercise tolerance, 2) that sympathetic hyperactivity...
in late-stage HF can aggravate skeletal muscle myopathy, leading to skeletal muscle atrophy, capillary rarefaction, oxidative stress, and exercise intolerance; and 3) that exercise training can prevent skeletal muscle myopathy in α2A/α2C-ARKO mice.

METHODS

Study population. A cohort of male congenic α2A/α2C-ARKO mice in a C57Bl6/J genetic background and their wild-type (WT) controls were studied at 3 and 7 mo of age. At 3 mo of age, α2A/α2C-ARKO mice display normal cardiac function (30), but at 7 mo of age, they present severe cardiac dysfunction associated with exercise intolerance and increased mortality rate (42). To further investigate the isolated effect of sympathetic nerve activity on skeletal muscle, we used a different set of congenic FVB mouse strain lacking β2-adrenoceptor (β2KO), which is the main subtype involved in muscular effects of norepinephrine (33), and their WT control (8). Mice were maintained in a 12:12-h dark-light cycle and a temperature-controlled environment (22°C) with free access to standard laboratory chow (Nuvital Nutrientes, Curitiba, Brazil) and tap water. This study was in accordance with ethical principles in animal research adopted by the Brazilian College of Animal Experimentation (www.cobea.org.br). In addition, this study was approved by the University of Sao Paulo Ethical Committee (CEP no. 058).

Graded treadmill exercise test. Exercise capacity, estimated by total distance run, correlates with skeletal muscle work capacity, and it is a method used for detecting exercise intolerance in HF. Exercise tolerance was evaluated using a graded treadmill exercise protocol for mice as previously described (15). Briefly, after being adapted to treadmill exercises over a week (10 min of exercise per session), mice were placed in the treadmill streak and allowed to acclimatize for at least 30 min. Intensity of exercise was increased by 3 m/min (6–33 m/min) every 3 min at 0% grade until exhaustion. The graded treadmill exercise test was performed in WT and α2A/α2C-ARKO mice at 3 and 7 mo of age.

Maximal lactate steady-state workload. In HF, lactate accumulation during exercise starts at lower than normal workload (19, 25). Therefore, we determined in WT and α2A/α2C-ARKO mice at 3 and 7 mo of age the maximal lactate steady-state workload (MLSSw), which consisted of the highest workload that could be maintained over 28 min of running without continual blood lactate accumulation (blood lactate varying by <1 mM from 7 to 28 min). Blood samples (25 μl) were taken from the tail vein every 7 min of running for further lactate measurements (YSI 2300 Sport) as previously described (15).

Cardiovascular measurements. Resting blood pressure (BP) and heart rate (HR) were determined noninvasively using a computerized tail-cuff system (BP-2000; Visitech Systems, Apex, NC) described elsewhere (7). Mice were acclimatized to the apparatus during daily sessions over 4 days, 1 wk before starting the experimental period.

Catecholamine measurements. Gastrocnemius and plasma norepinephrine were measured by HPLC using ion-pair reverse-phase chromatography coupled with electrochemical detection (0.5 V) as described by Monte et al. (31).

Skeletal muscle enzyme assay. For enzyme assays, red and white portions of gastrocnemius muscle and soleus muscle were previously frozen in liquid nitrogen. Muscles were homogenized (1:10 wt/vol) using a sampling homogenizer (Tecnal, Sao Paulo, Brazil) with the appropriate extraction medium for each enzyme. The activities of hexokinase (EC 2.7.1.1) and citrate synthase (EC 4.1.3.7) were determined as previously described (11). The extraction medium for hexokinase contained 50 mM Tris·HCl, 1 mM EDTA, 30 mM MgCl2, and 20 mM β-mercaptoethanol at pH 7.4. The extraction medium for citrate synthase contained 50 mM Tris·HCl and 1 mM EDTA at pH 7.4. Hexokinase was assayed by following the rate of change at a wavelength of 340 nm, and citrate synthase was determined at a wavelength of 412 nm. All spectrophotometric measurements were performed using an Ultrospec 3100 pro spectrophotometer (Amer sham Pharmacia Biotech, Freiburg, Germany) at 25°C. For all enzymes studied, preliminary experiments established that extraction and assay procedures produced maximum enzyme activities, as described previously (10). The enzyme activities are reported as micromoles per minute per gram of tissue fresh weight.

Muscle fiber typing and cross-sectional area. Twenty-four hours after the last exercise training session or the graded treadmill exercise test, sedentary and trained mice were killed and the calf muscles (soleus, plantaris, and red and white portions of gastrocnemius) were harvested, immediately frozen in melting isopentane, and stored in liquid nitrogen. Frozen muscles were cut into 10-μm cross sections from the proximal to distal region using a cryostat (Micron HM505E; Zeiss, Walldorf, Germany). Muscle sections were then incubated for myofibrillar ATPase activity after alkalai (myosin ATPase, pH 10.3) or acid preincubation (myosin ATPase, pH 4.6) as previously described (6). The myosin ATPase reaction was used to identify the muscle fiber type. Type I fibers reacted deeply after acid preincubation at pH 4.6 and lightly after formadehyde pretreatment and alkalai preincubation at pH 10.3. The inverse occurred with type II muscle fibers. Fiber typing and cross-sectional area were evaluated in whole muscles at ×200 magnification and further analyzed on a digitizing unit connected to a computer (Image-Pro Plus; Media Cybernetics, Silver Spring, MD). The total number of each fiber type was counted to calculate the numerical fiber type composition (I, IIA, IIX, and IIB). In plantaris, the fibers that could not be clearly identified as I, IIA, IIX, or IIB were classified as intermediary (they presented light stain at pH 10.3 and a small area). The cross-sectional area of each fiber type was measured for further calculation of averaged fiber cross-sectional area. All analyses were conducted by a single observer (A. V. N. Bacurau) blinded to mouse identity.

Capillary-to-fiber ratio. Capillary-to-fiber ratio of soleus, plantaris, and white gastrocnemius muscles was evaluated after myofibrillar ATPase histochemistry reaction at pH 10.3 as previously described (45). Briefly, capillary-to-fiber ratio was quantified by a 10 × 10 grid optically superimposed on each of 5 nonoverlapping fields at ×400 magnification, distributed in a random manner using a computer-assisted morphometric system (Quantimet 500; Leica, Cambridge, UK). For calculating capillary-to-fiber ratio, the total number of capillaries was divided by the total number of fibers counted in the same field. Only vessels with a diameter <10 μm were counted, which would largely comprise capillaries but might also include terminal arterioles or venules. All analyses were conducted by a single observer (A. V. N. Bacurau) blinded to mouse identity.

Skeletal muscle redox status. Because reactive oxygen species are produced in skeletal muscle and related to injury in skeletal myocytes (23), we evaluated the reduced glutathione-to-oxidized glutathione ratio (GSH/GSSG) and lipid peroxidation in soleus and plantaris of all mice studied. GSH/GSSG was determined by HPLC as described elsewhere (40). In brief, muscle samples were homogenized in cold buffer (1:20 wt/vol) containing 0.32 M sucrose, 10 mM HEPES, and 1 mM EDTA at pH 7.4 and immediately centrifuged at 10,000 g for 20 min at 4°C. Crude homogenates were mixed with ice-cold 10% (wt/vol) metaphosphoric acid, incubated for 30 min on ice, and centrifuged for 20 min at 14,000 g at 4°C. Supernatants were transferred to autosample vials and injected into a HPLC system, where samples were separated using a reversed-phase C18 column (5 μm, 4.6 ×150 mm). After HPLC, GSH and GSSG were detected with an electrochemical detector (Coulochem III, ESA, Chelmsford, MA) equipped with a two-channel analytical cell. Redox status was described as calculated GSH/GSSG.

Skeletal muscle lipid hydroperoxidation measurement. Lipid hydroperoxides were evaluated using the ferrous oxidation-xylenol orange technique (FOX2) as previously described (37). Soleus and plantaris samples were homogenized (1:20 wt/vol) in phosphate-buffered saline (PBS; 100 mM, pH 7.4) and immediately centrifuged at 12,000 g for 20 min at 4°C. Briefly, the homogenate was precipitated with trichloroacetic acid (10% wt/vol) and centrifuged at 12,000
Skeletal muscle protein expression. Immunooblots of 3- and 7-mo-old WT, α2A/α2C ARKO, and exercise-trained α2A/α2C ARKO soleus and plantaris muscle homogenates were performed according to Towbin et al. (47). Briefly, muscles frozen in liquid nitrogen were homogenized in a buffer containing 1 mM EDTA, 1 mM EGTA, 2 mM MgCl2, 5 mM KCl, 25 mM HEPES, pH 7.5, 100 μM PMSF, 2 mM DTT, 1% Triton X-100, and protease inhibitor cocktail (1:100; Sigma, St. Louis, MO). Samples were loaded and subjected to SDS-PAGE in polyacrylamide gels (10%). After electrophoresis, proteins were electrotransferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Equal loading of samples (25 μg) and even transfer efficiency were monitored with the use of 0.5% Poncet S staining of the blotted membrane. The blotted membrane was then incubated in a blocking buffer (5% nonfat dry milk, 10 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated overnight at 4°C with rabbit iNOS antibody (1:1,000; Abcam, Cambridge, MA). Binding of the primary antibody was detected with the use of peroxidase-conjugated secondary antibodies (anti-rabbit, 1:3,000, for 1.5 h at room temperature) and developed using enhanced chemiluminescence (Amersham Biosciences) detected by autoradiography. Quantification analysis of blots was performed with the use of Scion Image software (Scion based on NIH Image). Targeted bands were normalized to α-tubulin antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA).

Exercise training protocol. To verify whether exercise training could prevent skeletal muscle myopathy in 7-mo-old α2A/α2C ARKO mice, we performed exercise training in α2A/α2C ARKO mice aging from 5 to 7 mo of age. Exercise training consisted of 8 wk of running on a motor treadmill (ESD model 01; FUNBEC, Sao Paulo, Brazil), 5 days/wk, for 60 min at MLSSw intensity, as described elsewhere (15). All untrained mice were exposed to treadmill exercise (5 min) three times a week to become accustomed to exercise protocol and handling.

Isoproterenol treatment. Using osmotic pumps implanted subcutaneously (Alzet model 2002; Durect, Cupertino, CA), we treated WT and β2KO mice (3 mo of age) for 14 days with isoproterenol (Sigma) delivered at a rate of 15 μg·kg⁻¹·day⁻¹.

Statistical analysis. All values are means ± SE. Two-way analysis of variance (ANOVA) with post hoc Duncan’s testing (Statistica software; StatSoft, Tulsa, OK) was used to compare the effect of genotype (WT and α2A/α2C ARKO mice or β2KO mice when appropriate) and cardiomyopathy stage (3 and 7 mo of age) on distance run, MLSSw, cardiovascular measurements, citrate synthase and hexokinase maximal activities, muscular norepinephrine levels, capillary-to-fiber ratio, muscular fiber type distribution and cross-sectional area, and skeletal muscle redox status. One-way ANOVA with post hoc Duncan’s testing (Statistica) was used to verify the effect of exercise training on skeletal muscle biochemical and morphological variables in α2A/α2C ARKO mice. Statistical significance was considered achieved when the value of P was <0.05.

RESULTS

Physiological parameters of mice studied are presented in Table 1. α2A/α2C ARKO mice presented resting tachycardia with no significant change of resting BP compared with age-matched WT mice. At 3 mo of age, body weight (26 ± 0.5 vs. 25 ± 0.4 g, P > 0.05), wet-to-dry lung weight ratio (5.0 ± 0.1 vs. 5.3 ± 0.1, P > 0.05), muscular norepinephrine levels (0.38 ± 0.1 vs. 0.36 ± 0.1 pg/g, P > 0.05), and exercise tolerance were similar between WT and α2A/α2C ARKO mice, respectively. In contrast, plasma norepinephrine levels (4.617 ± 332 vs. 7.121 ± 630 pg/ml, P < 0.05) were significantly higher in α2A/α2C ARKO than in WT mice. However, at 7 mo of age, α2A/α2C ARKO mice displayed reduced body weight (30 ± 0.3 vs. 28 ± 0.8 g, P < 0.05), increased wet-to-dry lung weight ratio (5.6 ± 0.1 vs. 6.6 ± 0.5, P < 0.05), and increased muscular norepinephrine (0.47 ± 0.1 vs. 13.14 ± 3.8 pg/g, P < 0.05) and plasma norepinephrine levels (4.989 ± 525 vs. 8.175 ± 797 pg/ml, P < 0.05), as well as exercise intolerance, represented by decreased total distance run and maximal lactate steady-state workload (Table 1).

Metabolic enzymes and skeletal muscle capillary-to-fiber ratio. To verify the glycolytic capacity in white muscle of WT and α2A/α2C ARKO mice, we evaluated the hexokinase maximal activity. At 3 mo of age, α2A/α2C ARKO mice presented an increased hexokinase maximal activity in the white portion of gastrocnemius compared with age-matched WT mice (28.8 ± 3.0 vs. 23.2 ± 0.7 μmol·min⁻¹·g tissue fresh weight⁻¹, P < 0.05). At 7 mo of age, hexokinase activity was not different between α2A/α2C ARKO and WT mice. Exercise training did not change hexokinase activity in α2A/α2C ARKO mice. To verify the oxidative capacity in red and white muscles, we evaluated the citrate maximal activity of WT and α2A/α2C ARKO mice. Citrate synthase maximal activity was similar within mice studied at 3 mo of age. In contrast, 7-mo-old α2A/α2C ARKO mice presented lower maximal citrate synthase activity in soleus and red gastrocnemius muscles than age-matched WT mice (Fig. 1, A and B). This response was restricted to red muscles, since no change was observed in citrate synthase maximal activity in white gastrocnemius muscle (Fig. 1C). Exercise-trained α2A/α2C ARKO mice presented increased (70.4%) citrate synthase activity in red gastrocnemius muscle compared with untrained α2A/α2C ARKO mice (Fig. 1B), whereas no significant change was observed in white muscle.

Table 1. Physiological parameters in WT and α2A,α2C ARKO mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>ARKO</th>
<th>3 mo</th>
<th>7 mo</th>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>561 ± 7.6 (29)</td>
<td>642 ± 7.3* (15)</td>
<td>581 ± 8.4 (16)</td>
<td>690 ± 7.2†‡ (15)</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>110 ± 1.9 (29)</td>
<td>111 ± 3.1 (15)</td>
<td>112 ± 2.2 (16)</td>
<td>111 ± 1.7 (15)</td>
</tr>
<tr>
<td>Distance run, m</td>
<td>403 ± 28 (9)</td>
<td>362 ± 19 (21)</td>
<td>354 ± 18 (16)</td>
<td>235 ± 21†‡ (14)</td>
</tr>
<tr>
<td>MLSSw, m/min</td>
<td>15 ± 0.8 (8)</td>
<td>15 ± 0.7 (10)</td>
<td>15 ± 0.3 (12)</td>
<td>12 ± 0.3†‡ (11)</td>
</tr>
</tbody>
</table>

Values are means ± SE of heart rate (HR), blood pressure (BP), and maximal lactate steady-state workload (MLSSw) in wild-type (WT) and α2A/α2C-adrenoceptor knockout (α2A/α2C ARKO) mice (ARKO group). In addition, the exercise training effect was evaluated in ARKO mice (ARKO group). The number of animals studied in each group is shown in parentheses. *P < 0.05 vs. 3-mo-old WT. †P < 0.05 vs. 3-mo-old ARKO. ‡P < 0.05 vs. 7-mo-old WT. §P < 0.05 vs. 7-mo-old ARKO.
gastrocnemius muscle (Fig. 1C). This response was paralleled by an improved exercise tolerance in α2A/α2C-ARKO mice compared with age-matched untrained α2A/α2C-ARKO mice (377 ± 6 vs. 235 ± 2 m, respectively, P < 0.05).

Capillary-to-fiber ratio was evaluated in soleus, plantaris, and white gastrocnemius muscle. At 3 mo of age, no difference was observed in capillary-to-fiber ratio in soleus muscle of α2A/α2C-ARKO mice (Fig. 1D), whereas a capillary rarefaction could already be observed in plantaris and white gastrocnemius (Fig. 1, E and F).

The reduced skeletal muscle oxidative activity in red muscles of 7-mo-old α2A/α2C-ARKO mice was paralleled by capillary rarefaction in all muscles studied (Fig. 1, D–F), and exercise training increased capillary-to-fiber ratio of α2A/α2C-ARKO to values similar to those observed in WT mice.

**Fiber-type distribution and cross-sectional area.** The staining patterns of the fibers are shown in plantaris and soleus muscles (Fig. 2, A–D). In soleus muscle, there was no difference in percentage of type I, IIA, and IIX fiber distribution between 3-mo-old WT and α2A/α2C-ARKO mice (Fig. 3A). In contrast, 7-mo-old α2A/α2C-ARKO mice presented decreased type I and IIX fibers but increased type IIA fiber, which suggests a shift toward type II fiber in advanced-stage cardiomyopathy (Fig. 3A). In plantaris muscle, the shift toward more glycolytic fibers in α2A/α2C-ARKO could be observed as early as 3 mo of age (Fig. 3B). α2A/α2C-ARKO mice presented a decreased percentage of type IIA and increased type IIX and intermediary fiber types compared with age-matched WT mice (Fig. 3B). However, no further differences in plantaris muscle composition were observed between WT and α2A/α2C-ARKO mice at 7 mo of age (Fig. 3B). Exercise training prevented changes in soleus fiber type composition of α2A/α2C-ARKO mice (Fig. 3A) and reduced type IIB fibers in plantaris while increasing type IIX fibers, which suggests a shift back toward less glycolytic fibers (Fig. 3A).

Cross-sectional area of fibers I, IIA, and IIX in soleus muscle was not different between WT and α2A/α2C-ARKO mice at 3 mo of age (Fig. 4, A–C). However, in soleus muscle of 7-mo-old α2A/α2C-ARKO mice with severe cardiomyopathy, cross-sectional area was lower than that of age-matched WT mice (Fig. 4, D and E). Conversely, 7-mo-old α2A/α2C-ARKO mice displayed plantaris IIA and IIX fiber atrophy compared with age-matched WT mice (Fig. 4, D and F). The same pattern of response in α2A/α2C-ARKO mice (hypertrophy at 3 mo followed by atrophy at 7 mo of age) was observed when the whole plantaris muscle cross-sectional area was evaluated (data not shown). Exercise training prevented soleus and plantaris muscles atrophy, since...
it significantly increased cross-sectional area of most fibers studied of both muscles (Fig. 4).

**Isoproterenol treatment.** To investigate the isolated effect of sympathetic nerve activity on skeletal muscle morphology, we treated WT and \( \beta_2 \) KO mice with isoproterenol for 14 days. We hypothesized that early skeletal muscle hypertrophy, observed in 3-mo-old \( \alpha_2 \Lambda \Lambda A \) \( \alpha_2 \Lambda \Lambda C \) ARKO mice, is mediated by \( \beta_2 \)-adrenoceptor, which is the main subtype involved in muscular effects of norepinephrine. In fact, \( \beta_2 \) KO mice displayed reduced soleus and plantaris mass paralleled by decreased cross-sectional area of type I and IIA fibers compared with age-matched WT mice (Table 2). In addition, \( \beta_2 \)-adrenoceptor deletion markedly suppressed skeletal muscle enlargement and type I and IIA fiber hypertrophy observed in WT mice treated with isoproterenol (Table 2), suggesting a positive role of \( \beta_2 \)-adrenoceptor on skeletal muscle hypertrophy.
reduced lipid hydroperoxides to levels in WT mice (Fig. 5, C and D).

iNOS expression was increased in 3-mo-old α2A/α2CARKO mice compared with age-matched WT mice in both soleus and plantaris muscles. This increased iNOS expression remained in 7-mo-old α2A/α2CARKO mice (Fig. 5, E and F). Exercise training restored iNOS expression of α2A/α2CARKO mice to levels in age-matched WT mice (Fig. 5, E and F).

**DISCUSSION**

The chronic activation of the sympathetic nervous system has been postulated to contribute to exercise intolerance and skeletal muscle myopathy in HF (34). Excessive vasoconstriction by chronic sympathetic hyperactivity adversely affects muscle performance in HF by altering metabolic status and limiting oxygen supply to exercising muscle (22). Indeed, chronic imbalance of the autonomic nervous system is also present in early stages of cardiac dysfunction when no signs of HF are observed. In the present investigation, we used genetically modified mice based on the disruption of both α2A- and α2C-adrenoceptors to study the influence of sympathetic hyperactivity on disease-stage skeletal muscle changes and the relative contribution of exercise training in counteracting the skeletal muscle myopathy in advanced-stage HF.

The key findings of the present study are that sympathetic hyperactivity in early-stage cardiomyopathy leads to plantaris hypertrophy associated with preserved muscular norepinephrine levels and exercise tolerance but with capillary rarefaction, a shift toward fast fibers, decreased GSH/GSSG, and increased hexokinase maximal activity in white gastrocnemius. Conversely, chronic sympathetic hyperactivity associated with HF in α2A/α2CARKO mice led to soleus and plantaris atrophy paralleled by increased muscular norepinephrine levels, exercise intolerance, reduced MLSSw, changes in fiber type distribution, and reduced oxidative capacity in red muscles with capillary rarefaction and muscular oxidative stress. Remarkable was the fact that exercise training increased soleus and plantaris cross-sectional area, preventing skeletal muscle myopathy in advanced-stage cardiomyopathy of α2A/α2CARKO mice.

The sympathetic nervous system, through interaction of norepinephrine released from nerve terminals with β2-adrenoceptors, exerts anabolic action on skeletal muscle protein metabolism (16, 20, 32, 51). In fact, we provide direct evidence for the involvement of β2-adrenoceptor in skeletal muscle hypertrophy (Table 2). In addition, our findings show that hypertrophic effect observed in 3-mo-old α2A/α2CARKO mice might be associated mainly with increased circulating catecholamine levels, since no difference was observed in muscular norepinephrine levels compared with age-matched WT mice. Indeed, a moderated shift of the redox homoeostasis toward a prooxidant state presently demonstrated by reduced GSH/GSSG and increased hexokinase maximal activity in white gastrocnemius. Moreover, chronic sympathetic hyperactivity associated with HF in α2A/α2CARKO mice led to soleus and plantaris atrophy paralleled by increased muscular norepinephrine levels, exercise intolerance, reduced MLSSw, changes in fiber type distribution, and reduced oxidative capacity in red muscles with capillary rarefaction and muscular oxidative stress. Remarkable was the fact that exercise training increased soleus and plantaris cross-sectional area, preventing skeletal muscle myopathy in advanced-stage cardiomyopathy of α2A/α2CARKO mice. 

Markers of oxidative stress. Changes in muscle morphology and function in HF are associated with changes in skeletal muscle redox status (1). GSH/GSSG was decreased in 3-mo-old α2A/α2CARKO mice compared with age-matched WT mice in both soleus and plantaris muscles. This decrease in GSH/GSSG remained in 7-mo-old α2A/α2CARKO mice (Fig. 5, A and B). Exercise training restored redox status of α2A/α2CARKO mice to levels in age-matched WT mice (Fig. 5, A and B).

Lipid hydroperoxidation was similar between 3-mo-old α2A/α2CARKO and WT mice (Fig. 5, C and D). In contrast, at 7 mo of age, when cardiomyopathy was in an advanced stage, lipid hydroperoxidation was significantly increased in α2A/α2CARKO mice (Fig. 5, C and D). Of interest, exercise training
is probably due to the higher downregulation of \( \beta_2 \)-adrenoceptors in red than white muscles (17, 21). \( \beta_2 \)-Adrenoceptor activation, through \( \beta_2 \)-agonists, increases twitch-tension and glycogenolysis in fast fibers (20, 41). This may occur in HF, since 3-mo-old \( \alpha_{2A}/\alpha_{2C} \)ARKO mice showed increased CSA of plantaris muscle. In contrast, 7-mo-old \( \alpha_{2A}/\alpha_{2C} \)ARKO mice displayed atrophy in both soleus and plantaris muscles. Interestingly, exercise training prevented muscle atrophy in \( \alpha_{2A}/\alpha_{2C} \)ARKO mice. Data are means ± SE; the number of animals studied is indicated in the bar representing each group. *P < 0.05 vs. 3-mo-old WT. †P < 0.05 vs. 3-mo-old ARKO. ‡P < 0.05 vs. 7-mo-old WT. §P < 0.05 vs. 7-mo-old ARKO.

Table 2. Effect of 15 days of isoproterenol treatment on body mass, muscle mass, muscle cross-sectional area, and muscle fiber type distribution in WT and \( \beta_2 \)KO mice

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>WT</th>
<th>WTiso</th>
<th>( \beta_2 )KO</th>
<th>( \beta_2 )KOiso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28 ± 1.8 (9)</td>
<td>25 ± 0.9 (13)</td>
<td>33 ± 0.5 (3)</td>
<td>28 ± 0.5 (4)</td>
</tr>
<tr>
<td>Soleus mass, mg/g</td>
<td>0.25 ± 0.01 (5)</td>
<td>0.15 ± 0.01* (7)</td>
<td>0.31 ± 0.02* (3)</td>
<td>0.18 ± 0.02* (3)</td>
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<tr>
<td>Plantaris mass, mg/g</td>
<td>0.51 ± 0.01 (4)</td>
<td>0.36 ± 0.02* (7)</td>
<td>0.66 ± 0.03* (5)</td>
<td>0.39 ± 0.01 (6)</td>
</tr>
<tr>
<td>Soleus fiber type distribution, %</td>
<td>Type I</td>
<td>60 ± 1.4 (4)</td>
<td>62 ± 0.5 (4)</td>
<td>56 ± 1.5 (3)</td>
</tr>
<tr>
<td>Type IIA</td>
<td>38 ± 1.2 (4)</td>
<td>32 ± 2.2 (4)</td>
<td>37 ± 3.5 (3)</td>
<td>29 ± 3.6‡ (3)</td>
</tr>
<tr>
<td>Type IIX</td>
<td>1.7 ± 0.3 (4)</td>
<td>5.7 ± 2.4 (4)</td>
<td>5.1 ± 2.7 (3)</td>
<td>3.0 ± 1.7 (3)</td>
</tr>
<tr>
<td>Soleus cross-sectional area, ( \mu m^2 )</td>
<td>Type I</td>
<td>867 ± 49 (4)</td>
<td>670 ± 19* (4)</td>
<td>1,014 ± 34* (3)</td>
</tr>
<tr>
<td>Type IIA</td>
<td>695 ± 51 (4)</td>
<td>576 ± 32* (4)</td>
<td>865 ± 18* (3)</td>
<td>656 ± 4‡ (3)</td>
</tr>
<tr>
<td>Type IIX</td>
<td>759 ± 116 (4)</td>
<td>582 ± 38 (4)</td>
<td>742 ± 118 (3)</td>
<td>541 ± 95 (3)</td>
</tr>
</tbody>
</table>

Values are means ± SE from WT and \( \beta_2 \)-adrenoceptor knockout (\( \beta_2 \)KO) mice before and after treatment with isoproterenol (WT iso and \( \beta_2 \)KOiso). The number of animals studied in each group is shown in parentheses. *P < 0.05 vs. WT. †P < 0.05 vs. \( \beta_2 \)KO. ‡P < 0.05 vs. WTiso.
important to highlight that advanced-stage HF led to significant
global changes in both red and white muscles, such as muscle
bulk loss, capillary rarefaction, and reduced citrate synthase
activity in red muscles of 7-mo-old $\alpha_{2A}/\alpha_{2C}$-ARKO mice,
which suggests blunted oxidative metabolism paralleled by
fiber type composition toward faster fibers. Together, these
results suggest that 7-mo-old $\alpha_{2A}/\alpha_{2C}$-ARKO mice present
cardiac cachexia aggravated by reduced locomotion as ob-
served in an open-field test of 7-mo-old $\alpha_{2A}/\alpha_{2C}$-ARKO mice
(Supplemental Fig. 1). In fact, neurohumoral imbalance in
chronic HF together with increased circulating and local in-
flammation leads to increased iNOS expression and muscular
reactive oxygen species, which ultimately play an important
role in skeletal muscle wasting (23). It is possible that in-
creased skeletal muscle oxidative stress also contributes to the
typical sign of early muscle fatigue presently verified by
reduced exercise tolerance and MLSSw in 7-mo-old $\alpha_{2A}/\alpha_{2C}$-ARKO mice.

We have previously reported that exercise training in $\alpha_{2A}/$ 
$\alpha_{2C}$-ARKO mice reduces cardiac sympathetic tone (14), plasma
norepinephrine (42), and resting tachycardia paralleled by
increased ventricular function (14, 42). Presently, we report
that the beneficial effects of exercise training are extended to
skeletal muscle with different fiber type compositions, since
increased fiber cross-sectional area toward WT mice levels is
paralleled by reduced local oxidative stress in both red and
white muscles. This response might be related to decreased
sympathetic activity to skeletal muscle that could lead to a
reduced local oxidative stress and an increased $\beta_2$-adrenocep-
tor density. In fact, exercise training has been reported to exert
long-term antioxidative effects in mixed-fiber type vastus late-
ralis of HF patients (23) and to increase $\beta_2$-adrenoceptor
density in gastrocnemius and soleus of running-trained rats (50).

It is important to point out that the prevention of skeletal
muscle atrophy in $\alpha_{2A}/\alpha_{2C}$-ARKO mice occurs even with mice exer-
cising under a predominantly aerobic exercise training
regimen, which suggests a homeostatic role of exercise training
in restoring skeletal muscle mass when associated with muscle
atrophy states. The effect of aerobic exercise training in this
genetic model of HF is also observed as increased activity of
citrate synthase in red portion of gastrocnemius paralleled by
increased capillaries per muscle fiber. Altogether, these struc-

Fig. 5. Reduced glutathione-to-oxidized glutathione ratio (GSH/GSSG) in soleus (A) and plantaris muscles (B), lipid hydroperoxidation in soleus (C) and plantaris muscles (D), and inducible nitric oxide synthase (iNOS) protein expression levels in soleus (E) and plantaris muscles (F). Measurements were performed in 3- and 7-mo-old WT and $\alpha_{2A}/\alpha_{2C}$-ARKO mice. In addition, the effect of exercise training on muscle redox status and iNOS protein expres-
sion was evaluated in $\alpha_{2A}/\alpha_{2C}$-ARKO mice (ARKOT). Note that 3-mo-old $\alpha_{2A}/\alpha_{2C}$-ARKO mice already displayed decreased GSH/GSSG and increased iNOS protein expression compared with age-matched WT mice. At 7 mo of age, $\alpha_{2A}/\alpha_{2C}$-ARKO mice presented a global oxidative stress represented by a decreased
GSH/GSSG and increased lipid hydroperoxi-
dation and iNOS protein expression in both plantaris and soleus muscles. Interestingly, ex-
ercise training restored redox status in $\alpha_{2A}/$ 
$\alpha_{2C}$-ARKO mice. Data are means $\pm$ SE; the number of animals studied is indicated in the
bar representing each group. $^*P < 0.05$ vs. 3-mo-old WT. $^\dagger P < 0.05$ vs. 3-mo-old ARKO.
$^\ddagger P < 0.05$ vs. 7-mo-old WT. $^\S P < 0.05$ vs. 7-mo-old ARKO.
tural and metabolic changes induced by exercise training contribute to improved exercise capacity and later fatigue by increased MLSS in 7-mo-old α2A/α2C-ARKO mice.

**Study limitations.** Our study suggests that chronic sympathetic activation in advanced-stage cardiomyopathy of α2A/α2C-ARKO mice is associated with skeletal muscle atrophy. However, we cannot exclude the possibility that inactivity would be a potential factor aggravating skeletal muscle atrophy and changed phenotype in 7-mo-old ARKO mice with HF. Therefore, sympathetic hyperactivity can be considered a major factor aggravating HF-induced skeletal muscle myopathy.

In summary, the present findings provide evidence for the dichotomy involving the contribution of sympathetic hyperactivity on skeletal muscle plasticity. Although sympathetic activation associated with a moderated prooxidant state led to hypertrophy of plantaris with preserved exercise tolerance in 3-mo-old α2A/α2C ARKO mice, chronic activation of sympathetic nervous system in this genetic model was associated to red and white muscle atrophy, increased local norepinephrine levels, and oxidative stress associated with clinical signs of HF in α2A/α2C-ARKO mice at 7 mo of age. Finally, exercise training by restoring skeletal muscle mass, metabolism, redox state, and muscular phenotype can be considered an important therapeutic strategy for preventing or reversing skeletal muscle myopathy in HF.

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**REFERENCES**


