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Exercise can induce temporary mitochondrial and contractile dysfunction linked to impaired respiratory chain complex activity

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ABSTRACT

Exercise is considered to elicit a physiological response of the heart. Previous studies investigated the influence of repetitive exercise only at the end of the training period. We assessed the impact of 2 exercise protocols, differing in their treadmill inclination, on cardiac and mitochondrial function at different times during the training period. Within 10 weeks, animals trained with 16% incline developed hypertrophy (left ventricular posterior wall thickness: 1.6 ± 0.1 vs 2.4 ± 0.1 mm; $P < .05$) with normal function (ejection fraction: 75.2% ± 2.5% vs 75.6% ± 2.1%). However, at 6 weeks, there was temporary impairment of contractile function (ejection fraction: 74.5% ± 1.67% vs 65.8% ± 2.3%; $P < .05$) associated with decreased mitochondrial respiratory capacity (state 3 respiration: 326 ± 71 vs 161 ± 22 natoms/[min mg protein]; $P < .05$) and a gene expression shift from the adult (α) to the fetal (β) myosin heavy chain isoform. Although peroxisome proliferator-activated receptor gamma coactivator–1α expression was normal, nuclear respiratory factors (NRFs)–1 and –2 were significantly reduced (NRF-1: 1.00 ± 0.16 vs 0.55 ± 0.09; NRF-2: 1.00 ± 0.11 vs 0.63 ± 0.07; $P < .05$) after 6 weeks. These findings were associated with a reduction of electron transport chain complexes I and IV activity (complex I: 1016 ± 67 vs 758 ± 71 nmol/[min mg protein]; complex IV: 18768 ± 1394 vs 14692 ± 960 nmol/[min mg protein]; $P < .05$). Messenger RNA expression of selected nuclear encoded subunits of the electron transport chain was unchanged at all investigated time points. In contrast, animals trained with 10% incline showed less hypertrophy and normal function in echocardiography, normal maximal respiratory capacity, and unchanged complex activities at all 3 time points. Repetitive exercise may cause contractile and mitochondrial dysfunction characterized by impaired respiratory chain complex activities. This activity reduction is temporary and intensity related.

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1. Introduction

In heart muscle, 2 types of hypertrophy are distinguished. The first is pathological hypertrophy (eg, due to hypertension or aortic stenosis/constriction), ultimately leading to contractile dysfunction and heart failure. The second is physiological hypertrophy (eg, induced by treadmill running or swimming exercise), which is associated with limited hypertrophic growth and normal function without heart failure [1,2]. The underlying mechanisms for these 2 types of hypertrophy have not been fully investigated.

One major difference being discussed as an explanation for the pathological or physiological nature of hypertrophy is the chronicity or the repetitiveness of the stimulus. Interestingly, most studies using repetitive hypertrophic stimuli are located in the area of exercise and are considered to induce physiological hypertrophy. With chronic pressure overload, we recently identified mitochondrial dysfunction and heart failure based on significant proteomic remodeling of the mitochondria [3]. Other studies suggested the presence of myocardial damage and temporary contractile dysfunction even with exercise, specifically in athletes after acute intensive endurance exercise [4-6]. In animal studies, forced exercise has also been linked to detrimental effects by some investigators, including stress marker elevations such as corticosterone and adrenocorticotropic hormone [7,8], as well as contractile dysfunction [9]. However, most of these studies investigated the effect of an acute bout of exercise or investigated the animals at the end of the training/exercise period. No studies investigated heart and mitochondrial function at different time points during a period of repetitive exercise training.

We assessed the impact of endurance exercise during the development of presumably physiological hypertrophy in a rat model of treadmill running and assessed respiratory capacity of isolated mitochondria as well as individual respiratory complex activities. For this purpose, we modified a protocol taken from the literature, which had induced "physiological hypertrophy" before, associated with increased cardiac oxidative capacity after 10 weeks of training [10]. Contrary to expectations, after 6 weeks of exercise, we found temporary (ie, reversible) mitochondrial and contractile dysfunction associated with reduced complex activities. This dysfunction could be explained by impairments of respiratory chain complex activities and was completely absent when the intensity of the training was reduced.

2. Methods

2.1. Animal care

Male Sprague-Dawley rats were obtained from Charles River (Sulzfeld, Germany) and were fed ad libitum at 21°C with a light cycle of 12 hours. The use of animals was consistent with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NHI publication no. 85-23, revised 1996); and the experimental protocols were approved by the local Animal Welfare Committee of the University Leipzig, Germany.

2.2. Training protocol

Animals at 3 weeks of age were randomly assigned to either the training or the control group (sedentary). The rats in the training group were exercised on a treadmill over a period of 10 weeks with either a 16% or a 10% incline, a speed of 25 m/min, and 4 training episodes per week (Monday, Tuesday, Thursday, and Friday). Running times were incrementally increased as follows: 30 min/d in the first week, 45 min/d in the second week, 60 min/d in the third, 75 min/d in the fourth week, 90 min/d in the fifth week, 105 min/d in the sixth week, and 120 min/d in the last 4 weeks. After 2 and 6 weeks and at the end of the training protocol, animals were analyzed for echocardiographic and in vitro examination.

2.3. Echocardiography

Echocardiographic examination was performed after 2, 6, and 10 weeks of exercise. After anesthesia (fentanyl/midazolam hydrochloride/medetomidine hydrochloride, 0.005/2/0.15 mg/kg), the chest of the animals were shaved; and the rats were examined in supine position with a 12-MHz phased array transducer (Agilent/Philips, Böblingen, Germany). Two-dimensional short-axis views of the left ventricle at papillary muscle level were obtained [11]. Two-dimensional guided M-mode tracings were recorded with a sweep speed of 100 mm/s. The following parameters were measured: heart rate, left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and left ventricular posterior wall thickness in diastole (LVPWD). Based on these measurements, ejection fraction (EF) [12] and left ventricular endocardial fractional shortening (eFS) [13] were determined.

2.4. Tissue homogenization

For isolation of mitochondria, hearts were quickly excised and transferred into ice-cold cardioplegic solution containing 0.18 mol/L KCl and 10 mmol/L Tris/HCl, pH 7.4. Atria were removed, and ventricle weight was assessed. Ventricles were finely minced. One gram of heart muscle was added to 10 mL of CP2 isolation medium (1.0 mol/L KCl, 1.0 mol/L Mops, 0.1 mol/L $MgSO_4$, 0.1 mol/L EGTA, 5% bovine serum albumin [BSA], 1 mmol/L adenosine triphosphate [ATP]). The mixture was homogenized with an Ultra-Turrax at 9000 rpm for 2.5 seconds, followed by 2 down passes in a Potter-Elvejhem glass homogenizer by a motor-driven Teflon pestle at 600 to 800 rpm. All steps were carried out at 4°C.

2.5. Isolation of mitochondria

After homogenization of the heart muscle, mitochondria were isolated according to the method described by Palmer et al [14]. Isolated mitochondria were kept in KME buffer (containing 100 mmol/L KCl, 50 mmol/L Mops, and 0.5 mmol/L EGTA; pH 7.4; 4°C). Mitochondrial protein concentration was determined by the Bradford method using BSA as a standard. Mitochondrial citrate synthase (CS) activity was measured in fresh muscle homogenate and isolated mitochondria according to the protocol by Srere [15].

2.6. Mitochondrial respiration

Immediately after isolation, oxygen consumption of isolated mitochondria was measured at 25°C with a Clark-type oxygen electrode fitted to a water-jacketed reaction chamber of 1-mL volume. Mitochondrial preparations were added to oxygen electrode solution (100 mmol/L KCl, 50 mmol/L Mops, 1.0 mmol/L EGTA, 5.0 mmol/L Kpi, 1.0 mg/mL defatted BSA) to a final concentration of 1 U CS per milliliter in the reaction chamber. Using 10 mmol/L glutamate, 20 μmol/L palmitoylcarnitine/2.5 mmol/L malate, 5 mmol/L pyruvate/2.5 mmol/L malate, or 10 mmol/L succinate/3.75μmol/L rotenone as substrates, maximal oxygen consumption (state 3 respiration) was stimulated by adding 20 μL adenosine diphosphate (ADP) (10 mmol/L). Uncoupled respiration was measured by adding 2,4-dinitrophenol using glutamate and succinate/rotenone as substrates.

2.7. Quantitative real-time reverse transcriptase polymerase chain reaction

Myocardial mRNA was extracted from snap-frozen tissue samples using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). Synthesis of complementary DNA was performed with the cDNA synthesis kit from Fermentas (St Leon-Rot, Germany), and TaqMan quantitative real-time reverse transcriptase polymerase chain reaction was performed as previously described [16,17]. Forward and reverse primers were designed using the Universal Probe Library Assay Design Center. For each set of primers, a basic local alignment search tool search revealed that sequence homology was obtained only for the targeted gene. Polymerase chain reaction amplification was performed in triplicates in a reaction volume of 10 μL using AmpliTaq Gold (Applied Biosystems, Darmstadt, Germany) with the conditions suggested by the manufacturer. After initial denaturation and activation of enzyme for 10 minutes at 95°C, 40 cycles of denaturation at 95°C for 15 seconds, annealing, and extension at 60°C for 1 min were run. Results were normalized to the invariant transcript S29 ribosomal protein and are presented as fold change compared with sedentary animals, the value of which was set to 1.

2.8. Mitochondrial electron transport chain complex activity

Samples of fresh mitochondria were treated with 10 mg cholate per milligram mitochondrial protein, diluted to a final concentration of 1 mg/mL with MSM (5 mmol/L mannitol, 220 mmol/L sucrose, 5 mmol/L MOPS, pH 7.4)-EDTA buffer supplemented with 1 μL/mL mammalian protease inhibitor cocktail. For the assay, the samples were diluted with buffer 1:10 to measure complex I and III and 1:100 to measure complex IV activity. The protocols measuring complex I and II activity followed the same principle. Complex I activity was measured based on a protocol by Janssen et al [18], and complex II activity was measured according to Krahenbuhl et al [19] with small modifications. 2,6-Dichloroindophenol (DCIP) was used as a terminal electron acceptor. To measure complex I activity, NADH was added and oxidized by complex I. Released electrons reduce the artificial substrate decylubiquinone that subsequently delivers the electrons to DCIP. For measuring complex II activity, succinate was added, which was oxidized by complex II. Released electrons reduce the artificial substrate decylubiquinone that subsequently delivers the electrons to DCIP. The reduction of DCIP was followed spectrophotometrically at 600 nm. Complex III was blocked by the addition of antimycin A. Activity of complex III was measured according to Krahenbuhl et al [19]. Decylubiquinol is oxidized by complex III; and the released electrons reduce cytochrome *c*, which was added in the oxidized form. The reduction of cytochrome *c* can be followed spectrophotometrically at 550 nm. To avoid unspecific electron transfer, complex IV was inhibited by sodium azide. Cytochrome *c* oxidase activity was measured according to Wharton and Tzagoloff [20]. Reduced cytochrome *c* was oxidized by complex IV, and this reaction was followed spectrophotometrically at 550 nm.

2.9. Statistical analysis

Data are presented as mean ± SEM. Data were analyzed using a Student *t* test or a 1-way analysis of variance as appropriate. Post hoc comparisons among the groups were performed using the Holm-Sidak method. Differences among groups were considered statistically significant if $P < .05$.

3. Results

3.1. Treadmill running at 16% incline causes hypertrophy at 10 weeks, but temporary impairment of contractile function at 6 weeks

Table 1 shows body weight, heart weight, tibia length, and relative heart weight of sedentary and exercised rats (16% incline) at different time points. At 10 weeks of exercise, there was significantly less body mass in the exercised rats. Although absolute heart weight did not differ between sedentary and exercised rats at any time point, the heart to body weight ratio was significantly elevated after 2 and 10 weeks of exercise. Tibia length, as a marker of age, was not different between the 2 groups at each investigated time point. Heart weight to tibia length was increased at 2 weeks and unchanged at 6 and 10 weeks.

Table 2 shows standard echocardiographic parameters of sedentary and exercised (16% incline) rats at all investigated time points. The dimension of the posterior wall was normal after 2 and 6 weeks and significantly increased after 10 weeks of exercise. Contractile function and dimension of the left ventricle were normal after 2 weeks of exercise. Contrary to expectations, after 6 weeks, we found evidence for significant ventricular dilatation (increased LVEDD and LVESD) and impaired contractile function (significantly reduced EF and eFS). These dilatation and impairment in cardiac contractility had disappeared after 10 weeks of exercise.

3.2. Temporary contractile dysfunction at 6 weeks is accompanied by mitochondrial dysfunction

Fig. 1 shows the effects of endurance exercise on respiratory capacity of isolated mitochondria. Whereas maximal ADP-stimulated respiratory capacity (state 3 respiration) in cardiac

Table 1 – Body and heart weight as well as tibia length of sedentary rats and rats trained at 16% incline for 2, 6, and 10 weeks

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
BW (g)	143 ± 6	134 ± 5	249 ± 13	265 ± 4	339 ± 7	299 ± 8†
HW (mg)	473 ± 18	509 ± 18	792 ± 30	839 ± 17	917 ± 18	902 ± 29
HW/BW (mg/g)	3.86 ± 0.07	4.45 ± 0.09‡	3.71 ± 0.14	3.61 ± 0.06	3.08 ± 0.07	3.52 ± 0.08‡
TL (mm)	26.6 ± 0.4	27.1 ± 1.0	32.9 ± 0.9	34.1 ± 0.3	37.9 ± 0.7	37.8 ± 0.2
HW/TL (mg/mm)	19.9 ± 0.5	22.1 ± 0.8*	28.1 ± 0.6	28.0 ± 0.5	27.4 ± 0.6	27.8 ± 0.7

Data are mean ± SEM; n = 9 to 16 per group. SED indicates sedentary; TR, treadmill running; BW, body weight; HW, heart weight; TL, tibia length.
* $P < .05$ vs sedentary.
† $P < .01$ vs sedentary.
‡ $P < .001$ vs sedentary.

mitochondria of exercised rats was normal at 2 weeks, it was significantly reduced at 6 weeks. This reduction was present with complex I substrates glutamate, palmitoylcarnitine/malate, and pyruvate/malate, but not with complex II substrate succinate. The observed reduction in state 3 respiration appeared to be reversible. After 10 weeks, there was still a trend toward reduced state 3 respiration in the exercised groups. However, it was only significant with glutamate as substrate. Consistent with the reduction in state 3 respiration with complex I but not complex II substrates, there was reduced uncoupled respiration after 6 and 10 weeks with glutamate as substrate but not with succinate (Supplementary Table S6b).

Citrate synthase is considered a pace-making enzyme in the first step of the citric acid cycle and is commonly used as a marker enzyme to quantitate the presence of intact mitochondria. Table 3 shows citrate synthase activity in homogenate and isolated mitochondria from sedentary and exercised rats. The activity was not different from those measured in sedentary animals either in homogenate or in isolated mitochondria. Table 4 shows the ratio of ATP production to oxygen consumption (ADP/O ratio), commonly used as a marker for coupling of respiratory chain activity to ATP production. In contrast to the reductions in state 3 respiration at 6 and 10 weeks, ADP/O ratios were elevated at all time points for the complex I substrates (glutamate, palmitoylcarnitine, and pyruvate) and was normal for the complex II substrate succinate.

The reduction in state 3 respiration with complex I substrates suggests a defect at the level of complex I. Fig. 2 shows the individual enzyme activities of the 4 respiratory chain complexes of mitochondria isolated after 6 weeks of exercise with 16% incline. Consistent with the reduction of state 3 respiration with complex I substrates, complex I activity was significantly reduced in the exercised rats. Whereas the activities of complexes II and III were normal, there was also a significant reduction in complex IV activity in the exercised rats, which could explain the slight (but not significant) reduction in respiratory capacity with succinate as substrate. However, total activity of complex IV was 20-fold higher than that of complex I, suggesting that complex I is the rate-limiting step. To support these results, we analyzed mRNA expression of selected nuclear encoded subunits of electron transport chain (ETC) complexes (complex I, *NDUFA10*; complex II, *SdhB*; complex III, *UQCRC2*; complex IV, *CoxIV*; complex V, *ATP5A1*). However, no differences in the mRNA expression were found between sedentary and exercised animals at all investigated time points (Table 5).

Table 5 also shows the expression of genes involved in the regulation of mitochondrial biogenesis. Consistent with the temporary impairment of function at 6 weeks, there was a significant downregulation of the ETC transcription factors nuclear respiratory factor (NRF)–1 and –2, which act downstream of peroxisome proliferator-activated receptor gamma coactivator (PGC)–1α. Expressions of PGC-1α, estrogen-related receptor α, mitochondrial transcription factor A (Tfam), and uncoupling protein (UCP)–2 were unchanged at all investigated time points. Messenger RNA expression of adenine nucleotide translocator (ANT) was reduced at 6

Table 2 – Echocardiographic parameters of sedentary rats and rats trained at 16% incline for 2, 6, and 10 weeks

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
LVPWD (mm)	1.43 ± 0.13	1.19 ± 0.06	1.66 ± 0.10	1.76 ± 0.10	1.73 ± 0.09	2.35 ± 0.15‡
LVEDD (mm)	5.46 ± 0.24	5.49 ± 0.06	6.52 ± 0.16	7.16 ± 0.14†	7.19 ± 0.09	6.82 ± 0.20
LVESD (mm)	2.82 ± 0.17	2.60 ± 0.14	3.62 ± 0.17	4.44 ± 0.15‡	3.95 ± 0.12	3.69 ± 0.22
EF (%)	79.1 ± 1.7	82.2 ± 1.9	74.5 ± 1.7	65.8 ± 1.7‡	74.3 ± 1.5	75.6 ± 2.1
eFS (%)	48.6 ± 1.6	52.7 ± 2.3	44.9 ± 1.5	38.0 ± 1.5†	45.3 ± 1.4	46.4 ± 2.2

Data are mean ± SEM; n = 8 to 22 per group.
† $P < .01$ vs sedentary.
‡ $P < .001$ vs sedentary.

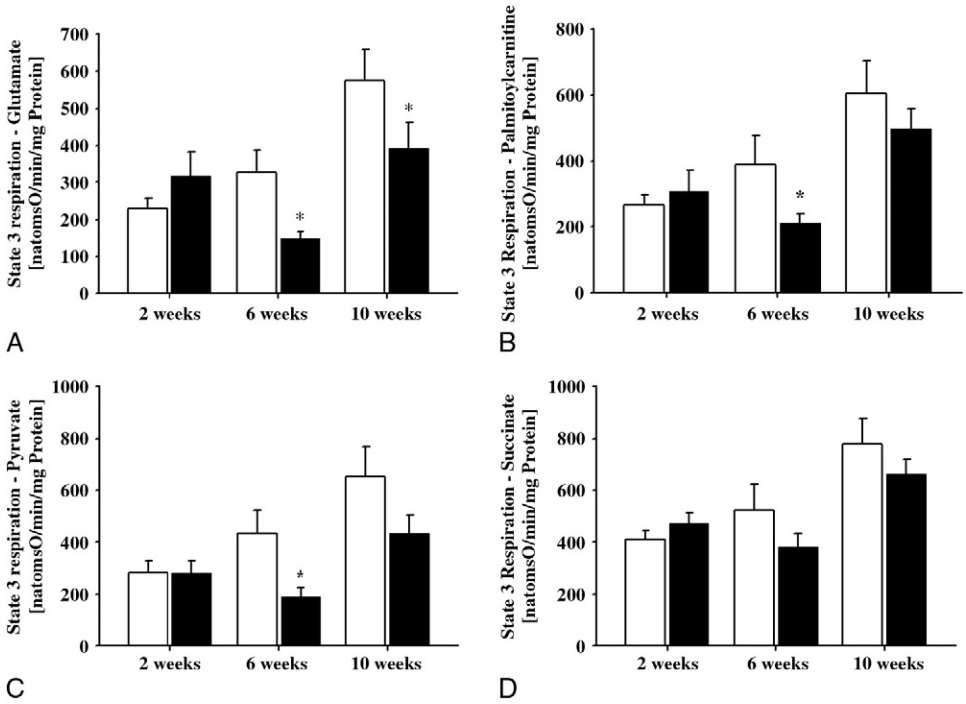

Fig. 1 – Effects of endurance exercise (16% incline, black bars) on respiratory capacity (state 3 respiration) of isolated rat heart mitochondria. State 3 respiration was induced by adding ADP in the presence of glutamate (A), palmitoylcarnitine/malate (B), pyruvate/malate (C), and succinate as substrates. Data are mean ± SEM; n = 6 to 12 per group. *P < .05 compared with sedentary (white bars).

weeks and normal at 10 weeks of exercise. Uncoupling protein–3 expression was significantly increased after 2 and 10 weeks and markedly decreased at 6 weeks of exercise. Consistent with decreased NRF-1/NRF-2 expression after 6 weeks of exercise, the expression of an important modulator of cellular metabolic processes, adenosine monophosphate–activated protein kinase (AMPK), was also reduced. In contrast, expression of glucose transporter type 4, whose expression and translocation are regulated by AMPK, was unchanged during exercise in heart muscle.

3.3. *Temporary mitochondrial dysfunction and reduced contractility are associated with a pathological myosin heavy chain shift*

An expressional shift of the myosin heavy chain (MHC) from the adult α- to the fetal β-form is considered indicative for pathological hypertrophy. Table 6 shows mRNA expression of α- and β-MHC as well as the expression of commonly accepted heart failure markers atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP). Consistent with the temporary reduction in mitochondrial and contractile function, a shift from α- to β-MHC expression was found, beginning at 2 weeks and being most pronounced at 6 weeks. At 10 weeks of treadmill running, the shift was no longer present. In contrast, ANF and BNP were not elevated with treadmill running. They were normal at 10 weeks and even reduced at 2 and 6 weeks.

3.4. *Temporary changes in cardiac and mitochondrial function are related to training intensity*

To get further insight into the underlying mechanism, we repeated the majority of the experiments with an identical training protocol but lowered the intensity of the training by reducing the incline of the treadmill from 16% to 10%. Rats trained with 10% incline showed normal body weight

Table 3 – Citrate synthase activity (units per milliliter sample) in homogenate and isolated mitochondria from rats trained at 16% incline for 2, 6, and 10 weeks

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
Homogenate	12.5 ± 1.12	11.4 ± 1.01	10.1 ± 1.68	13.3 ± 1.50	15.3 ± 1.93	15.8 ± 1.25
Mitochondria	23.7 ± 1.66	22.0 ± 1.77	28.6 ± 6.36	29.5 ± 2.15	38.6 ± 2.46	36.8 ± 2.31

Data are mean ± SEM; n = 6 to 12 per group.

Table 4 – ADP/O ratios of isolated mitochondria from rats trained at 16% incline for 2, 6, and 10 weeks

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
GLU	2.41 ± 0.13	3.14 ± 0.26†	2.15 ± 0.09	2.67 ± 0.09‡	1.94 ± 0.15	2.62 ± 0.07‡
PC/MAL	2.47 ± 0.13	3.03 ± 0.38	2.03 ± 0.14	2.48 ± 0.17*	1.93 ± 0.15	2.20 ± 0.17
PYR/MAL	2.20 ± 0.15	2.67 ± 0.13*	2.29 ± 0.14	2.69 ± 0.09*	2.07 ± 0.14	2.67 ± 0.06‡
SUCC	1.28 ± 0.11	1.46 ± 0.09	1.41 ± 0.25	1.24 ± 0.13	1.10 ± 0.09	1.38 ± 0.10

Data are mean ± SEM; n = 6 to 12 per group. GLU indicates glutamate; PC/MAL, palmitoylcarnitine/malate; PYR/MAL, pyruvate/malate; SUCC, succinate.
* $P < .05$ vs sedentary.
† $P < .01$ vs sedentary.
‡ $P < .001$ vs sedentary.

after 2 and 6 weeks but reduced body weight after 10 weeks. Tibia length was not different between sedentary and exercised animals. Absolute heart weight was significantly elevated after 6 and 10 weeks of exercise. Relative heart weight (heart weight/body weight, heart weight/tibia length) was significantly increased after 10 weeks of exercise, indicating hypertrophy (Supplementary Table S1). However, there was no evidence for cardiac hypertrophy in echocardiography (normal LVPWD). Furthermore, cardiac dimensions (LVEDD) and contractile function (EF, eFS) were unchanged at all investigated time points (Supplementary Table S2).

Fig. 3 shows respiratory capacity of isolated heart mitochondria from rats trained at 10% incline. State 3 respiration was not different from sedentary animals at all time points. Citrate synthase activity was slightly elevated after 2 and 6 weeks in homogenate and isolated mitochondria but normal after 10 weeks of exercise. Table S7 shows activity of individual respiratory chain complexes after 6 weeks of exercise. We found no activity changes between sedentary and exercised rats in all investigated complexes.

4. Discussion

We show here that repetitive exercise may cause temporary mitochondrial dysfunction and reduced myocardial contractility characterized by impaired respiratory chain complex activities. This activity reduction is temporary and intensity related.

The temporary reduction in contractile and mitochondrial function at 6 weeks of treadmill training at higher incline was surprising. Our findings show several similarities to changes usually considered "pathological": reduced contractile function, reduced respiratory capacity, and a shift from α- to β-MHC gene expression. A commonly considered difference between pathological and physiological hypertrophy is based on the chronic presumably

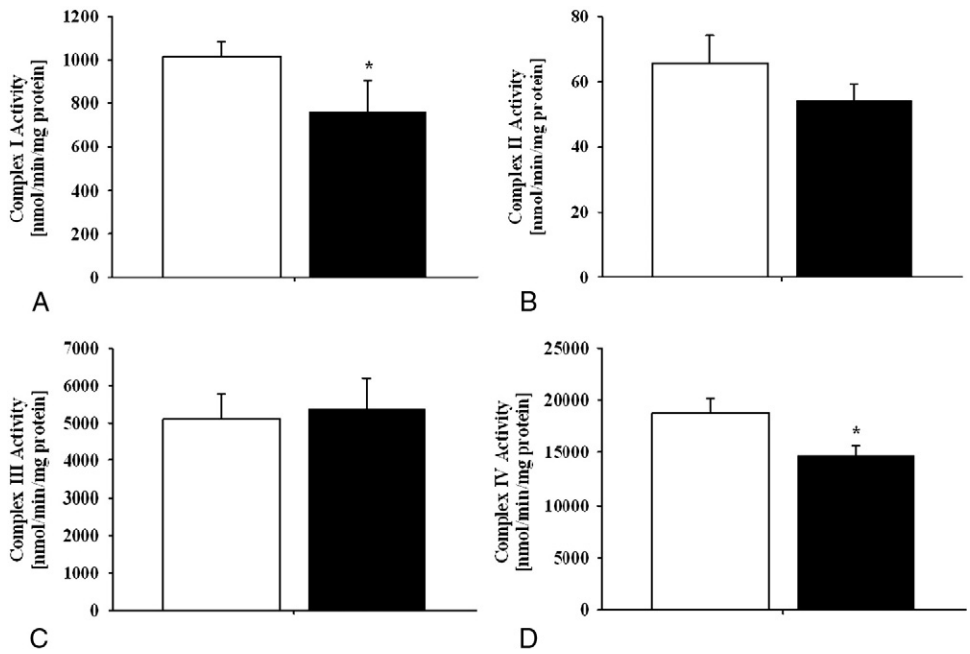

Fig. 2 – Activity of individual ETC complexes I, II, III, and IV in isolated rat heart mitochondria after 6 weeks of exercise (16% incline, black bars). Data are mean ± SEM; n = 6 to 7 per group. *P < .05 compared with sedentary (white bars).

Table 5 – Expression of genes related to mitochondrial biogenesis and subunits of the ETC complexes in heart muscle of sedentary and exercise rats (16% incline) at different time points

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
AMPK	1.00 ± 0.15	1.48 ± 0.23	1.00 ± 0.23	0.50 ± 0.06	1.00 ± 0.09	1.03 ± 0.11
PGC-1α	1.00 ± 0.14	0.98 ± 0.07	1.00 ± 0.12	1.25 ± 0.19	1.00 ± 0.10	0.98 ± 0.07
ERRα	1.00 ± 0.05	0.94 ± 0.10	1.00 ± 0.09	0.84 ± 0.06	1.00 ± 0.13	1.20 ± 0.10
NRF-1	1.00 ± 0.21	1.10 ± 0.29	1.00 ± 0.16	0.55 ± 0.09*	1.00 ± 0.13	0.95 ± 0.19
NRF-2	1.00 ± 0.13	1.04 ± 0.14	1.00 ± 0.11	0.63 ± 0.07*	1.00 ± 0.08	0.97 ± 0.05
Tfam	1.00 ± 0.14	0.90 ± 0.12	1.00 ± 0.06	1.14 ± 0.13	1.00 ± 0.08	1.14 ± 0.08
ANT	1.00 ± 0.21	0.56 ± 0.08	1.00 ± 0.19	0.44 ± 0.11*	1.00 ± 0.08	1.05 ± 0.03
Glut4	1.00 ± 0.17	1.32 ± 0.15	1.00 ± 0.20	1.07 ± 0.13	1.00 ± 0.07	1.17 ± 0.11
UCP-2	1.00 ± 0.16	1.52 ± 0.19	1.00 ± 0.12	0.66 ± 0.11	1.00 ± 0.09	0.78 ± 0.07
UCP-3	1.00 ± 0.20	4.32 ± 0.66†	1.00 ± 0.16	0.68 ± 0.18	1.00 ± 0.16	2.45 ± 0.26†
Complex I	1.00 ± 0.17	1.49 ± 0.26	1.00 ± 0.10	0.89 ± 0.13	1.00 ± 0.10	1.16 ± 0.07
Complex II	1.00 ± 0.13	1.29 ± 0.26	1.00 ± 0.09	1.64 ± 0.42	1.00 ± 0.09	1.19 ± 0.15
Complex III	1.00 ± 0.10	1.25 ± 0.13	1.00 ± 0.13	0.88 ± 0.07	1.00 ± 0.10	0.90 ± 0.05
Complex IV	1.00 ± 0.21	0.95 ± 0.12	1.00 ± 0.09	1.07 ± 0.16	1.00 ± 0.08	0.87 ± 0.04
Complex V	1.00 ± 0.07	1.22 ± 0.11	1.00 ± 0.05	0.84 ± 0.11	1.00 ± 0.11	1.08 ± 0.04

Data are mean ± SEM; n = 5 to 12 per group. ERRα indicates estrogen-related receptor α; Glut4, glucose transporter type 4.
* $P < .05$ vs sedentary.
† $P < .01$ vs sedentary.

"pathological" vs repetitive presumably "physiological" nature of the stimulus. We show here that repetitive exercise indeed can produce clear pathological alterations in both cardiac pheno- and genotype.

Although differences in hypertrophic signaling between physiological and pathological hypertrophy have been described, the arguments and findings are still contradictory. For instance, Wilkins et al [21] described the activation of calcineurin/nuclear factor of activated T-cells as pathological signaling, and Kemi et al [22] described Akt/mammalian target of rapamycin as physiological stimulus at the same time. Ni et al [23] reported that activation of Akt inhibits forkhead box protein O and consequently activates calcineurin/nuclear factor of activated T-cells. We did not aim to contribute to this controversy by assessing hypertrophic signaling in this study. However, we reasoned that hypertrophic growth needs to be balanced by mitochondrial function; and we therefore assessed respiratory capacity together with gene expression of mitochondrial biogenesis related genes and individual respiratory chain complex activities. We found that the temporary decrease in contractile function at 6 weeks was associated with significant reductions in complex I and IV activities, which in turn were consistent with reduced expression of the respiratory transcription factors (NRF-1 and NRF-2). Again, an analogy to pathological hypertrophy arises.

In pathological hypertrophy, reduced PGC-1α, as a mitochondrial master regulator, has been suggested as a key mechanism for impaired mitochondrial function in heart failure [24]. Our results of normal PGC-1α expression 24 hours after the last bout of exercise are consistent with those of others, who have demonstrated increased PGC-1α expression 12 hours after exercise but normal PGC-1α expressions 24 hours after the last bout of exercise [25]. Although we did not find reduced PGC-1α expression at the investigated time points, mRNA expression of NRF-1 and NRF-2 was reduced. NRF-1 and NRF-2 are transcription factors activating expression of genes regulating cellular growth, respiration, heme biosynthesis, and mitochondrial DNA transcription and replication [26]. NRF-1, together with NRF-2, mediates the

Table 6 – Messenger RNA expression of commonly accepted markers of heart failure in sedentary and trained rats (16% incline) at different time points

	2 wk		6 wk		10 wk	
	SED	TR	SED	TR	SED	TR
α-MHC	1.00 ± 0.29	0.65 ± 0.06	1.00 ± 0.18	0.42 ± 0.05*	1.00 ± 0.14	0.98 ± 0.06
β-MHC	1.00 ± 0.43	0.64 ± 0.22	1.00 ± 0.15	1.69 ± 0.73*	1.00 ± 0.13	1.12 ± 0.16
α-/β-MHC	1.00 ± 0.56	0.86 ± 0.18*	1.00 ± 0.10	0.59 ± 0.26*	1.00 ± 0.35	1.13 ± 0.19
BNP	1.00 ± 0.13	0.49 ± 0.04†	1.00 ± 0.29	0.24 ± 0.03*	1.00 ± 0.12	0.80 ± 0.10
ANF	1.00 ± 0.16	0.55 ± 0.09*	1.00 ± 0.11	0.59 ± 0.11*	1.00 ± 0.14	0.99 ± 0.16

Data are mean ± SEM; n = 5 to 12 per group.
* $P < .05$ vs sedentary.
† $P < .01$ vs sedentary.

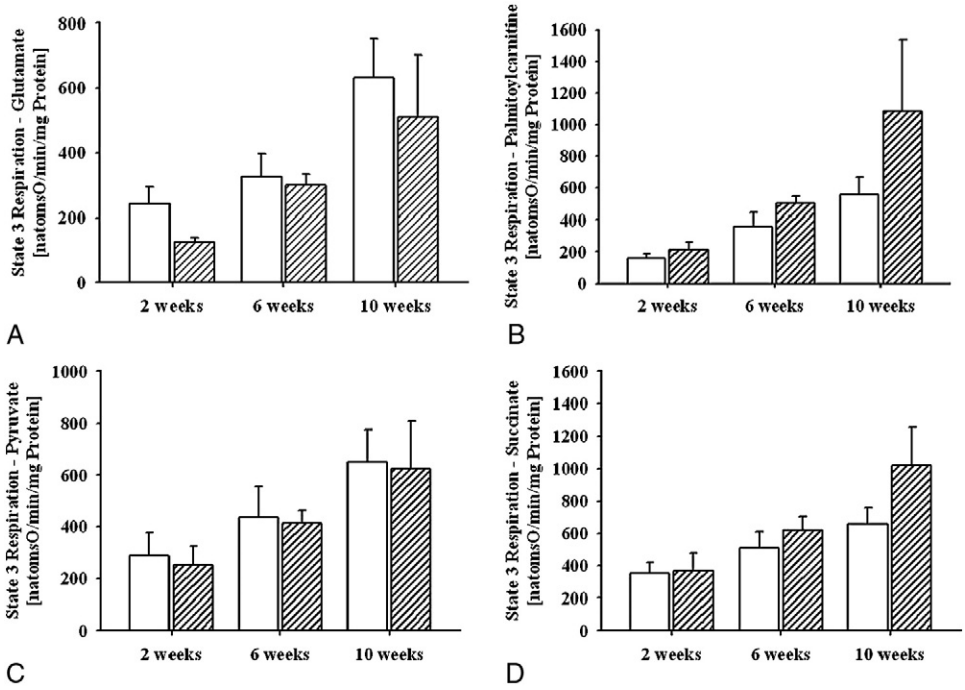

Fig. 3 – Effects of endurance exercise (10% incline, striped bars) on respiratory capacity (state 3 respiration) of isolated rat heart mitochondria. State 3 respiration was induced by adding ADP in the presence of glutamate (A), palmitoylcarnitine/malate (B), pyruvate/malate (C), and succinate as substrates. Data are mean ± SEM; n = 3 to 12 per group. *P < .05 compared with sedentary (white bars).

coordination between nuclear and mitochondrial genomes by directly regulating the expression of several nuclear-encoded ETC proteins and indirectly regulating the 13 mitochondrial-encoded subunits by activating Tfam, mitochondrial transcription factor B1, mitochondrial transcription factor B2, and mitochondrial transcription termination factor [27]. We measured mRNA expression of various nuclear encoded subunits of the ETC complexes and found no changes. However, we did not assess mitochondrial encoded subunits. Based on these facts, it is possible that mitochondrial biogenesis is not impaired at the level of the nuclear genome but rather at the level of the mitochondrial genome. Huang et al [9] found increased mitochondrial DNA deletion in rats trained with a similar training protocol. Messenger RNA expression of Tfam was normal in our rat model, but we did not assess the other parts of the mitochondrial transcription complex. Another supporting fact is that respiration with complex II substrate succinate and activity of complex II were unchanged in exercised animals because succinate dehydrogenase (complex II) is the only fully nuclear encoded ETC complex. In all other complexes, at least one subunit is encoded by the mitochondrial genome.

It is interesting to note that the ADP/O ratios for all complex I substrates and for all time points increased with exercise. This effect was not visible for complex II substrate succinate. Such an increase in ADP/O ratio would reflect better coupling between ATP production and oxygen consumption. We also analyzed UCP mRNA expression at different time points. We expected decreased UCP3 to correlate with the findings of increased coupling. However, our findings were inconsistent with increased UCP3 gene expression at 2 and 10 weeks and decreased expression at 6 weeks. Protein expression (analyzed at 10 weeks) was normal. Thus, the relevance of UCP3 in this context is questionable. It is a common notion that changes in state 4 respiration (Supplementary data: Table S5) would support the presumption of decreased or increased uncoupling in relation to UCP gene expression. State 4 respiration is used by many to calculate the respiratory control index. We are not certain whether state 4 respiration or respiratory control index is truly reflecting coupling of respiratory capacity to ATP production. We believe that ADP/O is a more appropriate parameter to assess this relationship, although we know that the ADP/O ratio may also have limitations. In any way, neither state 4 respiration nor ADP/O ratio was significantly altered among the groups, which did not further support our finding of increased UCP3 expression.

We also found reduced mRNA expression of ANT after 2 and 6 weeks of exercise. The role of the ADP/ATP translocator is to exchange ADP and ATP between the mitochondrial matrix and the cytoplasm. Adenine nucleotide translocator has also been suggested as a primary site of proton conductance in the inner mitochondrial membrane [28]. A decrease in the ANT content could also be a sign for a better coupling. In summary, one could conclude from this that ATP production seems to be more efficient and may counterbalance the temporary reductions in state 3 respiration.

In case of reduced ANT content, ATP would accumulate in the matrix and ADP in cytoplasm, which may hamper state 3 respiration independently of changes in the respiratory chain. This mechanism can be ruled out by measuring uncoupled respiration. By the addition of the uncoupler 2,4-dinitrophenol, we could measure uncoupled state 3 respiration without

accounting for the influences of the membrane potential, ATP synthase, and ATP/ADP translocator. The persistent decrease in respiratory capacity by collapsing the membrane potential supports the conclusion that mitochondrial respiration is restricted by defects localized in the ETC rather than in the components of the phosphorylation system.

Another surprising observation was the reduction of the heart failure markers BNP and ANF. These markers are considered sensitive indicators for the condition of contractile function [29]. We illustrate that changes in the contractile function are not paralleled by BNP expression. Exercise caused reductions in BNP expression in our study. This was also true at the 6-week time point, when contractile function was decreased and increased BNP expression would have been expected. We did not assess circulating BNP levels because the available BNP assays did not work in our rats. However, if our expression levels would parallel circulating BNP levels, the validity of BNP in animals or even humans performing repetitive exercise may be questioned.

Exercise is known to increase oxidative capacity in skeletal muscle [10,30-33]. In heart muscle, little is known about changes in oxidative capacity. Our findings are consistent with published data indicating that CS activity is not altered after endurance training in rat cardiac muscle [34-37]. It was suggested that myocardium has sufficient preexisting oxidative capacity to supply the energy requirement during exercise. Furthermore, most of the studies that examined cardiac muscle during exercise also found unchanged or reduced state 3 respiration [38,39].

Irrespective of the final explanation, most studies reporting physiological and pathological effects of exercise demonstrate results only at one time point, specifically at the end of the investigation [9,10,40]. Attempts to compare results are often hampered by the difference in protocol and investigated time point [9,10,40,41,38,39]. It is possible that a phase of contractile dysfunction may have been overlooked by not assessing it. For example, with protocols similar in intensity to ours, Marcil et al [38] report normal respiratory capacity at 10 weeks; and Terblanche et al [39] reported reduced oxidative capacity at 6 weeks. Both results are consistent with our findings; however, it is not clear whether function is reversible and whether the normal respiratory capacity of Marcil et al was preceded by impaired mitochondrial function. Similarly, in the group with lower intensity (10% incline), our study compares to that of Ascensao et al [41]. These authors showed an improvement of respiratory capacity after 14 weeks. No earlier time points of investigation were shown; therefore, it is not clear when this improvement had developed. Based on our results, shown in Fig. 3, it appears that extending our protocol by another 4 weeks may have also resulted in increased respiratory capacity in the exercised group. Taken together, these considerations illustrate that protocols affecting conditions with a chronic nature need to be examined at more than one time point. It currently cannot be excluded that many of the protocols described as inducers of physiological hypertrophy are associated with a temporary phase of dysfunction as shown in this article.

In conclusion, we demonstrate here that repetitive exercise may cause impaired contractility of the heart and mitochondrial dysfunction, characterized by impaired respiratory chain complex activities. This activity reduction is temporary and intensity related.

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Conflict of Interest

There is no conflict of interest.

REFERENCES

[1] Kemi OJ, Haram PM, Loennechen JP, Osnes JB, Skomedal T, Wisloff U, et al. Moderate vs. high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. Cardiovasc Res 2005;67:161-72.
[2] Kemi OJ, Loennechen JP, Wisloff U, Ellingsen O. Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. J Appl Physiol 2002;93:1301-9.
[3] Bugger H, Schwarzer M, Chen D, Schrepper A, Amorim PA, Schoepe M, et al. Proteomic remodelling of mitochondrial oxidative pathways in pressure overload-induced heart failure. Cardiovasc Res 2010;85:376-84.
[4] Douglas PS, O'Toole ML, Hiller WD, Hackney K, Reichek N. Cardiac fatigue after prolonged exercise. Circulation 1987;76:1206-13.
[5] Mousavi N, Czarnecki A, Kumar K, Fallah-Rad N, Lytwyn M, Han SY, et al. Relation of biomarkers and cardiac magnetic resonance imaging after marathon running. Am J Cardiol 2009;103:1467-72.
[6] Neilan TG, Yoerger DM, Douglas PS, Marshall JE, Halpern EF, Lawlor D, et al. Persistent and reversible cardiac dysfunction among amateur marathon runners. Eur Heart J 2006;27:1079-84.
[7] Contarteze RV, Manchado Fde B, Gobatto CA, De Mello MA. Stress biomarkers in rats submitted to swimming and treadmill running exercises. Comp Biochem Physiol A Mol Integr Physiol 2008;151:415-22.
[8] Moraska A, Deak T, Spencer RL, Roth D, Fleshner M. Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol 2000;279:R1321-9.
[9] Huang CC, Lin TJ, Chen CC, Lin WT. Endurance training accelerates exhaustive exercise-induced mitochondrial DNA deletion and apoptosis of left ventricle myocardium in rats. Eur J Appl Physiol 2009;107:697-706.
[10] Burelle Y, Wambolt RB, Grist M, Parsons HL, Chow JC, Antler C, et al. Regular exercise is associated with a protective metabolic phenotype in the rat heart. Am J Physiol Heart Circ Physiol 2004;287:H1055-63.
[11] Doenst T, Pytel G, Schrepper A, Amorim P, Farber G, Shingu Y, et al. Decreased rates of substrate oxidation ex vivo predict

the onset of heart failure and contractile dysfunction in rats with pressure overload. Cardiovasc Res 2010;86:461-70.
[12] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.
[13] Perlini S. Determinants of left ventricular mass in hypertensive heart disease: a complex interplay between volume, pressure, systolic function and genes. J Hypertens 2007;25:1341-2.
[14] Palmer JW, Tandler B, Hoppel CL. Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J Biol Chem 1977;252:8731-9.
[15] Srere P. Citrate synthase. Methods Enzymol 1969;13:3–11.
[16] Bugger H, Chemnitius JM, Doenst T. Differential changes in respiratory capacity and ischemia tolerance of isolated mitochondria from atrophied and hypertrophied hearts. Metabolism 2006;55:1097-106.
[17] Schwarzer M, Faerber G, Rueckauer T, Blum D, Pytel G, Mohr FW, et al. The metabolic modulators, etomoxir and NVP-LAB121, fail to reverse pressure overload induced heart failure in vivo. Basic Res Cardiol 2009;104:547-57.
[18] Janssen AJ, Trijbels FJ, Sengers RC, Smeitink JA, van den Heuvel LP, Wintjes LT, et al. Spectrophotometric assay for complex I of the respiratory chain in tissue samples and cultured fibroblasts. Clin Chem 2007;53:729-34.
[19] Krahenbuhl S, Chang M, Brass EP, Hoppel CL. Decreased activities of ubiquinol:ferricytochrome c oxidoreductase (complex III) and ferrocytochrome c:oxygen oxidoreductase (complex IV) in liver mitochondria from rats with hydroxycobalamin[c-lactam]–induced methylmalonic aciduria. J Biol Chem 1991;266:20998-1003.
[20] Wharton DC, Tzagoloff A. Cytochrome oxidase from beef heart mitochondria. Methods Enzymol 1967;10:245-50.
[21] Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, et al. Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. Circ Res 2004;94:110-8.
[22] Kemi OJ, Ceci M, Wisloff U, Grimaldi S, Gallo P, Smith GL, et al. Activation or inactivation of cardiac Akt/mTOR signaling diverges physiological from pathological hypertrophy. J Cell Physiol 2008;214:316-21.
[23] Ni YG, Berenji K, Wang N, Oh M, Sachan N, Dey A, et al. Foxo transcription factors blunt cardiac hypertrophy by inhibiting calcineurin signaling. Circulation 2006;114:1159-68.
[24] Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator–1 promotes cardiac mitochondrial biogenesis. J Clin Invest 2000;106:847-56.
[25] Ikeda S, Kizaki T, Haga S, Ohno H, Takemasa T. Acute exercise induces biphasic increase in respiratory mRNA in skeletal muscle. Biochem Biophys Res Commun 2008;368:323-8.
[26] Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev 2004;18:357-68.
[27] Scarpulla RC. Nuclear control of respiratory gene expression in mammalian cells. J Cell Biochem 2006;97:673-83.
[28] Brustovetsky N, Klingenberg M. The reconstituted ADP/ATP carrier can mediate H+ transport by free fatty acids, which is further stimulated by mersalyl. J Biol Chem 1994;269:27329-36.
[29] Abassi Z, Karram T, Ellaham S, Winaver J, Hoffman A. Implications of the natriuretic peptide system in the pathogenesis of heart failure: diagnostic and therapeutic importance. Pharmacol Ther 2004;102:223-41.
[30] Holloszy JO, Oscai LB, Don IJ, Mole PA. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. Biochem Biophys Res Commun 1970;40:1368-73.
[31] Naito H, Powers SK, Demirel HA, Aoki J. Exercise training increases heat shock protein in skeletal muscles of old rats. Med Sci Sports Exerc 2001;33:729-34.
[32] Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. J Appl Physiol 1996;80:2250-4.
[33] el Midaoui A, Tancrede G, Nadeau A. Effect of physical training on mitochondrial function in skeletal muscle of normal and diabetic rats. Metabolism 1996;45:810-6.
[34] Oscai LB, Mole PA, Holloszy JO. Effects of exercise on cardiac weight and mitochondria in male and female rats. Am J Physiol 1971;220:1944-8.
[35] Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, et al. Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. Am J Physiol 1998;275:R1468-77.
[36] Siu PM, Donley DA, Bryner RW, Alway SE. Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. J Appl Physiol 2003;94:555-60.
[37] Zonderland ML, Bar PR, Reijneveld JC, Spruijt BM, Keizer HA, Glatz JF. Different metabolic adaptation of heart and skeletal muscles to moderate-intensity treadmill training in the rat. Eur J Appl Physiol Occup Physiol 1999;79:391-6.
[38] Marcil M, Bourduas K, Ascah A, Burelle Y. Exercise training induces respiratory substrate-specific decrease in Ca2+-induced permeability transition pore opening in heart mitochondria. Am J Physiol Heart Circ Physiol 2006;290:H1549-57.
[39] Terblanche SE, Gohil K, Packer L, Henderson S, Brooks GA. The effects of endurance training and exhaustive exercise on mitochondrial enzymes in tissues of the rat (*Rattus norvegicus*). Comp Biochem Physiol A Mol Integr Physiol 2001;128:889-96.
[40] Murakami T, Shimomura Y, Fujitsuka N, Sugiyama S. Differential adaptation to endurance training between heart and gastrocnemius muscle mitochondria in rats. Biochem Mol Biol Int 1995;36:285-90.
[41] Ascensao A, Magalhaes J, Soares JM, Ferreira R, Neuparth MJ, Marques F, et al. Endurance training limits the functional alterations of rat heart mitochondria submitted to in vitro anoxia-reoxygenation. Int J Cardiol 2006;109:169-78.