Exercise training changes autonomic cardiovascular balance in mice


1Hypertension Unit, Heart Institute (INCOR), University of São Paulo, Medical School, 05403-000 São Paulo; 2Nephrology Department, Federal University of São Paulo, 04023-900 São Paulo; 3São Judas Tadeu University, 03160-000 São Paulo, Brazil; and 4Department of Pharmacology and Toxicology, Wright State University School Medicine, Dayton, Ohio, 45435

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Exercise training changes autonomic cardiovascular balance in mice. J Appl Physiol 96: 2174–2178, 2004. First published January 16, 2004; 10.1152/japplphysiol.00870.2003.—Experiments were performed to investigate the influence of exercise training on cardiovascular function in mice. Heart rate, arterial pressure, baroreflex sensitivity, and autonomic control of heart rate were measured in conscious, unrestrained male C57/6J sedentary (n = 8) and trained mice (n = 8). The exercise training protocol used a treadmill (1 h/day; 5 days/wk for 4 wk). Baroreflex sensitivity was evaluated by the tachycardic and bradycardic responses induced by sodium nitroprusside and phenylephrine, respectively. Autonomic control of heart rate and intrinsic heart rate were determined by use of methylatropine and propranolol. Resting bradycardia was observed in trained mice compared with sedentary animals [485 ± 9 vs. 612 ± 5 beats/min (bpm)], whereas mean arterial pressure was not different between the groups (106 ± 2 vs. 108 ± 3 mmHg). Baroreflex-mediated tachycardia was significantly enhanced in the trained group (0.97 ± 0.97 vs. 1.6 ± 0.21 bpm/mmHg, trained vs. sedentary), whereas baroreflex-mediated bradycardia was not altered by training. The tachycardia induced by methylatropine was significantly increased in trained animals (139 ± 12 vs. 40 ± 9 bpm, trained vs. sedentary), whereas the propranolol effect was significantly reduced in the trained group (49 ± 11 vs. 97 ± 11 bpm, trained vs. sedentary). Intrinsic heart rate was similar between groups. In conclusion, dynamic exercise training in mice induced a resting bradycardia and an improvement in baroreflex-mediated tachycardia. These changes are likely related to an increased vagal and decreased sympathetic tone, similar to the exercise response observed in humans.

Bradycardia; autonomic nervous system; baroreflex; blood pressure increases in HR, maximum oxygen consumption (VO2 max), and respiratory exchange ratio, similar to that seen in larger species (11). A test of β-adrenergic stimulation suggested that sympathetic tone was not altered, whereas vagal input was reduced. Swimming (4-wk duration) in mice produced cardiac hypertrophy, a decrease in the HR response to a submaximal workload, and an increase in muscle succinate dehydrogenase activity (15). HR in an isolated vagal-atrial preparation was reduced in mice trained for 10 wk on a treadmill (7). The authors concluded that training-induced bradycardia was associated with an increase in vagal function (7). Niebauer et al. (25) reported a reduced chronotropic response to submaximal exercise in trained (12 wk on treadmill) compared with sedentary mice, an effect that was associated with increased oxidative enzyme capacity.

Questions remain as to the effects of exercise training on autonomic cardiovascular function in mice, specifically in the conscious state. This is an important issue with the development of new genetic mice models that may be applied to clinically important problems. The objective of the present study was to investigate the effect of exercise training in conscious C57/6J mice on arterial pressure (AP), HR, baroreflex sensitivity, and autonomic control of HR. We tested the hypothesis that exercise training induces resting bradycardia that is associated with increased vagal and decreased sympathetic tone. We propose that this will be a useful model for the study of exercise and cardiovascular interactions that will be relevant to the human condition.

METHODS

Male C57/6J mice (25–30 g) were obtained from the breeding facility of the University of São Paulo (São Paulo, Brazil). Mice received standard laboratory chow and tap water ad libitum and were housed in temperature-controlled rooms (22°C) under a 12:12-h dark-light cycle. All animal protocols were approved by the Experimental Animal Use Committee of the University of São Paulo. Mice were randomly assigned to sedentary (S, n = 8) or trained (T, n = 8) groups.

Exercise protocols. Exercise training was performed on a motor treadmill at low-moderate intensity (~50–70% maximal running speed) for 1 h a day, 5 days/week for 4 wk, with a gradual increase in speed from 0.3 to 1.2 km/h. All animals were adapted to the procedure (10 min/day; 0.3 km/h) for 1 wk before beginning the exercise training protocol. After the adaptation, the sedentary group was exposed to exercise only during the maximum treadmill test. However, they were placed on the stationary treadmill three times a week to provide a similar environment.

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Sedentary and trained mice were submitted to a maximal treadmill test as described in detail in a previous publication (11). The tests were made at the beginning of the experiment and in the second and fourth weeks of the training protocol. The purpose was to determine aerobic capacity and exercise training intensity.

**Cardiovascular measurements.** AP and HR were measured by tail-cuff plethysmography at 0 time and after 2 and 4 wk. After the last training session, mice were anesthetized (ketamine-xylazine 80:40 mg/kg ip) and a polyethylene-tipped Tygon cannulas (4 cm of PE-08 connected to 2 cm of PE-50, Clay Adams) filled with heparinized saline were inserted into the carotid artery and jugular vein for direct measurements of arterial pressure and drug administration, respectively. The free ends of the cannulas were tunneled subcutaneously and exteriorized at the top of the skull. Two days after the catheter placement, hemodynamic measurements were made in conscious, freely moving mice. The arterial cannula was connected to a transducer (Narco Bio-Systems Miniature Pressure Transducer RP 1500), and blood pressure signals were recorded for a 20-min period using a microcomputer equipped with an analog-to-digital converter (CODAS, 4-kHz sampling frequency, Dataq Instruments). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean arterial pressure (MAP) and HR.

Baroreflex sensitivity was evaluated by a mean index relating the tachycardic or the bradycardic responses for each 5-mmHg interval of MAP changes (~30–40 mmHg) induced by sodium nitroprusside (8 μg/kg body wt iv) or phenylephrine (8 μg/kg body wt iv) injections, respectively. Data were expressed as beats per minute (bpm) per mmHg. Maximal volume per injection was <20 μl.

Vagal and sympathetic function and HR were measured by determining the response to methylatropine (1 mg/kg iv) and propranolol (1 mg/kg iv) with a maximal injection volume of 40 μl in a 2-day protocol (35). Because the HR responses to methylatropine and propranolol reach their peak in 3 min, this time interval was used to quantify the drug-induced HR changes. On the first day of study, resting HR was recorded while the rats were in their home cages in an unrestrained state. After methylatropine injection, AP and HR were recorded for 3 min. Propranolol was injected 6 min after methylatropine, and the response was measured for 3 min. The HR was evaluated after the combined treatment with propranolol and methylatropine. On the second day, the sequence of injections was inverted, beginning with the propranolol injection. The methylatropine effect was evaluated as the difference between the maximum HR after methylatropine and the control HR. The propranolol effect was evaluated as the difference between the control HR and minimum HR produced after propranolol injection.

**Statistical analysis.** Data are expressed as means ± SE, and Student’s unpaired t-test was used to compare the values of both groups. Significance level was established at \( P < 0.05 \).

**RESULTS**

Body weight was not different between the groups at the beginning (23.6 ± 2.03 vs. 24 ± 2.8 g in S group) or the end of the training protocol (25.8 ± 0.18 vs. 26.5 ± 0.9 g in S group).

**Maximal exercise protocol.** Aerobic physical performance was evaluated by the response to the maximal treadmill test. At the beginning of the experiment, the physical capacity was similar between groups (1.61 ± 0.22 vs. 1.98 ± 0.27 km/h, T vs. S group). However, the animals submitted to exercise training showed an increase in the maximum speed of running compared with sedentary group after 2 and 4 wk of exercise training (2nd wk: 2.63 ± 0.31 vs. 1.87 ± 0.26; 4th wk: 2.70 ± 0.16 vs. 1.91 ± 0.15 km/h, S vs. T group).

**Cardiovascular measurements.** The time course of the HR and systolic arterial pressure (SAP) changes were followed via tail-cuff plethysmography (0, 2, and 4 wk). SAP was similar between the groups (111 ± 10; 104 ± 9; 102 ± 7 vs. 102 ± 4; 102 ± 5; 101 ± 7 mmHg in S vs. T group at 0, 2, and 4 wk). HR was not different between the groups at 0 and 2 wk (577 ± 10 and 559 ± 13 vs. 593 ± 9 and 578 ± 11 bpm in T and S groups, respectively). However, trained mice showed a significantly lower resting HR after 4 wk of training compared with sedentary mice (505 ± 9 vs. 591 ± 8 bpm, T vs. S).

At the end of the experiment, direct measurements of AP in conscious mice confirmed indirect cardiovascular measurements (Table 1). SAP, diastolic arterial pressure, and MAP were similar between the groups; however, resting HR was lower in trained mice (485 ± 9 bpm) compared with sedentary mice (612 ± 5 bpm) only after 4 wk of training. This indicates that the exercise training induced a resting bradycardia.

The baroreflex tachycardic response evoked by sodium nitroprusside was markedly increased in the trained group (4-fold increase) compared with the sedentary group. However, baroreflex bradycardia evoked by phenylephrine was not changed by exercise training (Table 1, Fig. 1).

Vagal and sympathetic activities in sedentary and trained mice are shown in Fig. 2. Sedentary mice showed a decreased response to methylatropine compared with propranolol effect on basal HR (40 ± 9 vs. 97 ± 11 bpm, respectively). Interestingly, when the mice were submitted to exercise training, the methylatropine effect became significantly higher compared with propranolol effect (139 ± 12 vs. 49 ± 11 bpm, respectively). Furthermore, the methylatropine effect was markedly enhanced (3-fold increase) and the propranolol effect (51%) was decreased in trained mice compared with sedentary mice (Table 1, Fig. 2). The exercise training did not change the IHR in mice (Table 1).

**DISCUSSION**

Using a conscious mouse model, we showed that exercise training produces alterations in autonomic control of the heart that are very similar to those observed in humans. Chronic aerobic exercise in mice induced resting bradycardia, probably related to an enhancement in cardiac vagal and a decrease in
cardiac sympathetic input. Furthermore, training improved baroreflex sensitivity as seen by the increase in the tachycardic response to hypotension. The results document that mice provide a clinically relevant model for the study of exercise-cardiovascular interactions.

Resting bradycardia usually occurs in trained humans (15, 31, 32) and exercise-trained rats (8, 20, 24, 28) and is considered to be a hallmark of exercise training improvement (8, 16, 20, 24, 28). Our results verify that bradycardia is also a characteristic of training in mice as seen in a previous study (25). The presence of the bradycardia is important because it documents the effectiveness of the training protocol. In this context, the HR values observed in the sedentary mice are similar to those previously observed in this and other mouse strains (14, 21, 27).

The mechanisms underlying the cardiac adaptive responses to exercise training are distinct in different species. In normal rats, the reduction in IHR (pacemaker mediated) after exercise training seems to be the causal mechanism for the resting bradycardia (24, 28). Studies demonstrate that both sympathetic and parasympathetic input were reduced after a program of physical activity in normotensive rats (20, 24). However, the alteration in resting HR after training may be produced by different mechanisms in pathological conditions such as hypertension, diabetes, aging, etc (8, 9). In humans, resting bradycardia is associated with a decrease in IHR (16) as well as altered autonomic balance, a parasympathetic dominance (31, 32). Similar to humans, the exercise-induced bradycardia seen in this study is probably related to a decrease in sympathetic and increase in parasympathetic input, also resulting in cardiac vagal dominance. This is in contrast to the cardiac sympathetic dominance, characteristic of sedentary mice. The importance of these findings is that improvement in indexes of parasympathetic activity has been linked to physical performance and has emerged as an independent protector against sudden cardiac death (6).

The mechanisms by which exercise produces changes in autonomic control are unknown. However, there is evidence for alterations in the central (21) and afferent and efferent pathways (3) or in the effector organs (receptor function) (12). Recently, Danson and Paterson (7) showed that a nitric oxide-dependent facilitation of vagal-induced bradycardia is augmented after exercise training. In vivo gene transfer of nitric oxide synthase I into the atrial wall mimicked the exercise-trained vagal phenotype (23). This suggests that nitric oxide synthase I may be a key protein for increasing cardiac vagal function.

The baseline AP values observed in the present study were similar to those seen in other mouse strains (14, 21, 27). In addition, there was no change in basal AP after exercise training in these normotensive mice. This is consistent with reports in normotensive humans and rats (8, 10, 24). In contrast, exercise training was shown to decrease basal AP in hypertensive humans (26, 33) and rats (30) or to increase basal AP in hypertensive diabetic rats (9). Whereas baseline AP was unaltered after training, the controlling mechanisms are changed, with evidence for increases in baroreflex gain (1, 3, 10, 24, 26, 30).

The mechanisms responsible for increased baroreflex sensitivity were not directly addressed in the present study. However, the results from the cholinergic blockade study may provide some clues. Methylation produced a greater increase in HR in trained compared with sedentary mice. This enhancement could represent an improvement in the vagal reserve required for the HR responses evoked by baroreceptor stimulation. Alternatively, Brum et al. (3) observed an increase in baroreceptor sensitivity in trained normotensive rats, suggesting that similar alterations can occur in normotensive mice.

![Fig. 1. Baroreflex sensitivity evaluated by tachycardic and bradycardic responses evoked by sodium nitroprusside and phenylephrine injections, respectively, in sedentary and trained mice. bpm, Beats/min. *P < 0.05 compared with sedentary mice.](image1)

![Fig. 2. Graphs showing heart rate (HR), propranolol effect (PRE), and methylatropine effect (ME) in sedentary and trained mice. Values are means ± SE. The PRE was evaluated as the difference between the control HR and the minimal HR after propranolol injection. The ME was evaluated as the difference between the maximum HR after the methylatropine injection and the control HR. *P < 0.05 compared with sedentary mice.](image2)
It was surprising that exercise training did not alter baroreflex-mediated bradycardia in mice, suggesting that the increase in the tachycardia response was not due to an alteration in baroreflex gain or that the increase in baroreflex gain were opposed by alterations occurring along the entire reflex arc that blunted an enhancement in baroreflex-mediated bradycardia. Chen et al. (5) observed an attenuation of baroreflex tachycardia in anesthetized rats, subjected to daily spontaneous running. Because the baroreflex gain sensitivity was similar in exercise-trained and sedentary rats, they attributed the baroreflex attenuation to changes in the central component of the reflex, rather than a change in baroreceptor discharge. Moreover, according to the mechanoreflex concept, in the presence of increased vascular compliance, the same pulse can result in increased baroreceptors activation (19). Because exercise training increases aortic compliance in rats (18) and humans (4), we postulate that the improvement in baroreflex-mediated tachycardia may be related to an increase in vascular compliance. Endothelial changes after exercise training is another attractive hypothesis to explain the baroreflex alterations, because both the magnitude and the frequency of shear stress on the endothelial cells during exercise enhance the release of endothelial factors. These factors could in turn increase arterial baroreceptors activity (4). Nonetheless, we cannot exclude the possibility that exercise is associated with alterations in the central and efferent components of the baroreflex pathway.

One question is whether the exercise protocol used in our study was effective in producing physical training in the mice. One index that has been used for measuring exercise response is true \( V_{O2}^{\text{max}} \); however, this measurement is difficult to obtain in mice. A significant association (\( r = 0.83 \)) between oxygen consumption and running velocity (range from 14.3 to 43.1 \( \text{mlkg}^{-1}\text{min}^{-1} \)) was reported in untrained rats (2). Schefer and Talan (29) demonstrated in C57/6j mice that changes in treadmill speed from 3 to 25 m/min resulted in a progressive increase in oxygen consumption. These studies taken together suggest that it is appropriate to characterize the exercise load as a percent of \( V_{O2}^{\text{max}} \). In the present study, trained mice showed a marked increase in the estimated aerobic physiological capacity as evaluated by their response to the maximal exercise test. Furthermore, the finding of a resting bradycardia is a good indication of the efficacy of the exercise training protocol to produce overall fitness.

In conclusion, dynamic chronic exercise in mice changes autonomic balance in the heart and improves baroreflex control of blood pressure. These hemodynamic adaptations are probably related to an increase in vagal and decrease in sympathetic outflow.

**REFERENCES**


