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Tonic and reflex cardiovascular autonomic control in trained-female rats

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Abstract

The effects of exercise training on cardiovascular and autonomic functions were investigated in female rats. After an aerobic exercise training period (treadmill: 5 days/week for 8 weeks), conscious female Wistar (2 to 3 months) sedentary (S, N = 7) or trained rats (T, N = 7) were cannulated for direct arterial pressure (AP) recording in the non-ovulatory phases. Vagal (VT) and sympathetic tonus (ST) were evaluated by vagal (atropine) and sympathetic (propranolol) blockade. Baroreflex sensitivity was evaluated by the heart rate responses induced by AP changes. Cardiopulmonary reflex was measured by the bradycardic and hypotensive responses to serotonin. Resting bradycardia was observed in T (332 ± 7 bpm) compared with S animals (357 ± 10 bpm), whereas AP did not differ between groups. T animals exhibited depressed VT and ST (32 ± 7 and 15 ± 4 bpm) compared to S animals (55 ± 5 and 39 ± 10 bpm). The baroreflex and cardiopulmonary bradycardic responses were lower in T (-1.01 ± 0.27 bpm/mmHg and -17 ± 6 bpm) than in the S group (-1.47 ± 0.3 bpm/mmHg and -41 ± 9 bpm). Significant correlations were observed between VT and baroreflex (r = -0.72) and cardiopulmonary (r = -0.76) bradycardic responses. These data show that exercise training in healthy female rats induced resting bradycardia that was probably due to a reduced cardiac ST. Additionally, trained female rats presented attenuated bradycardic responses to baro- and cardiopulmonary receptor stimulation that were associated, at least in part, with exercise training-induced cardiac vagal reduction.

Key words: Baroreflex; Cardiopulmonary reflex; Exercise training; Female; Heart rate

Introduction

Resting bradycardia induced by exercise training has been well documented in humans and animals; however, the underlying mechanisms are not yet understood. Studies in men have suggested that increased vagal activity is responsible for a decrease in heart rate (HR) (1-3). In contrast, resting bradycardia in young trained male rats has been more closely associated with alterations in cardiac pacemakers (4,5). Moreover, improvement in baroreflex control of HR observed in trained male animals (6-8) and humans (9-11) may also play a role in basal HR changes. Additionally, few studies have so far analyzed the effects of exercise training on cardiopulmonary reflex, which plays a role in the homeostasis of fluid balance. Studies have shown that cardiopulmonary reflex was tonically stimulated in trained male rabbits (12) or blunted in male athletes (13). The results cited above illustrate the fact that most published studies have been performed on male subjects.

Regarding HR regulation in the resting condition, men, in contrast to women of the same age, seem to have a predominant sympathetic tone (14,15). Furthermore, studies have demonstrated a lower cardiac sympathetic modulation by spectral analysis in healthy women compared to men (15). Chen and DiCarlo (16) demonstrated that female rats had a higher maximum baroreflex gain than male rats, and daily spontaneous running attenuated the arterial baroreflex regulation of HR in both sexes. However, questions remain about the effects of controlled (duration, frequency and intensity) exercise training on hemodynamics and autonomic cardiovascular control in female rats, specifically in the conscious state. Therefore, the objective of the present study was to investigate the effect of exercise training on arterial pressure (AP), HR, baroreflex and cardiopulmonary reflex sensitivities, and autonomic control of HR in conscious female rats.

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Material and Methods

Female Wistar rats (200-230 g) were obtained from the Animal Facilities of São Judas Tadeu University (São Paulo, SP, Brazil). The rats received standard laboratory chow and tap water *ad libitum* and were housed in a temperature-controlled room (22-24°C) under a 12/12-h dark/light cycle. All surgical procedures and protocols used were approved by the Ethics Committee of São Judas Tadeu University (Protocol #076/2004) and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were randomly assigned to sedentary (S, N = 7) or trained (T, N = 7) groups.

Exercise protocols

Exercise training was performed on a motor treadmill (Imbramed TK-01, Brazil) at low-moderate intensity (~50-60% maximum running speed) for 1 h a day, 5 days a week for 8 weeks, with a gradual increase in speed from 0.3 to 1.0 km/h. All animals were adapted to the procedure (10 min/day; 0.3 km/h) for 1 week before the beginning of the exercise training protocol (7,17-19). After adaptation, the sedentary group was exposed to exercise only during the maximum treadmill test. However, the animals were placed on the stationary treadmill three times a week to provide a similar environment.

Sedentary and trained rats were submitted to a maximum treadmill test as described in detail in a previous study (20). Tests were performed at the beginning of the experiment and in the 4th and 8th weeks of the training protocol. The purpose was to determine physical capacity and exercise training intensity.

Determination of the estrous cycle phases

Vaginal secretion was collected and observed under a light microscope for the determination of the estrous cycle phases (21). Given the short time of the ovulatory phase, all evaluations were performed during the non-ovulatory phases of the estrous cycle (metestrous and diestrous).

Cardiovascular measurements

After the last training session, rats were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg) to implant 2 polyethylene-tipped Tygon cannulas filled with heparinized saline into the right 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. To avoid detraining hemodynamics, measurements were made in conscious, freely moving rats in their home cage 24 h after surgery since at that time no significant differences have been observed in AP values (17-19). The arterial cannula was connected to a transducer (Blood Pressure XDCR, Kent® Scientific, USA), and blood pressure signals were recorded for a 30-min period using a microcomputer equipped with an analog-to-digital converter (CODAS, 2Kz, DATAQ Instruments, USA). The recorded data were analyzed on a beat-to-beat basis to quantify changes in systolic (SAP), diastolic (DAP) and mean AP (MAP) and HR.

Increasing doses of phenylephrine (0.5 to 2.0 µg/mL) and sodium nitroprusside (5 to 20 µg/mL) were given as sequential bolus injections (0.1 mL) to produce pressure responses ranging from 5 to 40 mmHg for both pressoric and depressoric responses (18). A 3- to 5-min interval between doses was necessary for blood pressure to return to baseline. Peak increases or decreases in MAP after phenylephrine or sodium nitroprusside injection and the corresponding peak reflex changes in HR were recorded for each drug dose. Baroreflex sensitivity was evaluated by fitting a regression line through the points relating the changes in HR to the changes in MAP (7).

The responses to stimulation of chemosensitive cardiopulmonary receptors (Bezold-Jarisch reflex) were determined by successive bolus injections of serotonin (2, 4, and 16 µg/kg) while the MAP and HR were recorded in the animals. Baseline values and peak changes of MAP and HR for each given dose were considered for data analysis. Injections were not repeated until the recorded parameters had returned to preinjection levels (22).

After the cardiovascular reflex evaluations, the vagal and the sympathetic tonus and intrinsic heart rate (IHR) were measured by determining the response to methylatropine (3 mg/kg, *iv*) and propranolol (4 mg/kg, *iv*) with a maximum volume of 0.2 mL per injection (17,19,23). Because the HR response to these drugs reaches its peak within 3 to 5 min, this time interval was allowed to elapse before the HR measurement. Propranolol was injected 10 min after methylatropine, and again the response was evaluated after simultaneous blockade with propranolol and methylatropine. On the subsequent day, the sequence of injections was inverted (first propranolol and then methylatropine) and the IHR was evaluated after simultaneous blockade with propranolol and methylatropine. Sympathetic tonus was determined as the difference between maximum HR after methylatropine injection and IHR. Vagal tonus was obtained by the difference between the lowest HR after propranolol injection and IHR (17,19,23).

Statistical analysis

Data are reported as means ± SEM. The Student unpaired *t*-test and two-way ANOVA were used to compare groups, followed by the Student-Newman-Keuls test. Correlations were determined by linear regression analysis. The level of significance was established at $P < 0.05$.

Results

Body weight was similar in both groups at the beginning of the protocol (S: 245 ± 11 and T: 242 ± 7 g). Exercise training induced a reduction in body weight gain (S: 288 ± 20 *vs* T: 265 ± 14 g; $P < 0.05$).

Maximum exercise protocol

Physical capacity was evaluated by the response to the maximum treadmill test. At the beginning of the experiment, physical capacity was similar in both groups (S: 1.83 ± 0.2 and T: 1.96 ± 0.44 km/h). However, the animals submitted to exercise training showed an increase in maximum running speed when compared with the sedentary group after 4 and 8 weeks of exercise training (4th week: 2.24 ± 0.35 *vs* 1.85 ± 0.29 km/h; 8th week: 2.5 ± 0.28 *vs* 1.8 ± 0.45 km/h, T *vs* S; P < 0.05).

Arterial pressure and heart rate

SAP, DAP, and MAP were similar in the sedentary and trained rats. Resting bradycardia was observed in the trained group in relation to the sedentary group (P < 0.05; Table 1).

Autonomic control of HR

The autonomic control of HR and IHR are shown in Table 1. The vagal tonus was significantly reduced in T rats as compared to S rats. The sympathetic tonus was also significantly diminished after exercise training (62%). The IHR obtained after methylatropine and propranolol blockade was similar in the two groups.

A positive correlation determined by linear regression was obtained between sympathetic tonus and resting HR, showing that reduced sympathetic tonus was related to diminished resting HR (r = 0.7, P < 0.05; Figure 1A); however, no correlation was observed between vagal tonus and basal HR (r = 0.13, P > 0.05; Figure 1B).

Baroreflex sensitivity

The reflex bradycardia elicited by the increase in AP was significantly reduced in the T group compared to the S group (-1.01 ± 0.27 *vs* -1.47 ± 0.3 mmHg; P < 0.05). No significant difference was observed between groups in the baroreflex tachycardia induced by AP falls (S: 4.36 ± 0.32 and T: 4.11 ± 0.19 mmHg; Figure 2).

Table 1. Cardiovascular and autonomic evaluations of the sedentary and trained female rats.

Parameters	Sedentary	Trained
MAP (mmHg)	108 ± 1	105 ± 2
SAP (mmHg)	125 ± 3	126 ± 2
DAP (mmHg)	92 ± 1	88 ± 2
Heart rate (bpm)	357 ± 10	332 ± 7*
Vagal tonus (bpm)	55 ± 5	32 ± 7*
Sympathetic tonus (bpm)	39 ± 10	15 ± 4*
Intrinsic heart rate (bpm)	368 ± 8	353 ± 6

Data are reported as means ± SEM for 7 rats in each group. MAP = mean arterial pressure; SAP = systolic AP; DAP = diastolic AP. *P < 0.05 compared to sedentary rats (Student unpaired *t*-test).

The vagal tonus (r = -0.72, P < 0.05; Figure 3A) and the basal HR (r = -0.6, P < 0.05; Figure 3B) were inversely correlated with the bradycardic response to AP increase in the studied rats, suggesting that the animals presenting reduced vagal tonus or resting bradycardia had an attenuated baroreflex.

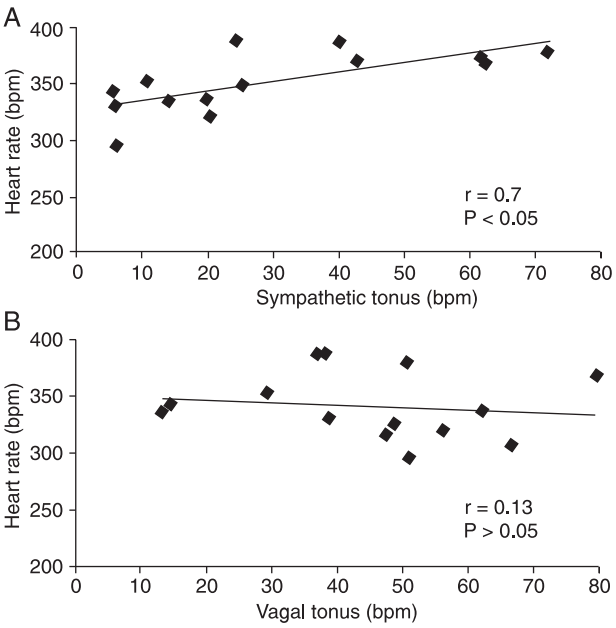

Figure 1. Correlations between (A) resting heart rate and sympathetic tonus (P < 0.05) and (B) resting heart rate and vagal tonus for both sedentary and trained rats. Correlations were determined by linear regression analysis.

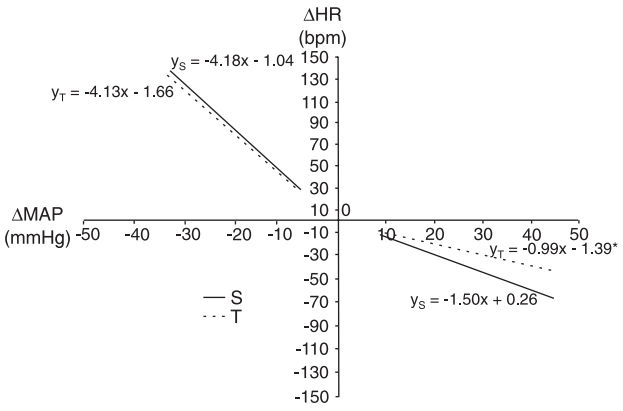

Figure 2. Regression lines of the bradycardic and tachycardic response to pressor changes induced by increasing doses of phenylephrine and sodium nitroprusside in sedentary (S) and trained (T) female rats (P < 0.05 *vs* S). HR = heart rate; MAP = mean arterial pressure. Correlations were determined by linear regression analysis.

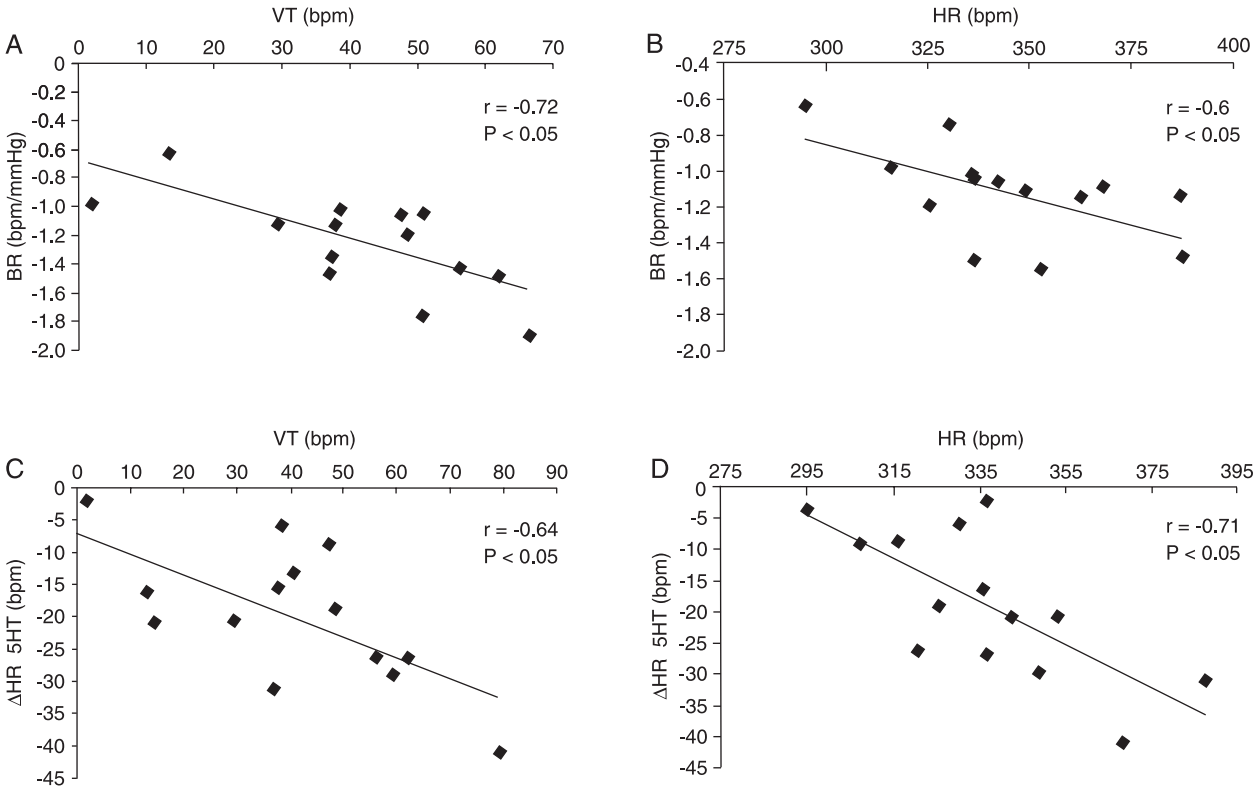

Figure 3. Correlations between (A) vagal tonus (VT) and bradycardic responses (BR) to falls in arterial pressure ($r = -0.72$, $P < 0.05$), (B) heart rate (HR) and BR ($r = -0.6$, $P < 0.05$), (C) VT and BR (ΔHR) to serotonin ($r = -0.64$, $P < 0.05$), and (D) HR and ΔHR to serotonin ($r = -0.71$, $P < 0.05$). 5HT = 5-hydroxytryptamine (serotonin). Correlations were determined by linear regression analysis of data for both sedentary and trained female rats.

Cardiopulmonary reflex sensitivity

The doses of 2 and 4 µg/kg serotonin induced similar bradycardic responses in the groups studied. The bradycardic response to serotonin at the dose of 16 µg/kg was significantly higher than that observed at the doses of 2 and 4 µg/kg in the S group. The bradycardic response to the higher dose of serotonin (16 µg/kg) was reduced in the T group compared to the S group. The hypotensive response to serotonin was similar in the two groups (Table 2).

In addition, vagal tonus ($r = -0.64$, $P < 0.05$; Figure 3C) and basal HR ($r = -0.71$, $P < 0.05$; Figure 3D) were inversely correlated with the bradycardic responses to serotonin, indicating that rats with reduced vagal tonus had an attenuated bradycardic response to the cardiopulmonary reflex evoked by serotonin injection.

Discussion

Although cardiovascular adaptation of males to dynamic exercise training has been extensively studied, the adaptations induced in females by exercise training are not well known. In the present study, we observed that aerobically trained female rats presented resting bradycardia, probably associated with sympathetic tonus reduction. Furthermore, the index for baro- and cardiopulmonary reflex sensitivity suggested an attenuation of bradycardic responses in the

Table 2. Cardiopulmonary reflex responses of the sedentary and trained female rats.

Cardiopulmonary response	Sedentary	Trained
Δ Heart rate (bpm)		
2 µg/kg serotonin	-17 ± 5	-7 ± 2
4 µg/kg serotonin	-19 ± 5	-14 ± 4
16 µg/kg serotonin	-42 ± 9^{+}	-17 ± 6*
Δ Arterial pressure (mmHg)		
2 µg/kg serotonin	-4.4 ± 1.5	-3.5 ± 0.6
4 µg/kg serotonin	-7.4 ± 2.3	-6.3 ± 1.5
16 µg/kg serotonin	-11.1 ± 3.5	-9.6 ± 1.7

Data are reported as means ± SEM for 7 rats in each group. $^{+}P < 0.05$ compared to 2 and 4 µg/kg in the same group; *$P < 0.05$ compared to sedentary rats (two-way ANOVA).

trained rats compared to the sedentary rats, which may have been due, at least in part, to changes in cardiac parasympathetic tonus.

Resting bradycardia usually occurs in trained humans (1-3,11,24) and exercise-trained male (5,17,23,25) and female (19,26) rats and is considered to be a hallmark of exercise training improvement (4,5,17,23,25). Our results confirmed the occurrence of resting bradycardia in female rats. Surprisingly, the vagal tonus was reduced in female rats after 8 weeks of exercise training. In fact, the mechanisms underlying the cardiac adaptive responses to exercise training seem to be different for different species and genders. In normal male rats and humans, the reduction in IHR (pacemaker mediated) after exercise training seems to be the causal mechanism of the resting bradycardia (4,23,25). Studies have demonstrated that sympathetic and/or parasympathetic inputs were reduced in trained normotensive male rats or humans (4,5,23,27,28).

If the resting bradycardia produced by training (as shown in this study) cannot be attributed to increased vagal activity, what could account for it? Given that IHR did not differ between groups, bradycardia may then be explained by the more pronounced reduction in cardiac sympathetic tonus (62%) than by the impairment in cardiac vagal tonus (42%) in trained female rats. In fact, we observed decreased sympathetic tonus in trained rats, which was correlated with changes in resting HR ($r = 0.7$; Figure 1), thus confirming the critical role of reduced sympathetic tonus in the resting bradycardia observed in trained female rats.

The AP values obtained in the present study did not change after exercise training in these female rats, in agreement with reports regarding normotensive humans and rats (7,17,23). Whereas baseline AP is unaltered after training, the controlling mechanisms are changed. There is evidence supporting the increase in baroreflex gain in trained subjects (6,7,9-11,23,29). Exercise training of normotensive male rats enhanced hypotension-induced tachycardia, whereas the bradycardic response was decreased or unchanged (7,23). Chen et al. (30) observed an attenuation of baroreflex tachycardia attributed to central changes in response to changes in AP induced in anesthetized rats submitted to daily spontaneous running.

Interestingly, in present study, the baroreflex bradycardia was more reduced in the trained group than in the sedentary one, whereas no significant difference between groups was observed in baroreflex tachycardic response. The unchanged tachycardic responses may be explained by the reduction in sympathetic and vagal tonus, each associated with opposite effects on the HR responses. The bradycardic response is more dependent on cardiac vagal stimulation than on sympathetic withdrawal and therefore the reduced vagal tonus was determinant in impairing this response. Indeed, in this study we observed an inverse correlation between vagal tonus and bradycardic response to AP changes ($r = -0.72$, $P > 0.05$). Our results corroborate the findings of Negrão et al. (23), who demonstrated resting bradycardia, depressed vagal and sympathetic tonus and reduced baroreflex bradycardia after 13 weeks of exercise training. These results were attributed mainly to the efferent vagal pathway, inasmuch as the bradycardia produced by vagal stimulation and methacholine injection was attenuated in trained male rats. Thus, the attenuation of baroreflex bradycardia in trained female rats may be due, at least in part, to the reduction in vagal tonus. However, we cannot exclude exercise-induced changes in afferent pathways or central regulation of the baroreflex (30,31).

Regarding the gender differences in untrained rats, our results showed reduced sympathetic tonus and improved baroreflex sensitivity in female rats compared to previous studies on male rats (7,23,32), corroborating human data that showed sympathetic predominance in men compared to women (14,15,33). In fact, Chen and DiCarlo (16) demonstrated that female rats had a higher maximum baroreflex gain (40%) than male rats, and daily spontaneous running attenuated the arterial baroreflex regulation of HR in both sexes. It is also important to emphasize that in the present study all evaluations were performed in females during non-ovulatory phases because previous studies have demonstrated changes in autonomic regulation during the cyclic hormonal changes of females (34). On this basis, we cannot exclude that some changes observed here in both groups may be related to sex hormone variations.

The decreased baroreflex sensitivity has also been attributed to heart hypertrophy and tonic stimulation of cardiopulmonary receptors in trained rabbits (12). In fact, previous studies have shown bradycardia and an increased heart weight/body weight ratio after 10 weeks of exercise training on a treadmill in female Sprague-Dawley rats (26). Meyrelles et al. (35) observed impairment of the Bezold-Jarish reflex in normotensive male rats with ventricular hypertrophy. In addition, significant blunting of cardiopulmonary reflexes has also been found in athletes with marked left ventricular hypertrophy but normal AP values (13). Unfortunately, we did not evaluate cardiac mass changes in trained female rats in our protocol, but we demonstrated that the stimulation of the cardiopulmonary receptors by serotonin caused a reduced bradycardia (Bezold-Jarish effect) at the highest dose (16 µg/kg) in trained female rats, while the hypotensive responses were similar for the two groups. The bradycardic response to serotonin is mainly dependent on cardiac vagal stimulation and the hypotensive response is a secondary response mainly dependent on peripheral sympathetic withdrawn. The unchanged hypotensive response may be due to unchanged sympathetic tonus for the vasculature in trained rats, since it is well known that sympathetic activity presents regional differences. Using correlation analysis, vagal impairment after training was demonstrated to be associated with reduced bradycardic responses to cardiopulmonary reflex activation. Thus, the reduced vagal tonus, the decreased baroreflex bradycardia, and the decreased

bradycardic response to cardiopulmonary reflex stimulation demonstrated that vagal activity is actually attenuated in female rats by exercise training.

Exercise training induced resting bradycardia in healthy female rats and this was probably due to a reduced cardiac sympathetic tonus. Additionally, trained female presented attenuated bradycardic responses to baro- and cardiopulmonary reflex stimulations that were related, at least in part, to the exercise training-induced cardiac vagal reduction. Our results also show that female rats can be a useful model for the study of exercise and cardiovascular interactions in female subjects and that this model can be relevant to studies in humans. Further studies are needed to better define the presumed sex-related differences in the effects of exercise training on neurohumoral control of the cardiovascular system in pathological conditions, thus contributing to improved prevention programs and therapies targeted at women.

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