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Combined effects of exercise training and high doses of anabolic steroids on cardiac autonomic modulation and ventricular repolarization properties in rats

Moacir Marocolo¹, Pedro L. Katayama², Anderson Meireles¹ Octávio Barbosa Neto³

¹Physiology and Human Performance research Group, Department of Physiology, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil
²Department of Physiology and Pathology, School of Dentistry, São Paulo State University, Araraquara, SP, Brazil
³Sport Sciences Department, Federal University of Triangulo Mineiro, Uberaba, MG, Brazil

Address for correspondence:
Moacir Marocolo
Physiology and Human Performance Research Group
Institute of Biological Sciences, Department of Physiology,
Federal University of Juiz de Fora - UFJF
R. José Lourenço Kelmer, s/n – Juiz de Fora – Brazil - 36036-900
Tel/Fax: + 55 322102-3211
E-mail: isamjf@gmail.com
Abstract

Several studies have reported that high doses of synthetic anabolic androgenic steroids (AAS) can have serious negative effects on health, including the cardiovascular system. The aim of this study was to evaluate the combined effects of AAS and exercise training on ventricular repolarization and cardiac autonomic modulation in rats. Male Wistar rats were allocated into four groups: CON-S: sedentary treated with vehicle, ND-S: sedentary treated with nandrolone decanoate, CON-T: swimming trained treated with vehicle, ND-T: swimming trained treated with nandrolone decanoate. Ventricular repolarization was evaluated by electrocardiographic analysis of QT interval and QT dispersion. Cardiac autonomic modulation was assessed by heart rate variability. Our results show that AAS increased QT interval and QT dispersion in sedentary rats (ND-S) as compared to sedentary rats treated with vehicle (CON-S), indicating AAS-induced ventricular repolarization abnormalities. When rats treated with nandrolone decanoate were subjected to concomitant exercise training (ND-T), ventricular repolarization was normalized. On the other hand, AAS-induced reduction in cardiac parasympathetic modulation was not prevented by exercise training. In conclusion, AAS produced cardiac autonomic dysfunction and ventricular repolarization disturbances in rats. Combining an exercise training protocol during the AAS treatment attenuated the ventricular repolarization abnormalities and did not prevent cardiac autonomic dysfunction.

Keywords: electrocardiography; exercise training; heart rate variability; nandrolone decanoate; spectral analysis


Introduction

Synthetic anabolic androgenic steroids (AAS) are substances that mimic naturally occurring testosterone, and are used in therapeutic doses for the treatment of several chronic conditions such as burn injuries, muscular diseases, HIV-wasting syndrome, chronic renal failure, obstructive lung disease, and osteoporosis (Mottram & George, 2000; Woerdeman & de Ronde, 2011). However, self-administration of high doses of AAS is a widespread practice among young athletes aiming to optimize muscle-building and strength and, also, among non-athletes with aesthetic purpose (Sjoqvist et al. 2008; Urhausen et al. 2004). Taking high doses of AAS can have serious negative effects on health, including the cardiovascular system. In this regard, the use of AAS has been associated with several cardiovascular disorders, such as ischemic stroke (Ferenchick & Adelman, 1992), dyslipidemia (Garevik et al. 2011; Hartgens & Kuipers, 2004), left ventricular hypertrophy (Andrade et al. 2011; Malhotra et al. 1990), impaired coronary arterial function (Tagarakis et al. 2000), reduced cardiac β-adrenoreceptor sensitivity (Norton et al. 2000), increased oxidative cardiac stress (Frankenfeld et al. 2014), and myocyte apoptosis (Zaugg et al. 2001). Also, the AAS-induced increased cardiac collagen content (Rocha et al. 2007), imbalance of cardiac autonomic regulation (Maior et al. 2013; Pereira-Junior et al. 2006) and disturbances in ventricular repolarization (Medei et al. 2010) are factors linked with the increased risk for malignant arrhythmias and sudden cardiac death (Saba et al. 2005a, 2005b; Zareba & Moss, 2003).

Cardiac autonomic impairment with a marked reduction in the parasympathetic activity was demonstrated in sedentary rats after AAS long-term treatment (Pereira-Junior et al. 2006). Another study suggested an alteration in the reflex control of heart rate that was seen at different stanozolol doses (Beutel et al. 2005). Accordingly, the imbalance in the autonomic nervous system modulation of the heart (i.e. reduced vagal tone and
sympathetic hyperactivity) increases incidence of cardiac arrhythmias and the analysis of heart rate variability (HRV) has been used as a tool for non-invasive assessment of cardiac autonomic balance in physiological and pathological conditions (Beutel et al. 2005; Maior et al. 2013; Marocolo et al. 2013; Medei et al. 2010; Saba et al. 2005a, 2005b; Zareba & Moss, 2003).

The QT interval is a global index of ventricular repolarization (Franz et al. 1991; Van & Durrer, 1964) and, changes in the QT interval and QT dispersion reflect the heterogeneity of ventricular repolarization. These parameters are considered predictors of increased risk for ventricular arrhythmias and sudden death in different diseases (Priori et al. 2003; Rossing et al. 2001; Salles et al. 2003; Schwartz & Wolf, 1978). Despite the known adverse cardiovascular effects of high-doses of AAS, few studies have used QT parameters (interval QT and dispersion of QT from electrocardiogram) to assess cardiovascular risk associated with AAS abuse. A study showed that sedentary rats chronically treated with supraphysiological doses of AAS presented disturbances in ventricular repolarization (Medei et al. 2010), in particular, increased action potential duration, low density of the transient outward potassium current and altered expression of potassium channels subunits resulting in prolonged QT interval (Medei et al. 2010). The occurrence of the increased QT interval and QT dispersion during AAS administration was also reported by a recent study (Marocolo et al. 2018).

If on one hand, the consumption of high doses of AAS is associated with cardiac arrhythmias (Ghorbani Baravati et al. 2015) and ventricular repolarization disturbances (Medei et al. 2010), on the other hand, the association of the exercise training with a high dosage of these drugs showed harmful (Ghorbani Baravati et al. 2015), controversial (Nasseri et al. 2015) or questionable (Frati et al. 2015) results. Thus, the aim of this study was to evaluate the combined effects of exercise training and chronic
treatment with a supraphysiological dose of nandrolone decanoate on ventricular repolarization and cardiac autonomic modulation in rats. Our hypothesis was that exercise training could avoid possible negative effects of AAS administration.

**Materials and Methods**

*Animals and Procedures*

Male *Wistar* rats (175.0 ± 9.0 g and 45 days of age) kept in a controlled environment of 24± 2 °C, with an inverted circadian cycle of 12 h (lights on at 7 p.m. and lights off at 7 a.m.) with water and food ad libitum. This study was following the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011, National Academies Press), and was approved by the Institutional Animal Care and use Committee (n. 202/2011).

Animals were randomly allocated into four experimental groups of 10 rats each: CON-S: sedentary rats treated with vehicle, ND-S: sedentary rats treated with nandrolone decanoate, CON-T: trained rats treated with vehicle, and ND-T: trained rats treated with nandrolone decanoate. Rats treated with AAS received 10 mg.kg⁻¹ of nandrolone decanoate (Norma Hellas S.A., Athens, Greece), administered by a single injection in the gluteus medium muscle once a week, for eight weeks. CON-S and CON-T groups received the same volume of vehicle, composed of peanut oil with benzyl alcohol (90:10, V/V).

*Exercise training protocol*

The exercise training protocol was performed in a glass tank with warm water at ~ 30° ± 1° C (Tanno et al. 2002). To familiarize with the liquid environment, the animals of trained groups were subjected to an adaptation phase for 1 week. The adaptation phase consisted in 20-min swimming on the first day, with daily increments of 10-min until reaching 60-min on the fifth day (Gobatto et al. 2001; Moraes et al. 2017). The exercise training was composed by 60-min swimming sessions, 5 days/week by 8 weeks. This
protocol is defined as endurance training and low-intensity, as the animals swam without additional workload. This method was adapted from previous study and match in the intensity below the anaerobic threshold in rats (Gobatto et al. 2001). The sedentary groups were placed in the swimming tank for 2-min in the same period of exercise training protocol to mimic the water stress with the experimental protocol.

Body weight was measured weekly (OAC-2.4, Taiwan). At the end of the 8th week, animals were euthanized by CO$_2$ inhalation and cervical dislocation. Then, the hearts were quickly removed, washed and weighed (Executive Pro Precisa 360 Ep, Dietikon, Switzerland). Finally, the index of cardiac hypertrophy was estimated by the heart weight/body weight ratio. Retroperitoneal fat was dissected out by one experienced researcher and weighed immediately after dissection to avoid weight loss by evaporation.

Electrocardiogram Acquisition and Measurement

ECG recordings (for HRV and QT analysis) were carried out by a noninvasive method (Pereira-Junior et al. 2010) applying a custom-made elastic cotton jacket with two platinum electrodes that directly contacted the animal’s skin in a lead close to DII, keeping the animals singly inside the cages. The ventral thoracic region of each animal was previously shaved and a conductive ECG gel was applied over each electrode. The two electrodes were connected to a differential A/C amplifier and the signal digitized by a 16 bit A/D interface converter (FE22400, ADInstruments, Sydney, Australia) at 2 kHz sample rate (LabChartPro, Australia). Data were stored in a computer for off-line processing. The ECG recording started 5 minutes after the animal was restrained, lasting for 10 minutes, being conducted in a constant environment, during the morning (08:00-10:00 h), without anesthesia, to prevent any physiological alterations in data analysis.

Ventricular repolarization analysis

QT interval was measured as the interval between the start of the QRS complex and the end of the T wave (at its intersection with the isoelectric line). Later, QT intervals
were corrected for heart rate using Bazett’s formula ($QTc = \frac{QT}{RR^{1/2}}$), RR being the interval between two consecutive R waves (in seconds).

Heart Rate Variability Assessment

The ECG signal was processed using custom-made algorithms written in Matlab v 6.5 release 13 (Mathworks, USA). Interbeat R-R intervals were extracted using the Pan-Tompkins algorithm (Meyer et al. 2006) and artifacts were de-emphasized using a variable threshold R wave filter previously described (Berntson et al. 1990). The following indexes were extracted in the time-domain: RR (mean RR interval), SDNN (standard deviation of RR intervals), RMSSD (square root of the mean squared differences of successive RR intervals) and pNN5 (percentage of successive RR interval differences greater than 5 ms) (Aubert et al. 1999).

The HRV in the frequency domain was assessed by autoregressive spectral analysis as described elsewhere (Malliani et al. 1991). Briefly, a modeling of the oscillatory components present in the time series of HR was calculated based on the Levinson–Durbin recursion, with the order of the model chosen according to Akaike's criterion (Malliani et al. 1991). This procedure allows an automatic quantification of the center frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labeled as very low (VLF), low (LF) or high frequency (HF) when their central frequency was located in a band of 0.01–0.25 Hz, 0.25–0.75 Hz or 0.75–2.50 Hz, respectively. The power of the LF and HF components of HRV was also expressed in normalized units, obtained by calculating the percentage of the LF and HF variability with respect to the total power (all components from zero to 2.5 Hz) after subtracting the power of the very-low-frequency component (frequencies 0.25 Hz). The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF and HF components of HRV (Malliani et al. 1991).
**Statistical Analysis**

The normal distribution of quantitative data was confirmed by the Kolmogorov-Smirnov test. Two-way ANOVA was used for inter groups comparisons, with Bonferroni’s test being applied as the *post hoc* or Mann-Whitney in agreement with the presence or not of distribution normality and/or homogeneity of the variance, respectively. Analyses were conducted using SigmaStat (Jandel Scientific Software; SPSS, Chicago, IL). Statistical significance was established at p<0.05.

**Results**

**Effects of exercise training and AAS on body weight, body fat and heart weight**

During the 8-week period of AAS treatment and/or exercise training, all groups presented body weight gain. At the end of 8 weeks, CON-S showed higher body weight compared to the other three groups, while ND-T and CON-T showed lower values compared to ND-S. No differences were found between trained groups. Exercise training and AAS treatment diminished the retroperitoneal fat measured at the end of 8 weeks (Table 1). Both absolute and relative heart weights were lower in CON-S compared to CON-T, ND-S and ND-T groups. ND-S group presented a higher absolute heart weight than CON-T. When we consider the heart weight/body weight ratio ND-T presented higher values than CON-T and ND-S (Table 1).

***Table 1 near here***

**Effects of exercise training and AAS on QTc and QTd**

Nandrolone decanoate treatment induced increases in QTc and QTd in sedentary rats (ND-S) as compared to sedentary rats treated with vehicle (CON-S) as shown in figures 1A and 1B from 4th week of treatment. When rats treated with nandrolone
decanoate were subjected to concomitant exercise training (ND-T), QTc and QTd prolongation were attenuated in comparison to ND-S and found similar to the values observed in CON-S rats (figures 1A and 1B). Trained rats treated with vehicle (CON-T) presented lower QTc and QTd in comparison to the other three groups (figures 1A and 1B).

***Figure 1 near here***

Effects of exercise training and AAS on time-domain measures of HRV

As shown in figure 2, nandrolone decanoate treatment decreased RR in sedentary rats (ND-S) as compared to sedentary rats treated with vehicle (CON-S) at 4th and 8th weeks (figure 2A). In addition, nandrolone decanoate treatment decreased SDNN, RMSSD and pNN5 in both sedentary (ND-S) and trained (ND-T) rats as compared to rats treated with vehicle (CON-S and CON-T) at 4th and 8th weeks (figures 2B, 2C and 2D). Conversely, exercise training in rats treated with vehicle (CON-T) increased RR, RMSSD and pNN5 as compared to sedentary rats (CON-S) as demonstrated in figures 2A, 2C and 2D. However, when comparing the effects of exercise training in rats treated with nandrolone decanoate (ND-T) to sedentary rats receiving nandrolone decanoate (ND-S) except for RMSSD and pNN5 at 8th week, all time-domain HRV parameters were found similar (Figure 2, A to D).

***Figure 2 near here***

Effects of exercise training and AAS on frequency-domain measures of HRV

Spectral analysis of HRV revealed that nandrolone decanoate treatment markedly decreased HF in both sedentary (ND-S) and trained (ND-T) rats as compared to rats treated with vehicle (CON-S and CON-T) at 4th and 8th weeks of treatment (figure 3B). Consequently, the LF/HF ratio, a marker of sympatho-vagal balance was found higher in
ND-S and ND-T rats in comparison to CON-S and CON-T rats (figure 3C). Exercise training in rats treated with vehicle (CON-T) increased HF and decreased LF/HF in comparison to sedentary rats treated with vehicle (CON-S) (figures 3B and 3C).

***Figure 3 near here***

**Discussion**

The main finding of this study was that combining an endurance swimming training protocol throughout the 8-week nandrolone decanoate treatment period, blunted the AAS-induced ventricular repolarization abnormalities but did not prevent the AAS-induced cardiac autonomic dysfunction in rats. The results have shown that rats subjected to nandrolone decanoate treatment (ND-S) presented a marked prolongation of QTc interval along with an increase in QTd. These ventricular repolarization alterations were attenuated when the nandrolone decanoate treatment was combined with a swimming endurance training protocol (ND-T). On the other hand, ND-S and ND-T rats presented a similar reduction in cardiac parasympathetic modulation as reflected by progressive decreases in RMSSD, pNN5, and HF during the nandrolone decanoate treatment, indicating that exercise training did not prevent the cardiac autonomic dysfunction resulting from the AAS treatment.

The prolongation of QTc interval induced by nandrolone decanoate treatment (ND-S) observed in the present study is in line with previous studies (Marocolo et al. 2018; Medei et al. 2010). Interestingly, in the present study, we found that combining the nandrolone decanoate treatment with swimming endurance training (ND-T) attenuated the prolongation of QTc. The mechanisms underlying the AAS-induced QTc prolongation are not entirely clear. In a previous study (Medei et al. 2010), rats treated with nandrolone decanoate presented a left ventricle action potential prolongation and a reduction in the transient outward potassium current density, likely caused by the
observed concomitant down-regulated gene expression of Kv1.4, Kv4.3 and KChIP2 channels in the left ventricle. The study from Medei et al. (2010) provided clear-cut evidence that a reduction in the density of the transient outward potassium current is involved in the genesis AAS-induced QTc prolongation. Since some studies have reported that moderate endurance exercise training can modulate the transient outward potassium current by increasing its density in physiological and pathological conditions (Jew et al. 2001; Roman-Campos et al. 2012), it is possible to hypothesize that the attenuated prolongation of QTc observed in the ND-T rats in the present study is a result of an exercise training-induced increase in the density of the transient outward potassium current, counterbalancing the AAS-induced decrease of this potassium current. On the other hand, intensive exercise training, such as that experienced by elite athletes, could lead to decreased cardiac potassium currents, repolarization disturbances and, ultimately, predispose to sudden cardiac death (Varró & Baczkó, 2010). In addition, D’Souza et al. (2014) reported that a very intense running training for 12 weeks in rats downregulated HCN4 channels in the sinus node and consequently decreased the density of its corresponding “funny” pacemaker current (If), resulting in a potentially pathological heart rate adaptation which might contribute to the paradoxical increased incidence of arrhythmias in athletes.

Additionally, the nandrolone decanoate-induced increase in QTd was also attenuated when the AAS treatment was combined with the swimming training protocol (ND-T). We did not find any report in the literature on the effects of AAS treatment over ventricular repolarization heterogeneity in animal models, but human data from our and other groups point towards an increase in QTd in AAS abusers (Maior et al. 2010, Stolt et al. 1999). Even though these subjects were involved in exercise training regimens, their exercise routines were focused on high intensity strength training (Maior et al. 2010, Stolt et al. 1999), differently from the endurance training used in the present study. Although previous studies have reported that endurance training does not induce changes in QTd of athletes (Stolt et al. 1999; Zoghi et al. 2002), cardiac rehabilitation with endurance exercise protocols significantly reduce QTd in patients under different pathological states,
such as myocardial infarction and the metabolic syndrome (Guiraud et al. 2010; Kalapura et al. 2003) and, therefore, could also exert beneficial effects over the increased ventricular repolarization heterogeneity observed with AAS use.

Regarding cardiac autonomic modulation, several studies reported that endurance exercise training increases the parasympathetic modulation of the heart in animals and humans (Barbosa-Neto et al. 2013; Dixon et al. 1992; Medeiros et al. 2004; Tulppo et al. 2003; Zaniquelli et al. 2014) which is considered a benefic adaptation and one of the main mechanisms underlying the exercise training-induced reduction in cardiovascular morbidity and mortality (Dixon et al. 1992; Levy et al. 1998; Tulppo et al. 2003). Consistently with these reports, in the present study, it was observed that control rats subjected to endurance swimming training (CON-T) presented an increase in HRV indexes of cardiac parasympathetic modulation such as RMSSD, pNN5 e HF when compared to sedentary animals (CON-S). Nevertheless, rats which underwent combined endurance swimming training and nandrolone decanoate treatment (ND-T), presented reductions in cardiac parasympathetic modulation as compared to CON-S and CON-T animals. These findings suggest that the benefic exercise training-induced increase in cardiac parasympathetic modulation is overwhelmed by the concomitant use of supraphysiological doses of AAS. Since cardiac autonomic dysfunction, often characterized by a reduction in parasympathetic modulation of the heart is an important predictor of arrhythmias and sudden cardiac death (Kleiger et al. 1987; Malik et al. 1996), our findings provide support to the notion that AAS, by itself or combined with exercise training can increase the risk of cardiac arrhythmias and sudden death.

The mechanisms involved in the AAS-induced cardiac autonomic dysfunction are not completely clear. Since it has been shown that androgens can cross the blood brain barrier (Kicman, 2008) and that androgen receptors are present in several brain areas involved in autonomic regulation (Sheridan et al. 1982; Sheridan & Weaker, 1982; Bao et al. 2006) it is likely that circulating AAS can access the central nervous system and affect cardiac autonomic control through its receptors in the brain stem and/or the hypothalamus. For example, it is well known that the paraventricular nucleus (PVN) of
the hypothalamus plays an important role in cardiovascular autonomic regulation (Guyenet, 2006) and in the autonomic adjustments to exercise training in rats (de Abreu et al. 2009). A previous study demonstrated that androgens can act on PVN down-regulating nitric oxide synthase (NOS) neuronal activity (Singh et al. 2000). Since it has been shown that exercise training increases the activity of neuronal NOS in the PVN of rats resulting in a reduction of sympathetic and an increase in parasympathetic modulation of the heart (de Abreu et al., 2009), it is possible to hypothesize that in the present study the increased circulating androgen levels in ND-T animals counteracted the exercise training-induced increase in PVN nitric oxide neurotransmission and parasympathetic modulation.

Finally, it is noteworthy that both absolute and relative heart weight were found higher in CON-T, ND-S and ND-T as compared to CON-S, indicating that the endurance exercise training, the AAS treatment or the combination of both, induced cardiac hypertrophy. We can consider that an additive effect of exercise training and AAS has occurred, since the relative heart weight of the ND-T group was higher than those observed in ND-S and CON-T groups. Our results differ from previous studies (Beutel et al. 2005; Pereira-Junior et al. 2006), in which the absolute weight of the heart showed no difference between groups and only the relative heart weight was significantly higher in the nandrolone decanoate treated groups. On the other hand, another study reported an increase in heart weight as well as in the heart/body weight ratio along with left ventricular hypertrophy, confirmed by morphological and histological analysis after 8 weeks of treatment with nandrolone decanoate in rats (Andrade et al. 2011).

It is important to recognize that the animal model (rat) used in the present study may represent a limitation in translating the results to humans. Studies have shown that some aspects of the cardiovascular system, including cardiac repolarization properties, differ between small rodents and humans, suggesting that other animals such as rabbits, dogs and pigs could be more appropriate models for cardiovascular research (Nerbonne, 2004; Tsang et al. 2016; Polyak et al. 2018). Nevertheless, a recent study reported that a running training protocol for 16 weeks increased cardiac parasympathetic modulation in
rabbits and dogs as suggested by HRV analysis (Polyak et al. 2018), similarly to the results observed in rats in the present study. On the other hand, Polyak and colleagues (2018) observed no differences in QTc between sedentary and trained rabbits or dogs, whereas we did find a shorter QTc in rats subjected to endurance training in comparison to sedentary rats. These discrepant results, however, might be attributed to the different training protocols rather than the species.

In conclusion, the administration of high doses of AAS induced cardiac autonomic dysfunction and ventricular repolarization disturbances in rats. More specifically, a nandrolone decanoate treatment for 8 weeks decreased the parasympathetic modulation of the heart and increased QT interval duration and dispersion, all factors associated with higher cardiovascular morbidity and mortality. Combining an endurance swimming training protocol during the AAS treatment attenuated the ventricular repolarization abnormalities but did not prevent cardiac autonomic dysfunction. These findings reinforce that AAS abuse results in adverse effects on cardiac health and provides further evidence that simultaneous endurance exercise training is not able to completely prevent these alterations.

**Conflicts of interest:** The authors declare no conflict of interest.
References


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Table 1. Effects of nandrolone decanoate treatment and swimming training on body, fat and heart weight.

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<th>Heart weight</th>
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<td></td>
<td></td>
<td>Absolute (g)</td>
<td>Relative (mg.g⁻¹)</td>
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<tr>
<td>CON-S</td>
<td>253 ± 2</td>
<td>4.71 ± 0.31</td>
<td>18.25 ± 0.89</td>
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<tr>
<td>ND-S</td>
<td>237 ± 4ᵃ</td>
<td>3.69 ± 0.27ᵃ</td>
<td>15.38 ± 0.57ᵃ</td>
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<tr>
<td>CON-T</td>
<td>211 ± 2ᵇ</td>
<td>3.09 ± 0.24ᵇ</td>
<td>13.72 ± 0.65ᵇ</td>
</tr>
<tr>
<td>ND-T</td>
<td>208 ± 3ᵇᵃᵇ</td>
<td>2.71 ± 0.34ᵇᵃᵇ</td>
<td>12.63 ± 0.51ᵇᵃᵇ</td>
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Values expressed as mean±standard error. Body weight is the final body weight at the end of the study. Relative retroperitoneal fat expressed as absolute retroperitoneal fat/final body weight. Relative heart weight expressed as absolute heart weight/ final body weight. CON-S: sedentary rats, ND-S: sedentary rats treated with nandrolone decanoate, CON-T: trained rats, ND-T: trained rats treated with nandrolone.  ᵃ_p<0.05 vs CON-S;  ᵇ_p<0.05 vs ND-S;  ᶜ_p<0.05 vs CON-T.
Figure legends

**Figure 1.** Effects of 8-week exercise training, nandrolone decanoate treatment, or both on (A) corrected QT interval and (B) QT dispersion. CON-S: sedentary rats, ND-S: sedentary rats treated with nandrolone decanoate, CON-T: trained rats, ND-T: trained rats treated with nandrolone decanoate, QTc: corrected QT interval, QTd: QT dispersion. \(^{a}p<0.05\) ND-S vs. three other groups; \(^{b}p<0.05\) vs CON-S; \(^{c}p<0.05\) vs ND-T. Values expressed as mean ± standard error of the mean.

**Figure 2.** Effects of 8-week exercise training, nandrolone decanoate treatment, or both on HRV parameters analyzed in the time domain. (A) mean RR interval, (B) SDNN, (C) RMSSD, and (D) pNN5. CON-S: sedentary rats, ND-S: sedentary rats treated with nandrolone decanoate, CON-T: trained rats, ND-T: trained rats treated with nandrolone decanoate, SDNN: standard deviation of successive RR intervals, RMSSD: square root of the mean squared differences of successive RR intervals, pNN5: percentage of successive RR interval differences greater than 5 ms. \(^{a}p<0.05\) vs CON-S in same week, \(^{b}p<0.05\) vs CON-T in same week; \(^{c}p<0.05\) vs ND-S in the same week. Values expressed as mean ± standard error of the mean.

**Figure 3.** Effects of 8-week exercise training, nandrolone decanoate treatment or both on spectral indexes of HRV. (A) low frequency power, (B) high frequency power, and (C) low frequency/high frequency ratio. CON-S: sedentary rats, ND-S: sedentary rats treated with nandrolone decanoate, CON-T: trained rats, ND-T: trained rats treated with nandrolone, LF: low frequency power, HF: high frequency power, LF/HF: low frequency/high frequency ratio. \(^{a}p<0.05\) vs CON-S in the same week, \(^{b}p<0.05\) vs CON-T in same week; \(^{c}p<0.05\) vs control ND-S in the same week. Values expressed as mean ± standard error of the mean.
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