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ORIGINAL RESEARCH

Long-term aquatic training triggers positive electrical alterations and other parameters in adult female rats

Treinamento aquático em longo prazo desencadeia alterações elétricas positivas e outros parâmetros em ratas adultas

El entrenamiento acuático en largo plazo produce alteraciones eléctricas positivas y otros parámetros en ratas adultas

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ABSTRACT | The purpose of this study was to assess cardiac electrical alterations and effects on body weight, water and food consumption, and relative weight of organs of adult female rats submitted to long-term swimming training. We used adult Wistar female rats, healthy, divided into n=10 groups, groups S (sedentary) and TR (trained), which had aquatic training for sixty minutes, three times a week, for 16 weeks. We evaluated body weight (W), water and food consumption, heart rate (HR), myocardial angle (SAQRS), intervals QRS, QTc, and PR, and relative weight of adrenal glands, heart, spleen, and kidneys. For statistical analysis, we used the statistical package "SPSS version 17.0"; data distribution was verified by the Kolmogorov-Smirnov normality test. For data with parametric distribution, we used student's t test for independent samples, while for data with nonparametric distribution we applied Mann-Whitney test ($p<0.05$). Rats from group TR had higher water and food consumption; however, body weight was similar between groups TR and S. Group TR had electrical alterations in HR, myocardial angle (SAQRS), S and T wave, demonstrating significant bradycardia at rest and possible cardiac hypertrophy, in addition to the difference found for relative weight of spleen and kidney. Training protocol used for adult female rats favored positive electrical changes in the heart, resulting in bradycardia during rest and improvement in cardiovascular fitness.

Keywords | Exercise; Swimming/statistics & numerical data; Heart Rate; Rats, Wistar; Adaptation.

RESUMO | A proposta deste estudo foi avaliar as alterações elétricas cardíacas e os efeitos sobre a massa corporal, consumo hídrico e alimentar, além do peso relativo de órgãos de ratas adultas submetidas a um treinamento de natação em longo prazo. Utilizou-se ratas Wistar adultas, saudáveis e dividas em grupos de n=10, sendo S (sedentárias) e TR (treinadas), que realizaram treinamento aquático de sessenta minutos, três vezes na semana, por 16 semanas. Foram avaliados o peso corporal (P), o consumo hídrico e alimentar, a frequência cardíaca (FC), ângulo do miocárdio (SÂQRS), os intervalos QRS, QTc e PR e o peso relativo dos glândulas adrenais, coração, baço e rins. Para análise estatística, utilizou-se o pacote estatístico SPSS versão 17.0; a distribuição dos dados foi verificada pelo teste de normalidade *Kolmogorov-Smirnov*. Para os dados com distribuição paramétrica, foi utilizado o teste T de *Student*, para amostras independentes, e aos dados com distribuição não paramétrica, foi aplicado o teste *Mann-Whitney* ($p<0,05$). As ratas do grupo TR apresentaram maior consumo hídrico e alimentar, entretanto, a massa corporal foi semelhante entre os grupos TR e S. O grupo TR apresentou alterações elétricas na FC, ângulo do miocárdio (SÂQRS), onda S e T, demonstrando significativa bradicardia em repouso e possível hipertrofia cardíaca, além da diferença encontrada no peso relativo

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do baço e dos rins. O protocolo de treinamento utilizado para as ratas adultas favoreceu as alterações elétricas positivas do coração, resultando em bradicardia durante repouso e melhora no condicionamento cardiovascular.

Descritores | Exercício; Natação/estatística & dados numéricos; Frequência Cardíaca; Ratos Wistar; Adaptação.

RESUMEN | Este estudio tuvo el propósito de evaluar las alteraciones eléctricas cardíacas y los resultados sobre la masa corporal, consumo de agua y alimentos, además del peso relacionado a los órganos de las ratas adultas sometidas a un entrenamiento de natación en largo plazo. Se utilizó de ratas Wistar, adultas, saludables, divididas en grupos de n=10, siendo S (sedentarias) y E (entrenadas), las que realizaron el entrenamiento acuático de 60 minutos, tres veces por semana, durante 16 semanas. Se evaluaron el peso corporal (P), el consumo de agua y alimentos, la frecuencia cardiaca (FC), el ángulo del miocardio (SAQRS), los intervalos QRS, QTc y PR y el peso relacionado a las glándulas adrenales, al corazón, al bazo y a los riñones. En el análisis estadístico se empleó el sistema estadístico SPSS versión 17.0; se evaluó la distribución de los datos a través del test de normalidad Kolmogorov-Smirnov. En los datos con distribución paramétrica se empleó el test t Student, en las muestras independientes y en los datos con distribución no paramétrica se aplicó el test Mann-Whitney ($p<0,05$). Las ratas del grupo E presentaron un mayor consumo de agua y de alimentos, no obstante, la masa corporal fue semejante entre los grupos E y S. El grupo E presentó alteraciones eléctricas en la FC, en el ángulo del miocardio (SAQRS), ondas S y T, lo que muestra una bradicardia en reposo y una posible hipertrofia cardiaca, además de la diferencia encontrada en el peso del bazo y de los riñones. El protocolo de entrenamiento empleado en las ratas adultas les favoreció las alteraciones eléctricas positivas del corazón, resultando en bradicardia durante reposo y en una mejora de su condición cardiovascular.

Palabras clave | Ejercicio; Natación/estadística y datos numéricos; Frecuencia Cardiaca; Ratas Wistar; Adaptación.

INTRODUCTION

Systemic changes in the body are observed which result from different stimuli, such as in response to physical activity or to natural conditions such as those that arise during the aging process[1].

Physical exercise before and/or during the aging process leads to changes in metabolic capacities and general physical mobility. Therefore, it is presumable that regular physical activity can mitigate the degenerative process of aging[2], reducing and/or preventing a number of functional declines[3,4].

In recent years, there has been a considerable increase in the incidence of heart failure (HF) in the population. The electrocardiogram (ECG), a simple and reproducible test, contributes to the effective diagnosis of heart disease, thus contributing to increased overall life expectancy[5].

It is known that, by combating any risk factor for cardiovascular disease, the possibility of developing heart problems is decreased; however, adequate myocardial vascularization is also necessary[6]. Thus, regular physical exercise has a favorable impact on almost all risk factors, in addition to maintaining or increasing the supply of blood to the heart[7].

However, the effectiveness of exercising in relation to an individual's life will depend on the type of exercise, its intensity, and the duration of the activity performed[8].

Regular aerobic exercises are the main origin of changes in the cardiovascular system. Reductions in heart rate and blood pressure are the main parameters for which such changes are evident[9,10].

Low-intensity exercise has a significant effect in reducing peripheral vascular resistance, marked by attenuation of vasoconstriction, expansion of endothelial function and structural changes in microcirculation[11].

Systemic adaptations stimulated by regular physical training also involve various organs, such as the heart, kidneys, spleen, and adrenal glands, which may change in volume as indication of effectiveness of exercise[12].

Most studies found in the literature directed to analyze the effectiveness of prior physical training in relation to different variables in animals utilized the physical exercise protocol of swimming, sixty minutes in length or more and five or six times a week[13-16]. This study aimed to analyze the cardiovascular effects of a long-term swimming training, with no load and performed only three times a week in healthy female adult rats, as well as to evaluate the effects of such training on body mass, water and food consumption, in addition to relative weight of adrenal glands, heart, spleen, and kidneys.

In considering the systemic changes provided by regular physical exercise, and, in an attempt to reproduce the practice indicated according to the

World Health Organization (WHO) for adults and seniors (18–64 years), which corresponds to aerobic physical activity, of moderate intensity, for at least 150 minutes during per week[4], the hypothesis of this study is that the type of training proposed may present positive changes in cardiac variables, identifiable through electrocardiographic examination.

METHODOLOGY

Experimental study with 20 adult female Wistar rats, healthy, eight months old, from the Vivarium of Methodist University of Piracicaba and kept in the Vivarium of the School of Health Sciences (FACIS-UNIMEP) under ambient temperature of 23°C ± 2°C, subjected to light/dark cycle of 12 h, with water and food ad libitum. The study was approved by the UNIMEP Ethics Committee on Animal Use under protocol No. 09/13.

Animal were randomly divided into two experimental groups named sedentary group (S, n=10 animals) and trained group (TR, n=10 animals).

Animals in group S were kept in large polyethylene cages, without performing any kind of activity, for a sixteen week period. Animals in group TR were submitted to physical swimming exercise three times a week, for sixty minutes and without additional load, during the same time period. At the end of the experiment, the adult female rats completed twelve months of age.

Animal training protocol

Based on recommendations from the WHO[4], seeking a close similarity in relation to humans, the training protocol for adult female rats in this study had, altogether, 180 minutes per week of physical activity (swimming) divided into three interspersed days of the week and two days of rest, each session with sixty minutes of duration[15].

Initially, in order to adapt to the aquatic environment, animals performed ten minutes of activity, with five extra minutes per session, up to a total of sixty minutes[15,17].

Training was conducted in a rectangular aquarium, 1 meter long and 45 cm wide, with thermal heating system and water drainage, so water could be replaced as needed, which had capacity for 5 animals per session. Water temperature was monitored by a thermometer in order to remain at 30°C ± 2°C, to minimize the stress associated with exposure to cold or hot water[18].

All training sessions were held in the afternoon and, after the exercise, animals were dried with hot air blast and then returned to the boxes[17]. There was no sample loss.

Electrocardiographic analysis

After the training period, animals were anesthetized with sodium pentobarbital (40mg/kg, ip) for electrocardiographic analysis. The choice of anesthetic agent was based on the context that the barbiturates do not alter the cardiovascular electric profile[19].

Electrodes were connected to the channels of the electrocardiography device (HeartWare® System) and recorded three bipolar derivations (DI, DII, and DIII) and three amplified derivations (aVR, aVL, and aVF), with sensibility N and speed of 50 mm/second (Figure 1).

QT interval was measured in ten consecutive beats, from the beginning of the QRS complex to the point of return of the isoelectric T wave defined as TP segment. QT interval was corrected by heart rate using the Bazett formula ($QTc = QT/\sqrt{RR}$).

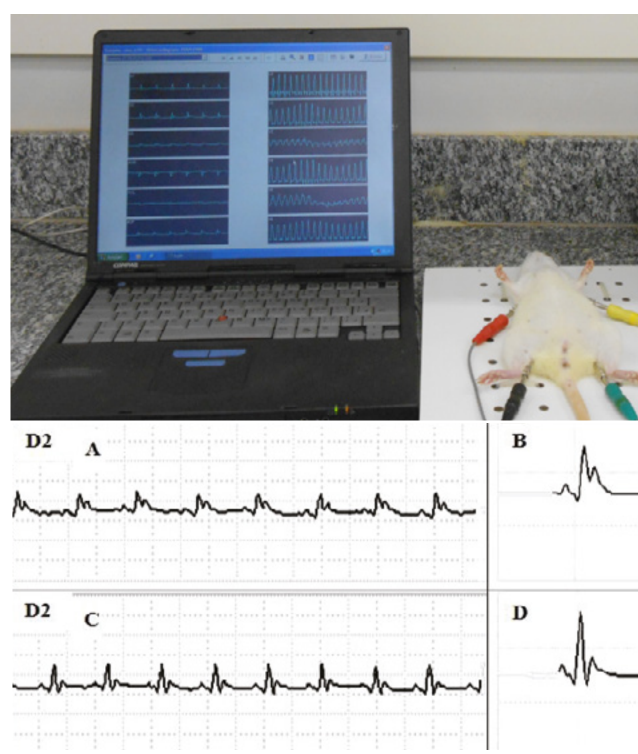

Figure 1. Representation of the HeartWare® system to obtain the ECG and electrocardiographic signals obtained from derivation DII. In A, we present the record of female rats in group S, and, in B, a complex of amplified waves. C presents the sign obtained from rats in group TR, and D presents a complex of amplified waves obtained from these same rats

Monitoring of body mass and water and food consumption

The animals' body mass weight was monitored through fortnightly assessments, using a digital scale (GEHAKA, BG 1000). Group TR was first weighed before beginning the first session of swimming.

Every three days, each box with four rats received 450 grams of food and 700 ml of water. At the end of the third day, the remaining food was weighed (digital scale, GEHAKA, BG 1000), calculating the proportional food consumption per animal, while water consumption was calculated by means of a test tube, in order to determine the volume consumed in each box.

Euthanasia of animals

In the following day after electrocardiographic analysis, animals were anesthetized with an intraperitoneal injection of a mixture of Ketalar® (50mg/mL) and Rompun® (2g/100mL), proportion of 1:1, dosage of 0.3mL/100g of body mass. After signals of general anesthesia, the following organs were removed and weighed: adrenal glands, heart, spleen, and kidneys.

To calculate relative weight of organs of animals, we followed the protocol described by Lana, Paulino and Gonçalves[13], which divided the weight of each organ (in grams) by the body weight of each animal on the collection day, and multiplied the value obtained by 100. Thus, the result was expressed in grams/100 grams of live weight (g/100g LW)

STATISTICAL ANALYSIS

The data collected were tabulated and subsequently analyzed using the statistical package "SPSS version 17.0." Statistical analysis was preceded by application of the Kolmogorov-Smirnov (KS) test to verify the normality of the data. Thus, for comparisons between groups the Student t test for independent samples was applied to data with parametric distribution, while the Mann-Whitney test applied to data with nonparametric distribution. In all cases, $p<0.05$ was adopted for statistical significance.

RESULTS

In comparing the average daily food and water consumption per animal, through the nonparametric statistical test Mann-Whitney, we observed that the value was significantly higher (p=0.002 and p=0,045) for group TR (21±2.9g / 45.3±6.5mL) compared to group S (17.3±2.1 g / 40.2±8mL).

However, as observed in Figure 2, the final body mass of the animals was similar between groups (Student t parametric test; p>0.05).

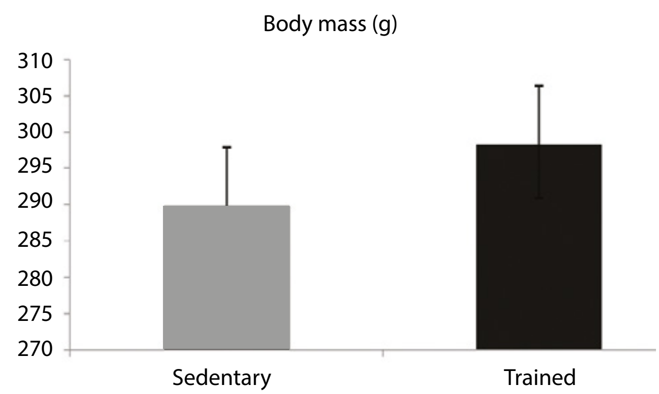

Figure 2. Body mass (g) of rats in the groups studied. The values correspond to mean ± SD, n=10

Concerning the relative weight of organs, comparison between groups using parametric Student's t test showed statistically significant increase ($p<0.05$) for kidneys and spleen of female rats submitted to training compared to the sedentary group. Weight of adrenal glands and heart was similar between groups (Figure 3).

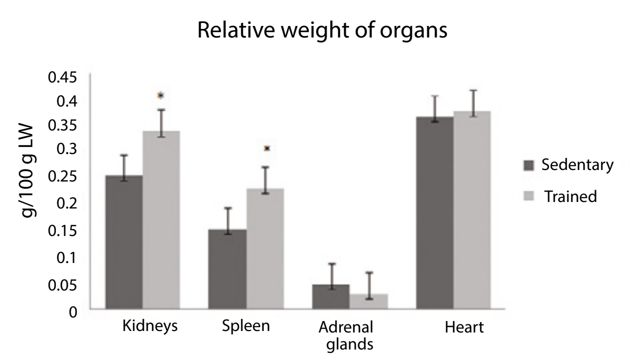

Figure 3. Relative weight of organs of female rats in the groups studied. Values correspond to mean ± SD, n=10. *p<0.05 differs from group S

In the electrocardiographic analysis, we started with heart rate, following the rule of difference between the peak of two consecutive R waves. Parametric Student's t test was applied, and statistically significant mean values that were 15% lower were observed for the TR group (Table 1).

Concerning results obtained for heart angle (SAQRS), we found that female rats from the TR group had a greater statistically significant angle (parametric Student's t test, $p<0.05$), with approximately 50.6° of difference in relation to rats in the S group, this being considered a more vertical heart (Table 1).

For QTc interval values, which corresponds to the time required to achieve myocardial repolarization, statistically significant differences were not observed (parametric Student's t test), although, in this case, the TR group had values 22.37% lower compared to the S group, as shown in Table 1.

For data with nonparametric distribution, IPR interval (ms) and SPR segment (ms), the Mann-Whitney test showed no statistically significant differences between groups.

Table 1. Heart rate (HR, beats/min), myocardium angle (SAQRS) and parameters of intervals and segments of electrocardiographic waves (ms) of sedentary (S) and trained (TR) female rats. Values are expressed in mean ± SD, n=10. *p<0.05 differs from group S

	HR (beats/min)	SAQRS	QTc (ms)	QRS (ms)	IPR (ms)	SPR (ms)
S	324.50±7.4	23.2±6.4	143.25±9.8	45.7±0.7	37.7±1.1	11.1±0.5
TR	275.58±9.0*	73.8±5.7*	111.20±8.6	47.1±1.1	38.3±1.5	12.4±1.4

Furthermore, the parametric Student's t test showed statistically significant differences in amplitude for the R wave, which corresponds to ventricular activation, and for S wave, which corresponds to the first negative deflection during ventricular depolarization; it was found that the voltage of the R wave was 94% higher, while the S wave was increased by 15% as a result of training (Figure 4).

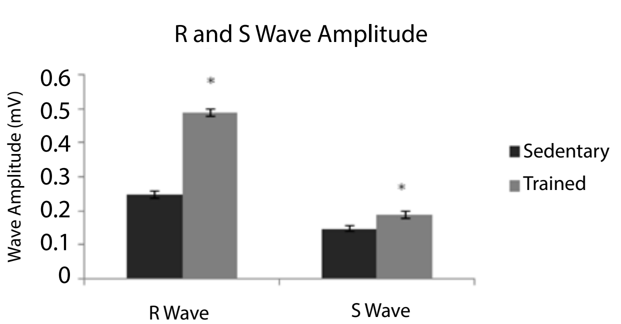

Figure 4. R and S wave amplitude (mV) for the groups studied. Values correspond to mean ± SD, n=10. *p<0.05 differs from group S

DISCUSSION

Long-term swimming training, with no load, three times a week, favored bradycardia at rest, heart verticalization, and increase in amplitude for the R and S waves, thus resulting in cardiovascular fitness.

Moreover, we observed that trained female rats consumed a greater amount of food and water; however, body mass was not increased. Training three times a week also promoted increase in mass for kidneys and spleen.

Regarding the animals' body mass weight, the fact that there were no statistically significant differences between the groups corroborates other studies[12–16], which observed no differences among the body mass weight of animals trained by swimming five times a week.

Reduction in heart rate at rest is among the main cardiovascular parameters subject to changes due to aerobic exercise, as evidenced by the hypertrophied heart of trained rats[10,13].

The expected heart rate of sedentary rats is approximately 344 bpm[20]. Results of this study show that the S group follows the expected pattern, while the TR group showed an average decrease of 15%, or 48 bpm.

Thus, beneficial cardiovascular anatomical adaptations were observed, causing the female rats submitted to training to have bradycardia at rest, corroborating the results of the study of Cardinot et al.[21], who evaluated the behavior of blood pressure and heart rate in mice submitted to preventive aerobic training.

Medeiros et al.[13], in a study with animals trained by swimming, with sixty-minute sessions, five times a week, for eight weeks, emphasize that bradycardia at rest is considered an efficient marker for the effect of aerobic physical training, and that swimming is a type of training which is effective in the adaptations of the cardiovascular system, considering the physical training in rats a good experimental model to study these adaptations.

In order to observe if left ventricular hypertrophy occurred, data of the present study are in accordance with the research described in the literature[21], reporting that hypertrophied left ventricles show an increase in amplitude for R wave, which evidences ventricular depolarization.

Although, indications of cardiac hypertrophy were observed in the ECG results from the present study, there was no statistically significant difference for relative weight of the heart.

The raise in the R wave voltage can be justified by the angle of the heart, since it is more vertical in the TR group, thus increasing the R wave amplitude in the D2 variation[22]. It is assumed that this verticalization is due to left ventricle hypertrophy, so the heart adapts anatomically to surrounding structures. Since the left ventricle internal dimensions uniformly increase due to the overload from exercise, there is an enlargement of the cavity which, subsequently, is followed by hypertrophy in the left ventricle myocardium[23].

The S wave is scarcely described in experimental studies; however, in humans, increase in voltage may be a signal of left ventricle hypertrophy[23]. Thus, its increased amplitude in long-term trained female rats suggests a high demand for electricity, in order depolarize a hypertrophied ventricle.

A smaller QTc interval indicates a short or early repolarization[24]. Thus, the reduction of QTc interval (QT interval corrected by Bazett formula) for the TR group, although not statistically significant, is considered an important finding in this study. Since the QRS interval between the groups is similar, it is possible to infer that there was a power adjustment in order to support the exercise load, starting the myocardial repolarization at an early stage.

Regarding the effect of regular exercise for prevention of cardiovascular diseases, such as acute myocardial infarction, there are differences among studies in the literature.

The study conducted by Veiga et al.[16] evaluated the effects of myocardial infarction (MI) in rats submitted to prior physical exercise for eight weeks, indicating that exercise by swimming did not attenuate the changes induced by MI in female rats.

In contrast, the study by Brown et al.[25] observed reduction in infarction area and preservation of coronary artery flow in female rats previously trained for 20 weeks through a running protocol. These data corroborate the study by Freimann et al.[14], who conducted physical training of swimming followed by induction of MI in male rats, observing considerable reduction in MI area, scar reduction, and increased density of arterioles.

While the objective of this study was not to induce MI, the electrical changes demonstrated by ECG in trained animals result in cardiovascular fitness and suggest the effectiveness of this type of training for the prevention of cardiovascular events.

With respect to the weight of organs, the literature shows increase in the relative weight of kidneys of animals only when they are submitted to high-intensity exercise, for an eleven-week period[12]. Thus, the increase in the relative weight of kidneys during physical exercise, even with no load, can be justified considering the longer training. According to Shizuru et al.[26], renal responses resulting from physical exercises are, in fact, related to their time and intensity.

The relative weight of spleen found in this study differs from results in the literature, since because of physical exercise the weight of the organ can be reduced as a result of increased expulsion of blood stored in the structure, when trained and immobilized animals are compared. However, rats had higher immune and endocrine activity, which can be induced by stress[27]. Aging associated with long-term exercise may have contributed to the relative increase in spleen size.

With regard to the weight of the adrenal gland, we observed no statistically significant difference, probably due to the type of training to which the animals were submitted, with no load, since data in the literature indicate that changes in the weight of adrenal glands occur more frequently in response to training with load and high intensity[12].

This research enabled better understanding of the aspects related to structural changes in the body of adult female rats, with emphasis on electrical variables of the myocardium, when submitted to physical training, with no load, performed only three times a week for a long period of time. A limitation of the experiment is that no data was collected concerning the blood pressure of animals in both groups.

CONCLUSION

We conclude that swimming, in the long term, only three times a week in adult female rats is effective to promote positive electrical changes, highlighting bradycardia at rest and vertical myocardial angle, characterizing an effective and preventive cardiovascular physical training.

Acknowledgements

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