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## The effects induced by swimming training on rats submitted to normal and hypercaloric diets

### Os efeitos induzidos pelo treino de natação em ratos sujeitos a dietas normal e hipercalórica

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#### ABSTRACT

The main aim of the present study was to analyse the effects induced by six weeks of swimming periodized training (SPT) on the aerobic capacity (AC) and body weight (BW), on rats fed with either normal or hypercaloric diets. Twenty-four Wistar rats (90 days old) were divided in two groups: Normocaloric (NG, n = 12) and Hypercaloric (HG, n = 12). The rats were fed with respective diets (NG or HG) for eight weeks and then underwent SPT for six weeks. Before (pre) and after (post) the SPT, the animals were submitted to an AC determination, using a "Chassain Test". There was no significant changes to AC in the HG group (pre =  $5.59 \pm 4.56\%$  BW vs. post =  $4.45 \pm 1.66\%$  BW), but in the NG group it increased significantly (pre =  $3.95 \pm 2.42\%$  BW vs. post =  $4.48 \pm 1.18\%$  BW).

**Keywords:** Obesity, Swimming, Aerobic Capacity, Lactate, Performance.

#### RESUMO

O objetivo do presente estudo foi investigar os efeitos de seis semanas de treinamento periodizado em natação (TPN) na capacidade aeróbia e peso corporal (PC) de ratos alimentados com dieta normal ou hipercalórica. 24 ratos Wistar (90 dias de idade) foram divididos em dois grupos: Normocalórico (NC, n = 12) e Hipercalórico (HC, n = 12). Os ratos foram alimentados com as respectivas dietas (NC e HC) por oito semanas e TPN por seis semanas. Antes (pré) e depois (pós) ao TPN os animais foram submetidos ao teste para a determinação da capacidade aeróbia (CA), usando o "Teste de Chassain". Não se observou mudanças significativas na CA no grupo HC (pré =  $5.59 \pm 4.56\%$  PC, post =  $4.45 \pm 1.66\%$  PC), mas no grupo NC, houve o aumento significativo (pré =  $3.95 \pm 2.42\%$  PC e pós =  $4.48 \pm 1.18\%$  PC) respectivamente

**Palavras-chaves:** Obesidade, Natação, Capacidade Aeróbia, Lactato, Desempenho

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## INTRODUCTION

A contemporary lifestyle influences eating habits, promoting a diet loaded with fat or carbohydrates, popularly known as the “westernized” or “fast food” diet (Cesaretti & Kohlmann Junior, 2006). As a result of a high fat intake, excessive body fat accumulation occurs, which predisposes to overweight and obesity, which in turn characterizes this type of diet as a risk factor for the development of chronic diseases (CD). This can lead to increased rates of mortality and a diminished quality of life, which makes it of paramount importance for global public health (Hardman, 1999; Mendonça & Anjos, 2004; Ribeiro Filho, Mariosa, Ferreira, & Zanella, 2006; Sociedade Brasileira de Cardiologia, 2005).

Nowadays, we have seen significant increases in the prevalence of overweight and obesity in both Brazil and the world because of endogenous (genetic, psychogenic, neurologic and endocrine) and exogenous (environmental factors, physical inactivity and poor dietary habits) factors (Araújo et al., 2009). Behavioral therapy has been used as a non-drug intervention aimed at controlling overweight and reducing cardiovascular risk, which involves exercise either allied with diet or not (Ward-Smith, 2010). Trying to reproduce human nutritional behavior, may elucidate this food approach, as well as make it possible to monitor the variables with greater precision and control, and, with this aim, there have been several experimental studies in rodents, promoting obesity by offering a high calorie diet (Cesaretti & Kohlmann Junior, 2006; Diniz et al., 2008; Estadella, Oyama, Dâmaso, Ribeiro, & Oller Do Nascimento, 2004).

Rats are an appropriate choice due to their ease of handling, small size and good response to exercise (Araujo, Papoti, Manchado, de Mello, & Gobatto, 2007; Gobatto et al., 2008). Recent studies have used swimming exercise (Bernardes, Manzoni, Souza, Tenório, & Dâmaso, 2004; Camargo-Filho et al., 2006; Cunha, da Cunha, Segundo, Moreira, & Simões, 2008; Estadella et al., 2004; Gobatto et

al., 2001; Zambon et al., 2009) in a rectangular training model, which uses a standard volume and intensity throughout the training period. Therefore there is a minimized risk of injury and overtraining, allowing for performance maximization (Araujo, Papoti, Manchado-Gobatto, Mello, & Gobatto, 2010).

However, there is lack of research regarding the application of periodized training using different types of exercise, in addition to applying normal and hypercaloric diets, especially with the use of physiological parameters such as blood acidosis. The main aim of the present study was to analyse the effects induced by six weeks of swimming periodized training (SPT) on the aerobic capacity (AC) and body weight (BW), on rats fed with either normal or hypercaloric diets.

## METHODS

### Animals

Twenty-four male Wistar rats (*Rattus norvegicus*) with 90 days old (body mass:  $364.1 \pm 34.8$  g) were placed in collective plastic cages (5 animals per cage) in a temperature controlled room ( $22 \pm 2^\circ$  C), with a relative humidity of 60% and lights from 7:00am to 7:00pm (12/12 hours). Throughout the experiment the animals received food and water *ad libitum*.

All the procedures followed the “Ethical Principles in Animal Experimentation” adopted by the Brazilian Society of Laboratory Animal Science (SBCAL) and received approval from the Ethics Committee of this institution under no. 2/2010.

### Experimental design

After environmental adaptation, the animals were paired according to body weight (g) and divided in two groups containing 12 animals each, differentiated by the type of diet. One group “normocaloric” (NG) was fed a normal calorie diet, which consisted of commercial food for rats (Primor) containing 4.07 kcal / g and the other group “hypercaloric” (HG) was fed with a high calorie diet, which

consisted of a mixture of hyper-energy foods totalling 5.12 kcal/g (Zambon et al., 2009). After the division of the groups, the feed was maintained until the end of the experiment.

Throughout the experiment, the animals were weigh up once a week and food intake was measured daily. Food consumption was calculated as the difference between feed offered and left over. To calculate the body mass evolution we used the following formula (Bernardes et al., 2004):

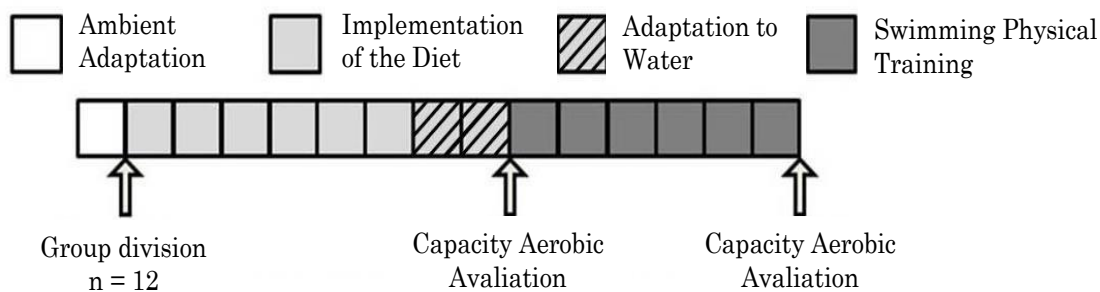
$$\Delta W = \frac{\text{final mass} - \text{initial mass}}{\text{initial mass}} \times 100$$

Although not being a usual activity, exercise performed in water (besides the activity itself) produces emotional stimuli by presenting the impossibility of escape and imminence of death (Camargo-Filho et al., 2006). For this reason, all the animals performed adaptation to the water for 10 days (Manchado, Gobatto, Voltarelli, & Mello, 2006) before the experi-

mental protocol, in order to reduce the animals' stress without, however, promoting physiological adaptations in response to physical training.

For the adaptation, we used a tank containing eight PVC cylindrical tubes (25 cm diameter x 120 cm depth). This tank was filled with water ( $31 \pm 1^\circ\text{C}$ ) reaching a maximum of 100 cm, so that the animals could not support their tail end on the bottom of the tank. During the final three days the animals performed swimming with an overload of 3% of body mass (BW), the intensity was maintained by using cloth bags containing lead, tied with elastic to each animal's thorax (Manchado et al., 2006).

After eight weeks of diet application (normal-and hypercaloric), the animals underwent a test for aerobic capacity determination (AC), a swimming periodized training (SPT) protocol and another test to determine AC after the training period (figure 1).



Each square represents one week of experiment

Figure 1. Experimental model utilized in the present study

#### Aerobic capacity

Aerobic capacity (AC) was assessed using the "Chassain Test" (non-exhaustive double efforts) method, performed in accordance with the double bout protocol validated by Manchado, Gobatto, Voltarelli, e Mello (2006) at two different times: before and after training, in environmental conditions similar to those of the adaptation period.

The protocol consisted of 3 to 4 continuous swimming tests with a randomly distributed overload of 4%, 6%, 7% and 8% of BW. The rats performed two loads per day with a six hour rest interval, which comprised two days for testing. The rats swam 2 x 5 min at the same intensity, with 2 min of passive recovery, resulting in a total duration of 12 min (figure 2).

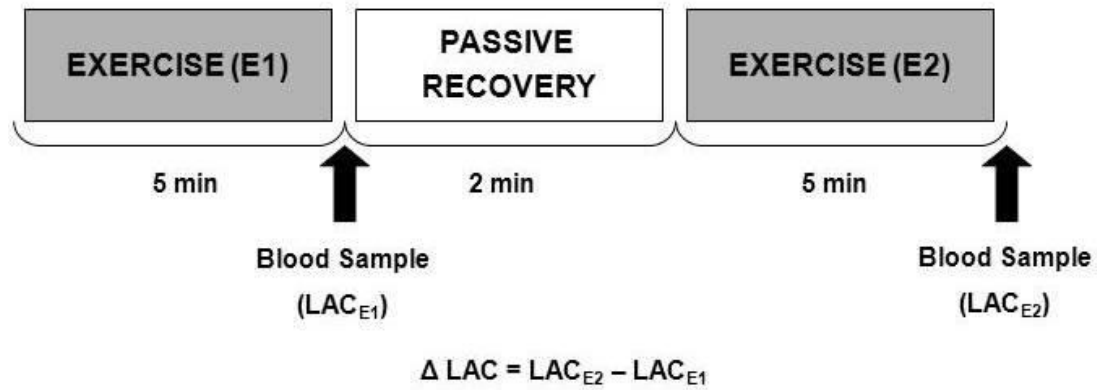


Figure 2. Aerobic Capacity Determination (AC) test, using the “Chassain Test” method performed in accordance with the double bout protocol validated by Manchado et al. (2006).

Blood samples were collected from a cut at the tail tip at the end of each bout of exercise. Samples (25 $\mu$ L) were placed in plastic tubes (Eppendorf – 1.5 mL capacity) with 50 $\mu$ L of Sodium Fluoride (1%) for further analysis. To avoid blood dilution by water, the rats were dried before blood collection and then returned to the water immediately after the collection. Lactate concentrations were determined in the lactate analyser model YSI 1500 Sport (Yellow Springs, Ohio, EUA).

For each intensity the change in lactate ( $\Delta$ LAC) was calculated by subtracting the concentration obtained after the first bout ( $\text{LAC}_{\text{E1}}$ ) from the concentration obtained at the end of the second bout ( $\text{LAC}_{\text{E2}}$ ). Then a linear interpolation was plotted, returning a null exercise load, equivalent to the critical load.

#### Swimming training programs

SPT was conducted for six weeks under the same conditions of AC, subdivided into three

levels (Araujo et al., 2010): Easy endurance (END1), Moderate (END2) and Intense (END3) related to the anaerobic threshold (Lan) estimated using the non-exhaustive method ( $\approx 4.8\%$  BW) (Manchado et al., 2006).

The END1 training sessions were performed over 60 min of continuous exercise at 80% of Lan. The END2 sessions were composed of continuous swimming at the intensity corresponding to Lan for 30 min. In the END3 sessions the rats were submitted to swimming intervals which lasted for five minutes with a recovery time of one minute and 15 min of exercise with an intensity of 120% Lan (table 1).

At the end of each session, the animals were dried with cotton and kept for 30 min in a wooden box heated by an incandescent light, then returned to their cages (Camargo-Filho et al., 2006).

Table 1

Training protocol organization established in six weeks

WEEK	MON	TUE	WED	THU	FRI	SAT
1	END1	END2	END2	END1	END2	OFF
2	END2	END1	END2	END1	END2	OFF
3	END2	END1	END3	END1	END2	OFF
4	END2	END3	END1	END1	END2	END3
5	END2	END3	END1	END1	END2	END3
6	END3	END2	END1	OFF	OFF	OFF

Note: Model of training protocol based on the periodization proposed by Araujo et al. (2010)

### Statistical Analyses

The results are expressed as mean  $\pm$  SD. Data normality was confirmed using the Shapiro-Wilk test. To compare data before and after training and between the HG and NG groups, the Student t test was used for paired and unpaired samples respectively. The “effect size” was calculated by Cohen’s index, subtracting the mean of the experimental group from that of the control group and dividing for

standard deviation ( $M_1 - M_2 / SD$ ). In all cases the significance was fixed at 95% ( $p < 0.05$ ).

### RESULTS

The mean values and standard deviation of the animals’ body mass in each group and their mass gain during the experimental diet and training are detailed in table 2. We noticed a significant increase in body weight (g) in both groups after diet and training.

Table 2

*Body weight (g) and Weight Gain (%).*

	W_PreD	W_PostD	$\Delta$ WD	(d)	W_PreT	W_PostT	$\Delta$ WT	(d)
NG	364.3 $\pm$ 36.4 <sup>a</sup>	445.89 $\pm$ 41.4 <sup>a</sup>	22.54 <sup>b</sup>	2.09	429.1 $\pm$ 44.6 <sup>a</sup>	460.6 $\pm$ 50.1 <sup>a</sup>	7.41	0.31
HG	363.9 $\pm$ 34.7 <sup>a</sup>	487.7 $\pm$ 61.7 <sup>a</sup>	33.75 <sup>b</sup>	2.56	490.9 $\pm$ 58.4 <sup>a</sup>	530.2 $\pm$ 61.7 <sup>a</sup>	8.07	0.64

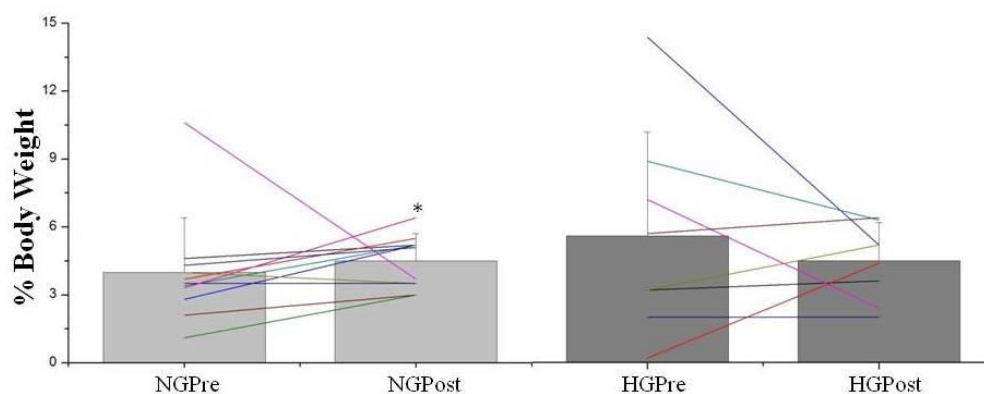
*W\_PreD = Body weight before Diet Administration; W\_PostD = Body weight after Diet Administration;  $\Delta$ WD = Weight Gain due to Diet; W\_PreT = Body weight before training; W\_PostT = Body weight after training period;  $\Delta$ WT = Weight Gain due to training protocol; (d): Effect size; <sup>a</sup> Significant difference in the animals’ weight; <sup>b</sup> Significant difference between the groups in body weight gain. Comparison realized with the “Student t test” with the significance level established at 5% ( $p < 0.05$ ).*

The animals’ dietary intake averaged 29.27  $\pm$  4.14 g for the NG group and 22.72  $\pm$  3.77 g for the HG, but with no significant difference ( $p > 0.05$ ). However, it was noticed that rats consuming the high calorie diet tended to decrease their food intake over time.

Deltas ( $\Delta$ LAC) obtained in each load of effort of AC determination ranged from 0.9% to 3.6% BW in trained animal. After training, negative values of  $\Delta$ LAC for both groups of 4%

BW intensity were identified, revealing a possible adaptation of the rats to SPT.

Results show that the NG group reached AC at an intensity of 3.94  $\pm$  2.42% BW before training, increasing to 4.48  $\pm$  1.18% BW after the training period, showing statistical significance. On the other hand, the HG group reached an AC of 5.59  $\pm$  4.56% BW before training and 4.45  $\pm$  1.66% BW after SPT (figure 3).



*Figure 3. Aerobic Capacity (% BW), NG – Normocaloric Group (light bars) and HG - Hypercaloric group (dark bars); Individual Load (% BW) of Anaerobic Threshold (thin lines), considering moments pre-and post-Swimming Physical Training.*

## DISCUSSION

The main finding of this study is that the training was able to contain the increase in the animals' body weight in both groups and increase the aerobic capacity of those who had access to standard food. It is known that the incidence of obesity is increasing in the world population, causing many health problems, including cardiovascular risk, diabetes and other chronic diseases, both in men and women (Araújo et al., 2009). Most diseases caused by obesity are associated with an increase in visceral adiposity, where excess fat intake and lack of physical activity are the likely causes of this accumulation (Zambon et al., 2009).

In this study, we chose the exogenous obesity model, which most closely resembles human obesity. For this, we administered a high calorie diet to animals by replacing their conventional diet with another made up of a combination of standard chow and high-calorie substances (chocolate, peanuts and cookies). We noticed a significant difference in body weight due to the administration of both diets, but the HG group showed the greatest variation in weight when compared to the NG, with an increase of 33.75% BW, which agrees with information in the literature regarding an increase in BW due to diet (Cesaretti & Kohlmann Junior, 2006; Duarte et al., 2006).

However, it was observed that the HG food intake was lower than the NG. This decrease suggests increased satiety by the HG group, because high-fat diets have reduced feed efficiency and increased metabolic efficiency due to high plasma levels of metabolic substrates such as glucose and triglycerides (Bernardes et al., 2004; Zambon et al., 2009).

Exercise has been used as a non-pharmacological treatment because of its ability to change the attitudes of some key enzymes in the metabolic system that prevent and mitigate metabolic syndrome effects (Moura et al., 2008). Using this information and knowing that AC level has an inverse relationship with the risk of developing cardiovascular disease (Moura et al., 2008), the training

model used in this study makes use of stimuli distributed in three different intensities to develop AC; merging the partial restoration of intramuscular glycogen content (END1), maintaining the highest lactate concentration without causing progressive lactacidemia accumulation throughout the session (END2) and the development of aerobic power (END3) (Araujo et al., 2010). Training at AC intensity is advantageous without reaching maximum oxygen consumption, thus diminishing cardiovascular risks (Cunha et al., 2008).

In this study, the three training intensities (80%, 100% and 120% of AC) were related to the anaerobic threshold (Lan) of 4.8% of BW, estimated by the non-exhaustive method (Manchado et al., 2006). Blood lactate measurement was used to evaluate training influence on AC. Originally, the protocol consisted of four tests with different random loads. However, this study showed a high level of failure at the higher intensity for the HG group, leading to an adjustment in the AC calculation, where all the values obtained with an 8% BW overload were discarded from both groups.

The change in lactate concentration found at the end of each bout performed at the same intensity is considered in the evaluation. Animals reached blood lactate stabilization at a load of approximately 4% BW for the NG group and 5% BW for the HG, before training. After SPT, this stabilization was approximately 5% BW for both groups. This intensity is similar to those obtained by Gobatto et al. (2001), Voltarelli, Gobatto, e Mello (2002), Manchado et al. (2006), Araujo, Papoti, Manchado, de Mello, e Gobatto. (2007), Gobatto et al. (2008), who denoted this stabilization at between 5 and 6% BW.

It was observed that for the HG group the load was maintained, but the variation magnitude between the animals decreased. These data concur with another study (Gobatto et al., 2001), where the same strain of sedentary animals showed stabilization in blood lactate

concentration during similar exercise with an overload of between 5 and 6% BW.

In their study, Chen et al. (2010) showed an inverse association between a high calorie diet, induced obesity and bone density, because free fatty acids can produce bone loss or reabsorption, thereby decreasing bone mineral density. Another study (Reis, 2010) showed that body density (BD) influences the exercise intensity in water, since exercise has a high negative correlation with BD and hydrostatic weighing (HW). In addition, the high calorie diet offered was able to make the animals physically heavier, larger and less dense, showing lower values for HW and BD while the relative intensity of MLSS presented lower values compared to the control group.

Based on this, it is assumed that because they are obese due to diet administration, the HG animals may have a decreased body density, which is suggested to have facilitated their buoyancy and made both training and testing easier to perform because they required less effort compared to the NG animals, with subsequent AC maintenance.

Thus, the present study collaborates with the literature in showing the effects of swimming physical training on body composition and aerobic capacity in Wistar rats. However, there are limitations related to the type of training. Experimental models utilizing running and jumping physical protocols and different intensities could contribute to this research.

### CONCLUSIONS

In short, we can conclude that in this study the proposed six weeks swimming periodized training protocol showed sensitivity to the lactate test and was able to halt the increase in body mass of both groups and improve the aerobic capacity of rats consuming a normal calorie diet. Taking into account that one of the ways of preventing and treating cardiovascular diseases is aerobic training, the proposed model could be applied to the assessment, prescription and control of the training inten-

sity, since it presents a non-exhaustive method.

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Nothing to declare

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### Conflicts of Interest:

Nothing to declare

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Nothing to declare

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