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CHANGES IN BODY COMPOSITION OF *WISTAR* RATS: EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING

Lucio Marques VIEIRA-SOUZA[1,2], Felipe J AIDAR[1,3], Jymmys Lopes DOS SANTOS[1], Eduardo KALININE[1], Jhennyfer Aline Lima RODRIGUES[4], José Uilien DE OLIVEIRA[3], Jéssica Denielle Matos DOS SANTOS[1], Márcio GETIRANA-MOTA[1], Waleska DOS SANTOS[1], Anderson Carlos MARÇAL[1]

[1] Post-Graduate Program in Physical Education, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil.
[2] Physical Education Course, Universidade Estadual de Minas Gerais, Passos, Minas Gerais, Brazil.
[3] Post-Graduate Program in Physiological Science, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil.
[4] School of Nursing of Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

Corresponding author:
Lucio Marques Vieira-Souza
profedf.luciomarkes@gmail.com

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Abstract
The present study aimed to investigate the effectiveness of high-intensity interval training (HIIT) in the body composition of *Wistar* rats. The HIIT protocol consisted of high-intensity swimming three times a week for four weeks. There were no differences between groups as to the Lee index. However, the weights of the perigonadal (p=0.001) and retroperitoneal (p=0.026) fats were significantly different between the Control Group (CG, n=10) vs. Trained Group (TG, n=10), respectively. There was also a significant increase in the body weight of the animals in TG (16.43%) and CG (7.19%) at the end of the experiment. These findings suggested that HIIT was not sufficient to improve significantly the body composition of rats.

Keywords: Body composition. High-intensity interval training. Physical activity. Swimming.

1. Introduction

The World Health Organization (WHO) states that regular physical exercise throughout life can promote benefits to mitigate or prevent the onset of several diseases, such as sarcopenia, obesity, cancer, and cardiovascular diseases (Kessler et al. 2012; Kushi et al. 2012; Marzetti et al. 2017). The advent of new technologies for social interaction caused physical exercise modalities to emerge and gain supporters. An example of this is high-intensity interval training (HIIT), which is characterized by brief and repeated sessions of intense activity interspersed with short periods of passive or active recovery of low-intensity exercises (Gibala et al. 2012; Batacan et al. 2016; Paes et al. 2020; Vieira-Souza et al. 2021).

Previous studies have shown that HIIT, compared to moderate-intensity continuous exercise, promotes similar adjustments in physiological parameters, such as improving cardiorespiratory fitness with a positive impact on health conditions (Nes et al. 2012; Gillen et al. 2016; Karlsen et al. 2017; Wewege et al. 2018; Martin-Smith et al. 2020). For a long time, low-intensity exercises were considered more beneficial than high-intensity exercises in reducing body fat mass due to a higher mobility and lipid oxidation during and after low-intensity physical exercises (Garber et al. 2011). High-intensity exercises might use energy from carbohydrate sources, especially for decreasing total body fat loss by providing a higher energy expenditure in short training sessions (Groussard et al. 2019).

The effects of high-intensity interval training on health parameters have been investigated, but the exercise modalities and intensities that induce higher benefits in body composition are still unknown. Although the scientific literature includes studies based on the positive effects of high-intensity interval training on cardiorespiratory fitness, the professional practice shows massive speculation about the potential benefits of high-intensity interval training to losing weight.

It is relevant to verify the influence of HIIT weeks on body composition, weight loss, and fat weight. The present study hypothesized that HIIT can assist in body weight loss because it is more intense, which may increase caloric expenditure during and after the training session. Therefore, the present study aimed to analyze the changes in the body composition of *Wistar* rats subjected to high-intensity interval training.

2. Material and Methods

This study was approved by the Animal Research Ethics Committee of the Federal University of Sergipe [*Universidade Federal de Sergipe*] – UFS (protocol 15/2017), and it complied with the guidelines of the Brazilian College of Animal Experimentation [*Colégio Brasileiro de Experiências com Animais*] (COBEA).

Twenty male *Wistar* rats were used. They were sourced from the sectoral vivarium of the Center for Intracellular Signaling Research [*Núcleo de Pesquisa em Sinalização Intracelular*] (NUPESIN) of the Federal University of Sergipe, randomly distributed, and housed in appropriate cages at a controlled temperature (22 ± 3ºC), with a 12-hour light/dark cycle (lights on 6 a.m. – 6 p.m.), and free access to a specific food for rodents and filtered water. The animals were divided into two groups: Control Group (CG, n=10), composed of animals not subjected to any intervention in the experimental period; and Trained Group (TG, n=10), with animals subjected to the high-intensity swimming interval training protocol (as described below).

The animals were subjected to a swimming exercise adapted from the protocol by De Araújo et al. (2016). The experiment used a black PVC cylinder (120-cm deep and 80-cm wide) with water at a depth of 50 cm and an average temperature of 25 ± 2°C, and the loads were attached to a vest on the back of the animals. To perform the exercise, the animals were placed individually in the PVC cylinders with water. The adaptation of the animals to the water environment consisted of swimming sessions of 20 minutes without load, three times a week for one week.

After the adaptation period, the HIIT protocol was implemented (Terada et al. 2001), consisting of 14 swimming periods of 20 seconds and 10-second intervals between each period. After ending the sessions, the animals were dried and housed again in their respective cages. This protocol lasted four weeks, and it was performed three times a week on alternate days, with a load of 14% of body weight.

After each training session, the animals were weighed to define the load percentage. The animals were weighed every two days throughout the experimental period. To determine weight gain, the initial weight was subtracted from the weight on the last day of the experiment. Twenty-four hours after ending the protocol, the animals were anesthetized with a mixture of ketamine and injectable xylazine (75 mg/kg + 10 mg/kg i.p.) for the subsequent euthanasia by bleeding under anesthesia. The perigonadal and retroperitoneal fats pads were removed, washed in saline solution (0.9%), and weighed. Fat weight was expressed relative to body weight (in grams). Furthermore, the animals had their body mass and naso-anal length measured biometrically (Águila et al. 2002). Afterward, these data were used to calculate the Lee index of each animal, which is the ratio of the cubic root of the body mass (g) by the naso-anal length (cm), multiplied by 10, which is equivalent to the body mass index (BMI) of humans (Bernardis and Patterson 1968).

The data obtained were described according to the descriptive statistical analysis method (mean and standard deviation). The Shapiro Wilk test was used to assess data distribution. The results were analyzed with the Student paired t-test for both periods (Pre-intervention vs. Post-intervention) in each group, while the Student unpaired t-test was used to compare the means between groups. The differences were statistically significant for $p<0.05$., The GraphPad Prism statistical software, version 7.0 (GraphPad Software, San Diego, CA, USA), was used for all procedures.

3. Results

Table 1 presents the Lee index between the groups without significant differences (p> 0.05).

Table 1. Analysis Lee index between control group and trained group.

Group	Mean	Median	Low	High	Standard Deviation	p*
CG	105.3	105.6	101.1	113.5	4.34	0.5773
TG	108.1	109.0	100.4	114.3	5.92	

Note: *Student t-test for independent samples. p<0.05. CG (Control Group), TG (Trained Group), n=10 per group.

Regarding fat weight, there was a significant difference for the weight of the perigonadal fat pad (CG = 1.4±0.46 g and TG = 2.7±0.72 g; p = 0.001) and the retroperitoneal fat pad (CG = 1.1±0.36 g and TG = 2.3±1.13 g; p=0.026) (Table 2).

Table 2. Analysis of perigonadal fat pad and retroperitoneal fat pad between different experimental groups.

Tissue	Group	Mean	Standard Deviation	p*
Perigonadal Fat pad	CG	0.45	0.15	0.0048*
	TG	0.77	0.20	
Retroperitoneal fat pad	CG	0.36	0.11	0.0491*
	TG	0.66	0.34	

Note: *Student t-test for independent samples. p<0.05. CG (Control Group), TG (Trained Group), n=10 per group.

As for body weight, there were significant differences in weight gain (Figure 1). The CG increased 7.19% (Pre CG vs. Post GC. p=0.05: 292 ± 11 g and 313±19 g, respectively), while the TG increased 16.43% (Pre TG vs. Post TG. p<0.0001; 292±13 g and 340±19 g, respectively). At the end of the HIIT protocol, Post TG showed a significant increase in body weight compared to the control group (Post CG) (p=0.017).

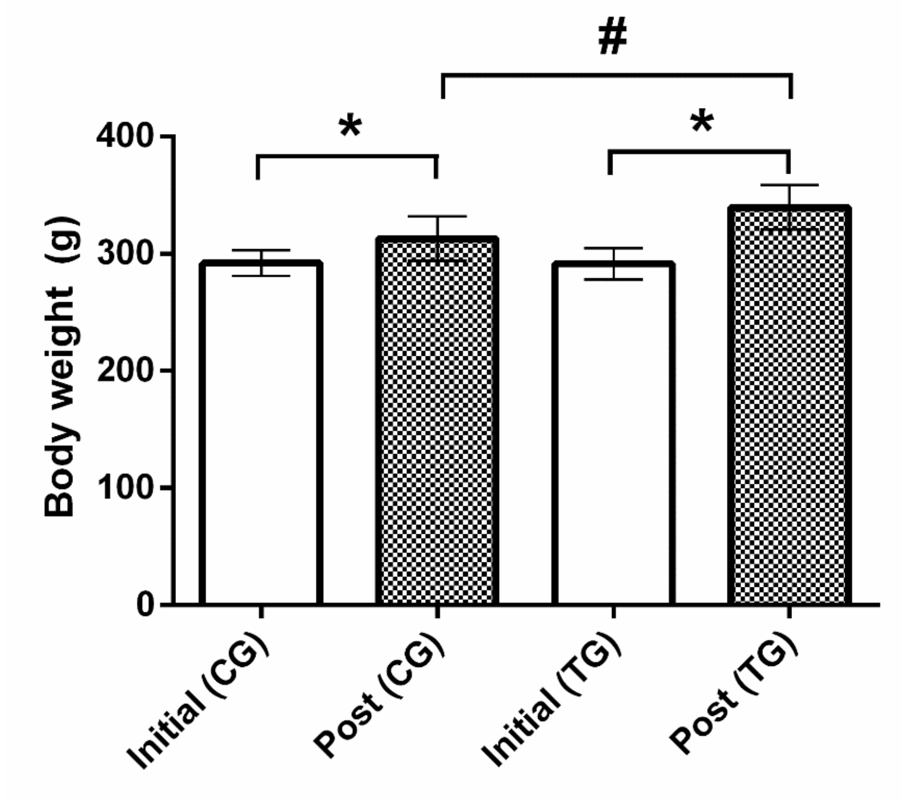

Figure 1. Effects of HIIT in body weight on *Wistar* rats.

4. Discussion

The main objective of the present study was to analyze changes in the body composition of *Wistar* rats subjected to high-intensity interval training (HIIT). HIIT can be efficient in improving several capacities, such as cardiorespiratory fitness, and can be advocated as a time-efficient strategy for eliciting fitness benefits comparable to traditional continuous exercise in inactive or overweight adults. However, the present study showed a lack of superiority of HIIT in improving body composition compared to the control group. It is worth noting that the workload is usually defined according to the parameters of the lactate minimum test and several others. Hence, an overload of 6% or more of the body weight of an animal under swimming training is considered a high-intensity exercise (De Araújo et al. 2016).

The fat metabolism increases after exercising, not only serving as a source of body fuel for any activity but also replenishing the glycogen depleted by high-intensity exercise, considering that fat metabolism can rise for up to 24 hours and depends on the length and intensity of the exercise (Hetlelid et al. 2015). Despite the four weeks of HIIT in the present study, the body weight increased between the groups. This may be explained by the classic study by Novelli et al. (2007), which highlights the direct responsibility of eating behavior and energy expenditure, considering that after 90 days of life, the body weight of animals increases, and from this age and maturation it is assumed that food and energy intake will change. Likewise, animals with high muscle mass may have a high Lee index despite low variations in the amount of fat (Nery et al. 2011).

Alkahtani et al. (2013) found similar results by examining the effect of four weeks of training on the response of fat oxidation in 10 overweight and obese individuals. Interestingly, they did not find differences in body composition but an increase in fat oxidation in both groups studied: HIIT (45% of VO2max) and HIIT (90% of VO2max). Furthermore, corroborating the results of the present study, Keating et al. (2014), with a sample of 38 inactive individuals who performed 12 weeks of HIIT, compared the response of total body mass, fat percentage, cardiorespiratory capacity, and work capacity in three different groups. The groups performed interval training (120% of VO2max), continuous training, or no training (placebo group), and the overall body composition did not change among the groups. In contrast, Ramos-Filho et al. (2015) used a protocol similar to the present study but with six weeks, an initial load of 9%, and an increase of 1% per week, and showed that the HIIT group reduced body weight and visceral fat compared to the control group. This means that the effects of HIIT might depend on the load and weeks of training.

5. Conclusions

Using HIIT is justified because the method does not require much time or refined motor skills, and it shows positive effects on cardiorespiratory fitness and the general health of subjects. However, because it is more intense, doubts remain as to whether HIIT could positively affect body composition, considering it is unknown whether the mobilization and oxidation of fatty acids occur during and/or after the training session. Consequently, the present study did not control food intake, but it is noteworthy that higher caloric intake may lead to weight gain.

Therefore, additional studies are required for assessing different physiological and biochemical analyses and parameters, and the studied variables, after a longer training period. In conclusion, the present study showed that HIIT was not sufficient to improve significantly the body composition of *Wistar* rats.

Authors' Contributions: VIEIRA-SOUZA, L.M.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; AIDAR, F.J.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; DOS SANTOS, J.L.: acquisition of data, and critical review of important intellectual content; KALININE, E.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; RODRIGUES, J.A.L.: analysis and interpretation of data, drafting the article, and critical review of important intellectual content; DE OLIVEIRA, J.U.: analysis and interpretation of data and drafting the article; DOS SANTOS, J.D.: analysis and interpretation of data and drafting the article; GETIRANA-MOTA, M.: analysis and interpretation of data and drafting the article; DOS SANTOS, W.: analysis and interpretation of data and drafting the article; MARÇAL, A.C.: critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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