

## Chronic effects of training and subsequent physical detraining on histology and morphometry of adipose tissue in adult Wistar rats

### Efectos crónicos del entrenamiento y posterior desentrenamiento físico sobre la histología y morfometría del tejido adiposo en ratas Wistar adultas

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**Abstract.** The objective of the study was to analyze the effects of physical exercise and subsequent detraining on histological and morphometric parameters of white adipose tissue (WAT) and brown adipose tissue (BAT). Also investigated were insulin and glucose tolerance. It was an experimental study with three groups: continuous moderate-intensity training (CMIT), high-intensity interval training (HIIT), and a control group (CG). Three assessments were carried out: pre-intervention, after 8 weeks of training, and after 4 weeks of detraining. A generalized estimation equation was performed for (group x moment), with Bonferroni post-hoc for group and moment in the analysis of adipocyte area and weight. A one-way ANOVA was performed to analyze the decay rate and the area under the curve between groups. For the intragroup study, repeated measures ANOVA with Bonferroni post-hoc was performed. An increase was observed between T2 and T3 in the area of perilumbar adipose tissue ( $747.3 \pm 28.4 \mu\text{m}^2$  vs.  $853.0 \pm 15.7 \mu\text{m}^2$ ,  $p \leq 0.01$ ) and perirenal ( $770.3 \pm 11.4 \mu\text{m}^2$  vs.  $830.9 \pm 18.6 \mu\text{m}^2$ ,  $p \leq 0.01$ ) regardless of the group, as well as an increase in the subscapular BAT area from T1 to T3 ( $419.9 \pm 38.5 \mu\text{m}^2$  vs.  $751.8 \pm 27.5 \mu\text{m}^2$ ,  $p \leq 0.001$ ). The weights of perirenal, perilumbar, and subscapular brown adipose tissues were lower in HIIT and CMIT compared to the CG ( $p \leq 0.001$ ). It was observed that after detraining, the calculation of the decline in glycemia showed a statistically significant difference ( $F = 8.79$ ;  $p = 0.005$ ) between CG and HIIT ( $0.78\%$  vs.  $1.82\%$ ), with a higher average percentage for HIIT. It is concluded that 8 weeks of CMIT and HIIT are efficient for weight control and adipose tissue area; however, this control is lost after 4 weeks of detraining, and even after this period, HIIT showed better insulin sensitivity.

**Keywords:** Exercise; Lipids; Adipose tissue; General adaptation syndrome; Supercompensation.

**Resumen.** El objetivo del estudio fue analizar los efectos del ejercicio físico y el posterior desentrenamiento sobre los parámetros histológicos y morfométricos del tejido adiposo blanco (TAB) y del tejido adiposo pardo (TAP). También se investigaron la tolerancia a la insulina y la glucosa. Fue un estudio experimental con tres grupos: entrenamiento continuo de intensidad moderada (ECIM), entrenamiento interválico de alta intensidad (EIAI) y grupo control (GC). Se realizaron tres evaluaciones: preintervención, tras 8 semanas de entrenamiento y tras 4 semanas de desentrenamiento. Se realizó una ecuación de estimación generalizada para (grupo por momento), con Bonferroni post-hoc para grupo y momento en el análisis del área y peso de los adipocitos. Para analizar la tasa de desintegración y el área bajo la curva entre grupos, se realizó ANOVA unidireccional. Para el estudio intragrupo, se realizó ANOVA de medidas repetidas con Bonferroni post-hoc. Se observó un aumento entre T2 y T3 en el área de tejido adiposo perilumbar ( $747,3 \pm 28,4 \mu\text{m}^2$  vs.  $853,0 \pm 15,7 \mu\text{m}^2$ ,  $p \leq 0,01$ ) y perirrenal ( $770,3 \pm 11,4 \mu\text{m}^2$  vs.  $830,9 \pm 18,6 \mu\text{m}^2$ ,  $p \leq 0,01$ ) independientemente del grupo, así como un aumento en el área TAP subescapular de T1 a T3 ( $419,9 \pm 38,5 \mu\text{m}^2$  vs.  $751,8 \pm 27,5 \mu\text{m}^2$ ,  $p \leq 0,001$ ). Los pesos de los tejidos adiposos pardos perirrenal, perilumbar y subescapular fueron menores en EIAI y ECIM en comparación con el GC ( $p \leq 0,001$ ). Se observó que después del desentrenamiento, el cálculo de la caída de la glucemia mostró una diferencia estadísticamente significativa ( $F = 8,79$ ;  $p = 0,005$ ) entre GC y EIAI ( $0,78\%$  vs.  $1,82\%$ ), con mayor porcentaje promedio para EIAI. Se concluye que 8 semanas de ECIM y EIAI son eficientes para el control de peso y área de tejido adiposo; sin embargo, este control se pierde después de 4 semanas de desentrenamiento, e incluso después de este período, el EIAI mostró una mejor sensibilidad a la insulina.

**Palabras clave:** Ejercicio; Lípidos; tejido adiposo; Síndrome de adaptación general; Supercompensación

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## Introduction

White adipose tissue (WAT) is responsible for regulating the body's energy balance, storing excess energy from food in the form of triglycerides (Schnaider & Borges, 2021). When the body needs energy, the triglycerides that make it up are released from adipocytes, broken down into fats and glycerol, and then used as a source of energy. WAT adipocytes also play roles in metabolic and hormonal regulation, secreting a wide variety of signals known as adipokines, which contribute to appetite control, glucose metabolism, regulation of the immune system, and inflammation (Rosenwald & Wolfrum, 2014). Understanding the dynamics of this adipose tissue in the face of stress is rele-

vant, as it can contribute to the organization of interventions that contribute to its management, including those aimed at weight loss (Del Vecchio et al., 2020). In turn, the role of brown adipose tissue (BAT) is to regulate thermogenesis, which is the process of heat production and plays a role in maintaining body temperature by burning stored fat to generate heat (Rosenwald et al., 2014). The number and area of adipocytes can vary between individuals, and an excessive increase in the number or area of adipocytes can lead to the accumulation of body fat and the development of obesity. This variation occurs for several reasons, including genetic, environmental, and lifestyle factors (Schnaider et al., 2021). Physical exercise is one of the exogenous factors capable of causing adaptive changes in adipose tissue, such

as increasing the rate of mobility and oxidation of triacylglycerides, causing a decrease in fat mass and, consequently, inducing negative energy balance, promoting weight loss, as well how to improve insulin sensitivity (Jesus et al., 2019).

The principle of adaptation suggests that when stress is suspended, or there is a period of recovery, the body readapts to such conditions to recover previous levels of homeostasis (Selye, 1936). In this sense, (Sertie et al., 2013) concluded that the cross-sectional area of adipocytes increased in a statistically significant way in animals that trained for eight weeks and detrained for four weeks compared to rats that continued training for the same total period and when compared to animals that remained sedentary (Sertie et al., 2013). Such findings would align with fat overshooting, in which the performance of a specific stimulus conditions the organism to overcome the induced stress, and its interruption would promote overcompensation (Del Vecchio et al., 2020).

Physical exercise is prescribed to prevent and treat various diseases and metabolic disorders, some related to insulin sensitivity (diabetes, obesity). Continuous moderate-intensity exercise increases insulin sensitivity, which promotes more significant fat mobilization and glucose uptake (Sertié et al., 2015). During detraining, there has yet to be a consensus in the literature on whether the changes caused by training are maintained. An animal study demonstrated that insulin sensitivity did not return to baseline levels after two weeks of cessation (10 weeks, 5 days per week of CMIT, 60 minutes/day) (Lehnen et al., 2010). In contrast, (Ryan et al., 2020) reported that obese, sedentary adults, after 4 days of interruption (12 weeks, 4 times a week, HIIT = 25 min/session and CMIT = 45 min/session) levels of sensitivity to insulin returned to pre-training values.

Knowing that different tissues, including adipose tissue (Sertie et al., 2013), can undergo a supercompensation process, it is essential to investigate whether other training methods generate different responses to this process. Thus, the study's objective was to measure the effects of high-intensity interval training (HIIT) and moderate-intensity continuous training (CMIT) and subsequent detraining on histological and morphometric parameters of adipose tissue and determination of insulin and glucose tolerance.

## Materials and methods

### Type of study

It is a randomized controlled study. The animal research ethics committee of the Federal University of Pelotas approved the project under number 52252-2019.

### Casuistic and environmental survival conditions

The statistical software WINPEPI 14.0 for Windows was used to calculate the sample size. Epididymal adipose tissue weight was assumed to be 5.18 g for sedentary, 4.2 g for trained, 5.03 g as the primary outcome for untrained,

and a difference of 7 grams in the weight of the animals between the HIIT and CMIT groups and untrained (Sertie et al., 2013). Considering a significance level of 5 % and power of 90 %, the sample calculation indicated 8 animals for each group. There were three groups (HIIT, CMIT, and sedentary) and three moments of analysis: pre-intervention, after eight weeks of training and after four weeks of detraining, and when considering the addition of an animal in each group to prevent losses, the final number of animals was 63. The standard deviations published in a similar experiment were used to calculate the sample size (Sertie et al., 2013).

The animals (male Wistar rats, 60 days old) came from the vivarium of the Federal University of Pelotas (UFPEL) and were placed in 40 x 30 cm boxes with 3 animals each. They were kept under a 12/12 hour light/dark schedule, with controlled temperature (22 and 23 °C), water, and standard balanced rodent food - Nuvilab® CR1 (with composition following the recommendations of the National Research Council and National Research Council). Institute of Health/USA for feeding laboratory rats) ad libitum. The animals' base weight values were 309, 296, and 305 grams. At the end of the intervention, the weights were 425, 430, and 445 grams, and at the end of the detraining period, the weights were 481, 476, and 492 grams in the HIIT, CMIT, and control groups, respectively (data not shown).

### Design

After the first euthanasia of 9 animals to acquire baseline values (T1), the remaining animals were randomly allocated into three groups: control group (CG, n = 18), continuous moderate-intensity training group (CMIT, n = 18), and high-intensity interval training group (HIIT, n = 18). Then, the training regime lasted 8 weeks. After the intervention with physical exercises, nine (9) animals from each group were euthanized (T2), and the groups were followed for another 4 weeks of detraining, during which the animals were kept under the same conditions previously described; however, without physical exercise. After this period, the animals were euthanized (T3) (figure 1). All euthanasia occurred in the morning, by cardiac exsanguination, after anesthesia with isoflurane. The peri-renal, peri-lumbar, and subscapular brown adipose tissues were collected and weighed, and their fragments were immediately added to formaldehyde for analysis.

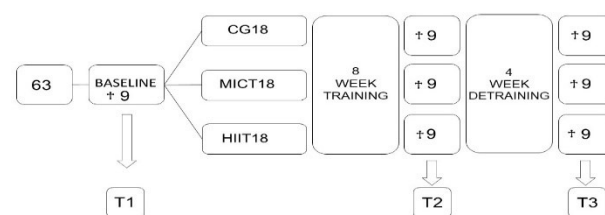


Figure 1. Experimental design of the study

CG = Control group; CMIT = Moderate intensity continuous training group; HIIT = High-intensity interval training group; † = euthanasia. T1 = Baseline euthanasia; T2 = Post-training euthanasia; T3 = Euthanasia post detraining.

### Intervention protocol and training programs

The rats were familiarized with treadmill training (Arktus®, Brazil), adapted for rodents, for 5 days (5 sessions) at 1 km/h lasting 10 min on the first day, with 5 minutes added more on each training day. After familiarization, the incremental test of maximum running capacity (CMC) was carried out following that proposed by (Koch & Britton, 2001) and adapted to the conditions of our laboratory, mainly regarding the minimum speed control sensitivity, which was 0.1 km/h, and electric shock stimuli were not applied (Koch et al., 2001). The treadmill was configured at the beginning of the test with an incline of 10 % and a speed of 1 km/h. After starting the trial, the speed was increased by 0.1 km/h every 2 min, and each animal exercised until exhaustion. The point of exhaustion was operationally defined when the animal could not follow the

treadmill's speed in three consecutive attempts after mechanical stimulation performed by the researcher. In the first week after familiarization, Kock et al. (2001) reported having found CMC of 1 to 1.8 km/h when applying a similar protocol (Koch et al., 2001). The test was used every two weeks to adjust workloads.

The CMIT performed continuous, moderate-intensity exercise at a speed of 80 % of CMC for 10 minutes on the first day and reached 25 minutes (increasing 5 minutes per day) on the last day of week 1, with a fixed incline of 10 %. The duration was increased throughout the training period, as was the intensity (table 1). The HIIT protocol consisted of interval exercise (week 1) of 15 sprints of 30 seconds with 30 seconds of active recovery. Over the weeks, the number of sprints progressively increased, with the intensity starting at 110 % of CMC in week 1, reaching values of 150 % of CMC (Table 1).

Table 1  
Training protocols.

Week	CMIT*		HIIT*			
	Session duration (min)	Intensity (% MRC)	Session duration (min)	Intensity (% MRC)	Duration (seconds)	
1	1° day: 10	80%	15	Effort (%)	Pause (%)	
	2° day: 15					
	3° day: 20			110	50	30
	4° - 5° days: 25					30
2	30	80%	20	110	50	30
3-4-5	40	80%	20	110	50	30
6-7-8	40	100%	20	150	50	30

\* treadmill inclination set at 10 % throughout the training period; min.= minutes; MRC= maximum running capacity; CMIT= Moderate intensity continuous training group; HIIT= high intensity interval training

### Determination of general insulin resistance

General insulin resistance was determined from the Glucose Tolerance Test (GTT) and the Insulin Tolerance Test (TTI). TTG was performed after 4 hours of fasting. After initial blood collection to determine baseline blood glucose values, an 80% glucose solution (2 g/kg of weight) was administered. Additional blood samples were collected after 15, 30, 60, and 120 minutes for the glucose tolerance test, while blood samples were collected at 5, 20, 35, 60, and 120 minutes for the insulin resistance test, with heparinized capillaries calibrated to 25µL, to determine glucose and insulin concentrations. Glucose concentrations were determined by collecting blood from the tail, which was added to a strip inserted into the glucometer. The results were analyzed by determining the areas under the serum glucose curves during the test using the trapezoidal method (Matthews et al., 1990). The TTI test was performed 48 hours after the TTG. The first blood collection was performed after 4 hours of fasting. After subcutaneously administering regular insulin solution (0.5 IU/kg), other supplies were carried out as described above. The analysis was carried out by calculating the glucose decay between 5 and 20 minutes after insulin application. For this, blood glucose values were normalized by the time value of 5 minutes, which was considered 100 %. The difference between 5 and 20 minutes was divided by 15 minutes, and the resulting value was expressed as % blood glucose decline/min.

### Determination of adipocyte areas and histological preparations

Histological processing of adipose tissues began with fixation in 10% formalin for 16 h at 4 °C (Lillie Ralph, 1965). The samples were then dehydrated in ethanol, cleared in xylene, and embedded in paraffin (Lillie Ralph, 1965). The microtomy was performed using a Leica microtome, with 3 sections measuring 5 micrometers thick and stained with hematoxylin and eosin (Lillie Ralph, 1965). The reading was performed using a microscope (Leipzig, model 5Xi-eLED, Motic camera, model Moticam 5, 5MP), with a 10x objective and photographic recording using the Motic Images Plus 2.0 software, capturing 4 images. Then, the morphometric measurement (area) was carried out using ImageJ software version 1.8.0\_172. Three hundred cells were analyzed, with areas between 200 and 2000 µm<sup>2</sup>, with intact membrane, randomly counted by the software, and the average of cells was used to determine the adipocyte length.

### Statistical analysis

Shapiro-Wilk test was used to test data normality. A Generalized Estimation Equation (GEE, group x moment) was performed, with Bonferroni *post-hoc* for group and moment, to analyze the area and weight of fatty tissues. The results are presented as mean and standard error. A one-way ANOVA investigated the decay index and area under the curve between groups. For intragroup analysis, repeated measures ANOVA was performed, with Bonferroni

*post-hoc*, and Mauchly's test of sphericity was applied. When the sphericity of the data was violated, the Greenhouse-Geisser correction was applied. Analyses with an error value below 5 % were considered significant. The tests were carried out using the SPSS 20 statistical software.

## Results

No significant group effect was found ( $W = 1.19$ ,  $GL = 2$ ,  $p \geq 0.05$ ), nor the interaction between time and group (figure 2, panel A;  $W = 1.15$ ,  $GL = 4$ ,  $p \geq 0.05$ ); however, there was a time effect ( $W = 12.3$ ,  $GL = 2$ ,  $p = 0.002$ ) on perilumbar area, with difference observed between T2 and T3 ( $747.3 \pm 28.4 \mu\text{m}^2$  vs.  $853.0 \pm 15.7 \mu\text{m}^2$ ), regardless of the group, revealing an increase after detraining period. When analyzing perilumbar adipose tissue weight, there was a significant group effect ( $W = 165.7$ ,  $GL = 2$ ,  $p \leq 0.001$ ), time ( $W = 8.16$ ,  $GL = 2$ ,  $p \leq 0.01$ ), and time x group interaction (figure 2, panel B;  $W = 119.2$ ,  $GL = 4$ ,  $p \leq 0.001$ ). The analysis of univariate effects showed that HIIT had lower mean values of perilumbar weight when compared to CG ( $1.94 \pm 0.73$  g vs.  $3.38 \pm 0.10$  g). The same occurred between CMIT and CG, with CMIT showing lower mean values ( $2.13 \pm 0.76$  g vs.  $3.38 \pm 0.10$  g). Furthermore, for the same variable, differences were found between T1 and T2 ( $2.25 \pm 0.05$  g vs.  $2.51 \pm 0.11$  g) as well as between T1 and T3 ( $2.25 \pm 0.05$  g vs.  $2.47 \pm 0.13$  g), regardless of the group. There was an interaction between time and group, showing a significant difference between CG and CMIT at T2, with the latter having a lower mean (difference between standards,  $-2.02$  g;  $p < 0.001$ ). The same occurred comparing CG and HIIT at T2, with the lower average in HIIT ( $-1.92$  g;  $p < 0.001$ ). Furthermore, CG differed from the HIIT and CMIT at T3 (difference between groups, respectively  $-2.73$  g;  $-2.12$  g;  $p < 0.001$ ).

Analyzing the subscapular brown adipose tissue area, there was no significant group effect ( $W = 0.55$ ,  $GL = 2$ ,  $p \geq 0.05$ ) nor the interaction between group and time (figure 2, panel C;  $W = 1.33$ ,  $GL = 4$ ,  $p \geq 0.05$ ). However, a time effect on adipose area was identified ( $W = 42.93$ ,  $GL = 2$ ,  $p \leq 0.001$ ), with increases from T1 to T2 ( $419.9 \pm 38.5 \mu\text{m}^2$  vs.  $752.7 \pm 46.1 \mu\text{m}^2$ ), and from T1 to T3 ( $419.9 \pm 38.5 \mu\text{m}^2$  vs.  $751.8 \pm 27.5 \mu\text{m}^2$ ). When analyzing the subscapular brown adipose tissue weight, there were effects of group ( $W = 35.7$ ,  $GL = 2$ ,  $p \leq 0.001$ ), time ( $W = 85.9$ ,  $GL = 2$ ,  $p \leq 0.001$ ), and interaction between group and time (figure 2, panel D;  $W = 13.1$ ,  $GL = 4$ ,  $p \leq 0.01$ ). Univariate analysis showed that HIIT had lower mean values than CG ( $0.36 \pm 0.01$  g vs.  $0.47 \pm 0.01$  g). Furthermore, CMIT mean values were lower than CG ( $0.40 \pm 0.02$  g vs.  $0.47 \pm 0.01$  g). A difference was also observed between T1 and T2 (respectively  $0.30 \pm 0.01$  g vs.  $0.53 \pm 0.02$  g) and between T1 and T3 ( $0.30 \pm 0.01$  g vs.  $0.43 \pm 0.02$  g). Furthermore, higher mean values were detected in T2 compared to T3 ( $0.53 \pm 0.02$  g vs.  $0.43 \pm 0.02$  g). Significant differences were also observed between CG, HIIT, and CMIT at T2, with higher means in the CG (differences between means,

respectively,  $0.27$  g and  $0.41$  g;  $p \leq 0.001$ ).

There was no significant effect of group ( $W = 0.90$ ,  $GL = 2$ ,  $p \geq 0.05$ ) nor the interaction between group and time (figure 2, panel E;  $W = 1.85$ ,  $GL = 4$ ,  $p \geq 0.05$ ) for perirenal adipose tissue area. However, a time effect on the area was observed ( $W = 8.92$ ,  $GL = 2$ ,  $p \leq 0.01$ ), and the difference was between T2 and T3 ( $770.3 \pm 11.4 \mu\text{m}^2$  vs.  $830.9 \pm 18.6 \mu\text{m}^2$ ), independent of the group, revealing an increase after detraining. When analyzing the weight of perirenal adipose tissue, there were effects of group ( $W = 78.7$ ,  $GL = 2$ ,  $p \leq 0.001$ ), time ( $W = 6.27$ ,  $GL = 2$ ,  $p \leq 0.04$ ), and significant interaction between time and group (figure 2, panel F;  $W = 20.8$ ,  $GL = 4$ ,  $p \leq 0.001$ ). The analysis of univariate effects showed that HIIT has lower mean values of perirenal adipose tissue weight when compared to CG (respectively  $0.58 \pm 0.02$  g vs.  $0.91 \pm 0.02$  g). The same happened between CMIT and CG ( $0.64 \pm 0.04$  g vs.  $0.91 \pm 0.02$  g). Furthermore, a difference was identified between T1 and T3 ( $0.63 \pm 0.03$  g vs.  $0.75 \pm 0.03$  g) regardless of group. Again, there was a difference between CG and HIIT at T2, with CG being  $0.60$  g ( $p \leq 0.001$ ) heavier. There was also a difference between CG and CMIT, the latter being  $0.54$  g lighter ( $p \leq 0.001$ ). Furthermore, the CG differed from the HIIT at T3, with the CG being  $0.46$  g heavier ( $p \leq 0.001$ ).

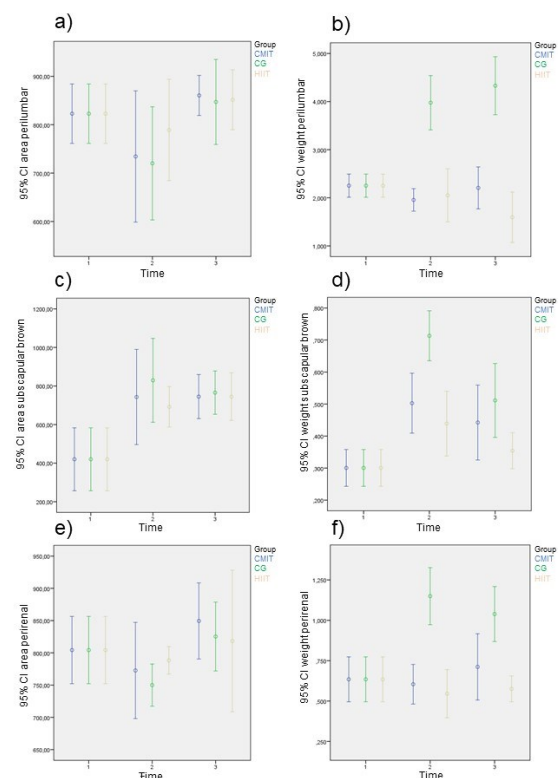


Figure 2. Interaction graph according to time and group for the variables weight and area of fatty tissues.  
CG = Control group; CMIT = Moderate intensity continuous training group; HIIT = High-intensity interval training group.

There was no statistical difference in the comparison of the area under the curve for the glucose tolerance test in the comparison between groups after 8 weeks of training ( $F = 1.59$ ;  $p = 0.24$ ) and after 4 weeks of detraining ( $F = 0.47$ ;  $p = 0.63$ ). There was no significant difference between groups of area under the curve considering the insulin resistance test after 8 weeks of training ( $F = 0.95$ ;  $p = 0.40$ ) nor in the calculation of glycemia decay ( $F = 0.06$ ;  $p = 0.06$ ). There was also no difference between groups of the area under the curve after 4 weeks of detraining ( $F = 0.76$ ;  $p = 0.48$ ), unlike the calculation of glycemia decay, which showed a statistically significant difference ( $F = 8.79$ ;  $p = 0.005$ ) between CG and HIIT (0.78 % vs. 1.82 %), with a higher mean percentage for HIIT when compared to CG. There was no intragroup statistical difference for the analysis of the area under the curve for time ( $F = 1.5$ ;  $p = 0.26$ ) and group ( $F = 0.0$ ;  $p = 0.99$ ) and for the calculation of the decay of glycemia in the analysis of time ( $F = 1.45$ ;  $p = 0.29$ ) and group ( $F = 2.49$ ;  $p = 0.18$ ).

## Discussion

The aim of the present study was to measure the effects of two types of training and subsequent detraining on different parts of adipose tissue and insulin sensitivity. The main findings were: i) increase in the area of perilumbar adipose tissue between T2 and T3; ii) lower weight of perilumbar fatty tissue in the HIIT and CMIT when compared to the control group; iii) difference in the area between T2 and T3 in the perirenal adipose tissue, regardless of the group; iv) HIIT and CMIT presented lower mean values of perirenal adipose tissue weight when compared to the CG. Furthermore, we observed an increase in the weight of the same tissue between T1 and T3 regardless of the group; v) an increase in the area from T1 to T3 of subscapular brown adipose tissue, regardless of the group; vi) the weight of subscapular brown adipose tissue was lower in HIIT and CMIT when compared to CG; vii) the percentage of blood glucose decline was more significant in the HIIT group when compared to the CG.

Adipose tissue is crucial in energy storage, metabolism, and various physiological processes (Schnaider et al., 2021). It is considered an endocrine organ that secretes hormones and cytokines, including leptin, responsible for satiety, and adiponectin, which promotes the oxidation of fatty acids and the entry of glucose into muscle cells. It can also improve insulin resistance in muscles and the liver (Carballo et al., 2020). However, the accumulation of white adipose tissue can be harmful, as it is associated with metabolic disorders, endocrine and heart diseases, and, more significantly, joint degeneration. Broadly speaking, it is reported that physical exercise promotes the reduction of this type of adipose tissue, contributing to an increase in energy expenditure and a greater energy flow, resulting in possible weight loss (Hill et al., 2013).

Our findings revealed an increase of 14 % in the perilumbar adipose tissue area and 7 % in the perirenal area

from moments T2 to T3, regardless of the group, thus characterizing a process of adipose tissue super-compensation after physical detraining. It may have occurred because perhaps the HIIT did not promote an increase in [La<sup>+</sup>] levels sufficient to inhibit lipolysis during training (Liu et al., 2020), as expected during the CMIT, thus not directing the supercompensation process towards the glycogen (Nikooie & Samaneh, 2016) and yes to adipose tissue. Therefore, during the detraining cycle, adipose tissue was overcompensated due to increased adipogenesis and decreased lipolysis. In this sense, Nikooie & Samaneh (2016) demonstrated that lactate derived from HIIT (2 min @ 80 % CMC for 1 min of passive recovery, 5 days/week) appears to be used significantly for oxidation and glycogen replacement, which reduces the oxidation of intramuscular triglycerides in the first stage of recovery after intense exercise. On the other hand, it appears that from 10 hours after ceasing activity and chronically (5 weeks), the oxidative capacity of fats is increased, as seen by the abundance of TGF- $\beta$ 1, which may have been regulated by exposure to high lactate concentrations (Nikooie & Samaneh, 2016). The literature also suggests that weight regain and some metabolic dysfunctions after a physical exercise program ends can be explained by the increase in the 11 $\beta$ -HSD1 enzyme in WAT stimulated by physical exercise (Teich et al., 2017). It, in turn, is responsible for regulating glucocorticoids (steroid hormones), which affect the signaling of the insulin cascade at muscle levels. It is also known that excess glucocorticoids induce skeletal muscle atrophy, especially in glycolytic fibers, and may thus impair insulin sensitivity (Teich et al., 2017).

In reducing BAD, the literature has discussed different strategies that involve changing lifestyles based on physical exercise (Dambha-Miller et al., 2020). Even though there are disagreements in favor of one type of physical exercise or another, most scientific production has recorded the absence of significant clinically relevant differences between continuous moderate and interval models, including high intensity (Bellicha et al., 2021). In the present study, the weight of adipose tissue reduced in the perilumbar (9 %; 13.5 %) and perirenal (14 %; 5 %) regions, respectively, in the HIIT and CMIT groups, after 8 weeks of intervention, which endorses evidence of partial of scientific literature. Such findings refute our initial hypotheses that HIIT could promote more significant fat loss than CMIT (Türk et al., 2017). Although there was no difference in weight loss between the two groups, it is worth highlighting the efficiency of HIIT in time and fat reduction, as the time spent in training sessions was shorter when compared to CMIT, which has twice the volume (from the third week onwards). The literature also highlights the importance of physical exercise for reducing visceral fat (Visser et al., 2013). HIIT is an efficient model for reducing such fat, sometimes superior to other training methods, such as strength training and CMIT. Still, it should be noted that this superiority can be neutralized when energy expenditure is equalized between

CMIT and HIIT protocols (Bellicha et al., 2021).

The HIIT and CMIT groups presented lower values of perirenal adipose tissue weight when compared to the CG; however, surprisingly, we observed an increase in the weight of the same tissue between T1 and T3, regardless of the group. This may have occurred because the animals were still growing, as they began training at 60 days, the transition phase to adulthood. However, it is crucial to raise some hypotheses about weight gain during physical training already reported in the literature. Some studies have demonstrated energy compensation and metabolic changes reacting under the stress of increased physical activity (Paravidino et al., 2021). Some theories have already been reported, such as the one exposed by Rowland (1998), where he says that there is a biological control center, in which whenever there is an imbalance, regulatory mechanisms are activated to restore a particular parameter, that is, according to this theory, an increase in the amount of physical activity at one time would be compensated with less physical activity at another time, to restore balance to the system. There is also the concept proposed by Pontzer (2015), who presented the “restricted model” to explain the relationship between energy expenditure and physical activity. According to this model, an individual's metabolism adapts in response to increased physical activity, and above a specific “tipping point,” increased volume of physical activity does not cause a concomitant increase in energy expenditure. According to the authors, this compensation can be explained by behavioral factors (more extended periods of sitting than standing throughout the day compared to a period without exercise) or metabolic changes (decrease in resting metabolic rate, increase in muscular efficiency for same activity demand, or even hormonal changes, such as a decrease in estrogen and testosterone production and a decrease in the activities of the immune system) (Pontzer, 2015). This compensatory phenomenon of physical activity is also observed in some species of birds and mammals. The “energy budget” of these animals is also limited, and any increase in energy expenditure to maintain basal metabolism would leave a smaller amount of energy available for other functions, such as flying, fighting, or hunting (Paravidino et al., 2021).

One of the main functions of BAT is thermogenesis, which is caused by the oxidation of fatty acids. Multilocular cells form brown fat capable of regulating energy expenditure through adaptive thermogenesis via uncoupling protein (UCP) (Schneider & Borges, 2021). This protein is responsible for uncoupling oxidative phosphorylation from electron transport; therefore, part of the oxidation energy is dissipated through heat and not through conversion into adenosine triphosphate (Schneider & Borges, 2021). Recently, the literature revealed that irisin is a myokine considered a potential mediator of exercise-induced energy metabolism, and its secretion is known to promote the darkening of white adipose tissue cells, forming new beige or brown cells (Trettel et al., 2023). Regardless of the modality practiced, there is the secretion of irisin. However,

modalities with greater intensity are more efficient in increasing the secretion of this exercise (Vecchiato et al., 2023). In the present study, we detected an increase in the subscapular BAT area from time T1 to time T3, regardless of the group, thus suggesting a possible supercompensation process, as also found in the perirenal and perilumbar adipose tissues, and attribute these results to the methods indicated by Liu et al. (2020), Nikooie & Samaneh (2016) and Teich et al. (2017), already explained. Furthermore, the weight of the subscapular BAT was lower in the HIIT and CMIT when compared to the control group. Corroborating our findings, Teich et al. (2017) revealed that after 3 weeks of physical training (free running/24 hours) accompanied by caloric restriction, male Sprague-Dawley rats reduced the absolute and relative weight of adipose tissues (epididymal, perirenal, and inguinal), an effect not observed with the sedentary group. The authors also report that, after a week of detraining, the rats in the trained group significantly increased the weight of the tissues mentioned above. However, our study did not detect any objective possibility of overcompensation in tissue weights, which may have occurred because we did not induce obesity at the beginning of the analysis (Teich et al., 2017).

The effects of exercise on insulin sensitivity are acute and temporary, and for lasting benefits, constant physical activity is necessary (Dimenna & Arad, 2021). In this sense, Shakoor et al. (2023), after placing 20 adults male Wistar rats on a CMIT program (treadmill running, 5 days/week, 60 minutes/day, 25 m/min), divided into two groups (trained for 4 weeks and untrained for 8 weeks, and prepared for 8 weeks and unfit for 4 weeks), found that the improvements acquired with physical training were not maintained after the detraining periods. Furthermore, Teich et al. (2017) also found that after 3 weeks of CMIT, the rats significantly improved their insulin sensitivity and glucose tolerance. However, after a week of detraining, these values returned to baseline values and were worse than those before the beginning of the intervention, assuming a super compensatory effect. Our study found no difference between groups or periods in insulin sensitivity after 8 weeks of intervention; however, it revealed that the percentage of blood glucose decline was more significant in the HIIT group than the CG after a detraining period. It may have occurred due to increased irisin (a measure not evaluated in our study).

Another point that may help explain our results is the findings of the meta-analysis conducted by Jelleyman et al. (2015), in which the authors report that HIIT is more effective in improving insulin resistance measures compared to CMIT and CG without exercise. According to the authors of the meta-analysis, this superiority can be explained by two factors: interval and high intensity. In addition, it is recognized that muscle glycogen is not significantly affected after CMIT, unlike what was observed after HIIT. This fact may explain why HIIT can increase insulin sensitivity in a more significant way than CMIT (Jelleyman et al., 2015) since the reduction in muscle glycogen induced by training

is a crucial factor in the post-training improvement in insulin sensitivity (Bogardus et al., 1983), which may explain our findings.

Our study is not free from limitations, making us look at some results cautiously. One of the essential points is that lactate concentration during training was not analyzed, but we controlled the intensity of activity using the CMC test. Another critical point is that the animals were not induced to become obese before the interventions began. Furthermore, no prior selection of born-running rats was carried out (Gobatto, 2007), which resulted in difficulties in making the animals run during training interventions. However, this was an innovative study where different training methods were used to analyze the super-compensation of fatty tissues, which until now, to the researchers' knowledge, was not available in the literature.

## Conclusion

Our findings revealed that the intervention groups had lower adipose tissue weight than the control group. Furthermore, the perirenal and perilumbar adipose tissues underwent a process of super-compensation during the detraining period, as well as an increase in the area of adipocytes in the subscapular BAT from T1 to T2. There was superior insulin sensitivity in the HIIT group compared to the control group after the detraining period. Therefore, it is concluded that 8 weeks of both interventions (CMIT and HIIT) are efficient for controlling weight and adipose tissue area; however, this control is lost after 4 weeks of detraining in terms of adipocyte diameter, thus suggesting adipocyte hypertrophy. Furthermore, HIIT is more efficient in improving insulin sensitivity than CMIT. More studies are needed to understand the supercompensation of fatty tissues better.

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