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AN EARLY STARTED OVERLOAD SWIMMING PROTOCOL ATTENUATED OBESITY BUT ACTED AS ANXIOGENIC IN WESTERN DIET-FED FEMALE RATS

UM PROTOCOLO DE NATAÇÃO DE SOBRECARGA INICIADO PRECOCEMENTE ATENUOU A OBESIDADE, MAS AGIU COMO ANSIOGÊNICO EM RATAS ALIMENTADAS COM DIETA OCIDENTAL

UN PROTOCOLO DE NATACIÓN CON SOBRECARGA INICIADO TEMPRANAMENTE ATENUÓ LA OBESIDAD PERO ACTUÓ COMO ANSIOGÉNICO EN RATAS HEMBRAS ALIMENTADAS CON UNA DIETA OCCIDENTAL

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ABSTRACT
Aim: To evaluate the influence of an early overload swimming training (OST) on the development of obesity and anxiety-like behavior in female rats fed a cafeteria diet (Caf).

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Main methods: Sixty-six Female Wistar rats (21 days old, 11/group) were grouped as: Chow-fed sedentary (Ch-S), Chow-fed exercised once per week (Ch-E1), chow-fed exercised 5 days/week (Ch-E5), Caf-fed sedentary (Caf-S), Caf-fed exercised once per week (Caf-E1), and Caf-fed exercised 5 days/week (Caf-E5). OST was initiated the next day (45 min/ 5% body weight overload). The Caf diet started at 31 days of life, with the addition of hypercaloric foods to the commercial rat chow and water. Both Caf and OST continued for 60 days. Key findings: Caf-S rats displayed increased calorie intake, body weight, abdominal fat deposits, triglyceridemia, and abdominal circumference. This increase in obesity markers was prevented by OST, with being E5 more efficient than E1. Caf did not alter behavior, but Caf-E1 and Caf-E5 spent less time in the open arms of the elevated plus maze than the Caf-S group, indicating that the association of Caf + exercise was anxiogenic. As there were no alterations in the number of entrances in the enclosed arm and no differences in mobility in the open field test, this cannot be attributed to a motor effect. Significance: OST was effective in reducing obesity markers that were increased by the cafeteria diet but was also anxiogenic. OST in females may induce chronic stress that should be considered during the interpretation of its anti-obesity effect.

Keywords: Overload Swimming Training. Cafeteria Diet. Anxiety. Obesity. Female Rats.

RESUMO

Objetivo: Avaliar a influência de um treinamento de natação com sobrecarga precoce (OST) no desenvolvimento de obesidade e comportamento ansioso em ratas alimentadas com dieta de cafeteria (Caf). Principais métodos: Sessenta e seis ratas Wistar (21 dias de idade, 11/grupo) foram agrupadas como: sedentárias alimentadas com ração (Ch-S), alimentadas com ração exercitadas uma vez por semana (Ch-E1), alimentadas com ração exercitadas 5 dias/semana (Ch-E5), sedentárias alimentadas com Caf (Caf-S), alimentadas com Caf exercitadas uma vez por semana (Caf-E1) e alimentadas com Caf exercitadas 5 dias/semana (Caf-E5). O OST foi iniciado no dia seguinte (45 min/sobrecarga de 5% do peso corporal). A dieta Café começou aos 31 dias de vida, com a adição de alimentos hipercalóricos à ração comercial e à água. Tanto Caf quanto OST continuaram por 60 dias. Principais descobertas: Os ratos Caf-S apresentaram aumento na ingestão calórica, peso corporal, depósitos de gordura abdominal, trigliceridemia e circunferência abdominal. Esse aumento nos marcadores de obesidade foi prevenido pela OST, sendo E5 mais eficiente que E1. Caf não alterou o comportamento, mas Caf-E1 e Caf-E5 passaram menos tempo nos braços abertos do labirinto em cruz elevado do que o grupo Caf-S, indicando que a associação de Caf + exercício foi ansiogênica. Como não houve alterações no número de entradas no braço fechado e nenhuma diferença na mobilidade no teste de campo aberto, isso não pode ser atribuído a um efeito motor. Significância: A OST foi eficaz na redução dos marcadores de obesidade que foram aumentados pela dieta de cafeteria, mas também foi ansiogênica. A OST em fêmeas pode induzir estresse crônico que deve ser considerado durante a interpretação de seu efeito antiobesidade.

Palavras-chave: Treinamento de Natação com Sobrecarga. Dieta de Cafeteria. Ansiedade. Obesidade. Ratas Fêmeas.

RESUMEN

Objetivo: Evaluar la influencia del entrenamiento temprano de natación con sobrecarga (OST) en el desarrollo de obesidad y comportamiento ansioso en ratas alimentadas con una dieta de cafetería (Caf). Métodos principales: Sesenta y seis ratas Wistar (21 días de edad, 11/grupo) se agruparon de la siguiente manera: alimentadas con pienso sedentario (Ch-S), alimentadas con pienso y ejercitadas una vez a la semana (Ch-E1), alimentadas con pienso y ejercitadas 5 días/semana (Ch-E5), alimentadas con Caf sedentaria (Caf-S), alimentadas con Caf y ejercitadas una vez a la semana (Caf-E1) y alimentadas con Caf y ejercitadas 5 días/semana (Caf-E5). El OST se inició al día siguiente (45 min/sobrecarga del 5% del peso corporal). La dieta Café comenzó a los 31 días de edad, con la adición de alimentos ricos en calorías al pienso comercial y al agua. Tanto la Caf como el OST se continuaron durante 60 días. Hallazgos principales: Las ratas Caf-S mostraron un aumento de la ingesta calórica, el peso corporal, la deposición de grasa abdominal, la trigliceridemia y la circunferencia abdominal. Este aumento en los marcadores de obesidad fue prevenido por OST, siendo E5 más efectivo que E1. Caf no alteró el comportamiento, pero Caf-E1 y Caf-E5 pasaron menos tiempo en los brazos abiertos del laberinto elevado en cruz que el grupo Caf-S, lo que indica que la combinación Caf + ejercicio fue ansiogénica. Dado que no hubo cambios en el número de entradas en el brazo cerrado ni diferencias en la movilidad en la prueba de campo abierto, esto no puede atribuirse a un efecto motor. Significación: OST fue eficaz para reducir los marcadores de obesidad que aumentaron con la dieta de la cafetería, pero también fue ansiogénico. OST en hembras puede inducir estrés crónico, lo que debe considerarse al interpretar su efecto antiobesidad.

Palabras clave: Entrenamiento de Natación Sobrecargado. Dieta de Cafetería. Ansiedad. Obesidad. Ratas Hembras.

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1 INTRODUCTION

Obesity is characterized by the excessive accumulation of fat tissue and is an important risk factor for the development of many chronic diseases [1]. Obesity is linked to several inflammatory and degenerative conditions, such as cardiovascular disease, metabolic disruption, and behavioral alterations, due to the release of pro-inflammatory and oxidant cytokines by adipocytes and the consequent disruption of the hypothalamus-pituitary-adrenal axis [2,3].

Obesity can be induced by genetic predisposition, high caloric intake associated with low physical activity, environmental factors, or an association of all these factors [4]. Consumption of a diet based on junk food or hyperpalatable or even ultra-processed food leads to high calorie intake, since it induces less satiety than natural food, and their attractive taste and texture activate the reward systems in the brain [5,6,7]. Therefore, this dietary habit can induce calorie intake that is so high that even an intense exercise regime cannot counterbalance.

On the other hand, physical exercise increases calorie deficit and basal metabolic rate, which can halt the storage of fat in adipose tissue, working as a protector against obesity by increasing blood flow and insulin sensitivity, while reducing pro-inflammatory markers and oxidative stress [8]. Both mechanisms are known to induce neuroplasticity and improve behavioral and cognitive function. Additionally, physical exercise can act as an adjuvant for the treatment of depression and anxiety [9,10].

Despite the development of obesity in both men and women, there are differences in the mechanisms of adipose tissue gain and loss. Testosterone plays an important role in this difference because it is increased by exercise and acts as an antioxidant and inducer of neuroplasticity [11, 12]. Despite this, in preclinical research, especially using rodents, far more studies focusing on behavioral alterations caused by obesity are performed in males than in females [13].

There has been growing interest in treating rodents with industrialized hyperpalatable food as a model of hypercaloric diet-induced obesity [4]. Both cafeteria and Western diet models have high face validity and can induce obesity. Therefore, both have been used to study treatments to prevent the effects of obesity, such as physical exercise [3]. Additionally, a cafeteria diet can induce behavioral alterations [14,15].

Modelling physical exercise in rodents is challenging. Many protocols have been validated, mostly using a treadmill or swimming. However, there is a great variability of training protocol in the literature because the studies use different intensities, volumes, durations, and starting points (before, with, or after the diet) [12,16 - 20]. Thereby, it is not yet fully understood how different training protocols could have different effects on female rat health. Thus, it is still necessary to investigate, as different exercise regimes can influence obesity and behavioral alterations. Therefore, the objective of this study was to test the effectiveness of an early start (before the diet) of two exercise regimes, once a week versus five times a week, on the development of obesity and its associated behavioral alterations.

2 MATERIALS AND METHODS

2.1 SUBJECTS

Female Wistar rats (n= 66) from the Universidade Estadual de Ponta Grossa (UEPG) breeding stock were kept at 3 per cage in an animal house at (22 ± 2º C) under a 12-hour light-dark cycle (lights at 7:00 a.m.). All rats were 21 days-old (25-35 g) at the beginning of the experiment and were fed rodent Nuvilab CR1 chow (NUVITAL, Curitiba, Brazil) and water ad libitum throughout the experiment. All animal handling and procedures were performed according to ethical principles, and the project was approved by the UEPG Animal Use Ethics Commission (protocol no. 349197/2020).

Using the GPower 3.1 program, sample size was calculated based on the mean standard deviation of 20%, test power of 90%, level of significance (alpha) was 0.05, Cohen d of 1.5 as significant, based on anxious-like behavior in rats literature.

2.2 EXPERIMENTAL DESIGN

Weaning female Wistar rats (21 days old) were distributed into six groups (n=11): Chow-fed and sedentary (Ch-S); Chow-fed and exercised once per week (Ch-E1); Chow-fed and exercised five times per week (Ch-E5); Cafeteria Diet-fed and sedentary (Caf-S); Cafeteria Diet-fed and exercised once per week (Caf-E1); and Cafeteria Diet-fed and exercised five times per week (Caf-E5). The following day, Ch-E1, Ch-E5, Caf-E1, and Caf-E5 rats were subjected to an overload swimming training protocol, which lasted until the end of the experiment, while sedentary rats remained in their home cages. From the 31st day of life, rats from the Caf-S, Caf-E1, and Caf-E5 groups were fed a western diet in their home cages until the end of the experiment. All rats were weighed weekly. On the 90th day of life, they were subjected to the Elevated Plus Maze test and on the next day, to the Open Field test. One day later, all rats were weighed, their length from head to tail and circumference

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were measured, and they were sacrificed for fat tissue quantification and blood collection for biochemical analysis. No criteria for excluding animals were set. Group members were codified, and analysis were double-checked by blind members of the group to avoid confounders. The first author was responsible for tracking group allocation at the different stages of the experiment.

2.3 OVERLOAD SWIMMING TRAINING PROTOCOL

During the first week of the experimental period (22 days old), the rats in the exercise groups adapted to water and load attachment. They were kept in a tank (100 × 100 × 100 cm) containing shallow water at 32 ± 2 °C for 15 min/day, followed by a daily crescent load of 0–5% / BW attached to their chest. The load consisted of a lead pellet tied with Lastex thread. The following week, the water level was raised so that they could not touch the floor, and they were subjected to swimming sessions of 45 min/day, with a 5% BW load attached to the chest, once (Ch-E1 and Caf-E1) or five (Ch-E5 and Caf-E5) days a week, until the end of the experiment, based on Wang 2020 [21]. Training sessions were always conducted at 10:00 AM in the same tank and at the same water temperature.

2.4 OBESITY INDUCTION BY CAF

Caf was offered as described before [18]. It consisted of a group of ultra-processed, highly palatable, hypercaloric foods and degassed cola soda (Ice Cola, Várzea Grande, MT, BRA), distributed in three menus over the week and displaced to the rats on the cage grid. The food offering pattern was as follows: Monday- cheese snacks (Tick Titos Alimentos, Sertanópolis, PR, Brazil) and chocolate wafer cookies (Casaredo, São Lourenço do Oeste, SC, Brazil); Wednesday, marshmallow (Fini Comercializadora Ltda, Jundiaí, SP, Brazil), and cooked ham (Frimesa, Medianeira, PR, Brazil); Friday-bacon snacks and corn-starch cookies (both Tick Titos Alimentos, Sertanópolis, PR, Brazil). All products were purchased from local supermarkets and maintained at the same brands throughout the experiment. Weekly consumption was registered in grams and converted to kilocalories (Kcal).

2.5 BEHAVIORAL TESTS

2.5.1 Elevated Plus Maze test

Anxious-like behavior was measured on a 50 cm high wooden plus maze, which consisted of two 50 cm long open and two enclosed arms, with the latter having 30 cm high walls. The rats were left facing one of the enclosed arms to explore the apparatus for 5 minutes. The procedures were filmed for later computing the time spent and the number of entrances on each arm by an observer who was blind to the rat's group. The floor was cleaned with a 5% ethanol solution between tests to eliminate possible bias caused by odors left by previous rats.

2.5.2 Open field test (OFT)

Locomotion was evaluated on a square wooden arena (1 m^2) with the floor divided into 25 small squares. The rats were placed in the center of the open field for 5 min to allow free exploration. Two motor parameters were recorded and quantified: the number of squares crossed (locomotion) and rearing frequency. The floor was cleaned with a 5% ethanol solution between tests to eliminate possible bias caused by odors left by previous rats. This test was video-recorded, and the images were analyzed as described for the elevated plus maze.

2.5.3 Biometric data

All animals were weighed weekly from the 30^{th} day of life until the day of euthanasia, to obtain the body growth curve. Euthanasia was performed by decapitation after a food deprivation period of 12 h. A laparotomy was then performed for removal of retroperitoneal, mesenteric, and periovarian fat stocks of all animals, which were dissected and weighed. In addition to the comparison of each fat stock among the groups, total abdominal adiposity was calculated from the sum of the three fat stocks/final body weight × 100.

2.5.4 Plasma biochemical parameters

Non-hemolyzed blood samples were collected in glass tubes containing EDTA and KFl and immediately centrifuged at 5000 × g to separate the plasma, which was stored at -20º C until analysis. On the test day, glucose, total cholesterol, and triglyceride concentrations were measured using colorimetric kits (Laborclin, Pinhais, Brazil) following recommendations from the manufacturer, as described elsewhere, and are presented as mg/dl [19].

2.5.5 Statistical analysis

Normality of the data was confirmed using the Kolmogorov-Smirnov test. Data are expressed as mean ± standard error (SE), analyzed by repeated measures ANOVA, followed by Bonferroni post-test (curves) or Student's t-test (bars), and considered significant when $p \leq 0.05$. All analyses were performed using the statistical software GraphPad Prism 5.01

3 RESULTS

3.1 BODY COMPOSITION

The weekly calorie intake of all groups is shown in Figure 1. Figure 1A shows all groups (without significantly different markers), while figures 1B, C, and D show selected groups to facilitate visualization and analysis. Caf-S showed a higher calorie consumption than Ch-S ($p< 0.05$), which was significantly higher from the 3rd week until the end of the experiment (fig. 1B). Figure 1C also shows that Caf-E1 and Caf-E5 consumed significantly more calories than Ch-S in weeks 3rd and the 4th week on ($p< 0.05$), respectively. From the 6th week onwards, Caf-E5 calorie intake was even higher than that of the Caf-E1 group ($p< 0.05$). In addition, Caf-E1 and Caf-E5 presented higher calorie intake than Ch-E1 and Ch-E5 from the 3rd week ($p< 0.05$), as can be seen in fig 2D.

Caf increased the gain of body weight (BW). As shown in figure 2, Caf-S animals were heavier than Ch-S animals from the 8th week of the diet ($p< 0.001$). Fig 2B represents the same data as in Fig 2A, but starting from the 7th week of the experiment, to facilitate visualization. The BW of Ch-E1 and Ch-E5 did not differ from that of Ch-S, indicating that exercise alone did not interfere with BW. However, exercise attenuated the weight gain of the Caf groups, as Caf-E1 and Caf-E5 BW curves were not different from those of Ch-S and were almost significantly different from those of Caf-S: Caf-S × Caf-E1 ($p = 0.068$) and Caf-S × Caf-E5 ($p = 0.056$).

The rats in the Caf group also had heavier fat stocks. Figure 3 shows that the mesenteric fat of Caf-S rats (3A, $p< 0.01$), periovarian (3B, $p< 0.01$), and retroperitoneal (3C, $p< 0.01$) fat stocks at least doubled the mass measured in the Ch-S group. The same pattern was observed in the calculation of the total abdominal adiposity (3D, $p< 0.01$). Caf-E1 and Caf-E5 mesenteric fat did not differ from the Caf-S group (3A), but figures 3B and C indicate that Caf-E5 periovarian and peritoneal fat stocks were smaller than those in the Caf-S group ($p< 0.05$), which was also the case for the sum of the three fat stocks (3D). In none of these cases, Caf-E1 was significantly different from Caf-S ($p> 0.05$).

As can be seen in figure 4, although glucose and cholesterol blood concentrations were not different among the groups (4B and C), the Caf-S group presented higher triglyceride (TG) levels than the Ch-S rats ($p< 0.01$, 4A). Exercise was able to lower

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triglyceride concentrations ($p<0.05$) with higher efficiency in the 5-day protocol, since Caf-E1 concentration was not different from Ch-S or Caf-S, but Caf-E5 was significantly different from Caf-S ($p< 0.01$) and similar to Ch-S.

3.2 BEHAVIORAL TESTS

In the elevated plus maze, there was no difference between Caf-S and Ch-S in the time spent either on the open arms (Fig 5A) or on the enclosed arms (Fig 5B). Exercise did not alter the behavior of the chow-fed groups ($p > 0.05$). On the other hand, Fig 5A shows that Caf-E1 and Caf-E5 on the open arm were not different from Ch-S, but significantly lower than Caf-S (exercise factor $F(2, 61)= 9.6$, $p< 0.01$). When considering the time spent in the enclosed arms (Fig 5B), an exercise effect was detected ($F(2, 62)=3,15$, $p< 0.05$), but no difference was found among the groups using the post hoc test. These differences cannot be attributed to an alteration in motility by obesity or exercise protocol, since there were no differences among groups in the number of entrances on the enclosed arm (Fig 5C), along with no differences in the number of lines crossed or in the frequency of rearing behavior in the open field test ($p> 0,05$ – data not shown).

4 DISCUSSION

In this study, we showed that an early start of physical exercise was efficient in preventing fat stocks and triglyceride levels caused by a cafeteria diet in female rats. However, high-intensity exercise induces an anxiogenic stimulus.

First, the cafeteria diet was effective in causing obesity in female rats. Our results indicate that it increased body weight, abdominal circumference, fat storage, and TG concentration in the blood, all markers related to obesity. WD or cafeteria diets have been widely used to induce obesity in rats, both in studies using male and female animals. [4, 14, 19].

Because of the lack of difference in blood glucose concentration, it was not possible to determine if a condition similar to diabetes was established. It is possible that metabolic syndrome was under development, with increased insulin secretion controlling glycemia and promoting cholesterol storage in the liver or adipose tissue, which would reduce blood concentrations, as seen in other studies [19]. This hypothesis was not tested in this study, but other authors have shown differences in the glycemic curve after 60 days of WD in rats, with no alteration in fasting glucose [22,23].

Increased weight, body fat, and blood TG were related to a higher caloric intake associated with a preference for a highly palatable and hypercaloric diet rich in sugars and fat. It has been reported that the Caf diet has a significant hedonic value that increases consumption, and these more attractive kinds of food are highly energetic and induce voluntary hyperphagia, with a craving behavior similar to that of drug addiction, which could be considered evolutionary advantageous regarding energy efficiency, but can cause health problems with modern ultra-processed food [24].

The results presented in this work indicate that Caf diet/obesity does not cause anxiety. Neither time spent on plus maze arms nor exploration of the open field differed between Caf-S and Ch-S rats. Other studies have indicated the development of depressive or anxiety-like behaviors with Caf or WD [2, 3, 4, 7]. However, the outcomes varied. Alonso-Caraballo and coworkers (2019) report that only obesity-prone rats become anxious with a Caf diet, but not outbred rats, both male and female [25].

The heterogeneity of the Caf and/or WD diets may pose another difficulty when comparing the results from different studies. The choice or availability of products can vary from one region to another, changing the composition of carbohydrates, fats, and proteins added to the regular commercial chow. In addition, rats can develop different preferences for one item over another, making it more difficult to precisely determine the exact composition of their diet.

Exercise did not reduce the body weight of the chow-fed groups but was efficient in attenuating the increase in obesity markers caused by Caf. Caf-E1 and Caf-E5 final body weights were not different from those of Caf-S but were also not different from those of Ch-S, indicating a mild effect. It can be assumed that they did not lose much weight because they developed lean muscular mass (not quantified). However, part of this weight could be attributed to higher water retention caused by the Caf diet (not quantified).

This hypothesis is supported by the fact that, when fat stocks are compared, it can be seen that exercise was efficient in reducing all three fat stocks in a volume-dependent fashion. All abdominal fat stock accumulations caused by the Caf diet were prevented by early exercise, and both groups, Caf-E1 and Caf-E5, were not different from Ch-S or Caf-S. Except for mesenteric fat, the frequency of exercising 5 days per week was more efficient in preventing abdominal fat accumulation than the once-a-week exercise protocol. This result was expected because the Caf-E5 protocol provided a higher exercise volume than Caf-E1, increasing energy expenditure (TG catabolism) and avoiding fat accumulation. This reduction

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in both fat accumulation and blood TG confirms the health benefits of exercise, since it reduces the risk of cardiovascular disease [4, 32].

Interestingly, both Caf-E1 and Caf-E5 ingested more calories than Caf-S rats throughout the experiment. It can be assumed that more calories were expended with the exercise protocols, leading to more intense hunger, as there is a need for more calorie consumption.

Obesity is often referred to as a condition of low-grade chronic systemic inflammation, which is directly linked to the development of depressive behavior [26, 27]. Once adipose tissue secretes pro-inflammatory cytokines, obesity is related to a higher concentration of these mediators in the blood. Increased levels of IL-1β, TNF-α, and IL-6 are associated with neuroinflammatory conditions and have been observed in neuropsychiatric patients [28].

These elevated cytokines may directly and indirectly impair serotoninergic transmission in the limbic areas, negatively modulating mood. Animal models of inflammatory depression have been developed based on this relationship [29]. Some cytokines are reported to acutely increase serotoninergic synapse efficacy [30], but in the long term, they reduce the expression of serotonin (5-HT) receptors and stimulate the enzyme indoleamine 2,3-dioxygenase, which reduces tryptophan availability for 5-HT synthesis [30, 31]. In addition, TNF-α and IL-1β may increase presynaptic 5-HT reuptake by either the induction of 5-HT transporter expression or their activity [30,31].

Although beneficial for obesity, the overload exercise protocol seemed to act as a stress-inducing stimulus in female rats in this study. Obesity or exercise was not enough to change behavior but caused anxiety-like behavior when combined. This was concluded by observing a reduction in time spent in the open arm of the Elevated Plus Maze caused by this association, which is an indication of anxiety-like behavior [33].

Da Costa Estrela and coworkers [31] showed a lack of behavioral effects associated with obesity, but when obesity was associated with a stress stimulus, it aggravated metabolic syndrome and caused behavioral changes [31]. In our study, a higher intensity of training could have worked as the stress stimulus necessary to trigger a higher inflammatory state or a more significant release of corticosterone, inducing anxiety-like behavior. In other words, obesity may render animals more sensitive to the behavioral manifestations of stress.

It was expected that exercise would work as anxiolytic as seen elsewhere [9, 10, 15, 34]. Eight weeks of high-intensity interval training (HIIT) reduced anxiety and improved memory in elderly male rats [35]. In this study, the longer length of each swimming session

(45 min), associated with the overload (5% BW), increased the intensity, which may have been stressful for female rats. This higher anxiety-like behavior may also be responsible for the increase in calorie consumption, as discussed above.

Overloaded swimming training in rats is a physical activity model widely used in preclinical experimentation with the aim of studying its effects on animal metabolism. However, our results indicate that this protocol may be stressful for animals, altering their behavior. This alteration could be linked to the activation of the sympathetic nervous system, leading to the release of noradrenalin or even increased activation of the hypothalamus-pituitary axis [9]. Therefore, the anxious state induced by training could modulate metabolism differently from the mild stress induced by physical exercise itself.

Some studies demonstrate that the effects of acute and chronic exercise on anxiety are uncertain for humans (Carraça et al., 2021; Connor et al., 2023; Stonerock, Gupta, Blumenthal, 2024). Extrapolation of the results of this study to humans should be viewed with caution due to the protocol used. Therefore, new studies should investigate the effects of exercise for the treatment of anxiety in obesity.

In conclusion, despite being beneficial for attenuating the rise in obesity markers, early high-intensity exercise has been shown to be stressful and anxiogenic in female rats. This result should be considered when designing experiments to evaluate the effects of different exercise protocols on rats. It also needs to be addressed whether males would respond in the same way.

The authors declare that they have no conflicts of interest.

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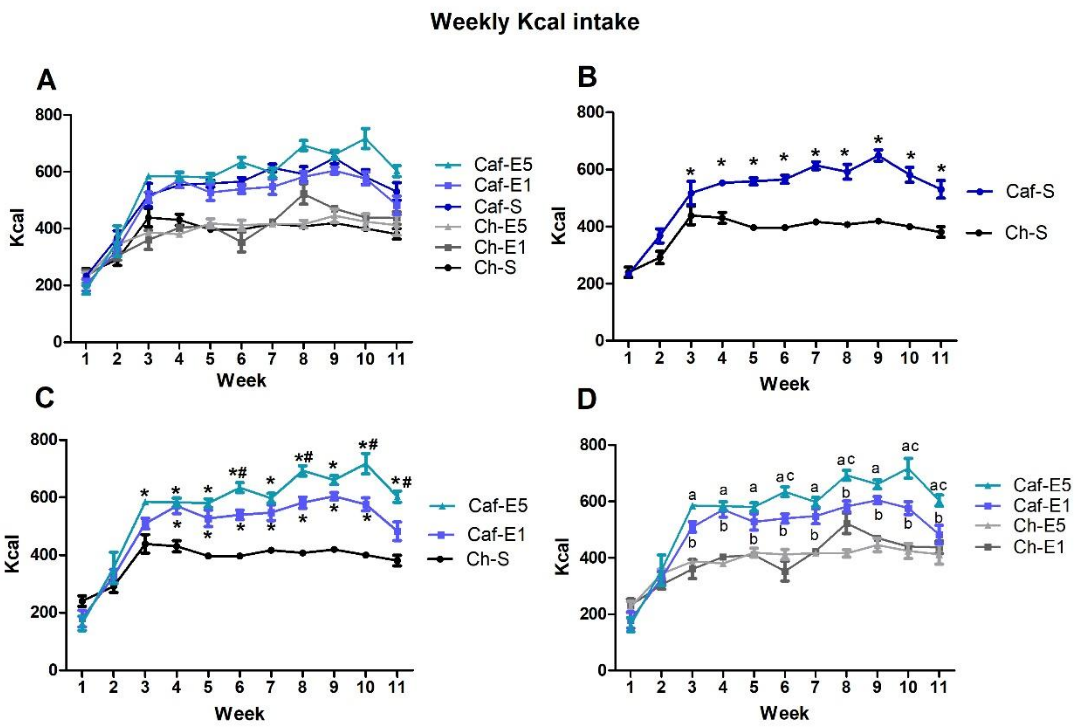
ANEX I - FIGURES

Figure 1

Weekly Kcal Intake



Data represent means ± SEM of weekly calorie intake per group along the experiment. The graph was divided to facilitate observation. A) all groups; B) sedentary groups; C) Caf-exercised groups compared to Chow-sedentary (control) groups; D) exercised groups. * Indicates a significant difference ($p < 0.05$) from the Ch-S group, # indicates a significant difference from the Caf-S group; a: $p < 0.05$, compared to the Ch-E5 group; b: $p < 0.05$, compared to the Ch-E1 group (n=11 per group; two-way ANOVA followed by Tukey's post-test).

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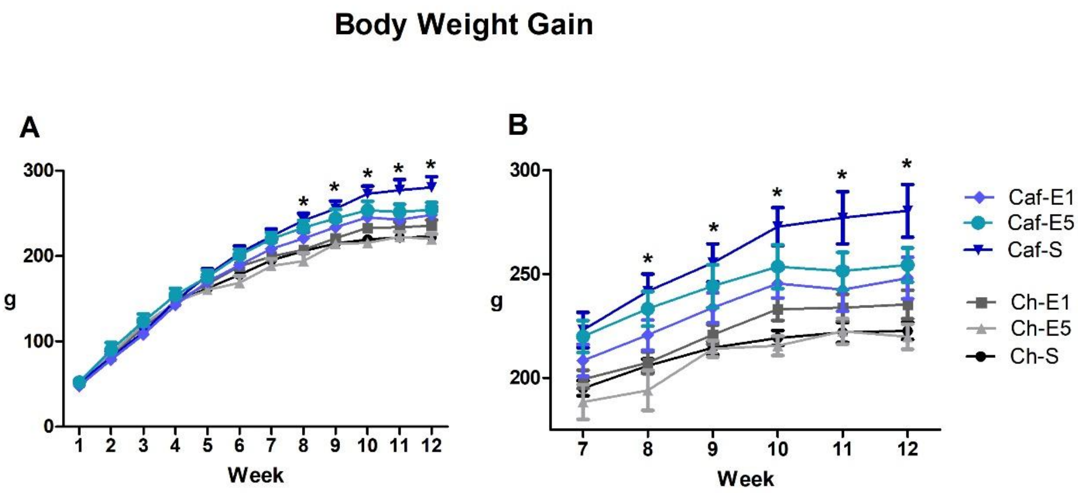
Figure 2

Body Weight Gain



The Caf diet increased the body weight gain, which was partially prevented by exercise. Data represent means ± SEM of body weight gain over the weeks of treatment with cafeteria diet and/or overload swimming exercise (A). B) represents the same curve as in A, but from the 7th week on for better visualization. * Indicates a significant difference ($p < 0.05$) from the Ch-S group (n=11 per group; repeated-measures ANOVA followed by Tukey's post-test).

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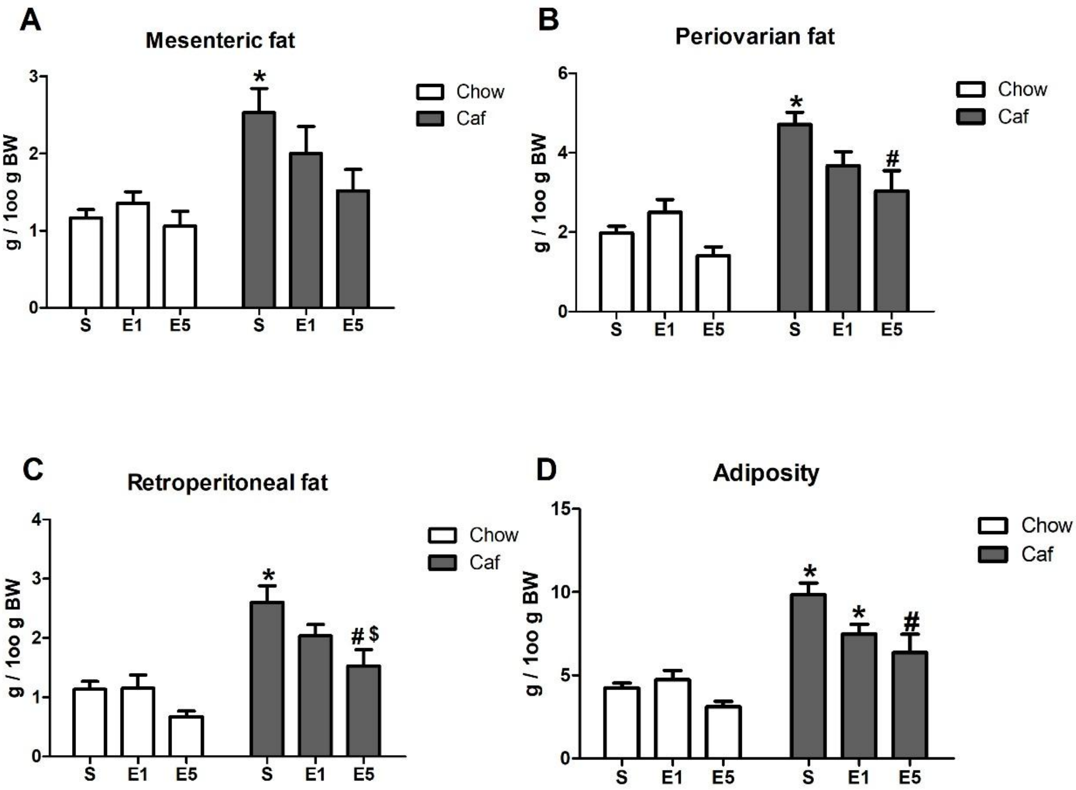
Figure 3



The Caf diet increased fat deposits, which were prevented by exercise. Data represent mean ± SEM of mesenteric (A), periovarian (B), and retroperitoneal (C) fat depots, measured at the end of the experiment. D) represents the sum of all the three fat deposits. * Indicates significant difference ($p < 0.05$) from the Ch-S group, # indicates significant difference from the Caf-S group, $ indicates significant difference from the Caf-E5 group (n=11 per group; two-way ANOVA followed by Tukey's post-test).

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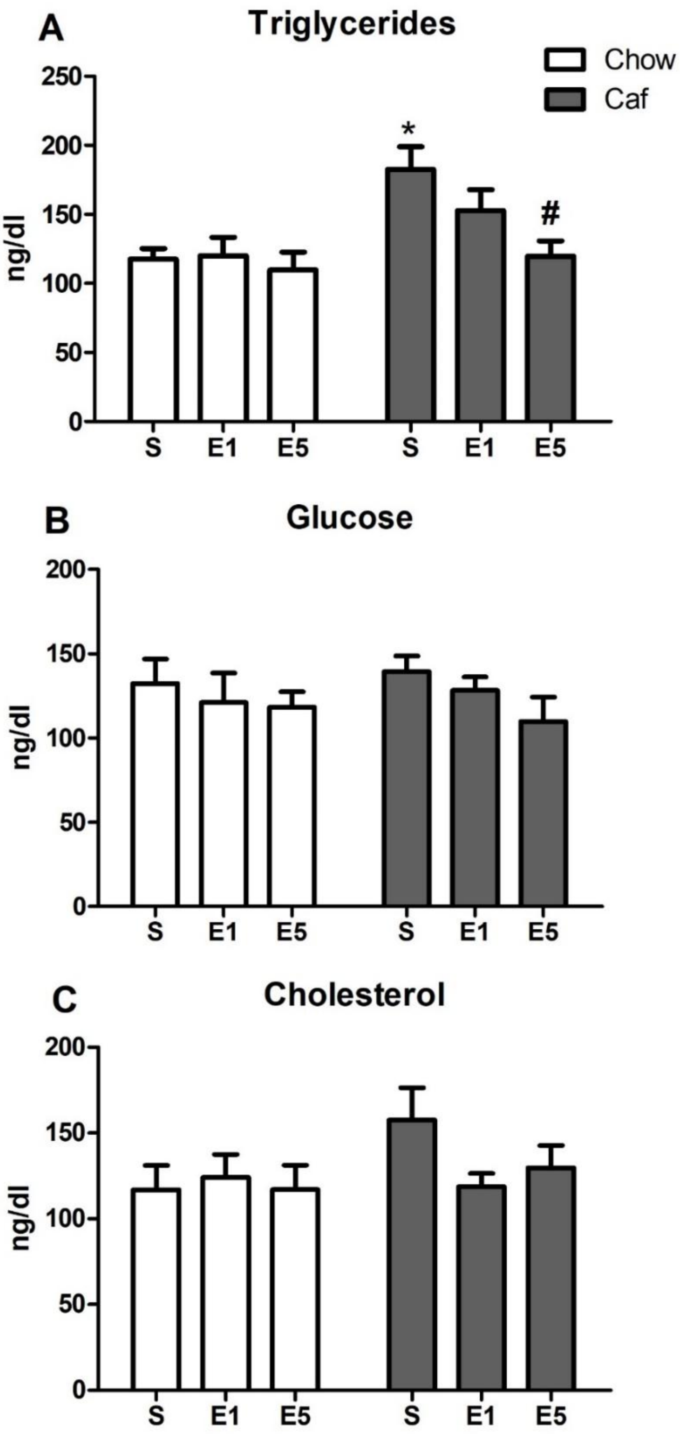
Figure 4



The Caf diet increased the blood triglyceride concentration, which was prevented by 5 days per week of exercise. Data represent mean ± SEM of blood concentrations of triglycerides (A), glucose (B), and cholesterol (C), measured at the end of the experiment. *

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Indicates a significant difference (p< 0.05) from the Ch-S group, # indicates a significant difference from the Caf-S group (n=11 per group; two-way ANOVA followed by Tukey's post-test).

Figure 5

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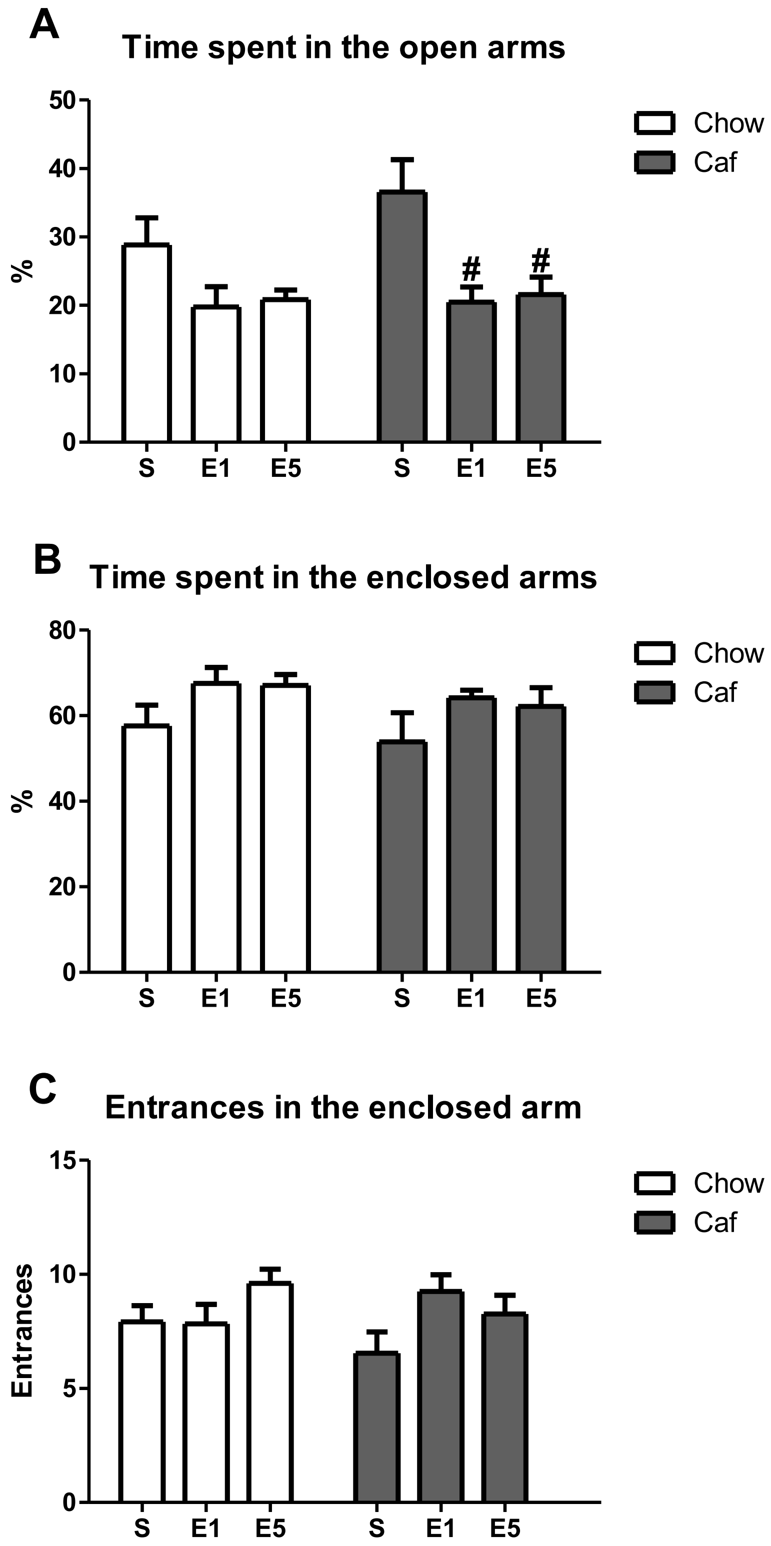

Anxious-like behavior on the Elevated Plus Maze test. Data represent mean ± SEM of (A) percentage of time spent in the open arms, (B) percentage of time spent in the enclosed arms, and (C) total number of entrances in the enclosed arm. #Indicates a significant

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difference from the Caf-S group (n=11 per group; two-way ANOVA followed by Tukey's post-test).