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Original Article

EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING ON FOOD INTAKE, RETROPERITONEAL ADIPOSE TISSUE, AND LIPID PROFILE IN WISTAR RATS: INTERACTIONS WITH AGE AND DIET COMPOSITION

EFEITOS DO TREINAMENTO INTERVALADO DE ALTA INTENSIDADE NA INGESTÃO ALIMENTAR, NO TECIDO ADIPOSO RETROPERITONEAL E NO PERFIL LIPÍDICO DE RATOS WISTAR: INTERAÇÕES COM A IDADE E A COMPOSIÇÃO DA DIETA

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RESUMO
A relação entre dieta, exercício e saúde é amplamente estudada devido ao impacto no equilíbrio metabólico e na prevenção de doenças crônicas. Dieta rica em gordura (HFD) está associada à obesidade, enquanto o treinamento intervalado de alta intensidade (HIIT) é conhecido por melhorar a composição corporal. A idade adiciona complexidade em resposta à dieta e ao exercício devido a mudanças fisiológicas ao longo do tempo. O objetivo deste estudo foi investigar os efeitos do HIIT na ingestão alimentar, tecido adiposo retroperitoneal e perfil lipídico em ratos Wistar. Os ratos foram divididos em grupos sedentários e treinados, com monitoramento semanal da ingestão de água e alimentos. Após 8 semanas de HIIT, os animais foram eutanasiados para análise de lipídios sanguíneos e gordura retroperitoneal. Animais mais velhos apresentaram reduções significativas na ingestão alimentar durante o HIIT (p<0.0001). Embora a massa corporal tenha aumentado com a idade (p<0.0001), o HIIT reduziu a massa corporal e a gordura retroperitoneal (p<0.0001) e reduziu o colesterol total e os triglicerídeos em animais adultos e de meia-idade (p<0.0001). O HIIT modula efetivamente a ingestão alimentar, adiposidade e metabolismo lipídico, com efeitos influenciados pela idade e pela dieta, enfatizando a importância de intervenções de exercícios personalizadas ao longo da vida.
Palavras-chave: HIIT; envelhecimento; dieta rica em gordura; lipídeos, composição corporal.

ABSTRACT
The relationship between diet, exercise, and health is widely studied due to its impact on metabolic balance and the prevention of chronic diseases. A high-fat diet (HFD) is associated with obesity, while high-intensity interval training (HIIT) is known to improve body composition. Age adds complexity to the response to diet and exercise due to physiological changes over time. The aim of this study was to investigate the effects of HIIT on food intake, retroperitoneal adipose tissue, and lipid profile in Wistar rats. The rats were divided into sedentary and trained groups, with weekly monitoring of food and water intake. After 8 weeks of HIIT, the animals were euthanized for blood lipid and retroperitoneal fat analysis. Older animals showed significant reductions in food intake during HIIT (p<0.0001). Although body mass increased with age (p<0.0001), HIIT reduced body mass and retroperitoneal fat (p<0.0001), and decreased total cholesterol and triglycerides in adult and middle-aged animals (p<0.0001). HIIT effectively modulates food intake, adiposity, and lipid metabolism, with effects influenced by age and diet, highlighting the importance of personalized exercise interventions throughout life.
Keywords: HIIT; aging; high-fat diet; lipids; body composition.

Introduction

Physical exercise is a key strategy in the prevention and treatment of obesity, contributing to energy balance, improved metabolic health, and reduced adiposity. Among the various exercise modalities, high-intensity interval training (HIIT) has gained prominence due to its time efficiency and potent physiological effects. Additionally, the relationship between diet and health has been largely investigated over the years, with substantial evidence pointing to the crucial role of diet and physical exercise in maintaining metabolic balance and preventing chronic diseases[1]. In addition, the growing increase in metabolic syndrome has led researchers to further explore the effects of diet in different contexts, including under the influence of age[2].

This is because the aging process brings with it systemic physiological changes that can influence how the body responds to different dietary patterns[3].

Additionally, overweight and obesity are associated with changes in plasma lipid levels, with an increase in the plasma concentration of low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG)[4]. Again, age represents an aggravating factor for the imbalance in cholesterol (COL) and TG homeostasis, increasing plasma levels by around 40% from 20 to 60 years old[5].

On the other hand, physical exercise has been largely used as an efficient therapy in changes associated with overweight, obesity and comorbidities associated with aging. Among the different types of exercises, HIIT training has been practiced as a different strategy of physical exercise that seeks to improve physical conditioning and body composition. HIIT consists of short bursts of intense activity alternated with periods of recovery, and has been shown to improve insulin sensitivity, lipid profile, and body composition. Its effectiveness in reducing visceral fat and promoting weight loss makes it a promising intervention for individuals with obesity, and can be more effective than Moderate Intensity Training (MIT) in several parameters, both in human beings and animal models[6-9].

In recent years, many researchers have been investigating the influence of several types of exercise on food consumption in humans and animal models. Foright et al.[10], for instance, studied this relationship during forced running. Tibana et al.[11] and Bloomer et al.[12] analyzed the influence of resistance exercises, while Quintanilha et al.[13] and Leigh et al.[14] assessed moderate intensity exercises. The effects of HIIT were also researched by Pekala et al.[15], Mendes et al.[16] and Arbus et al.[17].

Likewise, the interference of age on food consumption has also been investigated[3,18], however, the relationship between food consumption, exercise and age is scarce. Thus, although the literature has elucidated the effects of HFD in rodents, there is still a gap in understanding how these effects manifest at different ages, especially when combined with high-intensity exercise. The use of animal models is crucial to deepen this understanding, providing valuable information for scientific research with such models. In this context, this study aimed to investigate the effects of high-intensity interval training on food intake, retroperitoneal adipose tissue, and lipid profile in Wistar rats, considering interactions with age and diet composition.

Methods

Animals and experimental design

This study used adult (7and 9 months old), middle-aged (12 months old) and elderly (18 months old) male Wistar rats obtained from the Animal Facility of the State University of Maringá (UEM) and kept in the Animal Facility of the Department of Morphological Sciences, under controlled temperature conditions and with free access to water and rodent diet (Nuvilab®). All experimental procedures were approved by the Animal Use Ethics Committee (CEUA/UEM), under the protocol no. 5230050620.

Obesity was induced by offering a high-fat diet (HFD) for 16 weeks (Table 1), comprising eight weeks prior to and eight weeks concomitant with HIIT. The experiment lasted four months for all ages and began four months before the final age of each group. After eight weeks of inducing obesity, the rats were randomized and divided into 16 groups (n=7) according to their respective ages: T-SD (trained receiving standard diet), S-SD (sedentary receiving standard diet), T-HFD (trained receiving HFD) and S-HFD (sedentary receiving HFD), each group consisted of rats aged 7, 9, 12, and 18 months.

Table 1. Composition of the standard chow (Nuvilab®) and the high-fat diet (HFD)

Ingredients (grams)	**AIN-93M – Standard feed (1000 g)**	**High-fat (35% lard)**
Starch (q.s.)	465.7	115.5
Casein	140.0	200.0
Corn starch	155.0	132.0
Sucrose	100.0	100.0
Soybean oil	40.0	40.0
Lard	**0.0**	**312.0**
Microcrystalline cellulose	50.0	50.0
Mineral mix	35.0	35.0
Vitamin mix	10.0	10.0
L-Cystine	1.8	3.0
Choline bitartrate	2.5	2.5
Total sum (g)	1000.0	1000.0

Note: AIN-93M: American Institute of Nutrition (AIN) formulation, designed to provide nutrients at concentrations sufficient only to maintain populations of rats or mice.
Source: The authors.

Treadmill physical training protocol

All treadmill procedures were performed using a rodent-specific motorized treadmill (Panlab, Harvard Apparatus®) equipped with electrical shock stimuli (between 0.2-0.4 milliampere) to encourage running. After a 15-day adaptation period, the animals were submitted to a maximal running capacity test, which consisted of a 5-minute warm-up at 0.08 m/s. Following 1 minute of passive recovery, the animals underwent an incremental exercise protocol, with speed increased by 0.08 m/s every 4 minutes. Exhaustion was defined as the point at which the animal refused to continue running, even after tactile stimulation using a cone-shaped rolled newspaper applied to its back. This test was repeated after 4 weeks to adjust the training speed, and again at the end of the intervention.

The animals in the T-SD and T-HFD groups were then subjected to a high-intensity interval training (HIIT) protocol on the same treadmill. The protocol consisted of a 5-minute warm-up at 40% of the maximum running capacity, followed by six 4-minute bouts at 85–90% of maximum capacity, interspersed with 3-minute recovery periods at 50–60% of maximum capacity. Training sessions were conducted in the evening (6:00 p.m.), three times per week, totaling 24 sessions[19]

Food and water consumption and body mass monitoring

Food and water consumption were monitored every two days to calculate weekly average for the group throughout the experimental period. Quantities of food (SD e HFD) were measured using a digital scale (Filizola BP3), and the consumption was calculated as the difference between the amount given and the amount left in the cage. Similarly, water consumption was measured using a graduated beaker (NALGON de 1000 ml), calculating the difference between water supplied and water remaining in the bottle. In addition, the body mass of each animal was assessed weekly during the first eight weeks of obesity induction and during the training period.

Euthanasia, blood collection and biochemical analyses

Blood samples were collected from the inferior cava vein at the time of euthanasia, where the animals were anesthetized (Ketamine-Xylazine; 3mg + 0.6mg/100g of body weight) and thoracotomized. For TG and COL, we used commercial kits (Labtests; Gold Analisa, Brazil), with data expressed in mg/Dl[20]. In addition, at the moment of euthanasia, retroperitoneal fat and the liver were removed, dissected and weighed on an analytical balance (Shimadzu®) to obtain the total mass (g) and the relative mass (organ mass/final body mass*100, data expressed in %).

Statistical analysis

The data distribution and statistical tests were carried out using the GraphPad Prism 8® program. The area under the curve (AUC) analysis was used to evaluate food and water consumption during the experimental period. In addition, the analysis of variance ANOVA Two or Three-way was applied for interactions between the variables Age, Training and Diet. The Bonferroni post-test was used to compare the groups when necessary. In addition, to quantify the size of the effects identified by ANOVA, effect size measures in percentages (ETA squared) were presented. The significance criterion established was $p \leq 0.05$.

Results

AUC of food consumption

During the period of obesity induction, meaningful differences were observed for both age ($p<0.0001$) and diet ($p<0.0001$). In the SD groups, in addition to the differences between ages, there was a lower AUC of food at 18 months ($p<0.0001$). Additionally, there was a meaningful difference between the types of diet, where HFD groups had a lower AUC for food consumption compared to the SD groups ($p<0.0001$) (Figure 1B). Data presented in Figure 1E show that HIIT reduced food consumption at 12 and 18 months in animals fed a standard diet, and at all ages in HFD-fed animals ($p<0.0001$). In addition, age was the main factor associated with reduced consumption, especially at 18 months ($p<0.0001$).

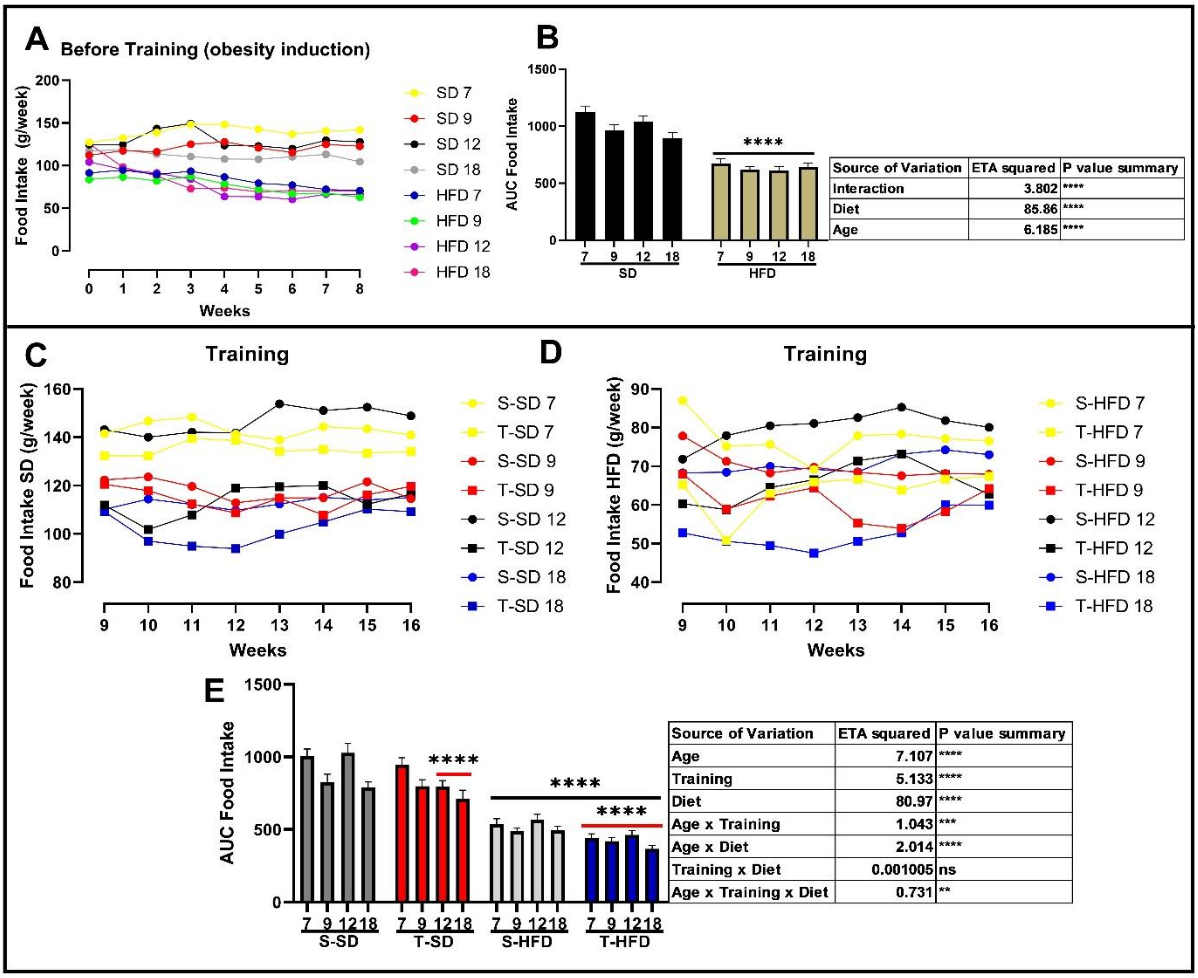


Figure 1. Weekly average food consumption (g) before the training period **(A).** Mean±SE graph graph of AUC of food consumption before the training period **(B).** Weekly average food consumption (g) during training in the SD **(C)** and HFD groups **(D).** Mean±SE graph of AUC of food consumption during training **(E)**. Black bars: significant difference for the effect of diet ($p<0.0001$); red bars: significant difference related to training ($p<0.0001$).

AUC of water consumption

In the period of obesity induction, besides a gradual reduction noticed in the water consumption because of age ($p<0.0001$), HFD exercised an additional reducing factor in this consumption in comparison with the SD groups ($p<0.0001$) (Figure 2B). During the training period, both HFD and age continued to represent a reduction factor in water consumption ($p<0.0001$). On the other hand, HIIT reduced consumption only at 12 and 18 months of animals fed standard diet, and at 7 months of animals fed HFD (Age x Training, $p=0.0012$) (Figure 2E).

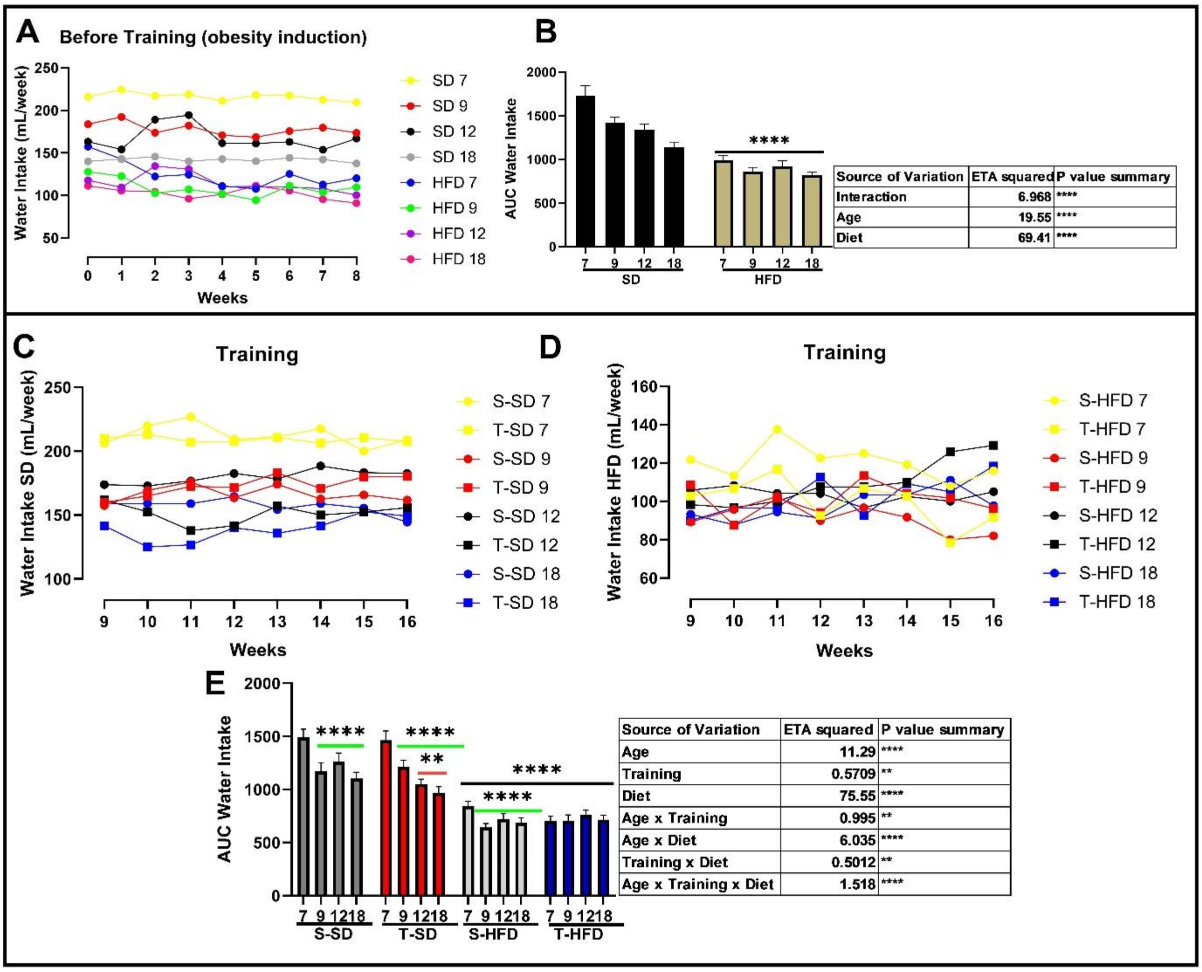


Figure 2. A: Weekly average water consumption (ml) before the training period **(A).** Mean±SE graph graph of AUC of water consumption before the training period **(B).** Weekly average water consumption (ml) during training in SD **(C)** and HFD **(D)** groups. Mean±SE graph of AUC of water consumption during training **(E)**. Black bars: significant difference for the effect of diet ($p<0.0001$); red bars: significant difference related to training ($p<0.005$) and green bars: significant difference for the effect of age ($p<0.0001$).

AUC of body mass

In the period of obesity induction there was a gradual rise of AUC of body mass, both in SD groups and HFD groups due to age ($p<0.0001$; Figure 3B). In addition, HFD groups showed higher AUC of body mass when compared to SD groups ($p<0.0001$; Figure 3B). On the other hand, in the training period, despite both age and HFD exercise a rise in body mass ($p<0.0001$), HIIT was effective in reducing this parameter ($p<0.0001$; Figure 3E). Nevertheless, the T-HFD groups showed a higher AUC compared to the T-SD groups (Training x diet; $p<0.05$).

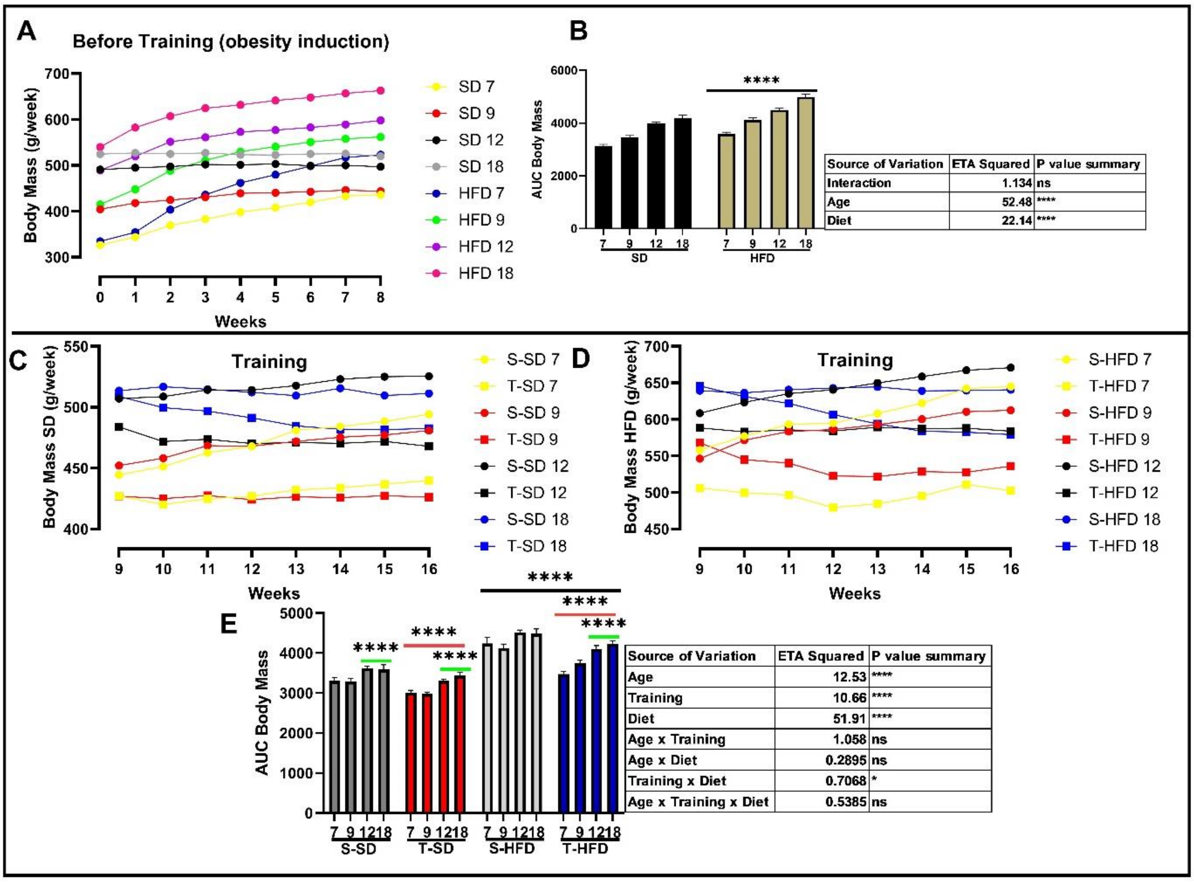


Figure 3. Weekly average of body mass (g) before the training period **(A).** Mean±SE graph graph of AUC of body mass before the training period **(B).** Weekly average of body mass (g) during training in SD **(C)** and HFD **(D)** groups. Mean±SE graph of AUC of body mass during training **(E)**. Black bars: significant difference for the effect of diet (p<0.0001); red bars: significant difference related to training (p<0.0001) and green bars: significant difference for the effect of age (p<0.0001).

Source: The authors.

Mass of retroperitoneal adipose tissue and liver

Results for retroperitoneal fat (Figure 4A) show that although the mass of this fat increased with diet (p<0.0001), and the 8-week HIIT program was effective in reducing it (p<0.0001). However, the T-HFD groups have greater fat mass than the T-SD groups (Training x Diet, p<0.0001). Regarding age, the 12- and 18-month-old animals in the trained groups also had higher fat mass, regardless of diet (Age x Training, p=0.0017). On the other hand, in the sedentary groups, this fat mass was higher in the 7- and 12-month-old animals compared to the 12- and 18-month-old animals, regardless of the diet offered (Age x Diet, p=0.0009).

Regarding liver mass (Figure 4C), HIIT was only effective in reducing it in the T-HFD groups (Training x Diet, p<0.0001). In addition, besides the increase with diet in S-HFD groups (p<0.0001), 12- and 18-month-old animals showed greater liver mass (Age x diet, p<0.0001). Different results were found for the relative mass of this organ (Figure 4D), mainly in relation to diet, where HFD consumption reduced this parameter (p<0.0001). However, HIIT promoted an increase in the relative mass of the liver in the SD groups (Training x Diet, p<0.0001).

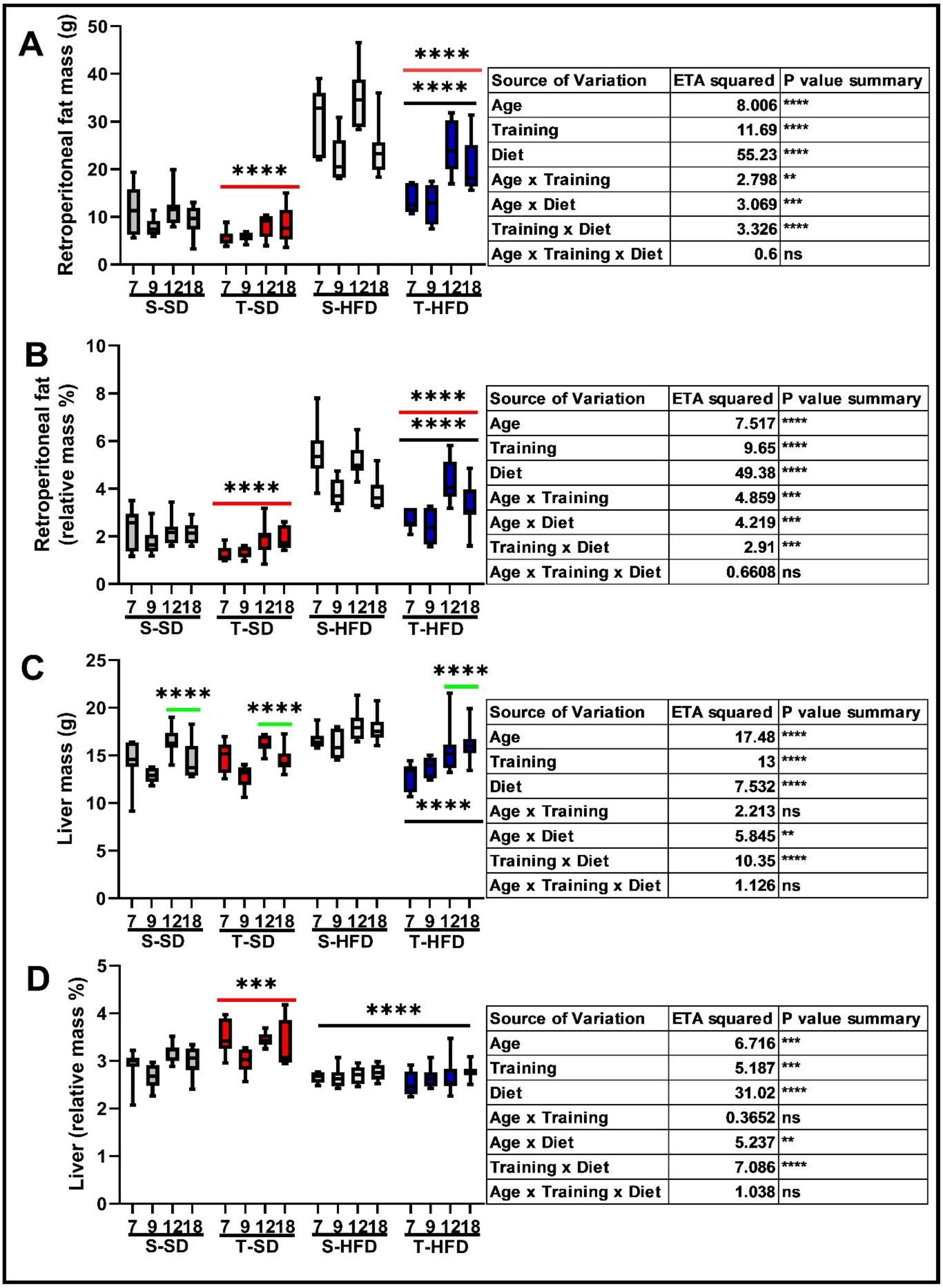
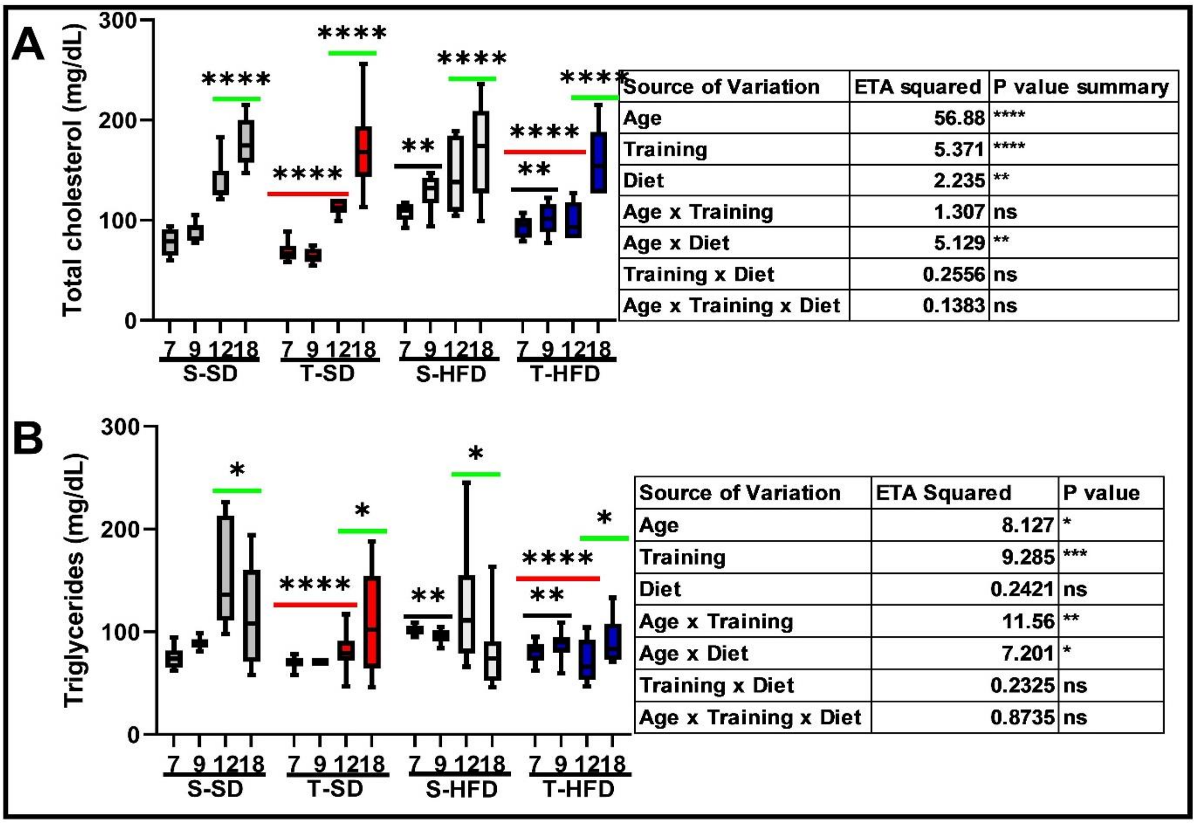


Figure 4. Box-plot graph in relation to total mass (g) of retroperitoneal fat (**A**), relative mass (%) of this fat **(B)**, total mass (g) of liver **(C),** relative mass (%) of this organ **(D)**. Red bars: significant difference related to training ($p<0.0001$), green bars: significant difference for the effect of age ($p<0.0001$; $p<0.005$), Black bars: significant difference training x diet ($p<0.0001$).
Source: The authors.

Lipid profile

Data analysis showed that age significantly increased the level of total plasma COL, especially at 18 months ($p<0.0001$). In relation to diet, there was an increase in total plasma COL only at 7 and 9 months (Age x Diet, $p=0.0013$). HIIT reduced total plasma COL levels at 7, 9 and 12 months of age in both the SD and HFD groups ($p<0.0001$; Figure 5A). Regarding the TG plasma levels, data show that while the diet increased their levels only at 7 and 9 months of age (Age x Diet $p=0.0181$), HIIT was effective in reducing this parameter at 7, 9 and 12 months ($p=0.0013$; Figure 5B). Furthermore age significantly increased the level TG plasma levels at 12 and 18 months ($p<0.05$).



Source of Variation	ETA squared	P value summary
Age	56.88	****
Training	5.371	****
Diet	2.235	**
Age x Training	1.307	ns
Age x Diet	5.129	**
Training x Diet	0.2556	ns
Age x Training x Diet	0.1383	ns

Source of Variation	ETA Squared	P value
Age	8.127	*
Training	9.285	***
Diet	0.2421	ns
Age x Training	11.56	**
Age x Diet	7.201	*
Training x Diet	0.2325	ns
Age x Training x Diet	0.8735	ns

Figure 5. Box-plot graph showing data relating to total plasma levels of total cholesterol (COL; mg/dL) **(A)** and triglycerides (TG; mg/dL) **(B)**. Red bars: significant difference related to training ($p<0.0001$; $p<0.05$), green bars: significant difference for the effect of age ($p<0.0001$; $p<0.05$), Black bars: significant difference age x diet ($p<0.05$).
Source: The authors.

Discussion

This study aimed at assessing the influence of HIIT on the food and water consumption and lipid profiles of rats of different ages and diets. Its importance is due to the fact that, although there are studies on food consumption at different ages and before physical exercise, there are still few that assess these variables in more intense training methods such as HIIT at various stages of life. Analysing the lipid profile is also important to better understand how HIIT acts on fat metabolism at different ages and under different diets.

Given the pleiotropic effects of exercise, the relationship between its practice and energy balance is complex and intricate with individual variability in the response to its interventions[10]. Additionally, research indicate that various factors such as insulin[21], leptin[22] and the motivational aspects of a more palatable diet[23] can interfere with the effects of exercise in relation to energy balance.

Food consumption. In relation to lower food intake in elderly animals, studies show that the level of hormones related to satisfaction can be altered with aging[18,22]. Malikovick et al.[3] noticed that older rats had lower consumption, comparatively to the young, that had higher consumption in order to compensate for the low availability of nutrients in a modified diet. This can, at least in part, explain our findings.

The consumption difference between standard diet and HFD is widely known[24]. Likewise, HIIT reduced the consumption of food mainly in middle-aged and elderly animals fed with standard diet and in all age groups of HFD groups. This reduction can be related to stress caused by high-intensity training, increasing serum levels of corticotropin-releasing hormone and cortisol[25], as well as a reduction in ghrelin[26]. Very recently, Arbus et al.[17], similarly to our findings, when using female rats, observed a reduction in consumption with HIIT in animals fed standard diet, but the opposite was observed in relation to HFD consumption. Moreover, different results from ours were observed by Teixeira Júnior et al.[20], in which mice showed increased food intake during moderate-intensity training, suggesting that food consumption may be related not only to the intensity of the training but also to the animal species used.

Water consumption. The reduction of water consumption with aging can be due to common hormonal changes to increasing age related to the perception of thirst[27] and increase of satiety[28]. Regarding reduction seen in HFD animals, Volcko et al.[29] rose the hypothesis that the reduction in water consumption in HFD animals can be related to a change in orosensory feedback, instead of a change in post-digestive feedback, as HFD alters sensitivity to GLP-1 (glucagon-like peptide 1) which also controls water consumption under central receptor activation.

Similarly, to food consumption, HIIT reduced water consumption in middle-aged and elderly animals of SD groups. A pioneering study by Classen[30] identified that water consumption occurs mainly during meals and is strongly linked to the quantity of food eaten. It is therefore possible that our findings are associated with the reduction of standard diet consumption observed in these groups. On the other hand, in HFD-fed animals, there was a reduction in water consumption only in 7-month-old animals submitted to HIIT. Due to the limited research that could explain this finding, we infer that this diet may be influencing water consumption more significantly only in early adulthood.

Body mass. In the period of obesity induction, a progressive rise in body mass was observed with age, which can be explained by somatic growth[28]. Regarding HFD, such gain was already expected due to the rise of lipogenesis, mainly in subcutaneous and visceral adipose tissue[15,24]. These data show that, beyond obesogenic profile related to HFD consumption, increasing age has further favored the development of obesity.

Research has shown that HIIT is as efficient in reducing body weight as moderate-intensity exercises[15]. Differently from our results, Arbus et al.[17] observed that HIIT was less efficient in the reduction of this parameter in female rats fed with this diet. It could be that this difference is due to the sex of the animals researched and the frequency of training adopted is this research. Corroborating, Costa et al.[19] also verified that HIIT did not significantly reduce body mass in obese animals. However, despite our study adopting the same training protocol of Costa et al.[19], these authors did not use electrical shocks to stimulate running, as we did in our research.

Retroperitoneal adipose tissue. Our results showed that HIIT suppressed the increase of this fat due to the consumption of HFD in all ages. Costa et al.[19] also observed an increase of this and other fat deposits with such a diet. However, differently from our results, they did not observe a meaningful reduction of adipose tissue with HIIT, even in retroperitoneal fat. We believe that the reason for the difference in these findings can be related to the stimulus during training, as cited before. However, corroborating to our findings, Sun et al.[31] and Mendes et al.[16] observed a reduction in body mass, visceral adipose mass and the area of these adipocytes with the practice of HIIT.

The present study showed that middle-aged and elderly animals showed greater mass of this fat in relation to adult animals, even when they practiced HIIT. In fact, aging is typically associated with the rise of adiposity and progressive redistribution of adipose tissue. Thus, it is possible that age can cause reduction in the use of fat as energy substrate during training[32,33].

Liver mass. Our data showed that HFD increased liver mass and that HIIT was able to reduce it. Research has shown that the increase in free fatty acid (FFA) due to HFD can cause insulin resistance in peripheral cells, developing hyperinsulinemia, deregulating the rate of hepatic uptake of FFA, increasing hepatic lipid content and making the liver fatter and heavier[34]. In addition, Zhang et al.[35] observed that in older rat's insulin resistance is even more significant, which may explain, or at least in part, the gradual increase in liver mass with age in the trained groups receiving HFD.

On the other hand, intense physical exercise such as HIIT can influence transcriptional expression in the liver. Wang et al.[6], for example, found that HIIT prevents lipid accumulation in the liver by restoring the mRNA levels of specific genes (SREBP1, ACC1 and, FAS) involved in hepatic lipogenesis in adult rats (six months) fed HFD. Regarding relative liver weight, our data showed that practicing HIIT for eight weeks increased this parameter in the SD groups. Silva et al.[36] observed this same result with long-term aerobic training in young rats (24 weeks), but were unable to explain the etiology of this finding.

Also, in relation to relative liver mass, our data showed that consumption of HFD was able to reduce it. Current studies have found it difficult to discuss this parameter [37,38]. According to Costa et al.[19], this finding can be partially explained by the increase in hepatic triglyceride concentrations with a consecutive reduction in liver glycogen levels. This accumulation shifts the predominance of fatty acids as the main energy substrate in organic reactions to the detriment of hepatic glycogen. In turn, the reduction in glycogen levels in the liver observed in the study by Costa et al. may be associated with the lower weight of this organ, since glycogen carries water molecules for its transportation.

Lipid profile. Age was one of the main factors associated with increased plasma levels of total COL and TG, regardless of the diet offered. In a study by Ghezzi et al.[39] it was found that aging triggered signs of metabolic syndrome in Wistar rats, where the older rats, beyond showing an increase in total COL, showed an increase in serum TG and LDL concentrations as well.

Although it is already known that the consumption of HFD is capable of increasing the levels of total COL and TG[40], our data show novelty in relation to the fact that adult animals were more susceptible to the increase in these variables compared to middle-aged and elderly animals. On the other hand, the eight weeks of HIIT reduced total COL and TG levels only in adult and middle-aged animals. Rahmati-Ahmadabad et al.[9], also studying eight weeks of HIIT, observed a plasma reduction in total COL compared to moderate-intensity training in obese male rats.

Finally, the main limitation of this study is the absence of histological analyses of adipose and liver tissues. Such microscopic analyses could have provided greater insights into the morphological differences observed macroscopically. Furthermore, although differences were observed between age groups, an in-depth discussion of these discrepancies was not possible due to the lack of answers to some questions.

Final considerations

This study demonstrated that HIIT significantly influences food intake, retroperitoneal adipose tissue, and lipid profile in Wistar rats, with effects modulated by age and diet composition. HIIT reduced food consumption, especially in older animals, as well as body mass, and decreased retroperitoneal adipose tissue mass regardless of the diet provided. Moreover, HIIT was more effective in reducing total plasma cholesterol and triglyceride levels in adult and middle-aged animals. These results highlight the importance of interactions between age, diet, and exercise in metabolic regulation.

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CRediT author statement

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