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ORIGINAL ARTICLE

High-intensity interval training and moderate intensity training with exogenous adenosine counteract development of obesity in rats

L'entraînement par intervalles à haute intensité et l'entraînement à intensité modérée avec un apport d'adénosine exogène préviennent le développement de l'obésité chez le rat

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KEYWORDS

Exogenous
adenosine ;

Summary

Objectives. – High-Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT) can combat the obesity epidemic. However, studies comparing their effects on obesity show controversial findings regarding weight loss. Adenosine has emerged as a possible, novel therapeutic

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High-Intensity Interval Training (HIIT) ;
Moderate Intensity Training (MIT) ;
Isocaloric ;
Exercise ;
Weight loss ;
Energy intake ;
Obesity ;
High Fat diet (HFD)

MOTS CLÉS

Adénosine exogène ;
Entraînement par intervalles de haute intensité ;
Entraînement d'intensité modérée ;
HIIT ;
MIT ;
Isocalorique ;
Exercice ;
Perte de poids ;
Apport énergétique ;
Obésité

agent to treat obesity, but more preclinical studies on its efficacy are needed. Therefore, the objectives of this study were to compare the effects of HIIT and MIT on obesity, and further to determine the dose-dependent effects of adenosine on body weight.

Equipment and methods. – We induced obesity in male Wistar rats with high-fat diet (HFD) and monitored development of obesity by comparing the weight to rats on normal diet (ND). After 13 weeks of feeding, exercise training and intraperitoneal adenosine or placebo injections started. The animals were divided into the intervention groups based on equal average weight: (1) ND-1, 2) ND-2, (3) HFD, (4) HFD + placebo, (5) HFD + adenosine, (6) HFD + adenosine + HIIT, (7) HFD + placebo + HIIT, (8) HFD + adenosine + MIT, and (9) HFD + placebo + MIT. During the first six weeks of interventions, adenosine was injected at a dose of 0.2 mg/ml/kg and the following six weeks at 0.4 mg/ml/kg. Weight and feed consumption were determined, and energy intake was calculated as kJ/day.

Results. – The HFD started to cause obesity after 19 weeks of feeding. Both HIIT and MIT were equally effective in counteracting the HFD-induced weight gain, while no differences in the effects were found between the two exercise modes. Adenosine, independent of the dose, also prevented weight gain but did not enhance the effects of HIIT or MIT on weight. The obesity-counteracting effects of the treatments were not due to lower energy intake. In conclusion, our study shows that HIIT and MIT are equally effective to combat obesity, and further, provide preclinical evidence of that adenosine injections could be used to treat obesity.

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Résumé

Objectifs. – L'entraînement par intervalles à haute intensité (HIIT) et l'entraînement à intensité modérée (MIT) peuvent lutter contre l'épidémie d'obésité. Cependant, les études comparant leurs effets sur l'obésité montrent des résultats controversés concernant la perte de poids. L'adénosine est apparue comme un nouvel agent thérapeutique possible pour traiter l'obésité, mais davantage d'études précliniques sur son efficacité sont nécessaires. Par conséquent, les objectifs de cette étude étaient de comparer les effets du HIIT et du MIT sur l'obésité, et de déterminer en outre les effets dose-dépendants de l'adénosine sur le poids corporel.

Équipement et méthodes. – Nous avons induit l'obésité chez des rats Wistar mâles avec un régime riche en graisses (HFD) et surveillé le développement de l'obésité en comparant leurs poids à ceux des rats suivant un régime normal (ND). Après 13 semaines d'alimentation, l'entraînement physique et les injections intrapéritonéales d'adénosine ou de placebo ont commencé. Les animaux ont été divisés en groupes d'intervention sur la base d'un poids corporel moyen égal: (1) ND-1, 2) ND-2, (3) HFD, 4) HFD + placebo, (5) HFD + adénosine, (6) HFD + adénosine + HIIT, (7) HFD + placebo + HIIT, (8) HFD + adénosine + MIT, and (9) HFD + placebo + MIT. Au cours des six premières semaines d'intervention, l'adénosine a été injectée à une dose de 0,2 mg/ml/kg et les six semaines suivantes à 0,4 mg/ml/kg. Le poids et la consommation d'aliments ont été déterminés, et l'apport énergétique a été calculé en kJ/jour.

Résultats. – Le HFD a commencé à provoquer l'obésité après 19 semaines d'alimentation. Le HIIT et le MIT étaient tous deux aussi efficaces pour contrer le gain de poids induit par le HFD, alors qu'aucune différence dans les effets n'a été trouvée entre les deux modes d'exercice. L'adénosine, indépendamment de la dose, a également empêché la prise de poids mais n'a pas renforcé les effets du HIIT ou du MIT sur le poids. Les effets des traitements contre l'obésité n'étaient pas dus à un apport énergétique plus faible. En conclusion, notre étude montre que le HIIT et le MIT sont tout aussi efficaces pour lutter contre l'obésité et, en outre, fournissent des preuves précliniques que les injections d'adénosine pourraient être utilisées pour traiter l'obésité.

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1. Introduction

Obesity has become a global health burden; the worldwide number of obese adults is already reaching 2 billions, and the prevalence is constantly increasing (WHO, 2017). Obesity, which is defined by body mass index (BMI) > 30, is a condition in which excess body fat has accumulated to the

extent that it can have adverse effects on metabolic health and increase the risk for cardiovascular diseases. To date, effective long-term treatment strategies for obesity remain a challenge [1]. The current strategies rely mainly on rather extreme lifestyle changes and diets that, unfortunately, only a minority of patients are able to follow, thus leaving the problem unresolved [2,3]. Therefore, it is important to

find several effective and easy to implement ways to treat obesity.

High intensity exercise with reduced training time could be an effective and time-saving way to combat obesity, but frequently obese individuals are unable to perform it due to physiological restrictions. Therefore, High-Intensity Interval Training (HIIT), which consists of alternating short bursts of high-intensity exercise and light exercise or passive recovery periods, has been considered as a more suitable alternative for obese individuals. Another better choice for obese could be Moderate Intensity Continuous Training (MICT), which usually consists of 30–60 min of aerobic exercise at 64–76% peak heart rate. The effects of HIIT and MICT on weight loss have been intensively studied separately, and also compared with each other in their effectiveness in reducing body weight and fat. However, the results have been rather controversial. One randomized controlled trial showed no effects of HIIT or Medium Intensity Training (MIT) on the body weight of obese participants, while both exercise modes, however, transiently decreased body fat percentage [4]. A study by Keating and et al, also failed to show effects of HIIT and MIT on the body mass, but reported that MIT reduced body fat, while HIIT did not [5]. Similarly, Sawyer et al did not find effects of HIIT or MIT on the body weight [6]. However, HIIT but not MICT decreased body fat percentage. On a contrary, Martins et al showed that both HIIT and MICT reduced body weight of obese participants being equally effective [7]. A randomized controlled trial conducted by Kong and et al, reported that MICT, but not HIIT significantly decreased body weight and fat percentage [8]. These contradictory findings might be partly due to environmental or lifestyle factors, such as diet. Yet, in most of the above-mentioned studies the diet was not monitored, though it is well known that when physical activity is increased, often spontaneous changes in diet occur. Therefore, we aimed to compare the effects of HIIT and MIT on obesity using rats that allow minimizing environmental factors and diet as confounding factors as the animals are kept in a controlled environment and fed with standardized diets.

In addition to exercise studied widely as a treatment for obesity, adenosine and inhibition of adenosine monophosphate (AMP) deaminase have emerged as possible, novel therapeutic agent and target, respectively, in the management of obesity and cardiovascular diseases. Adenosine is produced by the degradation of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and AMP. Adenosine exerts its effects through the receptors A_1 , A_{2A} , A_{2B} and A_3 that are shown to be involved in glucose homeostasis and type 2 diabetes [9], insulin resistance [10] as well as adipose tissue browning [11], for instance. While many studies have investigated the effects of activation and blockage of adenosine receptors with agonists and antagonists (for review [12]), the studies describing the effects of adenosine injections on systemic bodily functions are scarce. Exogenous adenosine infusion has been shown to increase glucose uptake in human skeletal muscle, while endogenous adenosine did not [13]. A study in obese rats showed that intravenous injection of adenosine at a dose of 0.2 mg/ml/kg reduced the expression of A_1 receptor in the heart, while the effects on weight were not reported [14]. The A_1 receptor has been shown to regulate lipolysis and lipogenesis in the adipose tissue of mice [15], suggesting that it plays a role in

obesity. Another study in rats detected no effects of intravenous injection of adenosine at a dose of 0.2 mg/ml/kg on body weight, but found an increase in the expression of uncoupling protein 1 (UCP1) in the visceral adipose tissue [16]. It has been shown that UCP1 knockout mice are obesity-resistant [17]. To our knowledge, intraperitoneal injections of adenosine have not been studied, and thus, we aimed at studying them to increase the knowledge on the effects of adenosine injections on obesity. Based on two previous existing works showing synergistic effect of adenosine and exercise [13,14], we also wanted to study the effects of their combination and their sole effects on obesity. To this end, we determined whether intraperitoneal adenosine injections alone and/or combined with HIIT or MIT could reduce HFD-induced obesity in rats. Moreover, as we did not find studies on the dose-dependent effects of adenosine, we also determined whether increasing the dosage of adenosine injection from 0.2 to 0.4 mg/ml/kg would cause more pronounced effects on the body weight of the rats.

2. Materials and methods

2.1. Animals

The study was approved by the ethics committee of sports science research institute, Tehran, Iran (Document no. IR.SSRI.REC.1395.115). The EU Directive 2010/63/EU and the European Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No123, Strasbourg 1985) were followed. The complete study protocol has been previously published [18]. Seventy-seven male Wistar rats were purchased from the Shahid Mirghani Research institute (Golestan, Gorgan, Iran). The rats were not genetically modified and were healthy. The sample size was calculated using G*Power (University of Dusseldorf, Germany) using ANOVA repeated measures as the statistical test. The statistical power was 82%. All rats were kept in similar laboratory environmental conditions at $22 \pm 3^\circ\text{C}$, housed four rats per cage and were kept in 12-hour light/dark cycle. During the study the cage locations were not changed to avoid causing extra stress to the animals.

All rats were fed with a normal diet (ND) until ~6 weeks of age (Phase 1). The diets were purchased from the Shahid Mirghani Research institute (Golestan, Gorgan, Iran). The ND contained 4.30 kcal per gram consisting of 3.87% fat (soy oil), 17.46% casein protein, 68.7% carbohydrates, 8.97% minerals and 1% vitamins. After the 6-weeks of ND, the animals were divided into the intervention groups based on equal average weight (and not randomized). This was done to ensure that all groups had the same average weight at the beginning of the intervention, which allows a faithful comparison of the groups in terms of the development of obesity. The groups were as follows: (1) ND-1, (2) ND-2, (3) High-fat diet (HFD), (4) HFD + placebo, (5) HFD + adenosine, (6) HFD + adenosine + HIIT, (7) HFD + placebo + HIIT, (8) HFD + adenosine + MIT, and (9) HFD + placebo + MIT (Fig. 1). The composition of the ND was as described above. The energy content of the HFD was 5.81 kcal per gram, and it contained 40% fat (20% soy oil and 20% animal subcutaneous fat oil), 14.1% casein protein, 36.58% carbohydrates, 8.4%

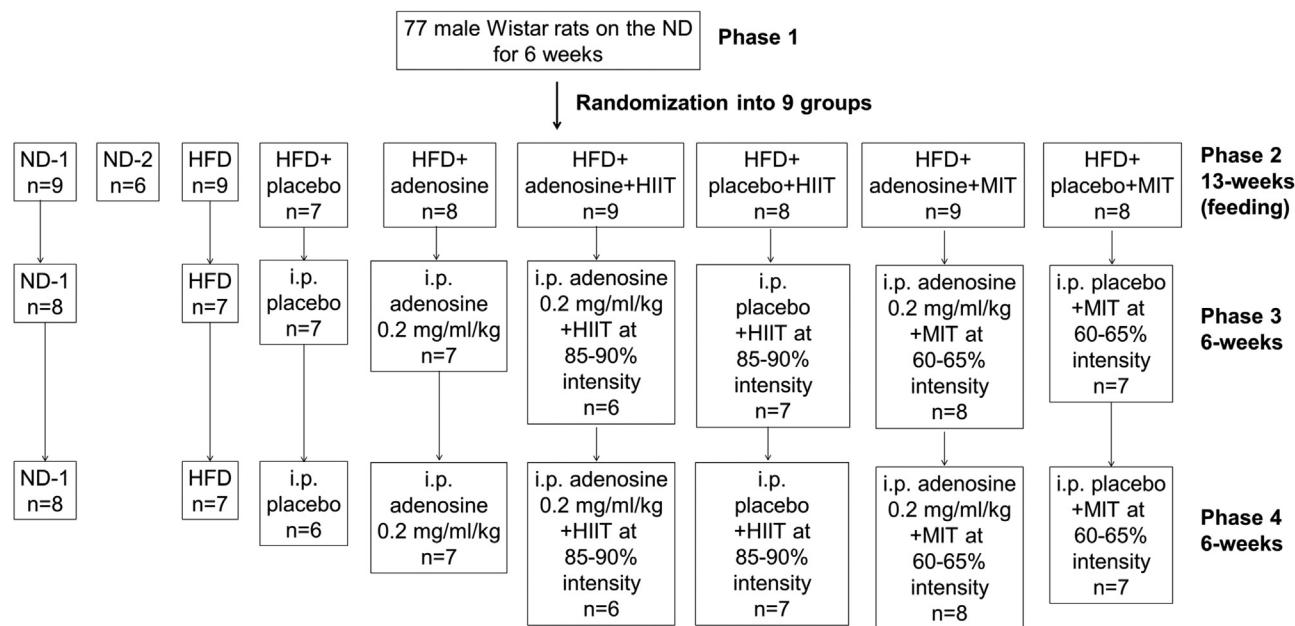


Figure 1 The experimental design, treatments, and interventions. The number of the animals (n) in each group corresponds to the n that finalized the phase. In the HFD groups, the number of animals decreased during the experiment because some of the animals needed to be euthanized because of digestive problems and infection. In the exercising groups, the number of animals decreased during the experiment because some of the animals needed to be euthanized because of skeletal muscle injuries. These exclusion criteria were established a priori. HFD: high-fat diet; ND: normal diet; HIIT: high intensity interval training; MIT, moderate intensity training; i.p.: intraperitoneal. The complete study protocol has been previously published [18].

minerals and 0.72% vitamins. This feeding phase (Phase 2) that aimed at inducing obesity in the HFD groups (Phase 2), continued for 13 weeks.

2.2. Exercise interventions and treatments

After the 13 weeks of feeding with the high-fat diet (HFD) and normal diet (ND), the exercise interventions as well as adenosine or placebo injections started for the first six weeks (Phase 3). At the Phase 3, adenosine was injected intraperitoneally at a daily dose of 0.2 mg/ml/kg. The adenosine was purchased from the College of Pharmacy, Tehran University of Medical Sciences and was dissolved in saline solution. Adenosine doses were based on LD50 and intravenous injection. We tried to reduce the mortality of the rats and side effects by using intraperitoneal injection, and therefore, we needed to use higher doses of adenosine compared to intravenous injection because of metabolism. The placebo-injected groups received daily the same volume of saline solution. Adenosine and placebo were always injected three hours prior to the exercise training. Before the exercise interventions started, the rats in the exercising groups were first habituated to the exercise. To this end, the rats were placed on animal treadmills to run at various speeds for one week daily as explained in the following sentences. First, the animals were running at a speed of 6 meters per min during the first session that lasted for 10 min. Then, the second session was performed at a speed of 8 meters per minute for 12 min. The third session was at a speed of 10 meters per minute for 12 min, and finally, the fourth session was performed at a speed of 10 meters per

minute for 15 min without a slope. After the last habituation session, every rat was placed on the treadmill to continue running at maximum speed until exhaustion point. Following the recording of the maximum speed during exhaustion for every rat, the mean value of speed of the exercising rats was calculated. The exhaustion test was repeated after every four weeks to obtain the new maximum speed to be used in the exercise training. Based on the results, the exercise protocols were designed and followed as shown in Table 1. The high intensity interval training (HIIT) was set at 85 to 90% intensity, whereas the moderate intensity training program (MIT) was set at 60 to 65% intensity. In each exercise session of HIIT and MIT, the warm-up period was 3 min of running at a speed of 10 m/min followed by a cool down period of 2 min of running at a speed of 15 m/min. The exercise training sessions and adenosine injections were always done daily in the same order for each cage and group.

After the first six weeks of exercise, the exercise continued with the same intensity levels and as otherwise specified in Table 1 (Phase 4) for additional six weeks. At this Phase 4, the daily dose of adenosine injections was increased into 0.4 mg/ml/kg. Again, adenosine was dissolved in saline solution and the placebo-injected groups received the same volume as the adenosine-injected groups.

2.3. Measurement of food consumption and energy intake

The weight and amount of feed were monitored daily using an electronic scale. After measuring the quantity of feed placed at 6 pm, the amount of uneaten food was measured

Table 1 Exercise intervention protocols. The complete study protocol has been previously published [18].

High Intensity Interval Training Protocol (HIIT)

Weeks	Bouts	Speed of running m/min	Time	Active Rest	Bouts	Speed of running m/min	Distance m
1st	7	31	1 min	1 min	6	15	402
2nd	8	31	1 min	1 min	7	15	448
3rd	8	35	1 min	1 min	7	17	494
4th	9	36	1 min	1 min	8	17	555
5th	9	41	1 min	1 min	8	19	616
6th	9	45	1 min	1 min	8	20	660
7th	10	45	1 min	1 min	9	22	743
8th	10	47	1 min	1 min	9	22	763
9th	10	49	1 min	1 min	9	23	792
10th	10	50	1 min	1 min	9	24	811
11th	10	52	1 min	1 min	9	24	831
12th	10	55	1 min	1 min	9	25	870

Moderate Intensity Training Protocol (MIT)

Weeks	Bouts	Speed of running m/min	Time	Active Rest
1st	1	20	15:21	402
2nd	1	20	17:39	448
3rd	1	21	19	494
4th	1	21	21:54	555
5th	1	22	23:41	616
6th	1	23	24:34	660
7th	1	24	27	743
8th	1	24	27:50	763
9th	1	24	29:3	792
10th	1	24	29:50	811
11th	1	24	30:38	831
12th	1	25	31	870

m: meters; min: minutes.

after the next 24 hours, and the amount of the remaining feed was deduced from the total initial feed that was put into the cage. The energy intake was calculated by multiplying the amount of consumed feed during 24 hours in grams by the kcal/g that the diet contained, and further by 4.184 to convert the results into kJ per day. All rats in the HFD and ND groups were provided with feed and water ad libitum during the first 13 weeks of the obesity-inducing (or not) feeding, and the first six weeks of exercise training. After that, all groups of rats (HFD and ND) were fed with the same amount of food (the grams of food were measured with an electronic scale daily) during the second six weeks of the exercise (or not) training phase based on the amount of food consumed by the two HFD control groups.

2.4. Statistical analyses

The statistical analyses were made using IBM SPSS Statistics 26 (Armonk, NY, USA). The normal distribution was determined using Shapiro Wilks test. The differences in

the variables between the time points were studied with repeated measures analysis of variance (ANOVA) using the GLM procedure. In addition, the interactive effects of the variables and group were analyzed. The differences in the variables between the groups at one time point were analyzed using ANOVA and Bonferroni as the post-hoc test.

3. Results

3.1. High-fat diet induced obesity in rats independent of energy intake in the groups of rats that were not exercising and/or injected with adenosine

To induce obesity in rats, we fed them with a HFD that contained 40% of energy fat. After the 13 weeks of feeding with the HFD and ND (Phase 2), all groups of rats gained weight ($p < 0.05$ for all), which was expected because the rats were aging and growing (Fig. 2). However, there was

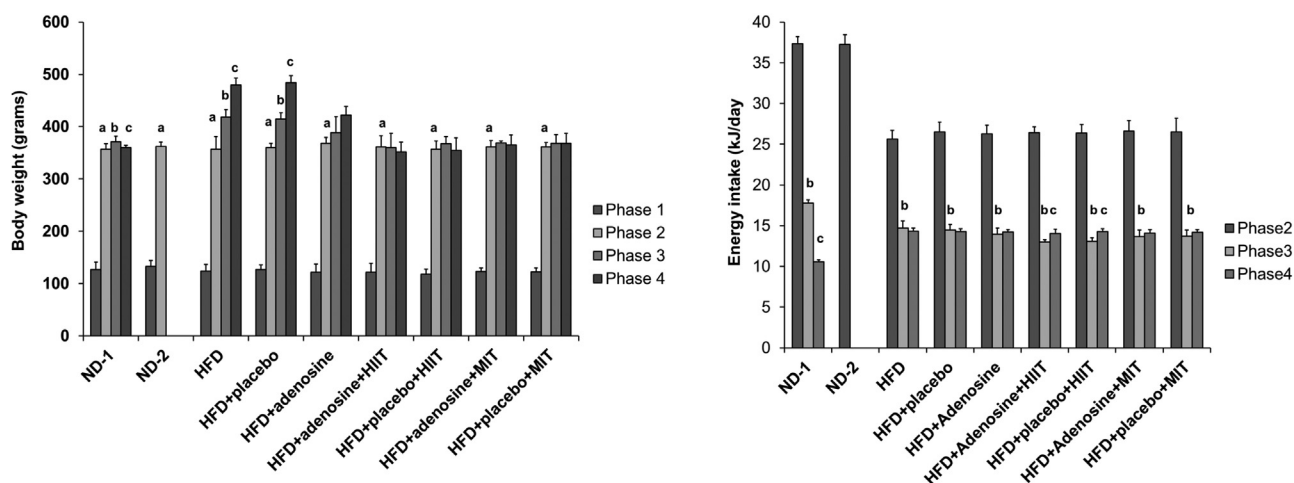


Figure 2 Body weight (grams) and energy intake (kJ/day) after the different phases in the experimental groups. The letters a, b and c indicate a p-value < 0.05, and the letter meanings are as follows: a= compared to Phase 1, b= compared to Phase 2, c= compared to Phase 3. HFD: high-fat diet; ND: normal diet; HIIT: high intensity interval training; MIT: moderate intensity training. Energy intake is the average value of all days of the phase. No experimental data points or units were excluded from the analyses.

no interactive effect of weight and group [F (7, 46)= 2.127, P=0.056]], indicating that the groups did not differ from each other in weight at that point (Table 2), indicating that the HFD had not yet caused significant obesity. After the 13 weeks of feeding (Phase 2), the ND-1 group had higher energy intake than all HFD groups (P=0.000 for all), while no differences between the HFD groups were found in the energy intake.

After continuing for six weeks more on the HFD (Phase 3), the weight increased in all groups [F (1, 46)= 48.747, P=0.000] (Fig. 2). In addition, there was an interactive effect of weight and group [F (7, 46)= 10.78, P=0.000]. At the Phase 3, the HFD and HFD + placebo groups weighed more than the ND-1 group (P=0.000 for both) (Table 2). The ND-1 group continued to have higher energy intake than all HFD groups (P=0.000 for all) (Fig. 2).

At the final of the intervention and 13 weeks on the HFD plus 12 weeks of exercise or not, the HFD, HFD + placebo and HFD + adenosine groups weighed more than the ND-1 group (P=0.000 for all) (Table 2). During the last six weeks of the intervention (Phase 4), there was an interactive effect of weight and group [F (7, 46)= 18.635, P=0.000]. At the Phase 4, the weight decreased in the ND-1 group [F (1, 7)= 10.469, P=0.014], and increased in the HFD [F (1, 6)= 68.397, P=0.000] and HFD + placebo [F (1, 5)= 55.062, P=0.000] groups (Fig. 2). At the final of the interventions, there was an interactive effect of weight and energy intake [F (7, 48)= 90.137, P=0.000]. The energy intake decreased in the ND-1 group [F (1, 7)= 5171.851, P=0.000] and increased in the HFD + adenosine + HIIT [F (1, 5)= 14.688, P=0.012], HFD + placebo + HIIT [F (1, 6)= 22.196, P=0.003] groups, but not in the non-exercising HFD groups (Fig. 2). To summarize the longitudinal results, during the interventions the HFD-induced increase in weight was not seen in the HFD + adenosine, HFD + placebo + HIIT, HFD + adenosine + MIT or HFD + placebo + MIT groups.

3.2. Both HIIT and MIT counteracted the high-fat diet-induced increase in body weight, which was not due to lower energy intake

Next, we analyzed whether the exercise training programs were able to reduce the HFD-induced obesity. After the first six weeks of exercise training (Phase 3), the HFD + placebo + HIIT and HFD + placebo + MIT groups weighed less than the HFD and HFD + placebo groups (P=0.000 for all) (Table 2). Further, the longitudinal analyses showed that there was an interactive effect of weight and group [F (7, 46)= 10.784, P=0.000]. The weight increased in the ND-1 [F (1, 7)= 13.464, P=0.008], HFD [F (1, 6)= 100.842, P=0.000] and HFD + placebo [F (1, 6)= 74.768, P=0.000] groups, but not in the exercising groups (Fig. 1). At this point, the HFD + placebo + HIIT group (P=0.000) had lower energy intake than the HFD group (Table 2). However, the longitudinal analyses showed that during the first six weeks energy intake decreased in all groups [F (1, 57)= 7751.975, P=0.000] (Fig. 2). In addition, there was an interactive effect of group and energy intake [F (7, 57)= 41.596, P=0.000].

After the second six weeks of exercise training (Phase 4), the HFD + placebo + HIIT and HFD + placebo + MIT groups continued to weigh less than the HFD and HFD + placebo groups (P=0.000 for all) (Table 2). According to the longitudinal analyses, there was an interactive effect of group and weight [F (7, 46)= 18.635, p=0.000]. At the Phase 4, the weight decreased in the ND-1 group [F (1, 7)= 10.469, P=0.014] and increased in the HFD [F (1, 6)= 68.397, P=0.000] and HFD + placebo [F (1, 5)= 55.062, p=0.001] groups during these six weeks (Fig. 2). Again, the weight did not change in the exercising groups, suggesting that both exercise modes efficiently counteracted the HFD-induced increase in body weight. There was interactive

Table 2 Mean values ± SD of the body weight (grams) and energy intake (kJ/gram) in the groups at each phase (P).

Variable	ND-1	ND-2	HFD	HFD+pla	HFD+ade	HFD+ade+HIIT	HFD+pla+HIIT	HFD+ade+MIT	HFD+pla+MIT
weight P1	127.20 ± 13.95	133.10 ± 11.37	124.12 ± 12.50	127.00 ± 9.01	121.50 ± 16.00	121.89 ± 16.80	115.28 ± 5.19	123.25 ± 7.02	122.82 ± 7.23
weight P2	356.88 ± 10.62	362.05 ± 8.45	356.92 ± 23.70	360.18 ± 7.95	368.17 ± 10.99	361.87 ± 20.32	357.36 ± 15.03	361.61 ± 12.15	361.63 ± 8.03
weight P3	371.50 ± 10.06		417.95 ± 14.50	414.76 ± 11.71	388.52 ± 30.20	360.32 ± 26.27	367.43 ± 13.53	369.28 ± 3.27	368.12 ± 16.34
weight P4	359.76 ± 5.11		479.21 ± 13.89	484.04 ± 13.56	422.15 ± 16.23	351.50 ± 18.97	355.05 ± 23.75	365.38 ± 18.83	368.20 ± 18.45
Energy intake P2	37.36 ± 0.87	37.24 ± 1.22	25.67 ± 1.02	26.52 ± 1.18	26.28 ± 1.03	26.43 ± 0.71	26.35 ± 1.07	26.61 ± 1.30	26.49 ± 1.68
Energy intake P3	17.80 ± 0.37		14.71 ± 0.85	14.48 ± 0.66	13.96 ± 0.76	12.96 ± 0.33	13.06 ± 0.45	13.67 ± 0.78	13.70 ± 0.77
Energy intake P4	10.57 ± 0.24		14.34 ± 0.36	14.26 ± 0.33	14.21 ± 0.29	14.03 ± 0.53	14.25 ± 0.37	14.07 ± 0.43	14.19 ± 0.34

P: phase; HFD, high-fat diet; ND: normal diet; HIIT: high intensity interval training; MIT: moderate intensity training; pla, placebo; ade, adenosine. Energy intake is the average value of all days of the phase.

effect of group and energy intake [F (7, 48)=90.137, P=0.000]. The energy intake decreased in the ND-1 group [F (1, 7)=5171.851, p=0.000], and increased in the HFD+placebo+HIIT group [F (1, 6)=22.196, P=0.003] (Fig. 2).

3.3. Adenosine counteracted high-fat diet-induced increase in body weight, but did not enhance the weight-decreasing effects of exercise

Next, we analyzed whether the intraperitoneal adenosine injections also prevented HFD-induced obesity and enhanced the effects of exercise on weight. After the first 13 weeks on the HFD, adenosine was injected first at a dose of 0.2mg/ml/kg for six weeks to five groups of rats as specified in the methods section (Phase 3). Placebo was injected to two groups that were on the HFD and performed MIT or HIIT. The group-wise comparisons showed no differences in weight between the HFD and HFD+adenosine, HFD+adenosine and HFD+adenosine+HIIT or HFD+adenosine+MIT, HFD+placebo+HIIT and HFD+placebo+MIT groups after the six weeks (Table 2), suggesting that the adenosine dose of 0.2mg/ml/kg did not enhance the effects of exercise on preventing HFD-induced weight gain. However, the longitudinal analyses showed that the weight did not change in the HFD+adenosine, HFD+adenosine+HIIT or HFD+adenosine+MIT groups during the six weeks of injections (Fig. 2), indicating that adenosine at the dose of 0.2mg/ml/kg alone and combined with exercise was able to counteract the effects of the HFD on weight. In all adenosine-injected groups the energy intake decreased during these first six weeks (P=0.000 for all) (Fig. 2). Thus, the lack of body weight change could be partly due to decreased energy intake.

After the first six weeks, adenosine was injected at a dose of 0.4mg/ml/kg for another six weeks (Phase 4), and the injections were combined or not with exercise. After the second six weeks, the HFD and HFD+placebo groups had higher body weight than all adenosine-injected groups (P=0.000 for all) (Table 2). These results suggest that adenosine at a dose of 0.4mg/ml/kg alone or combined with exercise had weight lowering effects. These suggestions were further confirmed by the longitudinal analyses showing that the weight did not change in the adenosine-injected groups (Fig. 2). At the Phase 4, the energy intake did not change in the adenosine-injected groups suggesting that the effects of adenosine on reducing body weight gain were not dependent on diet.

To determine whether the effects of adenosine on weight were dose-dependent, we calculated the fold changes in weight after the first and second six-weeks of injections, and then compared the fold changes inside the groups. No dose-dependent effects of adenosine on weight were found.

4. Discussion

In this study, we aimed to compare the effects of High Intensity Interval Training (HIIT) and Moderate Intensity Training (MIT) on the high-fat diet (HFD)-induced obesity in rats. We further determined whether intraperitoneal adenosine

injections dose-dependently alone or combined with exercise training could combat obesity. We found that the HFD effectively induced obesity in rats, and both HIIT and MIT counteracted HFD-induced increase in body weight. Further, independent of the dose, adenosine alone or combined with exercise also counteracted HFD-induced the increase in body weight. This effect was independent of energy intake and food consumption. However, adenosine did not enhance the weight-decreasing effects of HIIT or MIT. The HIIT and MIT were equally effective to prevent the onset of obesity in rats.

Similar to our previous study in Wistar rats [19], the HFD induced obesity compared to the normal diet (ND). However, in the present study obesity developed only after 19 weeks on the HFD, while in the earlier study the HFD rats started to weigh more than the low-fat diet rats already after around four weeks of diets [19]. This is likely due to the amount of fat in the feed, which was 40% of total energy in the present study, and 60% in the earlier [19]. In this study, we show that both HIIT and MIT equally efficiently counteracted the HFD-induced increase in body weight, and thus, were able to combat obesity. This effect was seen after the first six weeks of exercise training, and it continued until the end of the study, *i.e.*, 12 weeks of exercise training in total. As mentioned in the introduction, human studies comparing the effects of HIIT and MIT/MICT on weight loss have reported controversial outcomes. However, a recent systematic review and meta-analysis of overweight/obese humans subjected to exercise training [20] support our findings. From the 1,334 articles initially screened, the authors finally included 13 original research articles to their study, and concluded that both HIIT and MICT reduced weight, body fat mass and waist circumference of the participants. All 13 studies were conducted for around ten weeks and included three sessions per week of exercise training. In that paper, similar to what we found in obese rats, there were no significant differences between the HIIT and MICT for any of the body composition measures of the obese participants [20]. In a Pubmed search, we found eight studies in obese rats that have compared the effects of HIIT and MICT. Only one of the studies reported body weight after the exercise training showing no effects of HIIT or MICT on obesity [21]. It is not clear why the results are contrary to our results because the HIIT protocol was very similar and lasted for 12 weeks. Another study in obese rats compared the physiological effects of HIIT and MIT. In agreement with our results, both exercise modes decreased the body weight compared to the sedentary HFD-fed rats, while no differences in weight were observed between the HIIT and MIT groups [22].

Several animal studies have investigated the effects of adenosine receptor agonists mimicking adenosine on bodily functions, but studies on the effects of adenosine injections are scarce. Studies in rats have reported that adenosine injections decreased the expression of A_1 receptor in the heart tissue [14], and that adenosine decreased the expression of UCP1 in the visceral adipose tissue [16]. It has also been shown that adenosine dose-dependently increased lipolysis in cultivated human adipocytes and in the brown adipose tissue of mice, similar to what happened when the mice were treated with the A_{2A} and A_{2B} receptor agonists [11]. These reported effects of adenosine and adenosine receptor agonists are likely health beneficial.

However, despite the important lack of clinical and pre-clinical studies using adenosine, we found at least one private medical center that offers adenosine injections to their patients. Therefore, we believe that it is important to provide more preclinical data on the safety of adenosine injections as well as on the obesity-reducing effects of adenosine. In the present study, we show that intraperitoneally injected adenosine alone or combined with exercise counteracted the HFD-induced increase in body weight. This effect of adenosine was not dose dependent. However, adenosine did not enhance the weight-decreasing effects of HIIT or MIT. Similar to our results on adenosine, 5'-N-ethylcarboxamidoadenosine (NECA), a potent non-selective adenosine receptor agonist has been shown to reduce diet-induced obesity in mice [23]. The NECA-treated mice exhibited some improvements in glucose homeostasis associated with the observed weight loss [23]. In addition, a selective and high-affinity A_{2B} agonist Bay 60-6583 has been reported to counteract HFD-induced obesity [24]. The A_{B2} agonist also increased muscle and lean mass as well as improved glucose tolerance [24]. To summarize, accumulating data is showing that adenosine and activation of adenosine receptors can have obesity-reducing and metabolism-improving effects.

This study is not without limitations. Due to the lack of resources, we studied only male rats. In the future, it would be important to determine whether HIIT, MIT and adenosine similarly counteract HFD-induced increase in body weight also in female rats. While it has been shown that some adenosine receptor agonists increase muscle mass, we did not measure the weight of different muscles in our study. Future experiments should be conducted to study more in detail the effects of adenosine on body composition.

Disclosure of interest

The authors declare that they have no competing interests.

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Références

- [1] Takaki A, Kawai D, Yamamoto K. Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* 2014;15:7352–79.
- [2] Tanenbaum HC, Felicitas JQ, Li Y, Tobias M, Chou CP, Palmer PH, et al. Overweight perception: associations with weight control goals, attempts, and practices among chinese female college students. *J Acad Nutr Diet* 2016;116:458–66.
- [3] Younossi ZM, Reyes MJ, Mishra A, Mehta R, Henry L. Systematic review with meta-analysis: non-alcoholic steatohepatitis - a case for personalised treatment based on pathogenic targets. *Aliment Pharmacol Therapeut* 2014;39:3–14.

- [4] Fisher G, Brown AW, Bohan Brown MM, Alcorn A, Noles C, Winwood L, et al. High intensity interval- vs moderate intensity-training for improving cardiometabolic health in overweight or obese males: a randomized controlled trial. *PLoS One* 2015;10:e0138853.
- [5] Keating SE, Machan EA, O'Connor HT, Gerofi JA, Sainsbury A, Catterson ID, et al. Continuous exercise but not high intensity interval training improves fat distribution in overweight adults. *J Obes* 2014;2014:834865.
- [6] Sawyer BJ, Tucker WJ, Bhammar DM, Ryder JR, Sweazea KL, Gaesser GA. Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. *J Appl Physiol* (1985) 2016;121:279–88.
- [7] Martins C, Kazakova I, Ludviksen M, Mehus I, Wisloff U, Kul-seng B, et al. High-intensity interval training and isocaloric moderate-intensity continuous training result in similar improvements in body composition and fitness in obese individuals. *Int J Sport Nutr Exerc Metab* 2016;26:197–204.
- [8] Kong Z, Fan X, Sun S, Song L, Shi Q, Nie J. Comparison of high-intensity interval training and moderate-to-vigorous continuous training for cardiometabolic health and exercise enjoyment in obese young women: a randomized controlled trial. *PLoS One* 2016;11:e0158589.
- [9] Johnston-Cox H, Koupnova M, Yang D, Corkey B, Gokce N, Farb MG, et al. The A2b adenosine receptor modulates glucose homeostasis and obesity. *PLoS One* 2012;7:e40584.
- [10] Sacramento JF, Martins FO, Rodrigues T, Matafome P, Ribeiro MJ, Olea E, et al. A2 adenosine receptors mediate whole-body insulin sensitivity in a prediabetes animal model: primary effects on skeletal muscle. *Front Endocrinol (Lausanne)* 2020;11:262.
- [11] Gnad T, Scheibler S, von Kugelgen I, Scheele C, Kilic A, Glode A, et al. Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. *Nature* 2014;516:395–9.
- [12] Pardo F, Villalobos-Labra R, Chiarello DI, Salsoso R, Toledo F, Gutierrez J, et al. Molecular implications of adenosine in obesity. *Mol Aspects Med* 2017;55:90–101.
- [13] Heinonen I, Kempainen J, Kaskinoro K, Peltonen JE, Sipila HT, Nuutila P, et al. Effects of adenosine, exercise, and moderate acute hypoxia on energy substrate utilization of human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 2012;302:R385–90.
- [14] Farsi S, Ahmadi N, Azarbayjani MA. Effect of continuous and interval training with adenosine consumption on A1 and A2A adenosine receptors in heart tissue of obese rats. *Gene Cell Tissue* 2020;7:e97210.
- [15] Johansson SM, Lindgren E, Yang JN, Herling AW, Fredholm BB. Adenosine A1 receptors regulate lipolysis and lipogenesis in mouse adipose tissue-interactions with insulin. *Eur J Pharmacol* 2008;597:92–101.
- [16] Barjaste Yazdi A, Matinhomae H, Peeri M. The effect of endurance exercise and adenosine consumption on UCP-1 gene expression in the visceral adipose tissue of obese male rats. *Iran J Diab Obes* 2018;10.
- [17] Liu X, Rossmeisl M, McClaine J, Riachi M, Harper ME, Kozak LP. Paradoxical resistance to diet-induced obesity in UCP1-deficient mice. *J Clin Invest* 2003;111:399–407.
- [18] Mirghani SJ, Peeri M, Yaghoobpour Yekani O, Zamani M, Feizolahi F, Nikbin S, et al. Role or synergistic interaction of adenosine and vitamin D3 alongside high-intensity interval training and isocaloric moderate intensity training on metabolic parameters: protocol for an experimental study. *JMIR Res Protoc* 2019;8:e10753.
- [19] Lensu S, Pariyani R, Makinen E, Yang B, Saleem W, Munukka E, et al. Prebiotic Xylo-Oligosaccharides ameliorate high-fat-diet-induced hepatic steatosis in rats. *Nutrients* 2020;12.
- [20] Wewege M, van den Berg R, Ward RE, Keech A. The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes Rev* 2017;18:635–46.
- [21] Khalafi M, Mohebbi H, Symonds ME, Karimi P, Akbari A, Tabari E, et al. The Impact of moderate-intensity continuous or high-intensity interval training on adipogenesis and browning of subcutaneous adipose tissue in obese male rats. *Nutrients* 2020;12:925.
- [22] Rahmati-Ahmadabad S, Azarbayjani MA, Farzanegi P, Moradi L. High-intensity interval training has a greater effect on reverse cholesterol transport elements compared with moderate-intensity continuous training in obese male rats. *Eur J Prev Cardiol* 2019 [2047487319887828].
- [23] DeOliveira CC, Paiva Caria CR, Ferreira Gotardo EM, Ribeiro ML, Gambero A. Role of A1 and A2A adenosine receptor agonists in adipose tissue inflammation induced by obesity in mice. *Eur J Pharmacol* 2017;799:154–9.
- [24] Gnad T, Navarro G, Lahesmaa M, Reverte-Salisa L, Copperi F, Cordomi A, et al. Adenosine/A2B receptor signaling ameliorates the effects of aging and counteracts obesity. *Cell Metab* 2020;32:56–70, e7.