Iranian Journal of Pharmaceutical Sciences
2025: 21 (1): 89- 98
https://journals.sbmu.ac.ir/IJPS

Research Article

Adenosine and High-intensity Interval Training as Potential Therapies on Free Fatty Acids and Metabolic Factors in High-fat Diet-Induced Obesity in Male Wistar Rats

Zahra Eslami[a], Ayda Fallah[b], Abdorreza Eghbal Moghanlou [c], Giyasettin Baydaş [d], Shohreh Sharifian [e], Seyed Javad Mirghani[f*]

[a] *Department of Clinical Biochemistry, Hamadan university of medical science, Hamadan, Iran.*
[b] *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*
[c] *Department of Coaching Education, Istanbul Esenyurt University, Istanbul, Turkey.*
[d] *Istanbul Medeniyet Üniversity, Faculty of Medicine, Department of Physiology, Istanbul, Turkey.*
[e] *Department of Sport Studies and Planning, Central Organization, Islamic Azad University, Tehran, Iran.*
[f] *Shahid Mirghani Research Institute, Golestan, Iran.*

Received: October 19, 2024 **Last Revision:** November 30, 2024 **Accepted:** December 02, 2024 **Available online:** February 17, 2025

Abstract

Obesity, caused by an inequality between energy production and consumption, is characterized by lipid accumulation in adipose tissues. Currently, around 650 million adults and roughly 340 million children and adolescents (aged 5-19 years) are affected by obesity. This condition tends to be more common among women and older populations. It is imperative to develop uncomplicated therapeutic approaches to prevent obesity-related metabolic diseases in conjunction with lifestyle modifications. Forty-three rats were randomly divided into five groups: 1. normal diet (ND), 2. High-fat diet (HFD), 3. HFD + adenosine, 4. HFD + High-intensity interval training (HIIT) + adenosine, and 5. HFD + HIIT. Gene expression of CGI-58, HSL, and AMPK in subcutaneous adipose tissues and serum level of glucose, insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), free fatty acid (FFA), and lipid profile (Triglyceride (TG), Total cholesterol (TC), Low density lipoprotein (LDL), High density lipoprotein (HDL), and Very low density lipoprotein (VLDL)) were assessed. The rats were fed HFD-induced obesity for 13 weeks. Following, adenosine 0.2 mg/ml/kg and 0.4 mg/ml/kg, as well as HIIT, were administered over 12 weeks. CGI-58, HSL and AMPK gene expression showed significant expression in all groups. HFD+HIIT+adenosine, HFD+adenosine, and HFD+HIIT groups significantly increased all genes. Conversely, FFA and glucose serum levels were significantly reduced in intervention groups. Insulin had higher serum levels in ND, HFD + adenosine, and HFD+HIIT groups, and adenosine caused decreased glucose. Also, favorable effects of HIIT and adenosine on lipid profile were observed. HIIT and adenosine can affect lipid metabolism, improve insulin resistance, and increase lipolysis in adipose tissue. Furthermore, adenosine can boost the effect of HIIT on gene expression, triggering lipolysis to prevent obesity.

Keywords: High-fat diet; High intensity interval training; Adipose tissue; Adenosine; Lipid.

*** Corresponding Author**:
Seyed Javad Mirghani, Shahid Mirghani Research Institute, Golestan, Iran. E-mail: seyedgavadmirghani@yahoo.com.

Cite this article as: Eslami Z., Fallah A., Eghbal Moghanlou A., Baydaş G., Sharifian Sh., Mirghani S.J. Adenosine and High-intensity Interval Training as Potential Therapies on Free Fatty Acids and Metabolic Factors in High-fat Diet-Induced Obesity in Male Wistar Rats. Iran. J. Pharm. Sci., 2025, 21 (1): 89-98.

DOI: https://doi.org/10.22037/ijps.v21i1.46498

1. Introduction

Obesity, one of the world's greatest public health problems, has affected more than 35% and 40% of men and women, respectively, in the 21st century [1, 2]. The lipids store excess in adipose tissue that arises from the disproportion between the production and usage of energy, known as obesity [3]. Therefore, for the prevention and control of obesity and associated incidences, suppression of lipogenesis and stimulation of lipolysis and energy consumption may have advantageous effects [4].

Since obesity is a chronic condition and lifestyle modifications have had only modest success, finding safe medications must be a long-term treatment objective [5]. The following processes have been primarily used to produce a variety of drugs for the treatment of obesity: 1) decreasing hunger and, subsequently, energy intake, 2) limiting calorie absorption, and 3) encouraging activity levels [6]. Although at present, physical activity, diet, change of lifestyle, surgery, and pharmacological treatment have beneficial effects on obesity, they have some complications such as wasting price and time, adverse effects and comfortable weight regained followed by stopping medication usage [7]. Some investigations have examined intervention processes aimed at assisting individuals in achieving weight loss, particularly in reducing fat weight. Researchers have also uncovered previously unknown impacts of adenosine derived from adenosine triphosphate (ATP) [8].

Adenosine plays a role in glucose homeostasis, inflammation, lipogenesis, insulin sensitivity, and thermogenesis. Therapeutic modulation of adenosine receptors may have advantageous effects in treating obesity. The G protein-coupled receptors known as the A1, A2A, A2B, and A3 regulate the actions of adenosine in diverse ways depending on the tissue and stage of illness. For example, adipogenesis will be boosted, and lipolysis will be decreased by activating the A1 receptor. A2A receptor activation, however, will increase lipolysis [3]. The A1 receptor decreases cAMP, while the A2A receptor increases cAMP, inhibiting and activating hormone-sensitive lipase (HSL) and controlling the lipolysis pathway [9].

Another efficient and time-saving strategy to prevent obesity is limited-time high-intensity training. High-intensity interval training (HIIT), which includes modifying short periods of high-intensity exercise with mild activity or passive rest time, has been considered an option for obese people [10]. However, it is debatable how HIIT affects body weight and composition [11]. However, it has gained popularity in the past ten years as a hot issue in health promotion because of its notable benefits on intra-abdominal and liver fat reduction and amelioration of insulin resistance (IR) compared to traditional aerobic exercise [12]. A crucial element of HIIT is the catecholamine response, specifically the role of catechol amines such as epinephrine in stimulating lipolysis and facilitating the release of fat from subcutaneous and intramuscular fat reserves [13]. The positive correlation between HIIT and improved glucose and insulin levels has been scientifically proven. Evidence underlines the potential of HIIT to influence the reduction of postprandial glucose (PPG) and postprandial insulin (PPI) responses [14].

Adenosine performs a critical function by modulating insulin secretion from pancreatic β-cells, with low concentrations decreasing glucose-induced insulin secretion and high concentrations increasing it. Moreover, the biosynthetic and breakdown pathways of adenosine are influenced by glucose and insulin concentrations, affecting the extracellular levels of adenosine and inosine during ATP catabolism [15]. Overall, adenosine has a complex role in glucose and insulin regulation, with its impacts relying on the specific adenosine receptor subtype and the concentrations of adenosine, glucose, and insulin. Skeletal muscle requires free fatty acids and glucose as fuels to maintain the myofibril contractions during physical activity [16]. The primary regulator of TAG lipolysis, which hydrolyzes a TAG's first ester bond, is adipose triglyceride lipase (ATGL). Hormone-sensitive lipase (HSL) hydrolyzes the second ester bond, and finally, monoglyceride lipase breaks down monoacyl glycerol into glycerol and a free fatty acid. The connection between ATGL and its co-activator CGI-58 increases ATGL activity [17]. When the AMP/ATP ratio rises in response to an energy shortage, the main sensor of intracellular energy, adenosine monophosphate-activated protein kinase (AMPK), is activated, leading to restoring equilibration of energy by inhibiting the building-up aspect of metabolism, which uses ATP and promoting catabolism, give rise to ATP [18]. In adipocytes, AMPK may phosphorylate HSL at Ser565 to reduce HSL activity and significantly inhibit lipolysis [19]. However, one previous research indicates that activating AMPK during exercise did not prevent HSL Ser563 phosphorylation, activity, or lipolysis [20]. The effects of HIIT and exogenous adenosine on the fat loss signaling pathway are still poorly studied. For this purpose, we determined whether exogenous adenosine and/or HIIT could increase lipolysis and its effect on glycemic indices in HFD-induced obese rats.

2. Material and methods

2.1. Animals

Forty-three male Wistar rats were procured from the Shahid Mirghani Research Institute (Golestan, Iran). The rats were in a controlled environment at 22°C±3°C and a 12-hour day-night cycle. They were fed a regular diet for 5 to 6 weeks, resulting in an average weight gain of 182.32 grams. Following one week of acclimation, the rats were divided into five groups: (1) ND, (2) HFD, (3) HFD + adenosine, (4) HFD + adenosine + HIIT, and (5) HFD + HIIT. The study consisted of two phases: the first phase involved induction of obesity (40% fat) for 13 weeks, while the second phase included interventions over 12 weeks. During the initial 6 weeks of the second phase, the rats had unrestricted access to food and water. From the onset of the second 6-week period of the second phase, the sustenance provided to all groups was determined by the food consumption levels exhibited by the HFD group. The integrity of this study underwent scrutiny from the research ethics committee of the Sports Science Research Institute, and the designation IR was approved.SSRI.REC.1395.115.

2.2. Diet

The standard diet comprised 4.30 kcal/gr, consisting of 3.87% fat derived from soy oil, 17.46% casein protein, 68.7% carbohydrates, 8.97% minerals, and 1% vitamins. Conversely, the HFD contained 5.81 kilocalories per gram, with 40% fat comprised of 20% soy oil and 20% subcutaneous fat oil derived from animal fat, 14.1% casein protein, 36.58% carbohydrates, 8.4% minerals, and 0.72% vitamins [21].

2.3. Exercise protocol

The initial phase of the study involved subjecting the training groups to a week-long session on the animal treadmill, with speeds ranging from 6 to 10 m/min, to enhance their skills. Subsequently, each rat underwent treadmill sessions where the speed was progressively increased until the point of exhaustion was reached. The average speed of the training groups was then determined by analyzing the outcomes of the training protocol that had been carefully designed [22]. The HIIT incorporated an intensity level of 85% to 90% of the maximum velocity (Vmax). Before the HIIT sessions, a warm-up period consisting of a 3-minute run at 10 m/min was conducted, followed by a cool-down period involving a 2-minute run at 15 m/min. The HIIT intervention spanned 12 weeks.

2.4. Adenosine treatment

Injection of adenosine and training intervention started after 13 weeks of feeding HFD. Adenosine 0.2 mg/ml/kg was injected for the first 6 weeks, and 0.4 mg/ml/kg (dissolved in saline solution) was injected for the second 6 weeks (intraperitoneally, 3 hours before HIIT every day). We used intraperitoneal rather than intravenous injection because it had fewer side effects. However, we used a higher dose of adenosine during the second 6 weeks because of the high metabolization of it [21]. The adenosine was purchased from the College of Pharmacy, Tehran University of Medical Sciences.

2.5. Tissue preparation

After 24 hours of rest and 8 hours of fasting, the rats were anesthetized by pentobarbital sodium (40 mg/kg; IP). Then, blood samples were drawn directly from the heart and transferred into tubes for serum separation by centrifugation. The thigh fat (subcutaneous) samples were isolated in 2×2 mm size. After placing the samples in nitrogen for RNA extraction and gene analysis, they transferred to −80°C [21].

2.6. Quantitative polymerase chain reaction (q-PCR)

Adipose tissue specimens were homogenized in Trizol solution (Tissue-Lyser LT; Qiagen, Valencia, CA), whereby the RNA was subsequently extracted and converted into complementary DNA (cDNA) employing the high-capacity cDNA reverse transcription kit (Applied system; Applied Biosystems). To assess purity and concentration, total RNA was assayed using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). The primer was devised using the National Center for Biotechnology Information primer design tool and was synthesized by Pishgam (Pishgam, Iran). Each well of a 96-well plate was filled with a 20 µL reaction mixture incorporating 10 µL of SYBR Green Master mix (Amplicon), appropriate concentrations of gene-specific primers, and 1000 ng/µL of cDNA template. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were employed as an internal control. The primer sequences can be found in **Table 1**.

2.7. Biochemical analysis

Glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) determined by using an automated analyzer (Pars Azmoon, Tehran, Iran). According to the manufacturer's instructions, FFAs and insulin were measured with rat-specific ELISA kits. Homeostatic model assessment (HOMA-IR) is calculated using fasting glucose * fasting insulin /405.

2.8. Statistical analysis

The mean and standard deviation were computed using SPSS Statistics 16 (Armonk, NY, USA). The differences in the variables between the groups were analyzed using ANOVA.

3. Results and Discussion

3.1. Gene expression analysis

The mRNA levels of HSL, CGI-58, and AMPK were quantified to determine the effect of HIIT and adenosine on the genetic expression of adipose tissue. There is a statistically significant in HSL and AMPK ($p=0.009$) gene expression, as well as CGI-58 ($p=0.028$), in HFD + adenosine compared to HFD control. Furthermore, it was observed that HSL ($p=0.005$), AMPK ($p=0.004$), and CGI-58 ($p=0.003$) gene expression were significantly different in the HFD + adenosine + HIIT compared to the HFD. These results aligned with the HFD + HIIT group (**Figure 1**).

Table 1. Primer sequences of genes

Gene	Primer sequences
CGI-58	ATCAGCAGCAGCAGCCAA (forward) CCTGTTAGCAGAAGACAGTCCTTA (reverse)
AMPK	GCTTACCGATGACCACGATC (forward) TTCATTCACAGCGAGGTTGC (reverse)
HSL	TCACTGGTTTCAGCCTCTTCC (forward) ATGAGACAGCCCCGAGAT (reverse)
GAPDH	CATACTCAGCACCAGCATCACC (forward) AAGTTCAACGGCACAGTCAAGG (reverse)

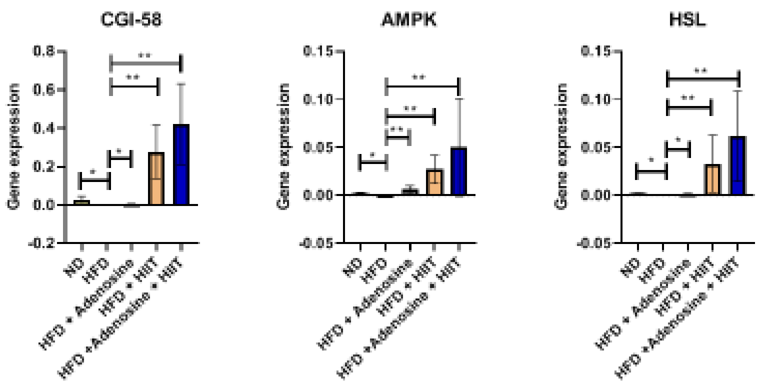

Figure 1. Gene expression of CGI-58, AMPK, and HSL in the study groups. The results are expressed as mean ± SD for each group. Individual data points within the columns are indicated with dots. The lines connecting the columns indicate that groups differ, and * denotes $p<0.05$, and ** denotes $p<0.01$.

3.2. FFAs analysis

Additionally, after collecting blood samples, the serum levels of FFAs were quantified. Due to the composition of the diet, the HFD group exhibited the most elevated concentration of FFAs, surpassing that of the other groups. Notably, the concentrations of FFAs in the HFD + adenosine, HFD + adenosine + HIIT, and HFD + HIIT were markedly lower in comparison to the HFD group (with respective p-values of 0.009, 0.006, and 0.004) (**Figure 2**).

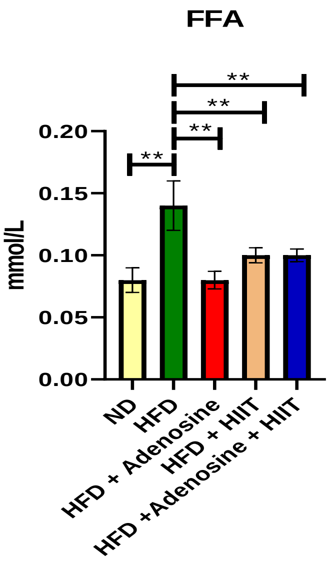

Figure 2. Serum concentration of FFA in the study groups. The results are expressed as mean ± SD for each group. Individual data points within the columns are indicated with dots. The lines connecting the columns indicate that groups differ, and * denotes p<0.05, and ** denotes p<0.01.

3.3. Glycemic indices

There is a significant difference in the serum level of glucose (P= 0.000) and HOMA-IR (P= 0.041). However, serum insulin levels did not differ significantly between groups (P= 0.293). Moreover, LSD post hoc test results showed that there is a significant difference in the serum level of glucose (P= 0.000) between the ND and HFD groups. Additionally, the serum level of glucose was lower in HFD + HIIT (P= 0.002) and HFD + HIIT + adenosine (P= 0.011) compared to the HFD. Also, HOMA-IR was significantly lower in HFD + HIIT + adenosine (P= 0.009) (**Figure 3**).

3.4. Lipid profile

The serum levels of TG (P= 0.000), LDL, and VLDL (P= 0.009) in the HFD were significantly increased compared to the ND group. Meanwhile, the serum level of HDL (P= 0.025) showed a significant decrease. Adenosine injection along with HFD improved serum levels of lipid profile (decrease of TG, TC (P= 0.000), LDL (P= 0.025), and VLDL (P= 0.009)) compared to HFD. These results were in line with the results of the HIIT + HFD, although the serum level of TC did not show a significant difference. It should be mentioned that the injection of adenosine and HIIT had similar results to the injection of it alone. Comparing each of the interventions alone, HIIT, although it led to improvements in HDL (P= 0.025), was associated with an increase in LDL (P= 0.025) and TC (P= 0.000) serum levels (**Figure 4**).

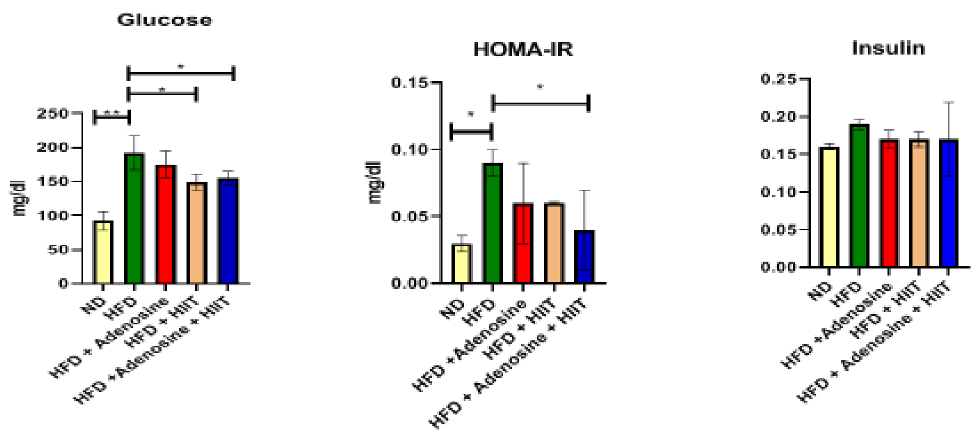

Figure 3. Serum concentration of glycemic indices (glucose, HOMA-IR, and insulin) in the study groups. The results are expressed as mean ± SD for each group. Individual data points within the columns are indicated with dots. The lines connecting the columns indicate that groups differ, and * denotes p<0.05, and ** denotes p<0.01.

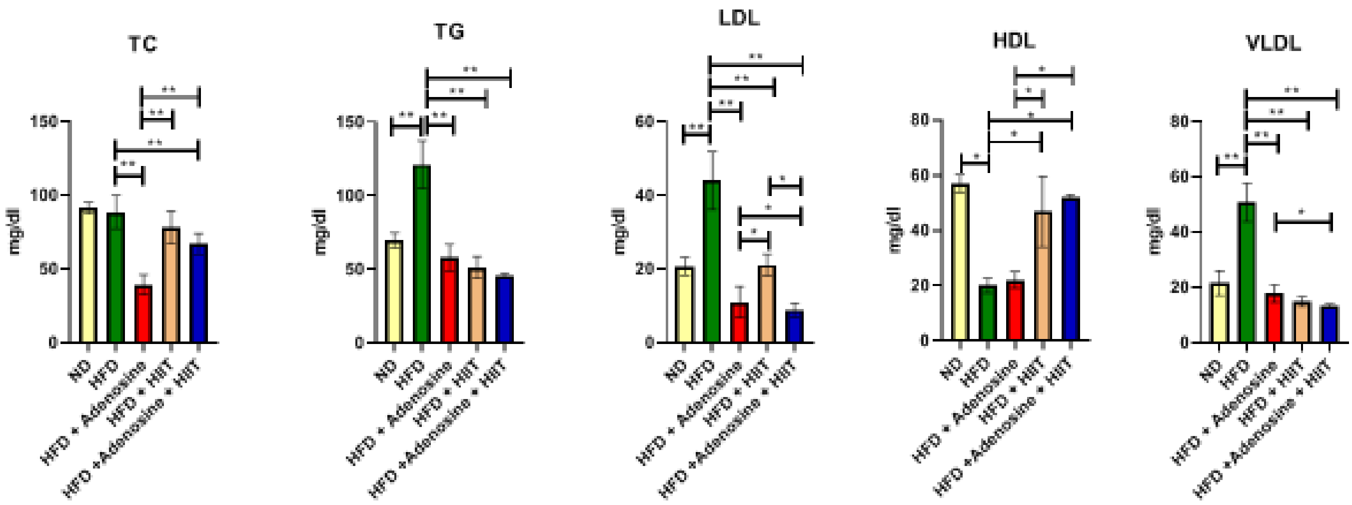

Figure 4. Serum concentration of lipid profile in the study groups. The results are expressed as mean ± SD for each group. Individual data points within the columns are indicated with dots. The lines connecting the columns indicate that groups differ, and * denotes p<0.05, and ** denotes p<0.01.

Obesity is a prevalent health issue that has been rapidly spreading worldwide. The lipid accumulation in adipose tissue, resulting from an imbalance between production and energy utilization, characterizes this condition. While lifestyle modifications have a limited impact on reducing obesity and current treatments, they present certain challenges, so identifying a suitable treatment approach can prove beneficial. Research has indicated that adenosine can influence fat metabolism by regulating lipogenesis and lipolysis. Additionally, HIIT led to obesity prevention by playing a role in this process. Consequently, we want to investigate the effect of HIIT and adenosine, either alone or in combination, in obese rats fed HFD.

Our results determined that intervention with adenosine, HIIT, and combination led to an elevation AMPK, HSL, and CGI-58 gene expression, facilitating fat lipolysis in the adipose tissue in rats fed HFD. These findings were in line with earlier studies that also reported an augmentation in HSL expression subsequent to endurance exercise [23]. Additionally, another study assessed the effect of HIIT on HSL expression in the adipose tissue in mice. The results showed that HIIT increased the HSL phosphorylation at Ser563 and Ser660 compared to the non-training group [24]. The effect of HIIT on the expression of key genes-related lipid metabolic processes in adipose tissue of obese elderly individuals was examined. The findings revealed a significant elevation in the expression of the CIDEA (an important regulator of fat metabolism) gene.

In contrast, no significant alternations were observed in the expression of the HSL and CGI-58 genes [25]. These non-significant results may be due to the relatively short period of the training protocols, which may not have been adequate to induce notable adaptations in the HSL gene. Few studies have been revealed on the effects of exogenous adenosine compared to the extensive research on the effects of adenosine receptor agonists, which mimic adenosine in biological processes. Furthermore, it has been demonstrated that adenosine promotes lipid catabolism in cultured human adipocytes and in the brown fat of mice, similar to the effects observed when the mice were administered with A2A and A2B receptor agonists [26].

It has been found that the activation of A1 adenosine receptors inhibits lipolysis and decreases circulating FFA concentrations by suppressing adenylyl cyclase activity and reducing cAMP production [27]. In this regard, a study revealed that adenosine injections cause a reduction in the expression of the A1 receptor, contributing to an increase in adipogenesis and a decrease in lipolysis in heart tissue [28]. A compound known as CVT-3619, which acts as a partial agonist for the A1 adenosine receptor, has been demonstrated to selectively inhibit lipolysis and decrease the levels of circulating FFAs [29]. Furthermore, our findings showed that intervention with adenosine, HIIT, or both of them can cause a decrease in the serum levels of FFA. HIIT additionally resulted in a decline in circulating FFAs in the obese rats. This decrease is attributed to the enhancement of fat oxidation and its uptake for metabolic purposes. The increase in fatty acid β-oxidation can be attributed to the adaptive response of the environment, which involves improvements in mitochondrial content and function. These adaptations encompass an augmentation in mitochondrial volume and the transfer of FFAs to mitochondria and muscle. The transportation process at each location involves numerous proteins in the plasma and mitochondrial membranes, such as FABP and FAT/CD36. The augmentation of fatty acid transfer proteins correlates to a partial increase in the oxidation of fatty acids in somatic muscle post-exercise. HIIT can potentially enhance FABP and FAT/CD36, thereby increasing somatic muscle fatty acid oxidation (29).

Furthermore, HIIT can also boost the production of mitochondrial enzymes. Moreover, the enhancement in mitochondrial oxidative capacity can potentially ameliorate the ability to oxidize lipids by activating AMP-activated PKA. Our findings are in contrast to a recent investigation that was carried out to establish the impact of HIIT on the breakdown of adipose tissue in aged rats. This study exemplified that HIIT augmented the levels of circulating FFAs and stimulated the expression of ATGL and HSL mRNA and protein in the adipose tissue surrounding the kidneys [23]. Correspondingly, a prior investigation examining the effect of HIIT on postprandial lipemia in healthy males found that HIIT did not notably influence the concentrations of FFAs [30], which is in alignment with a study conducted on obese Zucker rats that demonstrated negligible alterations in FFA concentrations due to physical exercise [31]. The HSL gene expression increased in HFD + HIIT and HFD + Adenosine + HIIT groups, although the serum level of FFA decreased. This may be due to the regulatory mechanism of HSL, which can be influenced by post-translational modifications (e.g., phosphorylation). High gene expression does not necessarily translate to high enzyme activity. Moreover, the decrease in FFA might result from their utilization following the interventions. During exercise, adenosine levels often rise, which may reduce plasma FFAs as energy sources shift towards glucose and other substrates [32, 33]. Indeed, Adenosine may enhance energy expenditure through its action in other organs, such as somatic muscle. Increased energy expenditure may lead to reduced availability of FFAs by promoting their energy utilization rather than allowing them to accumulate in the plasma.

The results demonstrated that interventions improve serum levels of glucose. However, this effect was greater in HIIT groups. Although the systematic review showed that HIIT was more efficient than moderate-intensity continuous training (MICT) in reducing TC, no significant alternations were observed in the other indicators. Additionally, the findings indicated that training programs lasting from medium to long duration (over 8 weeks) and at moderate to high intensity (80%-89%) had a more pronounced impact on most glycolipid metrics [34]. Previous research has not provided consistent findings regarding the effect of HIIT on glycolipid metabolism in individuals with type 2 diabetes. These discrepancies may arise from insufficient data concerning the HIIT intervention protocols (such as training intensity, frequency, and total duration) and patient characteristics (including age and diabetes duration) [34, 35].

In contrast, Winding et al. demonstrated that HIIT reduced HbA1c, fasting blood glucose, postprandial blood glucose, glycemic variability, and HOMA-IR [36]. Hayes et al. determined the effects of HIIT on fasting insulin, glucose, and insulin resistance in inactive older men. They reported that HIIT following aerobic preconditioning did not have a notable effect on insulin levels [37]. On the other hand, Søgaard D et al. found that after six weeks of HIIT, there was an elevation in insulin sensitivity and a reduction in HbA1c, but this effect was observed only in males [38]. HIIT improved peripheral insulin sensitivity by raising GLUT-4, increasing aerobic enzyme capacity and mitochondrial synthesis [39], and research examining the effects of HIIT on individuals with prevalent metabolic diseases has reported no alterations in HOMA-IR [40]. However, our findings showed non-significant differences in insulin levels.

On the other hand, Adenosine is linked to both lipid catabolism and glucose utilization. It has been suggested that adenosine acts on its receptors in adipose tissue to enhance insulin sensitivity. As a result, studies using adenosine-based pharmacological agents have shown improvements in glucose tolerance [41, 42]. Indeed, activating the adenosine A1 receptor initiated Ca2+-mediated glycogen metabolism, while the adenosine A2A receptor stimulation promoted cAMP-mediated glucose biosynthesis. Additionally, activation of the adenosine A3 receptor raised intracellular calcium levels ($[Ca^{2+}]_{cyt}$) and lowered cAMP, resulting in only slight modifications to glycogen metabolism [43]. Adenosine inhibits lipolysis and may improve insulin sensitivity through the activation of PKA. Insulin inhibits the activation of HSL triggered by glucagon or norepinephrine. In human adipocytes, the presence of adenosine enhances insulin's ability to block the lipolytic effect of norepinephrine. Likewise, activating the adenosine A1 receptor in rat adipocytes using pharmacological agents demonstrated similar effects on enhancing insulin sensitivity and consequently inhibiting lipolysis [44].

In this study, we observed that HIIT and adenosine alone or both of them can improve the lipid profile. Contrary to our results, previous studies have mentioned that exercise does not have a notable effect on lipid profile [45-47]. On the other hand, it was also mentioned that HIIT caused a significant decrease in TG and TC but had no effect on HDL and LDL serum levels [48]. It has been shown that the severity of exercise, such as HIIT, can increase growth hormones, adrenaline, and fat oxidation, the first two of which have lipolytic effects and can be considered favorable effects of HIIT [49-51]. Also, the high intensity of the exercise causes growth hormone elevation, which can exert more favorable effects on the lipid profile [50]. It has also been mentioned that HIIT can improve TC through its effect on 3-hydroxyacyl CoA dehydrogenase (β-HAD), a mitochondrial enzyme involved in lipid oxidation [52].

4. Conclusion

It appears that the combination of HIIT and adenosine administration can have a beneficial impact on lipid metabolism by enhancing the process of lipolysis in adipose tissue. Additionally, adenosine can enhance the effects of HIIT on gene expression, resulting in the initiation of lipolysis as a means to prevent obesity.

Ethics approval

This study has been approved by the research ethics committee of the Sports Science Research Institute (IR.SSRI.REC.1395.115).

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

Acknowledgment

The authors thank the staff of Shahid Mirghani Research Institute in Golestan province.

Data availability

The data that support the findings of this study are available on request from the corresponding author

Athors Contributions

Conception and design of the study: Zahra Eslami and Seyed Javad Mirghani

Analysis and interpretation of data: Ayda Fallah, Abdorreza Eghbal Moghanlou, Giyasettin Baydaş

Drafting the article: Zahra Eslami, Ayda Fallah, and Shohreh Sharifian

Revising the article: Zahra Eslami, and Seyed Javad Mirghani

Supervisor the article: Seyed Javad Mirghani

Athors Orcid numbers:

Zahra Eslami: 0000-0002-3187-3977
Ayda Fallah: 0009-0001-1656-9117
Abdorreza Eghbal Moghanlou: 0000-0003-1238-0541
Giyasettin Baydas: 0000-0002-9206-3177
Shohreh Sharifian: 0000-0003-4399-6189
Seyed Javad Mirghani: 0000-0002-4846-3077

Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

References

1. Chen, S., et al., *Suppression of high-fat-diet-induced obesity in mice by dietary folic acid supplementation is linked to changes in gut microbiota.* Eur J Nutr, 2022. **61**(4): p. 2015-2031.

2. Kong, Z., et al., *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**(7): p. e0158589.
3. Pardo, F., et al., *Molecular implications of adenosine in obesity.* Mol Aspects Med, 2017. **55**: p. 90-101.
4. Xu, H., et al., Distinct AMPK-Mediated FAS/HSL Pathway Is Implicated in the Alleviating Effect of Nuciferine on Obesity and Hepatic Steatosis in HFD-Fed Mice. Nutrients, 2022. 14(9).
5. Steinbeck, K.S., et al., Treatment of adolescent obesity. Nat Rev Endocrinol, 2018. 14(6): p. 331-344.
6. Tak, Y.J. and S.Y. Lee, Long-Term Efficacy and Safety of Anti-Obesity Treatment: Where Do We Stand? Curr Obes Rep, 2021. 10(1): p. 14-30.
7. Jin, Y.J., et al., Anti-Obesity Effects of Polymethoxyflavone-Rich Fraction from Jinkyool (Citrus sunki Hort. ex Tanaka) Leaf on Obese Mice Induced by High-Fat Diet. Nutrients, 2022. 14(4).
8. Zabielska, M.A., et al., Inhibition of AMP deaminase as therapeutic target in cardiovascular pathology. Pharmacol Rep, 2015. 67(4): p. 682-8.
9. D'Antongiovanni, V., et al., Managing Obesity and Related Comorbidities: A Potential Pharmacological Target in the Adenosine System? Front Pharmacol, 2020. 11: p. 621955.
10. Pekkala, S., et al., High-intensity interval training and moderate intensity training with exogenous adenosine counteract development of obesity in rats. Science & Sports, 2022.
11. Martins, C., 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(3): p. 197-204.
12. Liu, Y., et al., Comparison of visceral fat lipolysis adaptation to high-intensity interval training in obesity-prone and obesity-resistant rats. Diabetol Metab Syndr, 2022. 14(1): p. 62.
13. Issekutz Jr, B., Role of beta-adrenergic receptors in mobilization of energy sources in exercising dogs. Journal of applied physiology, 1978. 44(6): p. 869-876.
14. Khalafi, M., et al., The impact of high-intensity interval training on postprandial glucose and insulin: A systematic review and meta-analysis. Diabetes Res Clin Pract, 2022. 186: p. 109815.
15. Szkudelski, T. and K. Szkudelska, Regulatory role of adenosine in insulin secretion from pancreatic β-cells--action via adenosine A_1 receptor and beyond. J Physiol Biochem, 2015. 71(1): p. 133-40.
16. Laurens, C., et al., Influence of Acute and Chronic Exercise on Abdominal Fat Lipolysis: An Updat*e.* Front Physiol, 2020. **11**: p. 575363.
17. Wojcik, B., et al., *Effect of tachycardia on mRNA andf protein expression of the principal compon*ents of the lipolytic system in the rat's heart ventricles. J Physiol Pharmacol, 2017. 68(5): p. 731-736.
18. Rodríguez, C., et al., AMPK, metabolism, and vascular function. Febs j, 2021. 288(12): p. 3746-3771.
19. Wang, Q., et al., AMPK-Mediated Regulation of Lipid Metabolism by Phosphorylation. Biol Pharm Bull, 2018. 41(7): p. 985-993.
20. Roepstorff, C., et al., Regulation of hormone-sensitive lipase activity and Ser563 and Ser565 phosphorylation in human skeletal muscle during exercise. J Physiol, 2004. 560(Pt 2): p. 551-62.
21. Mirghani, S.J., 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(1): p. e10753.
22. Ferreira, J.C., et al., Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol, 2007. 34(8): p. 760-5.
23. Sun, L., et al., Effects of high-intensity interval training on adipose tissue lipolysis, inflammation, and metabolomics in aged rats. Pflugers Arch, 2020. 472(2): p. 245-258.
24. Liu, Y., et al., Post-exercise Effects and Long-Term Training Adaptations of Hormone Sensitive Lipase Lipolysis Induced by High-Intensity Interval Training in Adipose Tissue of Mice. Front Physiol, 2020. 11: p. 535722.
25. Youssef, L., et al., Clinical and Biological Adaptations in Obese Older Adults Following 12-Weeks of High-Intensity Interval Training or Moderate-Intensity Continuous Training. Healthcare (Basel), 2022. 10(7).
26. Gnad, T., et al., Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. Nature, 2014. 516(7531): p. 395-399.
27. Dhalla, A.K., et al., Pharmacology and therapeutic applications of A1 adenosine receptor ligands. Curr Top Med Chem, 2003. 3(4): p. 369-85.
28. Farsi, S., N. Ahmadi, and M.A. Azarbayjani, Effect of continuous and interval training with adenosine consumption on A1 and A2A adenosine receptors in heart tissue of obese rats. Gene, Cell and Tissue, 2020.
29. Dhalla, A.K., et al., A1 adenosine receptor partial agonist lowers plasma FFA and improves insulin resistance induced by high-fat diet in rodents. Am J

Physiol Endocrinol Metab, 2007. 292(5): p. E1358-63.
30. Wilhelmsen, A., et al., Chronic effects of high-intensity interval training on postprandial lipemia in healthy men. J Appl Physiol (1985), 2019. 127(6): p. 1763-1771.
31. Maillard, F., et al., High intensity interval training promotes total and visceral fat mass loss in obese Zucker rats without modulating gut microbiota. PLoS One, 2019. 14(4): p. e0214660.
32. Dworak, M., et al., Intense exercise increases adenosine concentrations in rat brain: Implications for a homeostatic sleep drive. Neuroscience, 2007. 150(4): p. 789-795.
33. Muscella, A., et al., The Regulation of Fat Metabolism During Aerobic Exercise. Biomolecules, 2020. 10(12).
34. Feng, J., et al., Effects of high-intensity intermittent exercise on glucose and lipid metabolism in type 2 diabetes patients: a systematic review and meta-analysis. Frontiers in Endocrinology, 2024. 15.
35. Liu, J.-x., et al., Effectiveness of high-intensity interval training on glycemic control and cardiorespiratory fitness in patients with type 2 diabetes: a systematic review and meta-analysis. Aging clinical and experimental research, 2019. 31: p. 575-593.
36. Winding, K.M., et al., The effect on glycaemic control of low-volume high-intensity interval training versus endurance training in individuals with type 2 diabetes. Diabetes, Obesity and Metabolism, 2018. 20(5): p. 1131-1139.
37. Hayes, L.D., et al., High intensity interval training (HIIT) produces small improvements in fasting glucose, insulin, and insulin resistance in sedentary older men but not masters athletes. Exp Gerontol, 2020. 140: p. 111074.
38. Søgaard, D., et al., High-intensity interval training improves insulin sensitivity in older individuals. Acta Physiol (Oxf), 2018. 222(4): p. e13009.
39. Simoneau, J.A., et al., Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. The FASEB Journal, 1995. 9(2): p. 273-278.
40. Robinson, E., et al., Short-term high-intensity interval and moderate-intensity continuous training reduce leukocyte TLR4 in inactive adults at elevated risk of type 2 diabetes. Journal of applied physiology, 2015. 119(5): p. 508-516.
41. Crist, G.H., et al., Tissue-specific effects of in vivo adenosine receptor blockade on glucose uptake in Zucker rats. The FASEB journal, 1998. 12(13): p. 1301-1308.
42. Xu, B., et al., A1 adenosine receptor antagonism improves glucose tolerance in Zucker rats. American Journal of Physiology-Endocrinology and Metabolism, 1998. 274(2): p. E271-E279.
43. González-Benítez, E., et al., Regulation of glycogen metabolism in hepatocytes through adenosine receptors. Role of Ca2+ and cAMP. European Journal of Pharmacology, 2002. 437(3): p. 105-111.
44. Koupenova, M. and K. Ravid, Adenosine, adenosine receptors and their role in glucose homeostasis and lipid metabolism. J Cell Physiol, 2013.
45. Ghasemi, E., M.E. Afzalpour, and A. Zarban, Effect of a 10 week high intensity interval training supplemented with green tea on lipid profiles and body composition in overweight women. Journal of Birjand University of Medical Sciences, 2016. 23(3): p. 198-210.
46. Khammassi, M., et al., Impact of a 12-week high-intensity interval training without caloric restriction on body composition and lipid profile in sedentary healthy overweight/obese youth. J Exerc Rehabil, 2018. 14(1): p. 118-125.
47. Gharari Arefi, R., M. Hemati Nafar, and M.R. Kordi, The Effect of a High Intensity Interval Training Program on Lipid Profile in Sedentary Young Men. Journal of Sport Biosciences, 2014. 6(3): p. 259-272.
48. Hemmatinafar, A., M. Fathi, and M.M. Ziaaldini, Effect of 8 weeks of HIIT on hepatic enzyme levels, lipid profile and body composition in overweight young men. Obesity Medicine, 2020. 18: p. 100233.
49. Bahr, R., et al., Effect of exercise on recovery changes in plasma levels of FFA, glycerol, glucose and catecholamines. Acta Physiol Scand, 1991. 143(1): p. 105-15.
50. Pritzlaff, C.J., et al., Catecholamine release, growth hormone secretion, and energy expenditure during exercise vs. recovery in men. J Appl Physiol (1985), 2000. 89(3): p. 937-46.
51. Soria, M., et al., Fat Oxidation Rate as a Function of Plasma Lipid and Hormone Response in Endurance Athletes. J Strength Cond Res, 2020. 34(1): p. 104-113.
52. Burgomaster, K.A., et al., Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol, 2008. 586(1): p. 151-60.