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## Effect of Eight Weeks of High-Intensity Interval Training (HIIT) on Serum Asprosin Levels and Body Composition in Overweight and Obese Men



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### ABSTRACT

**Objective:** This study aimed to investigate the impact of 8 weeks of high-intensity interval training (HIIT) on serum Asprosin levels and body composition in overweight and obese men.

**Methods and Materials:** A total of 36 overweight and obese men with a body mass index (BMI) of 25 to 34.9 aged between 30 and 35 years, were voluntarily selected and randomly assigned to either the HIIT group or a control group in a semi-experimental, pre-post study design. The HIIT intervention lasted for eight weeks, consisting of three sessions per week. Asprosin was measured using ELISA, while body composition was assessed via InBody 230 analysis. Statistical comparisons were performed using ANCOVA with significance set at  $\alpha < 0.05$ .

**Findings:** Following the intervention, the HIIT group exhibited significant reductions in serum asprosin levels ( $F = 134.06, p < 0.01$ ), body fat percentage ( $F = 186.79, p < 0.01$ ), BMI ( $F = 25.36, p < 0.01$ ), lean body mass ( $F = 9.88, p = 0.004$ ), and waist-to-hip ratio ( $F = 9.88, p < 0.01$ ).

**Conclusion:** These findings support the role of HIIT as an effective non-pharmacological strategy for reducing serum Asprosin levels and improving body composition in overweight and obese populations.

**Keywords:** High-intensity Interval Training, Asprosin, Body Composition, Obese Men

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## 1. Introduction

Obesity has become a pervasive global health concern over recent decades, with its prevalence rising alarmingly worldwide (1). According to the World Obesity Atlas 2023, the prevalence of overweight and obesity was nearly 38% in 2020 and is projected to escalate 42% by 2025, 46% by 2030, and 51% by 2035 (2). Obesity significantly increases the risk of numerous health complications, including type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), metabolic syndrome, chronic kidney disease, hyperlipidemia, hypertension, nonalcoholic fatty liver disease (NAFLD), certain types of cancer, obstructive sleep apnea, osteoarthritis, and depression (3). The World Health Organization classifies overweight and obesity as abnormal or excessive fat accumulation that poses a health risk (4). Initially perceived solely as an energy reservoir, adipose tissue is now recognized as an active endocrine organ that secretes a wide range of bioactive molecules, including adipokines. These substances influence metabolism through autocrine, paracrine, and endocrine mechanisms (5, 6).

In recent years, considerable interest has emerged regarding adipose-derived hormones that modulate skeletal muscle and systemic metabolism. One such hormone is Asprosin, a newly discovered adipokine released during fasting (7, 8). Asprosin travels to the liver, where it triggers gluconeogenesis via a cAMP-mediated pathway activated through G protein-coupled receptors (GPCRs) (9, 10). This cascade raises intracellular cAMP levels, stimulating protein kinase A (PKA), and subsequently promoting hepatic glucose release into the bloodstream. Beyond its hepatic effects, Asprosin can cross the blood-brain barrier and act on the central nervous system. It stimulates appetite under fasting conditions and helps regulate energy balance (8, 9, 11). However, in conditions such as obesity, insulin resistance, and metabolic syndrome, Asprosin levels are pathologically elevated, exacerbating hyperphagia and disrupting energy homeostasis (11-13).

Asprosin's association with obesity and insulin resistance has drawn attention to its therapeutic potential. Although genetic predisposition plays a role in obesity, lifestyle and environmental factors are now considered more significant (14). The appropriate exercise selection is a critical component of lifestyle modification and an effective strategy for managing and treating obesity (15). Recent research has shown that high-intensity interval training (HIIT) induces a transient state of anorexia, delaying the

onset of hunger for a short period after exercise; however, this effect is temporary (15, 16). Physical activity creates a negative energy balance because of increased energy expenditure (15, 17). Some studies have shown that HIIT is one of the most effective forms of exercise for improving aerobic fitness, metabolic health, and cardiovascular health (18-20). Based on their research findings, some researchers have emphasized the importance of performing HIIT as bouts of very high-intensity exercise followed by low-intensity active recovery, compared with continuous exercise (21).

Despite having a lower overall training volume than continuous exercise, HIIT may have similar or even better effects on inflammation, body composition, and insulin sensitivity. However, this claim has not been fully substantiated (22). By focusing on HIIT, we can potentially observe changes in energy intake through alterations in hunger and appetite signals, as well as regulatory peptides influencing short-term and long-term signaling (19). Given the novelty of Asprosin and the limited data regarding its response to exercise, this study aimed to evaluate whether eight weeks of HIIT could significantly alter serum Asprosin levels and improve body composition in overweight and obese men.

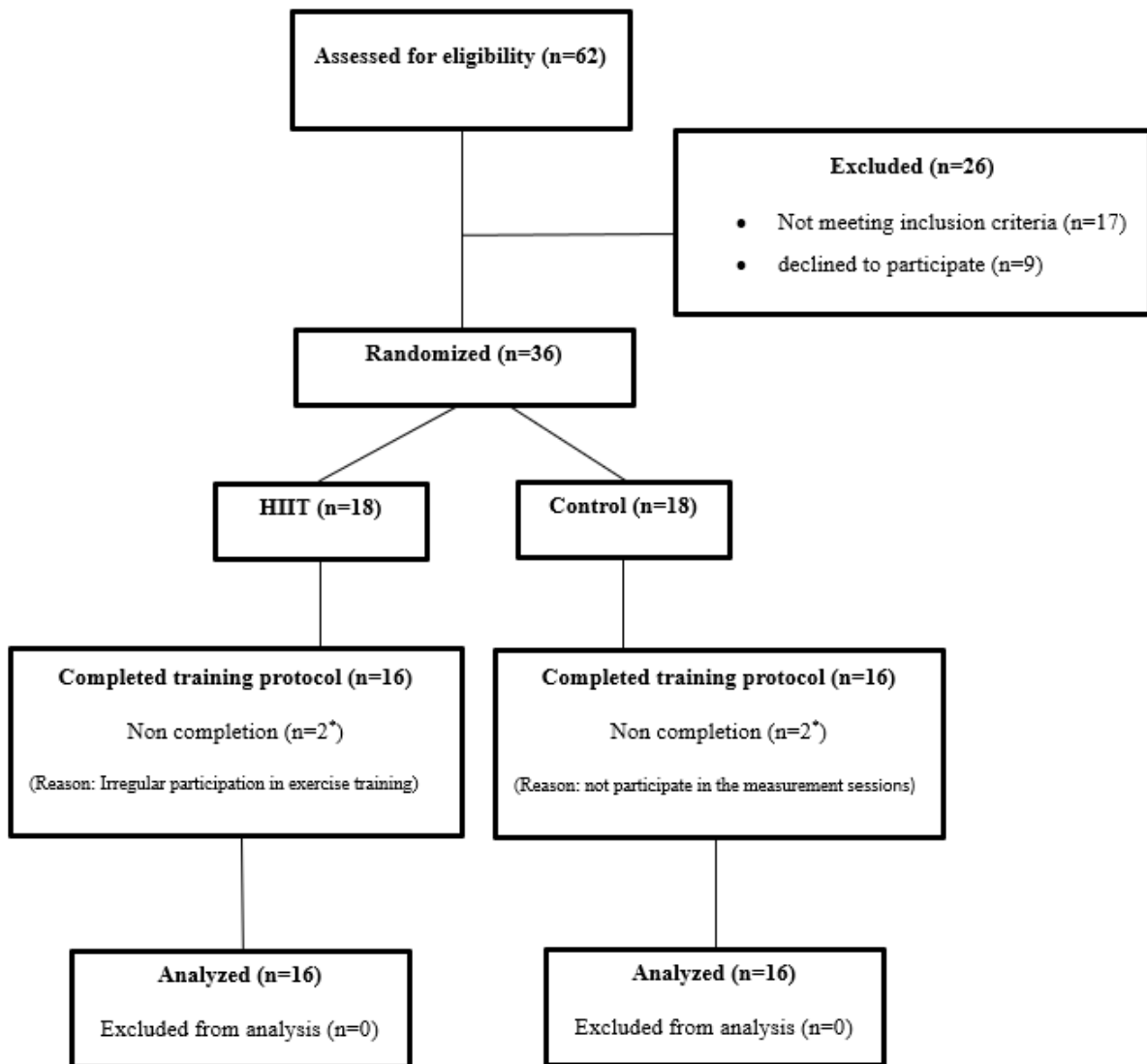
## 1. Methods and Materials

This study employed a quasi-experimental design with a pretest-posttest control group structure. Ethical approval was obtained from the University of Tabriz Ethics Committee (ID: IR.TABRIZU.REC.1403.111). The target population included sedentary Iraqi men aged 30 to 35 years residing in Karbala, Iraq, with a body mass index (BMI) of 25 to 34.9, corresponding to overweight and Class I obesity.

Inclusion criteria were: absence of chronic illnesses (e.g., cardiovascular disease, hypertension), no recent surgeries, no use of weight loss or anti-inflammatory medications, stable body weight during the past year, absence of neurological conditions or head trauma, and no contraindications to physical activity. Participants were recruited via public announcements. The required sample size was calculated using G\*Power version 3.1 (University of Düsseldorf, Germany). Based on an expected medium effect size ( $f = 0.25$ ),  $\alpha = 0.05$ , power  $(1-\beta) = 0.80$ , and ANCOVA with two groups and one covariate, the minimum required sample size was estimated to be 34 participants. To accommodate potential dropouts, 36 participants were

recruited. Following screening, 36 eligible volunteers were randomly assigned to the HIIT (n = 18) or control (n = 18) group. Randomization was performed at the individual level using Randomizer software. Allocation concealment was ensured by using opaque, sealed envelopes. Group assignments were coded, and the codes were securely held by an independent monitor until the completion of the allocation process.

Prior to the intervention, participants provided written informed consent, completed a general health questionnaire, and submitted a 24-hour dietary recall. Exclusion criteria included medical complications, injury preventing exercise participation, absence from more than two sessions, or failure to perform exercises properly. Consequently, four participants (two from each group) were excluded, resulting in 16 participants per group in the final analysis (Fig.1).



**Figure 1.** Study flow diagram

The training intervention consisted of a two-week familiarization period followed by an eight-week HIIT program, with three sessions per week. All sessions were supervised and conducted under the guidance of the investigator. During the first week of the familiarization period, participants performed one set of three running repetitions, each lasting 10 seconds at 50–70% of maximal

oxygen uptake velocity (vVO<sub>2</sub>max), with a 60-second rest interval between repetitions. In the second week of familiarization, participants completed two sets of HIIT, each set comprising three running repetitions of 10 seconds at 70–90% vVO<sub>2</sub>max with 90 seconds of rest between repetitions and sets. From the third week onward, the main eight-week HIIT program was implemented, consisting of

2–4 sets per session, with five repetitions of 20-second runs at 100–140% vVO<sub>2</sub>max (Table 1). Rest intervals between sets (active and passive) and between repetitions (passive) were 180 seconds and 60–80 seconds, respectively (Table 1).

Also, exercise intensity was monitored using the Borg Rating of Perceived Exertion (RPE) scale (23).

Blood samples (10 mL) were drawn from the antecubital vein in a seated position by a trained technician. Collections were performed at 7:30–8:00 AM following a 12-hour overnight fast, both at baseline and 48 hours after the final training session. Serum Asprosin levels were measured using a commercial ELISA kit (EH4176, Wuhan Fine Biotech Co., Ltd., China), with an intra-assay coefficient of variation of less than 8%. Fasting blood glucose levels were measured using a kit (cat. no. 3L82-22), and HbA1C was measured using a kit (cat. no. 4P52-20) with an Abbott Architect™ C 4000 automated analyzer (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA).

Body composition was evaluated in fasting conditions using an InBody 230 bioelectrical impedance analyzer at a constant room temperature of 23°C.

Statistical analysis was conducted using SPSS version 26. Descriptive statistics were reported as

means ± standard deviations. Normality of data distribution was assessed using the Shapiro-Wilk test. Baseline differences between the HIIT and control groups were examined using an independent samples t-test. Analyze of Covariance (ANCOVA) was used to assess between-group differences. Statistical significance was set at  $\alpha < 0.05$ .

## 2. Results

No adverse events were reported in athletes from either group. Initially, 18 participants were allocated to each group. Ultimately, 16 participants in each group completed the study (Fig 1). Two participants from each group were excluded from the final analysis due to irregular participation in the training sessions and failure to attend the assessment session.

**Table 1.** High-Intensity Interval Training (HIIT) Protocol

Week	Sessions per week	Sets per session	Repetitions per set	Repetition duration (second)	Rest between repetitions (second)	Work-to-rest ratio	Rest between sets (seconds)	Total running duration per session (second)	Percentage of vVO <sub>2</sub> MAX (m.s)
1	3	2	5	20	80	0.25	180	200	100-110
2	3	2	5	20	80	0.25	180	200	110-120
3	3	3	5	20	80	0.25	180	300	120-130
4	3	3	5	20	70	0.28	180	300	120-130
5	3	3	5	20	70	0.28	180	300	120-130
6	3	4	5	20	70	0.28	180	400	130-140
7	3	4	5	20	60	0.33	180	400	130-140
8	3	4	5	20	60	0.33	180	400	130-140

Table 2 presents the descriptive and inferential statistics comparing anthropometric and biochemical variables before and after the intervention. ANCOVA revealed statistically significant between-group differences in weight (F = 105.61,  $p < 0.01$ , Partial Eta Squared = 0.79), FBS (F = 82.49,  $p < 0.01$ , Partial Eta Squared = 0.74), HbA1c (F = 201.32,  $p < 0.01$ , Partial Eta Squared = 0.87), triglycerides (F = 196.52,

$p < 0.01$ , Partial Eta Squared = 0.87), total cholesterol (F = 228.93,  $p < 0.01$ , Partial Eta Squared = 0.88), and LDL-C (F = 224.71,  $p < 0.01$ , Partial Eta Squared = 0.88), and HDL-C (F = 9.59,  $p = 0.04$ , Partial Eta Squared = 0.24). A significant decrease was observed in weight, fasting blood glucose, x. and also HDL-C increased

**Table 2.** Descriptive characteristics of the participants

Groups	Baseline	8 weeks	Percentage change	F	P <sup>#</sup>	Partial Eta Squared ( $\eta^2p$ )
<b>Weight (kg)</b>						
HIIT	94.11±12.22	92.97±12.17	-1.211	105.61	0.001	0.79
Control	91.66±9.88	91.74±9.92	0.087			
<b>FBS (mg/dL)</b>						
HIIT	91.81±6.51	86.06±5.96	-6.263	82.49	0.001	0.74
Control	89.31±5.92	89.94±6.30	0.705			
<b>HbA1c</b>						
HIIT	4.87±0.23	4.67±0.22	-4.107	201.32	0.001	0.874
Control	4.77±0.21	4.88±0.20	2.306			
<b>Triglycerides (mg/dL)</b>						
HIIT	165.19±22.75	145.75±20.24	-11.77	196.52	0.001	0.871
Control	160.44±20.10	162.63±19.64	1.36			
<b>Total Cholesterol (mg/dL)</b>						
HIIT	154.56±30.76	146.06±28.89	-5.50	228.93	0.001	0.888
Control	144.75±26.92	145.44±26.88	0.48			
<b>LDL-C (mg/dL)</b>						
HIIT	85.94±17.65	77.88±16.97	-9.38	224.71	0.001	0.886
Control	91.88±12.87	92.44±12.55	0.61			
<b>HDL-C (mg/dL)</b>						
HIIT	36.13±3.76	36.69±3.34	1.55	9.59	0.004	0.249
Control	36.06±3.17	34.94±2.99	-3.11			

Data are presented as Mean ± SD, <sup>#</sup> Inter-group comparisons with ANCOVA.

Table 3 presents the results for Asprosin and body composition variables. The results of the independent samples t-test indicated no statistically significant differences between the HIIT and control groups in baseline characteristics ( $p > 0.05$ ). According to ANCOVA results, there were significant differences between the HIIT and the control group in Asprosin ( $F = 134.06$ ,  $p < 0.01$ , Partial Eta

Squared = 0.82), lean body mass ( $F = 9.88$ ,  $p = 0.04$ , Partial Eta Squared = 0.25), body fat percentage ( $F = 186.79$ ,  $p < 0.01$ , Partial Eta Squared = 0.86), BMI ( $F = 25.36$ ,  $p < 0.01$ , Partial Eta Squared = 0.46), and waist-to-hip ratio ( $F = 53.22$ ,  $p < 0.01$ , Partial Eta Squared = 0.64). These results suggest that HIIT significantly improved metabolic and body composition markers compared to the control group.

**Table 3.** Changes in study variables at pre- and post-test (Mean ±SD)

Groups	Baseline	P*	8 weeks	Percentage change	F	P <sup>#</sup>	Partial Eta Squared ( $\eta^2p$ )	95% CI	
								Lower	Upper
<b>Asprosin (ng/mL)</b>									
HIIT	15.91±5.39	0.476	12.29±4.92	-22.753	134.06	0.001	0.822	15.48	16.19
Control	19.01±8.47		19.39±8.49	1.999					
<b>Lean Body Mass (kg)</b>									
HIIT	66.10±7.24	0.304	65.91±7.36	-0.278	9.88	0.004	0.254	65.10	65.26
Control	64.42±5.38		64.46±5.39	0.062					
<b>Percent Body Fat (%)</b>									
HIIT	29.48±4.37	0.990	26.19±4.17	-11.160	186.79	0.001	0.866	27.60	28.11
Control	29.43±4.26		29.53±4.44	0.340					
<b>BMI (kg/m<sup>2</sup>)</b>									
HIIT	29.73±2.39	0.620	29.43±2.51	-1.009	25.36	0.001	0.467	29.60	29.73
Control	29.88±2.85		29.90±2.87	0.067					
<b>Waist-hip ratio</b>									
HIIT	0.962±0.039	0.561	0.950±0.039	-1.247	53.22	0.001	0.647	0.94	0.95
Control	0.944±0.033		0.946±0.034	0.212					

Data are presented as Mean ± SD, \* Independent T-test, <sup>#</sup> Inter-group comparisons with ANCOVA.

### 3. Discussion and Conclusion

The results of the between-group comparison test also demonstrated a significant difference in the mean scores of the exercise and control groups for the Asprosin variable. The HIIT group experienced a significant decrease in serum Asprosin levels. The eta squared value indicated that the effect size of the research intervention (HIIT exercise) was substantial, and eight weeks of high-intensity interval training had a significant effect on Asprosin in overweight and obese men. HIIT has been identified as one of the most effective strategies for managing overweight and obesity, particularly in males (24). With global rates of overweight and obesity surpassing 2.5 billion individuals in 2022 (25), there is an increasing demand for effective interventions to mitigate associated health complications, including metabolic disorders such as insulin resistance and type 2 diabetes (26, 27). Asprosin, a recently discovered hormone, has been found to play a crucial role in appetite regulation and hepatic glucose release, thereby contributing to elevated plasma glucose levels (10, 28). Studies have indicated that Asprosin acts as a central appetite stimulant, or a fasting-induced glucogenic protein hormone (11), via a signaling pathway similar to ghrelin, also known as lenomorelin or the "hunger hormone" (29). Asprosin deficiency is observed in patients with neonatal progeroid syndrome (NPS), whereas excess asprosin production is observed in conditions of insulin resistance and obesity (10, 28). In individuals with class I, II, and III obesity, Asprosin levels were two, three, and four times higher, respectively, than in the group with normal body mass (13). Therefore, it can be stated that Asprosin levels are associated with both glucose and fat metabolism, and its reduction improves insulin sensitivity (30). Liu et al. (2022) mentioned in their article that Asprosin, in cooperation with ghrelin, is beneficial for cachexia (severe weight loss) and complex metabolic syndromes (27). These findings suggest that Asprosin plays a crucial role in a range of metabolic-related diseases. Exercise has beneficial effects on the outcomes of metabolic disorders, including energy substrate redistribution, fat mass loss, and inflammation reduction (31). Exercise significantly influences Asprosin release, which may regulate related metabolism (32). Consistent with the results of the present study, Delfan et al. (2024), investigating the effect of high-intensity interval training on asprosin levels in obese men, found that after 12 weeks of training, a significant decrease in asprosin levels was observed (26). Dolatabadi et al.

(2023) investigated the effects of HIIT and high-intensity circuit training (HICT) on serum Asprosin levels in overweight and obese women. They concluded that after ten weeks of training, a significant decrease in Asprosin levels was observed in both the HIIT and HICT groups. The reduction in Asprosin was 49% in the HIIT group and 43% in the HICT group. This research also found that indices such as triglycerides, total cholesterol, LDL (significant only in the HIIT group), VLDL, weight, body mass index, waist circumference, hip circumference, body fat percentage, and fat mass showed significant reductions in the exercise group compared to pre-exercise levels (14, 19). In another study, Jahangiri et al. (2021) concluded that all three types of resistance training (traditional, circuit, and interval) led to a significant decrease in Asprosin levels in sedentary obese men and also improved body composition parameters (body weight, body mass index, body fat percentage, and WHR) compared to the control group. Interval resistance training had the greatest impact on reducing Asprosin levels and improving body composition outcomes (33). Kantorowicz et al. (2021), in a study of obese women with metabolic disorders, also concluded that Nordic walking (NW) led to a significant decrease in Asprosin (a 27% reduction after four weeks and a 41.9% reduction after eight weeks), WHR, waist-to-height ratio, and body adiposity index (BAI) (30). In contrast, the findings of some studies were not consistent with the results of the present research. In the research by Schumann et al. (2017), a significant increase in Asprosin was observed following anaerobic exercise (34). In the study by Wiecek et al. (2018), a non-significant increase was observed in men and a significant increase in women following acute anaerobic exercise (35). Considering that Asprosin causes the release of glucose from liver cells, and its removal represents a significant disruption in this process (10), blood glucose levels in women after anaerobic exercise may be a factor in inducing its secretion. The lowest glucose levels coincided with the highest Asprosin concentrations in the blood (35). Differences in findings may be attributed to the type of exercise protocol and the sex of the participants.

Given that body composition changes following weight gain and fat accumulation, physical activity is considered one of the most important factors in improving body composition (36). Considering the reduction in body composition indices observed in this study, the decrease in serum Asprosin levels can also be attributed to the reduction in body fat percentage, weight, and body mass. This is supported by several studies have demonstrated a relationship between serum levels of this variable and body

composition factors (7). To explain the effect of this variable, it can be stated that an increase in adipose tissue mass and dysfunction of adipose tissue (inefficient adipose tissue) leads to systemic fat overflow and low-grade inflammation through altered secretion of adipokines and cytokines, contributing to metabolic dysfunction (37, 38). Furthermore, increased fatty acid flow from adipose tissue may contribute to increased fat storage in the liver and skeletal muscles (ectopic fat deposition), which leads to altered hepatokine secretion, mitochondrial dysfunction, and impaired insulin signaling in skeletal muscles (37).

The interpretation of the findings of this study should be considered in light of its limitations. First, dietary intake was not continuously monitored or strictly controlled during the intervention period. Although a 24-hour dietary recall was obtained at baseline, variations in participants' dietary patterns throughout the 8-week intervention may have influenced asprosin levels and body composition. Second, other methodological limitation of this study is the absence of direct measurement or estimation of  $\text{VO}_2\text{max}$ . Consequently, training intensity was prescribed based on  $\text{vVO}_2\text{max}$  which may have influenced the precision of the training intensity determination. Third, in the present study, numerous paired t-tests were used to analyze the data. This approach may have increased the risk of Type I error and should be considered when interpreting the findings. Finally, in the present study, lean body mass decreased in the HIIT group after the intervention. This reduction may be partly attributed to limitations of the assessment method, as bioelectrical impedance analysis is sensitive to variations in hydration status and glycogen levels, which can influence the accuracy of lean mass estimation, particularly following high-intensity exercise. Therefore, the results should be interpreted with caution.

#### 4. Conclusion

This study demonstrates that high-intensity interval training (HIIT) significantly reduces serum Asprosin levels and improves key indicators of body composition in overweight and obese men. These effects are likely mediated through a combination of metabolic, inflammatory, and neuroendocrine mechanisms. Given the emerging role of Asprosin in energy regulation and metabolic disease, these findings underscore the therapeutic potential of HIIT as a non-pharmacological intervention for mitigating obesity-related complications. Future research should include long-term follow-up and explore the molecular mechanisms

underlying Asprosin regulation in response to various exercise modalities.

#### Authors' Contributions

All authors: conception and design of the work. AA, RA, and JV: data collection. AA and RA: data analysis. All authors: interpretation of data. AA and RA: Draft of the paper. All authors: critical review and revision of the article.

#### Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

#### Transparency Statement

Data sharing is available upon request from the corresponding author.

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#### Declaration of Interest

The authors report no conflict of interest.

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According to the authors, this article has no financial support.

#### Ethical Considerations

This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the research ethics committee (REC) of the University of Tabriz (ID: IR.TABRIZU.REC.1403.111).

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