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Both High-Intensity Interval and Moderate-Intensity Continuous Training Decrease Fetuin-A Levels in High Fat Diet Fed Male Rats

Tanto el entrenamiento en intervalos de alta intensidad como el continuo de intensidad moderada disminuyen los niveles de fetuina-A en ratas macho alimentadas con dieta rica en grasas

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Abstract. Background: Fetuin-A is a hepatokine that increases in obesity, and a high-fat diet (HFD) contributes to this condition. Obesity is characterized by increased body mass index (BMI) and is correlated to insulin resistance. This study aims to analyze the difference between High-Intensity Interval Training (HIIT) and Moderate-Intensity Continuous Training (MICT) on fetuin-A, insulin, fasting blood glucose (FBG) levels, and BMI in HFD-fed rats. Methods: Twenty-four male Wistar rats were divided into four groups: CD (standard diet), HFD (HFD only), HFD-IT (HFD and HIIT), and HFD-CT (HFD and MICT). HFD consisted of a standard diet with an additional 2 mL/200-gram body weight of lard oil daily. In the HFD-IT group, swimming was performed with a 9% body weight load with short duration and intermittent rest periods, while the HFD-CT group was given a 6% body weight load and continuous swimming. Swimming was conducted five days a week for four weeks. Fetuin-A and insulin levels were measured using enzyme-linked immunosorbent assay (ELISA) method, and FBG levels were measured using a glucometer. Results: Fetuin-A levels were significantly lower in the HFD-IT and HFD-CT groups compared to the HFD group ($p<0.05$). The HFD-CT group had a significant decrease in FBG levels ($p<0.05$), but the HFD-IT group did not. There were no differences in BMI and insulin levels between groups after four weeks of treatment ($p>0.05$). Conclusion: HIIT and MICT have similar effectiveness in reducing fetuin-A levels. In addition, MICT also managed to reduce FBG levels.
Keywords: interval training, continuous training, high-fat diet, fetuin-A, insulin, healthy lifestyle

Resumen. Antecedentes: La fetuína-A es una hepatocina que aumenta en la obesidad, y una dieta rica en grasas (HFD) contribuye a esta condición. La obesidad se caracteriza por el aumento en el índice de masa corporal (IMC) y se relaciona directamente con la resistencia a la insulina. Este estudio tiene como objetivo analizar la diferencia entre la eficacia del entrenamiento de intervalos de alta intensidad (HIIT) y del entrenamiento continuo de intensidad moderada (MICT) sobre la fetuína-A, la insulina, los niveles de glucosa en sangre en ayunas (FBG) y el IMC en ratas alimentadas con HFD. Métodos: Veinticuatro ratas Wistar macho se dividieron en cuatro grupos: CD (dieta estándar), HFD (sólo HFD), HFD-IT (HFD y HIIT) y HFD-CT (HFD y MICT). La HFD consistía en una dieta estándar con 2 ml/200 gramos de peso corporal adicionales de aceite de grasa de cerdo al día. En el grupo HFD-IT, se utilizó como ejercicio la natación con una carga del 9% del peso corporal, con períodos de descanso intermitentes y de corta duración, mientras que el grupo HFD-CT recibió una carga del 6% del peso corporal y natación continua. La natación se realizó cinco días a la semana durante cuatro semanas. Los niveles de fetuína-A e insulina se midieron mediante el método de ensayo inmuno-absorbente ligado a enzimas (ELISA), y los niveles de FBG se midieron con un glucómetro. Resultados: Los niveles de fetuína-A fueron significativamente inferiores en los grupos HFD-IT y HFD-CT en comparación con el grupo HFD ($p<0,05$). El grupo HFD-CT presentó un descenso significativo de los niveles de FBG ($p<0,05$), pero no así el grupo HFD-IT. No hubo diferencias en los niveles de IMC e insulina entre los grupos tras cuatro semanas de tratamiento ($p>0,05$). Conclusiones: El HIIT y el MICT tienen una eficacia similar en la reducción de los niveles de fetuína-A. Además, el MICT también consiguió reducir los niveles de FBG.
Palabras clave: entrenamiento a intervalos, entrenamiento continuo, dieta rica en grasas, fetuína-A, insulina, estilo de vida saludable

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Introduction

High-fat diets (HFD) contain excessive calories, contributing to weight gain and obesity through a complex metabolic process. Obesity is a complex chronic disease marked by excessive accumulation of body fat caused by the complex interplay of multiple genetic, metabolic, behavioral, and environmental factors (da Silva et al., 2022; Devi et al., 2023; Wharton et al., 2020). Based on the World Health Organization data, the worldwide prevalence of obesity in adults increased by 138% between 1975 and 2016, with a 21% increase between 2006 and 2016 (World Health Organization, 2022). Several studies illustrate the correlation between obesity, metabolic syndrome, and other chronic diseases such as cardiovascular disease, type 2 diabetes mellitus (DM), and Non-Alcoholic Fatty Liver Disease (NAFLD) (Boutari & Mantzoros, 2022; Meiliana & Wijaya, 2009; Zanetti et al., 2022). Lifestyle changes characterized by decreased physical activity and a high-fat diet play an important role in causing obesity. One of the therapies for obesity besides calorie restriction and pharmacological intervention is to increase physical exercise (Herawati et al., 2019; Vigriawan et al., 2022). Two types of popular exercise are known: Moderate-Intensity Continuous Training (MICT) and High-Intensity Interval Training (HIIT) (Ruslan et al., 2022). MICT, better known as endurance training, is performed over a longer duration with moderate exercise intensity. In contrast, HIIT is characterized by high exercise intensity with shorter duration and rest periods or low-intensity exercise in between (Maturana et al., 2021; Petridou et al., 2019; Syamsudin et al., 2023).

Fetuin-A is a multifunctional hepatokine that plays a role in several endocrine signaling pathways. Fetuin-A can suppress adiponectin production by adipocytes, induce pancreatic beta cell toxicity, and have pro-inflammatory effects that can cause insulin resistance and increased blood glucose levels (Ahn et al., 2021). Increased levels of fetuin-A are found in obese children and adolescents, which are linked to several metabolic diseases such as DM and NAFLD (Ahn et al., 2021). A study by Etienne et al. (2022) reported hepatic steatosis and increased fetuin-A levels in male mice fed a high-fat diet for four weeks.

A previous study by Khabiri et al. (2023) reported that eight weeks of aerobic exercise reduced fetuin-A levels, body weight, and insulin resistance in male rats fed a high-fat diet. Saberi et al. (2024) reported that eight weeks of HIIT training also reduced fetuin-A levels in diabetic male rats that received two months of a high-fat diet and a single dose of streptozotocin. Studies in humans have also shown that aerobic exercise can reduce fetuin-A levels and improve insulin resistance in men and women (Malin et al., 2014; Ramírez-Vélez et al., 2019). Despite previous studies, limited research has compared the effectiveness of HIIT and MICT on fetuin-A, insulin, and other obesity-related parameters in a short period. Some individuals have different preferences for the method of exercise performed, so it is important to know the difference in effectiveness between the two exercises to provide maximum results in a short time, especially for patients with metabolic diseases such as obesity and type 2 DM (Azhir et al., 2022; J. Liu et al., 2020). Based on the discussion above, this study aims to analyze the effects of four weeks of HIIT and MICT on fetuin-A, insulin, fasting blood glucose levels, and BMI in male rats fed a high-fat diet. We hypothesize that both HIIT and MICT for four weeks have the same ability to reduce these parameters in high-fat diet fed rats.

Material & Methods

This study was an experimental study and all research procedures have been approved by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya (No.181/EC/KEPK/FKUA/2023).

Experimental Animals

Twenty-four healthy Wistar male rats (*Rattus norvegicus*) aged 8-12 weeks and weighing 150-200 grams were used in this study. The rats were randomly divided into four groups, and each group consisted of six rats. The four groups consisted of CD (standard diet without treatment), HFD (HFD only without treatment), HFD-IT (combined HFD and HIIT), and HFD-CT (combined HFD and MICT).

This study was conducted at the animal laboratory of the Veterinary Medicine Faculty, Airlangga University. All animals were kept in a room with a temperature of 26±2ºC and a 50-60% humidity. The rats were housed in groups of six, according to their treatment groups. Standard food and water were provided ad libitum. Lighting was set to resemble a 12-hour light/12-hour dark cycle. Before treatment, the animals were acclimatized to the food, laboratory conditions, and swimming protocol for one week. Body weight and body length measurements were also taken before treatment to calculate BMI. BMI was calculated using the Lee index formula ($\sqrt[3]{}$body weight (g)/naso-anal length (cm) × 1000), and a value of more than 310 indicates obesity in rats (Francis et al., 2022). In addition to the BMI measurement, fasting blood glucose levels were also examined after 12 hours of fasting before starting treatment by collecting capillary blood from the rat's tail and measured using the Easy Touch GCU glucometer (Bioptik Technology, Inc., Miaoli County, Taiwan). Fasting blood glucose levels in rats are normal if less than 200 mg/dL (Fajardo et al., 2014).

Procedure Intervention

Rats in the control group (CD) received a standard diet consisting of 12% water, 20% protein, 4% fat and 4% fiber. In the HFD, HFD-IT, and HFD-CT groups, rats were given a standard diet with an additional lard oil (Yong's Deli Pork, Madiun, Indonesia), which was administered by oral gavage in a dose of 2 mL/200-gram body weight every morning for four weeks (Widianingsih et al., 2009). Rats in the CD and HFD groups were only given diet according to the group and without swimming treatment. Rats in the HFD-IT and HFD-CT groups were exercised according to the swimming protocol of Rahayu et al. (2021) and Riyono et al. (2022) with modifications, in a 60 cm diameter plastic tub with 55 cm water depth and 28-32ºC water temperature. In the HFD-IT group, rats swam for 3 minutes with a 9% load of body weight tied to the tail, followed by a rest period of 1 minute 45 seconds, counted as one set. In the first week, the set was repeated twice per session, repeated four times per session during the second week, and repeated six times per session during the third and fourth weeks. In the HFD-CT group, rats swam with a 6% load of body weight tied to the tail and were conducted without a rest period for 10 minutes in the first week, 20 minutes in the second week, and 30 minutes in the third and fourth weeks. The choice of applying different loads to rats' tail during swimming was based on the study done by Gobatto et al. (2001), which reported that rats given a 6% load reached the maximal lactate steady state or anaerobic threshold, while rats given an 8% load illustrate higher exercise intensity. In addition, Rahayu et al. (2021) used a 6% load to represent moderate-intensity exercise and a 9% load to represent high-intensity exercise, which was adapted into this study. The swimming session was conducted five days a week at 3 p.m. for four weeks. The body weight of the rats was weighed at the beginning of each week to adjust the load. At the same time, although the CD and HFD groups were not given the swimming treatment, they were still exposed to water at the level of the rats' feet.

Data Collection

At the end of week four of treatment, after 48 hours since the last swimming session and the rats were fasted for 12 hours, fasting blood glucose levels were measured along with body weight and body length measurements for Lee

Index calculation. Fasting blood glucose levels were measured using capillary blood from the rat's tail using the Easy Touch GCU glucometer (Bioptik Technology, Inc., Miaoli County, Taiwan). Body weight was measured using a digital scale, and body length was measured using measuring tape. The rats were then anesthetized, and 5 mL of blood was drawn from the heart. Serum was retrieved by centrifugation at 3000 rpm for 10 minutes and stored at -20°C for further testing. Fetuin-A levels in serum were measured using a BT-Lab ELISA kit (Catalog No: E0580Ra; Zhejiang, China), while insulin levels were measured using an Elabscience ELISA kit (Catalog No: E-EL-R2466; Texas, USA), according to the manufacturer-supplied instructions.

The quantitative measurement of fetuin-A levels in serum was performed using the ELISA kit with the sandwich ELISA principle. The ELISA plate provided inside the kit has been pre-coated with an antibody specific to rat fetuin-A. Fetuin-A present in the sample is added to the ELISA plate wells and binds with the antibodies. Then, a biotinylated rat fetuin-A antibody followed by Streptavidin-Horseradish Peroxide (HRP) is added to each well and incubated. After incubation, unbound Streptavidin-HRP is washed away during the washing step. A substrate solution is added to each well, and color develops, which describes the amount of fetuin-A. A stop solution is added to terminate the reaction, and the optical density (OD) is measured using a microplate reader at a wavelength of 450 nm. The same principle is used for the quantitative measurement of insulin levels in serum. The OD value is proportional to the concentration of fetuin-A or insulin. The concentration of fetuin-A or insulin in the sample is calculated by comparing the OD of the sample to the standard curve.

Data Analysis

The data analysis was conducted using SPSS software version 26 (IBM Corporation, Armonk, NY, USA). The normality test was performed using the Shapiro-Wilk test, while the homogeneity test was conducted using the Lavene test. Paired T-test was used to analyze pretest and post-test results of fasting blood glucose and BMI within each group, while One-Way ANOVA was conducted to analyze fetuin-A and insulin results between groups, which was examined at post-test only. Statistical tests were followed by Fisher's Least Significant Difference (LSD) post hoc test if significant differences were found between groups. Data were displayed as mean ± standard error of the mean (SEM), and results were defined as significant if the p-value was < 0.05.

Results

Based on the Lee index calculation of BMI at the beginning of treatment, none of the rats in this study were obese. There was no significant difference in BMI in each group after four weeks of treatment (p>0.05) based on Paired-T test results displayed in Table 1. All rats had normal fasting blood glucose levels before treatment (less than 200 mg/dL). Paired T-test in Table 1 shows that there was a significant decrease in blood glucose levels in the HFD (p<0.05) and HFD-CT (p<0.05) groups after being treated for four weeks.

Table 1.
Pre and Post Test Results for Body Mass Index and Fasting Blood Glucose

Groups	Body Mass Index (g/cm)		Fasting Blood Glucose (mg/dL)	
	Pre	Post	Pre	Post
CD	301.61 ± 2.39	284.36 ± 6.82	94 ± 5.43	93.50 ± 3.51
HFD	291.43 ± 4.21	280.31 ± 3.42	96.83 ± 3.10	67.67 ± 3.49*
HFD-IT	282.28 ± 1.75	278.01 ± 1.45	97.33 ± 4.01	90.17 ± 3.56
HFD-CT	297.43 ± 2.40	284.56 ± 5.02	110.50 ± 2.18	77.83 ± 4.82*

Description: CD: standard diet; HFD: high-fat diet only; HFD-IT: combination of HFD and HIIT; HFD-CT: combination of HFD and MICT. Data are displayed as mean ± SEM. *: p<0.05 compared to Pre, analyzed by Paired T-test.

The data in Figure 1 shows that the group fed a high-fat diet alone for four weeks (HFD) had the highest fetuin-A concentration and was significantly different from the CD, HFD-IT, and HFD-CT groups (p<0.05). This indicates that HIIT and MICT for four weeks can reduce fetuin-A levels compared to the group that received a high-fat diet alone without exercise. LSD post hoc test revealed no significant difference between the HIIT and MICT groups (p = 0.595). Both had the same effectiveness in reducing fetuin-A levels.

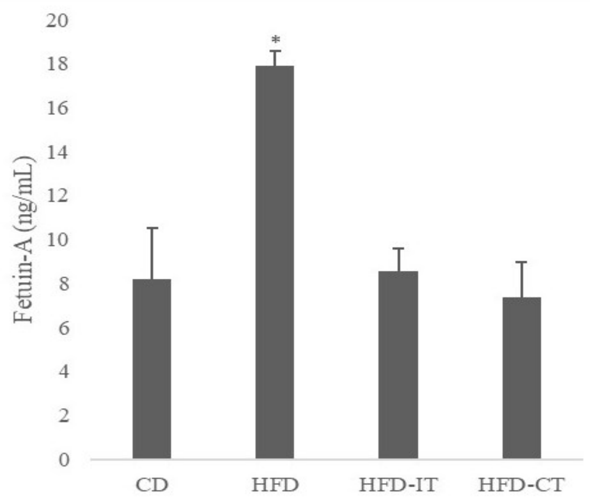

Figure 1. Fetuin-A levels after four weeks of treatment. Description: Data are displayed as mean ± SEM. *: p<0.05 vs. CD, HFD-IT, and HFD-CT were analyzed by One-Way ANOVA followed by LSD Post Hoc test.

Based on the data in Figure 2, no significant difference was found in insulin levels between groups using the One-Way ANOVA test (p>0.05). The administration of a high-fat diet and HIIT or MICT for four weeks showed no significant difference in insulin levels between groups.

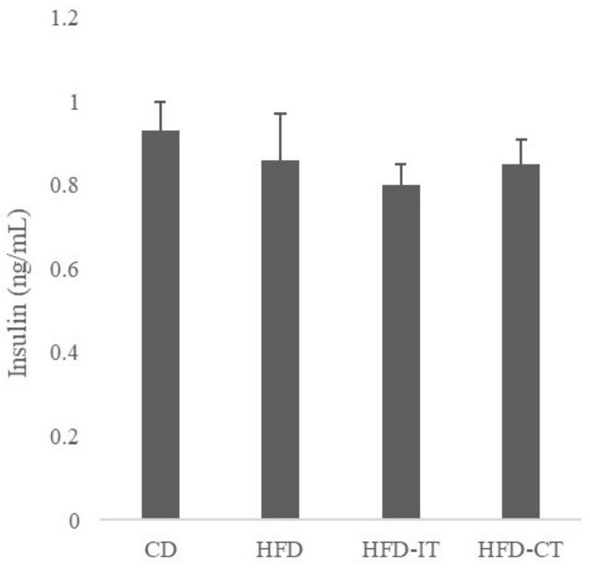

Figure 2. Insulin levels after four weeks of treatment. Description: Data are displayed as mean ± SEM and analyzed by One-Way ANOVA.

Discussion

The diet of this study was a standard diet consisting of 12% water, 20% protein, 4% fat, 4% fiber, and HFD consisting of standard diet and additional lard oil (Yong's Deli Pork, Madiun, Indonesia) in a dose of 2 mL/200-gram body weight every morning (Widianingsih et al., 2009). Rats' calorie intake is approximately 60 calories per day from a standard diet (Rising & Lifshitz, 2006). The lard oil used in this study consists of 95% fat, and the total energy value is 4,95 calories/gram, which is equivalent to an additional 8 – 9% calories from rats' standard daily intake. That value is equivalent to 3 tablespoons of sugar in an adult human.

In this study, there was no significant difference in BMI in each group after treatment for four weeks. The absence of significant changes in BMI in this study may be explained by the relatively short duration of the intervention, which was four weeks. D'Amuri et al. (2021) reported a significant BMI decrease in the HIIT and MICT groups after 12 weeks of intervention in obese men and women. Results from this study reported that the HFD-CT group had a more significant BMI reduction than the HFD-IT group, although there was no significant difference. These results are in line with research conducted by Schjerve et al. (2008), which states that both MICT and HIIT can reduce BMI after three times training per week for 12 weeks in obese men and women, but MICT has a greater reduction in BMI than HIIT ($p<0.007$ vs. $p<0.04$). Tjønna et al. (2008) also reported similar results, both continuous training and interval training three times per week for 16 weeks can reduce body weight in men and women with metabolic syndrome, but continuous training has superior results compared to interval training (-3.6 vs. -2.6 kg). Previous studies reported that slight changes in body mass in the HIIT group of obese men and women may be influenced by an increase in muscle mass (Blue et al., 2018; Rohmansyah et al., 2023). Although not significant, the decrease in BMI in the high-fat diet group could be due to the increase in cholecystokinin. Cholecystokinin works by suppressing appetite, limiting overeating and weight gain (Miller et al., 2021). Increased fasting plasma cholecystokinin was observed after high-fat feeding for 21 days in men (Little et al., 2008). Ahmed et al. (2020) also reported that weight loss was observed in overweight and type 2 DM patients who received a low-carbohydrate, high-fat diet for three months because of lipolysis and the use of non-esterified fatty acids as an alternative energy source. A decrease in body mass index in the control group, although not significant, was also obtained in this study. This can be caused by reduced physical activity, causing muscle atrophy or decreased muscle mass (Riyono et al., 2022). Zhang et al. (2018) reported that physical inactivity for two weeks caused skeletal muscle atrophy in male rats, and the underlying mechanism was activation of the AMP-activated kinase-Forkhead box O3 (AMPK/FoxO3) signaling pathway, which plays a role in the induction of atrophic gene expression, protein degradation, and final atrophy of skeletal muscle.

The group given a high-fat diet alone without exercise for four weeks increased fetuin-A levels significantly compared to the other groups in this study. This result is supported by the study from Etienne et al. (2022), which showed a significant increase in fetuin-A levels after administering a high-fat diet for four weeks in male mice. Increased levels of free fatty acids in the circulation caused by a high-fat diet lead to an increase in NF-κB binding to the fetuin-A promoter, which increases fetuin-A expression (Bhattacharya et al., 2012). Fetuin-A plays a role in inhibiting autophosphorylation of tyrosine kinase enzymes and insulin receptor substrate proteins (IRS-1), resulting in disturbances in the regulation of insulin signaling pathways and other cellular functions such as glucose storage and transport (Singh et al., 2012). In addition, fetuin-A acts as an endogenous ligand of Toll-like Receptor 4 (TLR4) that induces insulin resistance, macrophage infiltration, and inflammation in adipocytes (Ennequin et al., 2019).

This study found that the HFD-IT and HFD-CT groups had lower fetuin-A levels and were significantly different compared to the group that only received a high-fat diet (HFD). Ramírez-Vélez et al. (2019) reported that aerobic or anaerobic exercise at high or moderate intensity with a volume of 60 minutes per session and a frequency of at least four to seven sessions per week can significantly reduce fetuin-A levels in overweight or obese men and women. Malin et al. (2014) also reported that 12 weeks of aerobic exercise at 85% VO2max for 60 minutes per session can reduce fetuin-A levels in obese and insulin-resistant men and women.

There was no significant difference in fetuin-A levels between HIIT and MICT groups after four weeks of treatment in this study. Studies comparing the effectiveness of HIIT and MICT, specifically on fetuin-A, are still very limited. However, a meta-analysis done by J. Liu et al. (2020) showed that both HIIT and MICT were beneficial in lowering cardiometabolic risk factors such as BMI, body weight, and blood pressure reduction in obese children, and there were no significant differences between the two groups. The meta-analysis by Wewege et al. (2017) also reported that HIIT and MICT averaged for ten weeks with a frequency of three times a week contributed to the reduction of body fat levels and waist circumference in overweight and obese men and women, but there was no significant difference between the two groups, indicating that HIIT could be an alternative to the conventional MICT.

Despite no difference between HIIT and MICT results in some previous studies, the underlying physiological conditions are different. Moderate-intensity exercise, which generally has a longer duration per session, involves increased fat burning as a substrate, characterized by an increase in free fatty acids released and oxidized for energy (Hall, 2015; Rejeki et al., 2023). Meanwhile, high-intensity exercise is correlated with an increased secretion of catecholamines and growth hormone, which can increase the rate of adipose lipolysis (J. Liu et al., 2020). Fetuin-A secretion is influenced by a high-fat diet and an increase of free fatty acids that bind NF-κB to the fetuin-A promoter, so it is suspected that increased oxidation of free fatty acids due to HIIT and MICT plays a role in reducing fetuin-A levels in circulation.

All groups experienced a decrease in blood glucose levels after four weeks of treatment, but a significant reduction was only found in the HFD and HFD-CT groups. There was no significant difference in insulin levels between groups in this study. A study conducted by Lundsgaard et al. (2019) reported that high-fat diet administration in healthy and overweight men for six weeks decreased fasting insulin levels and insulin sensitivity was maintained, which may be the reason why there was no difference in insulin levels in the high-fat diet group compared to the control and exercise group in this study. In addition, the absence of significant differences in insulin levels between all groups could be due to the relatively short duration of the high-fat diet administration. Flanagan et al. (2008) reported that insulin resistance in female rats began to appear after the administration of a high-fat diet for nine weeks, which is marked by elevated insulin levels in serum. Another study also reported an increase in body weight, increased fasting blood glucose levels, and insulin resistance in male mice after 12 weeks of high-fat diet (Z. Liu et al., 2015).

The decrease in fasting blood glucose levels in the high-fat diet group in this study may be due to several factors, including the shorter duration of high-fat diet administration compared to previous studies, so it is suspected that insulin resistance, which causes an increase in fasting blood glucose levels has not yet occurred. A study by Lundsgaard et al. (2019) also reported that feeding a high-fat diet for six weeks in male rats showed suppression of phosphoenolpyruvate carboxykinase (PEPCK), which is a key enzyme in gluconeogenesis and led to decreased glucose production, which supported the decrease of blood glucose in the high-fat diet group in this study. In addition to this, Ahmed et al. (2020) reported that three months administration of a low-carbohydrate and high-fat diet can improve glycemic control, as described by a decrease in HbA1c levels in the type 2 DM population. Yang et al. (2021) also reported a reduction in blood glucose levels as well as improved glucose tolerance and insulin sensitivity in diabetic model male mice given a ketogenic diet for 12 weeks, but the diet also caused an increase in hepatic fat levels, which can have adverse effects if the high-fat diet is continued in the long term. This is in line with the significant rise in fetuin-A after four weeks of a high-fat diet in this study, although there were no signs of insulin resistance and increased fasting blood glucose levels yet.

This study revealed that the HFD-CT group showed a reduction in fasting blood glucose levels after four weeks of treatment compared to the HFD-IT group. This result is consistent with a meta-analysis done by Maturana et al. (2021), which stated that MICT is superior for improving long-term glucose metabolism compared to HIIT in men and women. Robinson et al. (2015) compared the effectiveness of HIIT and MICT for ten days in overweight and obese women, and the results also showed that MICT was superior in reducing fasting blood glucose levels. Despite these results, other previous studies have shown different findings. Rohmansyah et al. (2023) revealed that 16 weeks of HIIT significantly reduced fasting blood glucose compared to the MICT group in elderly women. A study by Kong et al. (2016) showed that both HIIT and MICT, for five weeks, reduced fasting blood glucose in overweight and obese young women, and there was no difference between both groups. Based on these different findings, further exploration related to different exercise intensities and fasting blood glucose is needed. During HIIT, there is an increase in several hormones, such as catecholamines, cortisol, and growth hormone, which play a role in glycogenolysis and the rise in blood glucose levels after exercise (Boutcher, 2011; Riddell et al., 2019). Individuals with type 1 DM may experience hyperglycemia after exercise, usually after high-intensity exercise (Riddell et al., 2019). HIIT has the potential to cause discomfort for some people because of its high intensity; thus, an adequate rest period between high-intensity training sessions is needed to prevent negative effects and maintain long-term exercise compliance (Atakan et al., 2021).

This study has several limitations, including the relatively short duration of the study, which limits the ability to analyze the long-term effects of high-fat diets and exercise in different intensities. A more in-depth calculation of the amount of food consumed by each group of rats can be done in further research to observe intake changes after being fed a high-fat diet. Also, more parameters related to obesity and insulin resistance should be evaluated to support the current results of this study.

Conclusion

Four weeks of HIIT and MICT were equally effective in reducing fetuin-A levels in high-fat diet-fed rats. In addition, MICT was also able to reduce fasting blood glucose levels. HIIT and MICT for four weeks had no significant effect on rats' BMI and insulin levels. The significant changes in fetuin-A levels in a short period compared to other parameters may make this parameter an early indicator of metabolic diseases. Further research is recommended, with a more prolonged diet and intervention duration, to obtain maximal results on obesity-related parameters.

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Conflicts of interest

The authors declare no conflict of interest.

References

Ahmed, S. R., Bellamkonda, S., Zilbermint, M., Wang, J., & Kalyani, R. R. (2020). Effects of the low carbohydrate, high fat diet on glycemic control and body weight in patients with type 2 diabetes: experience from a community-based cohort. BMJ Open Diabetes Research & Care, 8(1), e000980. https://doi.org/10.1136/bmjdrc-2019-000980

Ahn, M. B., Kim, S. K., Kim, S. H., Cho, W. K., Suh, J. S., Cho, K. S., Suh, B. K., & Jung, M. H. (2021). Clinical significance of the fetuin-a-to-adiponectin ratio in obese children and adolescents with diabetes mellitus. Children, 8(1155). https://doi.org/10.3390/children8121155

Atakan, M. M., Li, Y., Koşar, Ş. N., Turnagöl, H. H., & Yan, X. (2021). Evidence-based effects of high-intensity interval training on exercise capacity and health: A review with historical perspective. International Journal of Environmental Research and Public Health, 18(13). https://doi.org/10.3390/ijerph18137201

Azhir, S., Alijani, E., Martinez-Huenchullan, S., Amni, H., Baker, J. S., & Farhani, F. (2022). Effects of Exercise Intensity on Soleus Muscle Myostatin and Follistatin Levels of Hyperglycaemic Rats (Efectos de la intensidad del ejercicio sobre la miostatina y folistatina del músculo sóleo de ratas hiperglicémicas). Retos, 44, 889–896. https://doi.org/10.47197/retos.v44i0.91770

Bhattacharya, S., Kundu, R., Dasgupta, S., & Bhattacharya, S. (2012). Mechanism of Lipid Induced Insulin Resistance: An Overview. Endocrinology and Metabolism, 27(1), 12. https://doi.org/10.3803/enm.2012.27.1.12

Blue, M. N. M., Smith-ryan, A. E., Trexler, E. T., & Hirsch, K. R. (2018). The effects of high intensity interval training on muscle size and quality in overweight and obese adults. Journal of Science and Medicine in Sport, 21(2), 207–212. https://doi.org/10.1016/j.jsams.2017.06.001

Boutari, C., & Mantzoros, C. S. (2022). A 2022 update on the epidemiology of obesity and a call to action: as its twin COVID-19 pandemic appears to be receding, the obesity and dysmetabolism pandemic continues to rage on. Metabolism: Clinical and Experimental, 133(155217). https://doi.org/10.1016/j.metabol.2022.155217

Boutcher, S. H. (2011). High-intensity intermittent exercise and fat loss. Journal of Obesity, 2011. https://doi.org/10.1155/2011/868305

D'Amuri, A., Sanz, J. M., Capatti, E., Vece, F. Di, Vaccari, F., Lazzer, S., Zuliani, G., Nora, E. D., & Passaro, A. (2021). Effectiveness of high intensity interval training for weight loss in adults with obesity : a randomised controlled non-inferiority trial. BMJ Open Sport & Exercise Medicine, 1–10. https://doi.org/10.1136/bmjsem-2020-001021

da Silva, G. H. C., Marques, D. C. de S., Santos, I. C., de Oliveira, F. M., Marques, M. G. de S., Júnior, R. B. dos S., Pendić, L., & Branco, B. H. M. (2022). Effects of a multidisciplinary approach on the anthropometric and body composition responses of obese adolescents (Efectos de un abordaje multidisciplinario sobre las respuestas antropométricas y de composición corporal de adolescentes obesos). Retos, 2041(46), 323–329. https://doi.org/10.47197/retos.v46.93066

Devi, A. I., Rejeki, P. S., Argarini, R., Shakila, N., Yosnengsih, Y., Ilmi, S. B. Z., Karimullah, A., Ayubi, N., & Herawati, L. (2023). Response of TNF-α Levels and Blood Glucose Levels after Acute High-Intensity Intermittent Exercise in Overweight Women. Retos, 48, 101–105. https://doi.org/10.47197/retos.v48.94305

Ennequin, G., Sirvent, P., & Whitham, M. (2019). Role of exercise-induced hepatokines in metabolic disorders. American Journal of Physiology - Endocrinology and Metabolism, 317(1), E11–E24. https://doi.org/10.1152/ajpendo.00433.2018

Etienne, Q., Lebrun, V., Komuta, M., Navez, B., Thissen, J. P., Leclercq, I. A., & Lanthier, N. (2022). Fetuin-A in Activated Liver Macrophages Is a Key Feature of Non-Alcoholic Steatohepatitis. Metabolites, 12(7). https://doi.org/10.3390/metabo12070625

Fajardo, R. J., Karim, L., Calley, V. I., & Bouxsein, M. L. (2014). A review of rodent models of type 2 diabetic skeletal fragility. Journal of Bone and Mineral Research, 29(5), 1025–1040. https://doi.org/10.1002/jbmr.2210

Flanagan, A. M., Brown, J. L., Santiago, C. A., Aad, P. Y., Spicer, L. J., & Spicer, M. T. (2008). High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. Journal of Nutritional Biochemistry, 19(8), 505–513. https://doi.org/10.1016/j.jnutbio.2007.06.005

Francis, U. A., Melford, U. E., Hope, K. O., Chikodili, A. M., Kennedy, C. O., Isaiah, O. A., Eghosa, E. I., & and, D. C. N. (2022). Obesity related alterations in kidney function and plasma cytokines: Impact of sibutramine and diet in male Wistar rats. African Journal of Pharmacy and Pharmacology, 16(10), 161–172. https://doi.org/10.5897/ajpp2022.5305

Gobatto, C. A., de Mello, M. A. R., Sibuya, C. Y., de Azevedo, J. R. M., dos Santos, L. A., & Kokubun, E. (2001). Maximal lactate steady state in rats submitted to swimming exercise. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130(1), 21–27. https://doi.org/10.1016/s1095-6433(01)00362-2

Hall, G. Van. (2015). The Physiological Regulation of Skeletal Muscle Fatty Acid Supply and Oxidation During Moderate-Intensity Exercise. Sports Medicine, 45(1), 23–32. https://doi.org/10.1007/s40279-015-0394-8

Herawati, L., Lukitasari, L., Rimbun, R., Purwanto, B., & Sari, G. M. (2019). The combination of exercise and ascorbic acid decrease blood glucose level and tend to ameliorate pancreatic islets area on high carbohydrate diet rats.

International Journal of Applied Pharmaceutics, 11(Special Issue 3), 20–24. https://doi.org/10.22159/ijap.2019.v11s3.M1019

Khabiri, P., Rahimi, M. R., Rashidi, I., & Nedaei, S. E. (2023). Impacts of an 8-week regimen of aged garlic extract and aerobic exercise on the levels of Fetuin-A and inflammatory markers in the liver and visceral fat tissue of obese male rats. Clinical Nutrition ESPEN, 58, 79–88. https://doi.org/10.1016/j.clnesp.2023.09.004

Kong, Z., Sun, S., Liu, M., & Shi, Q. (2016). Short-Term High-Intensity Interval Training on Body Composition and Blood Glucose in Overweight and Obese Young Women. Journal of Diabetes Research, 2016, 10–12. https://doi.org/10.1155/2016/4073618

Little, T. J., Feltrin, K. L., Horowitz, M., Meyer, J. H., Wishart, J., Chapman, I. M., & Feinle-Bisset, C. (2008). A high-fat diet raises fasting plasma CCK but does not affect upper gut motility, PYY, and ghrelin, or energy intake during CCK-8 infusion in lean men. American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 294(1), 45–51. https://doi.org/10.1152/ajpregu.00597.2007

Liu, J., Zhu, L., & Su, Y. (2020). Comparative effectiveness of high-intensity interval training and moderate-intensity continuous training for cardiometabolic risk factors and cardiorespiratory fitness in childhood obesity: A meta-analysis of randomized controlled trials. Frontiers in Physiology, 11(April), 1–18. https://doi.org/10.3389/fphys.2020.00214

Liu, Z., Patil, I. Y., Jiang, T., Sancheti, H., Walsh, J. P., Stiles, B. L., Yin, F., & Cadenas, E. (2015). High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. PLoS ONE, 10(5), 1–16. https://doi.org/10.1371/journal.pone.0128274

Lundsgaard, A. M., Holm, J. B., Sjøberg, K. A., Bojsen-Møller, K. N., Myrmel, L. S., Fjære, E., Jensen, B. A. H., Nicolaisen, T. S., Hingst, J. R., Hansen, S. L., Doll, S., Geyer, P. E., Deshmukh, A. S., Holst, J. J., Madsen, L., Kristiansen, K., Wojtaszewski, J. F. P., Richter, E. A., & Kiens, B. (2019). Mechanisms Preserving Insulin Action during High Dietary Fat Intake. Cell Metabolism, 29(1), 50–63. https://doi.org/10.1016/j.cmet.2018.08.022

Malin, S. K., Del Rincon, J. P., Huang, H., & Kirwan, J. P. (2014). Exercise-induced lowering of fetuin-A may increase hepatic insulin sensitivity. Medicine and Science in Sports and Exercise, 46(11), 2085–2090. https://doi.org/10.1249/MSS.0000000000000338

Maturana, F. M., Martus, P., Zipfel, S., & NIEß, A. M. (2021). Effectiveness of HIIE versus MICT in Improving Cardiometabolic Risk Factors in Health and Disease: A Meta-analysis. Medicine and Science in Sports and Exercise, 53(3), 559–573. https://doi.org/10.1249/MSS.0000000000002506

Meiliana, A., & Wijaya, A. (2009). Peroxisome Proliferator–Activated Receptors and The Metabolic Syndrome. The Indonesian Biomedical Journal, 1(1), 4. https://doi.org/10.18585/inabj.v1i1.79

Miller, L. J., Harikumar, K. G., Wootten, D., & Sexton, P. M. (2021). Roles of Cholecystokinin in the Nutritional Continuum. Physiology and Potential Therapeutics. Frontiers in Endocrinology, 12(June), 1–7. https://doi.org/10.3389/fendo.2021.684656

Petridou, A., Siopi, A., & Mougios, V. (2019). Exercise in the management of obesity. Metabolism: Clinical and Experimental, 92, 163–169. https://doi.org/10.1016/j.metabol.2018.10.009

Rahayu, F. K., Dwiningsih, S. R., Sa'adi, A., & Herawati, L. (2021). Effects of different intensities of exercise on folliculogenesis in mice: Which is better? Clinical and Experimental Reproductive Medicine, 48(1), 43–49. https://doi.org/10.5653/cerm.2020.03937

Ramírez-Vélez, R., García-Hermoso, A., Hackney, A. C., & Izquierdo, M. (2019). Effects of exercise training on Fetuin-a in obese, type 2 diabetes and cardiovascular disease in adults and elderly: A systematic review and Meta-analysis. Lipids in Health and Disease, 18(1), 1–11. https://doi.org/10.1186/s12944-019-0962-2

Rejeki, P. S., Pranoto, A., Rahmanto, I., Izzatunnisa, N., Yosika, G. F., Hernaningsih, Y., Wungu, C. D. K., & Halim, S. (2023). The Positive Effect of Four-Week Combined Aerobic–Resistance Training on Body Composition and Adipokine Levels in Obese Females. Sports, 11(4), 1–13. https://doi.org/10.3390/sports11040090

Riddell, M. C., Pooni, R., Yavelberg, L., Li, Z., Kollman, C., Brown, R. E., Li, A., & Aronson, R. (2019). Reproducibility in the cardiometabolic responses to high-intensity interval exercise in adults with type 1 diabetes. Diabetes Research and Clinical Practice, 148, 137–143. https://doi.org/10.1016/j.diabres.2019.01.003

Rising, R., & Lifshitz, F. (2006). Energy expenditures & physical activity in rats with chronic suboptimal nutrition. Nutrition and Metabolism, 3(11), 1–9. https://doi.org/10.1186/1743-7075-3-11

Riyono, A., Tinduh, D., Othman, Z., & Herawati, L. (2022). Moderate intensity continuous and interval training affect visceral fat and insulin resistance model in female rat exposed high calorie diet. Comparative Exercise Physiology, 15(5), 403–411. DOI 10.3920/CEP220013

Robinson, E., Durrer, C., Simtchouk, S., Jung, M. E., Bourne, J. E., Voth, E., Little, J. P., & Short-term, L. J. P. (2015). 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, 119(5), 508–516. https://doi.org/10.1152/japplphysiol.00334.2015

Rohmansyah, N. A., Praja, R. K., Phanpheng, Y., & Hiruntrakul, A. (2023). High-Intensity Interval Training Versus Moderate-Intensity Continuous Training for Improving Physical Health in Elderly Women. Inquiry, 60, 1–13. https://doi.org/10.1177/00469580231172870

Ruslan, S., Ilias, N. F., Azidin, R. M. F. R., Omar, M., Ghani, R. A., & Ismail, H. (2022). Effect of high intensity interval training and moderate intensity continuous training on blood pressure and blood glucose among T2DM patients. Journal of Physical Education and Sport, 22(10), 2334–2339. https://doi.org/10.7752/jpes.2022.10297

Saberi, S., Askaripour, M., Khaksari, M., Amin Rajizadeh, M., Abbas Bejeshk, M., Akhbari, M., Jafari, E., & Khoramipour, K. (2024). Exercise training improves diabetic renal injury by reducing fetuin-A, oxidative stress and inflammation in type 2 diabetic rats. Heliyon, 10(6), e27749. https://doi.org/10.1016/j.heliyon.2024.e27749

Schjerve, I. E., Tyldum, G. A., Tjønna, A. E., Stølen, T., Loennechen, J. P., Hansen, H. E. M., Haram, P. M., Heinrich, G., Bye, A., Najjar, S. M., Smith, G. L., Slørdahl, S. A., Kemi, O. J., & Wisløff, U. (2008). Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. Clinical Science, 115(9), 283–293. https://doi.org/10.1042/CS20070332

Singh, M., Sharma, P. K., Garg, V. K., Mondal, S. C., Singh, A. K., & Kumar, N. (2012). Role of fetuin-A in atherosclerosis associated with diabetic patients. Journal of Pharmacy and Pharmacology, 64(12), 1703–1708. https://doi.org/10.1111/j.2042-7158.2012.01561.x

Syamsudin, F., Qurnianingsih, E., Kinanti, R. G., Vigriawan, G. E., Putri, E. A. C., Rif'at Fawaid As'ad, M., Callixte, C., & Herawati, L. (2023). Short Term HIIT increase VO2max, but can't decrease Free Fatty Acids in Women Sedentary Lifestyle. Retos, 50, 380–386. https://doi.org/10.47197/retos.v50.99573

Tjønna, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., Loennechen, J. P., Al-Share, Q. Y., Skogvoll, E., Slørdahl, S. A., Kemi, O. J., Najjar, S. M., & Wisløff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: A pilot study. Circulation, 118(4), 346–354. https://doi.org/10.1161/CIRCULATIONAHA.108.772822

Vigriawan, G. E., Putri, E. A. C., Rejeki, P. S., Qurnianingsih, E., Kinanti, R. G., Mohamed, M. N. A., & Herawati, L. (2022). High-intensity interval training improves physical performance without C-reactive protein (CRP) level alteration in overweight sedentary women. Journal of Physical Education and Sport, 22(2), 442–447. https://doi.org/10.7752/jpes.2022.02055

Wewege, M., Berg, R. Van Den, Ward, R. E., & Keech, A. (2017). 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. Obesity Reviews, 18(June), 635–646. https://doi.org/10.1111/obr.12532

Wharton, S., Lau, D. C. W., Vallis, M., Sharma, A. M., Biertho, L., Campbell-Scherer, D., Adamo, K., Alberga, A., Bell, R., Boulé, N., Boyling, E., Brown, J., Calam, B., Clarke, C., Crowshoe, L., Divalentino, D., Forhan, M., Freedhoff, Y., Gagner, M., … Wicklum, S. (2020). Obesity in adults: A clinical practice guideline. Canadian Medical Association Journal, 192(31), E875–E891. https://doi.org/10.1503/cmaj.191707

Widianingsih, W., Salamah, N., & Maulida, F. Q. (2009). The effects of ethanolic extract of green algae (ulva lactuca l.) on blood cholesterol levels in male rats induced by a high fat diet. Jurnal Kedokteran Dan Kesehatan Indonesia, 7(5), 181–186. https://doi.org/10.20885/jkki.vol7.iss5.art3

World Health Organization. (2022). Obesity in the WHO Region. https://cdn.who.int/media/docs/librariesprovider2/euro-health-topics/food-safety/europeanobesityreport-2022-fs-(1).pdf?sfvrsn=fcf36c2c_5&download=true

Yang, Z., Mi, J., Wang, Y., Xue, L., Liu, J., Fan, M., Zhang, D., Wang, L., Qian, H., & Li, Y. (2021). Effects of low-carbohydrate diet and ketogenic diet on glucose and lipid metabolism in type 2 diabetic mice. Nutrition, 89, 111230. https://doi.org/10.1016/j.nut.2021.111230

Zanetti, M. M., Lima e Silva, L. de, Sena, M. A. de B., Neves, E. B., Ferreira, P. F., Keese, F., Nunes, R. A. M., & Fortes, M. D. S. R. (2022). Correlation between anthropometric parameters and cardiometabolic risk in military (Correlación entre parámetros antropométricos y riesgo cadiometabólico en militares). Retos, 44, 1099–1103. https://doi.org/10.47197/retos.v44i0.91559

Zhang, S. F., Zhang, Y., Li, B., & Chen, N. (2018). Physical inactivity induces the atrophy of skeletal muscle of rats through activating AMPK/FoxO3 signal pathway. European Review for Medical and Pharmacological Sciences, 22(1), 199–209. https://doi.org/10.26355/eurrev-201801-14118

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