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Moderate-Intensity exercise has most beneficial effect on inflammatory response in fructose-induced mice (*Mus musculus*)

El ejercicio de intensidad moderada tiene el efecto más beneficioso sobre la respuesta inflamatoria en ratones inducidos por fructosa (Mus musculus)

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Abstract. Carbohydrates are the most important source of energy for most of the population. However, consuming high levels of carbohydrates, especially from processed sources, is associated with an increased incidence of obesity. Obesity causes adiponectin levels to decrease, which has implications for the activation of inflammatory signaling pathways, increasing chronic inflammation and the risk of developing cancer cells. Exercise is reported to be an alternative that can be used to maintain an anti-inflammatory environment. However, the effect of exercise intensity on reducing TNF-α levels and increasing adiponectin has not been clearly explored. Therefore, this study aims to prove the effect of various types of exercise on changes in the inflammatory response in mice induced by fructose (Mus musculus). This study is a true experimental study with a randomized control group posttest-only design using 28 male mice (Mus musculus), eight weeks old, with body weight 20 ± 5 grams and randomly divided into four groups: control group (CTR), low-intensity exercise (LIE), moderate-intensity exercise (MIE), and high-intensity exercise (HIE). Mice were induced orally with a 20% fructose solution at a dose of 1.86 grams/kg body weight from day 1 to day 60. Swimming training is carried out with a frequency of 3x/week for 8 weeks. Blood samples were taken 24 hours after the last exercise, while adiponectin and TNF-α levels were measured using the ELISA method. Data analysis techniques using one-way ANOVA and Tukey's HSD post-hoc test. Results of analysis of TNF-α levels in CTR, LIE, MIE, and HIE (103.43 ± 42.21 vs 93.82 ± 60.87 vs 34.52 ± 15.35 vs 68.14 ± 26.14 ng/mL and p = 0.004). Adiponectin in CTR, LIE, MIE, and HIE (27.39±7.48 vs 66.74±7.81 vs 235.13±47.94 vs 147.92±19.46 pg/mL and p=0.000). Exercise intervention with three different types of intensity for 8 weeks increased adiponectin levels, while TNF-α levels were only found to decrease in the moderate-intensity exercise group.
Keywords: Anti-inflammatory, obesity, pro-inflammatory, swimming exercise

Resumen. Los carbohidratos son la fuente de energía más importante para la mayoría de la población. Sin embargo, el consumo de altos niveles de carbohidratos, especialmente de fuentes procesadas, se asocia con una mayor incidencia de obesidad. La obesidad hace que los niveles de adiponectina disminuyan, lo que tiene implicaciones en la activación de vías de señalización inflamatorias, aumentando la inflamación crónica y el riesgo de desarrollar células cancerosas. Se informa que el ejercicio es una alternativa que puede utilizarse para mantener un ambiente antiinflamatorio. Sin embargo, no se ha explorado claramente el efecto de la intensidad del ejercicio sobre la reducción de los niveles de TNF-α y el aumento de la adiponectina. Por tanto, este estudio tiene como objetivo demostrar el efecto de varios tipos de ejercicio sobre los cambios en la respuesta inflamatoria en ratones inducida por fructosa (Mus musculus). Este estudio es un verdadero estudio experimental con un diseño de posprueba de grupo de control aleatorio utilizando 28 ratones macho (Mus musculus), de ocho semanas de edad, con un peso corporal de 20 ± 5 gramos y divididos aleatoriamente en cuatro grupos: grupo de control (CTR), grupo bajo. -ejercicio de intensidad (LIE), ejercicio de intensidad moderada (MIE) y ejercicio de alta intensidad (HIE). Los ratones fueron inducidos por vía oral con una solución de fructosa al 20% a una dosis de 1,86 gramos/kg de peso corporal desde el día 1 hasta el día 60. El entrenamiento de natación se llevó a cabo con una frecuencia de 3 veces por semana durante 8 semanas. Se tomaron muestras de sangre 24 horas después del último ejercicio, mientras que los niveles de adiponectina y TNF-α se midieron mediante el método ELISA. Técnicas de análisis de datos mediante ANOVA unidireccional y prueba post-hoc HSD de Tukey. Resultados del análisis de niveles de TNF-α en CTR, LIE, MIE y HIE (103,43 ± 42,21 vs 93,82 ± 60,87 vs 34,52 ± 15,35 vs 68,14 ± 26,14 ng/mL y p = 0,004). Adiponectina en CTR, LIE, MIE y HIE (27,39±7,48 vs 66,74±7,81 vs 235,13±47,94 vs 147,92±19,46 pg/mL y p=0,000). La intervención de ejercicio con tres tipos diferentes de intensidad durante 8 semanas aumentó los niveles de adiponectina, mientras que los niveles de TNF-α solo disminuyeron en el grupo de ejercicio de intensidad moderada.
Palabras clave: Antiinflamatorio, obesidad, proinflamatorio, ejercicio de natación

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Introduction

Carbohydrates are the most important source of energy for most of the population (Jebb et al., 2015). However, consuming high levels of carbohydrates (at least 60% of total energy), especially from processed sources (such as white rice and white bread), is associated with an increased risk of total mortality (Clemente-Suárez et al., 2022). Makarem et al. (2014) reported that the quantity and quality of carbohydrates consumed are associated with the risk and development of several chronic diseases, including cancer, cardiovascular disease (CVD), and type 2 diabetes mellitus. Meanwhile,

consuming refined grains, sugar, and drinks sweetened with sugar has also been reported to be associated with an increased risk of cancer (Drake et al., 2012; Cust et al., 2007), CVD, metabolic syndrome (Malik et al., 2010; Schulze et al., 2004), type 2 diabetes mellitus (DiNicolantonio et al., 2015), and weight gain (Malik et al., 2006). Increased consumption of refined carbohydrates is also reported to increase the incidence of obesity (Sartorius et al., 2018). This is because increased consumption of refined carbohydrates can cause increased fluctuations in plasma insulin and plasma glucose as well as post-prandial reactive hypoglycemia, which can harm metabolism (Alam et al., 2022). Excessive intake of refined carbohydrates, particularly those with a high glycemic index such as sugar and processed grains, leads to rapid increases in blood glucose levels, triggering a corresponding surge in insulin secretion (Ma et al., 2022). This frequent elevation in insulin promotes fat storage and eventually contributes to insulin resistance, a condition in which the body's cells become less responsive to insulin's effects (Wondmkun, 2020). As insulin resistance develops, the body struggles to regulate glucose levels, leading to persistent hyperglycemia and further worsening of fat accumulation, a key factor in obesity (Lee et al., 2022). Obesity itself is linked to a range of pathological conditions, including chronic inflammation, which is driven by excess fatty tissue releasing pro-inflammatory cytokines like TNF-α (Jin et al., 2023). This chronic inflammation increases the risk of developing metabolic disorders such as type 2 diabetes and cardiovascular diseases, as well as other serious conditions like cancer (Furman et al., 2019). Thus, the overconsumption of refined carbohydrates contributes directly to obesity and indirectly to various associated pathological conditions through its effects on metabolism and inflammation.

Adipose tissue is an endocrine organ that produces adipokines such as leptin, visfatin, alatin, resistin, and adiponectin, which modulate metabolic processes in the body (Singla et al., 2010). An increase in fat mass causes an imbalance in the release of these hormones, thereby having various metabolic effects (Singla et al., 2010). In conditions of obesity, adiponectin levels have been found to decrease (Ouchi et al., 2011), this is because adiponectin expression is inhibited by hypoxia, oxidative stress (Hosogai et al., 2007), insulin resistance, interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) (Fasshauer et al., 2002), catecholamine resistance (Reilly & Saltiel, 2017), fetuin-A (Odegaard & Chawla, 2013), and selenoprotein P (SeP) (Thorogood et al., 2011). Decreased adiponectin production will activate the inflammatory signaling pathway (Jackson et al., 2016), which has an impact on chronic inflammation (Bashashati et al., 2017), and increases the risk of developing cancer cells (Heikkilä et al., 2008). Increased levels of TNF-α evidence this as a pro-inflammatory indicator in obese individuals compared to non-obese individuals (De Lorenzo et al., 2016). High levels of TNF-α are characteristic of many malignant cancers, including breast cancer, and are often associated with cancer cell aggressiveness and poor prognosis (Wolczyk et al., 2016).

Exercise is an effective strategy for maintaining an anti-inflammatory environment (Pranoto et al., 2023a; Rejeki et al., 2023; Pranoto et al., 2023b; Gonzalez-Gil et al., 2020). This is because exercise can increase adiponectin levels as an anti-inflammatory marker (Achari & Jain, 2017; Bouassida et al., 2010) and reduce pro-inflammatory cytokines such as TNF-α (Jahromi et al., 2014), so exercise can be used to maintain a balance in inflammation levels. However, previous studies on the effects of exercise on inflammation levels have found controversial results. For example, research conducted by Racil et al. (2013) reported that high-intensity interval training and moderate-intensity interval training increased adiponectin levels in obese women. However, the results of the Meta-Analysis conducted by Hayashino et al. (2014) reported that no significant changes were found in adiponectin levels in response to physical exercise. Resistance training for 8 weeks significantly reduced pro-inflammatory cytokines, such as TNF-α (Jahromi et al., 2014). Likewise, training with an intensity of 70% VO_2max for 6 weeks significantly reduced TNF-α levels (Smart et al., 2011). Conraads et al. (2002) reported that the combination of endurance/resistance training did not affect TNF-α levels. Likewise, research by Sugama et al. (2013) reported that TNF-α levels increased after endurance exercise. Based on previous findings, there is still controversy regarding the results of research on the effects of exercise on the inflammatory response. Therefore, this study aims to prove the effect of various types of exercise on changes in the inflammatory response in mice that are induced by fructose (Mus musculus). The results of this research will provide a further scientific basis for preventing the negative effects of a high-carbohydrate, high-calorie diet, which is the lifestyle of the majority of the Indonesian population, especially at the level of inflammation.

Materials and Methods

Animals, diets, and experimental protocol

This research was experimental laboratory research with a randomized post-test-only control group design using 28 male mice (*Mus musculus*), 2 months old, 20±5 grams, healthy physical condition, and no abnormalities. All groups of mice were given standard food and drank a 30% fructose solution ad Libitum. In the Control group (CTR; $n = 7$) no swimming intervention was given, Low-intensity exercise (LIE; $n = 7$) was given a light-intensity swimming intervention (load 3% of body weight), Moderate-intensity exercise (MIE; $n = 7$) was given a moderate intensity swimming intervention (load 6% of body weight), and High-intensity exercise (HIE; $n = 7$)

was given a heavy intensity swimming intervention (load 9% of body weight). The swimming was given 80% of the maximum duration with a frequency of 3x/week for 8 weeks and started at 5.00 p.m (Sari et al., 2024). Details of the exercise program for mice can be seen in Table 1. The research was conducted at the Laboratory of Experimental Animal, Department of Medical Physiology and Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia within 8 weeks. Experimental animals were placed at room temperature 25 ± 2°C with 50-60% humidity (Sari et al., 2024). The lighting was regulated by a light-dark cycle with 12 hours of light and 12 hours of darkness (08.00-20.00) (Sari et al., 2024). Mice cages sized 60x30x30 cm made of wood with wire mesh equipped with a place to eat and drink bottles, each cage is filled with 5 mice. The food and drink were given at 06.00 a.m. These research procedures have been approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Airlangga Surabaya (No: 165/EC/KEPK/FKUA/2021).

Table 1.
Exercise protocol for mice

Components	CTR	LIE	MIE	HIE
Frequency	Without swimming exercise intervention	3x/weeks		
Intensity		3% of body weight	6% of body weight	9% of body weight
Type		Continuous		
Time		80% of the maximum swimming duration		
Length		8 weeks		

Blood sampling and biochemical analysis

Blood was drawn from the mice's left ventricle as much as 1 ml. Blood sampling was performed 12 hours after the swimming was performed with a fasting condition. Blood was centrifuged for 10 minutes at 3000 rpm. The serum was separated and stored at -80°C for analysis of TNF-α levels the following day. Measurement of TNF-α levels using the ELISA BT-E0117Mo Kit (Bioassay Technology Laboratory, Inc., Shanghai, China) with a standard curve range of 5-1000 ng/mL and a sensitivity of 2.49 ng/mL. Measurement of kadar adiponectin using the ELISA Kit (Cat.No E-EL-M0002; Elabscience Biotechnology Co. Ltd., USA) with a standard curve range 15.63-1000 pg/mL and a sensitivity of 9.38 pg/mL.

Statistical analysis

The statistical analysis used the Statistical Product and Service Solutions (SPSS) Software. The normality test used the Shapiro-Wilk test, while the homogeneity test used the Levene test. The parametric test used One way-ANOVA and continued with Tukey's Honest Significant Difference (HSD) post hoc test. Data that are not normally distributed are to non-parametric tests used Kruskal Wallis test and continued with Mann-Whitney U test. All data was displayed with mean ± standard deviation (SD). All statistical analyzes used a significant level of 5%.

Results

From the data that has been collected, descriptive tests and different tests were carried out on pre and post-intervention body weight before and after 8 weeks of intervention. The results of the descriptive test containing the mean pre and post-intervention weight values are presented in Figure 1.

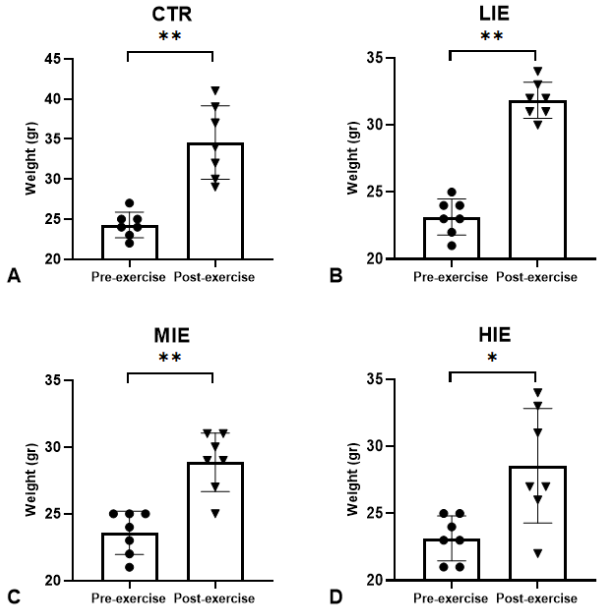

Figure 1. Mean of weight between pre-exercise and post-exercise in each group
Description: (*) Significant at pre-exercise in each group (p ≤ 0.05)

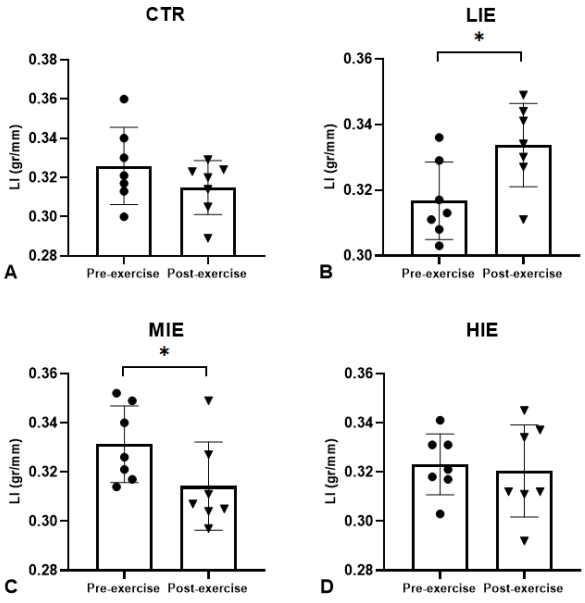

Figure 2. Mean of Lee index between pre-exercise and post-exercise in each group.
Description: (*) Significant at pre-exercise in each group (p ≤ 0.05)

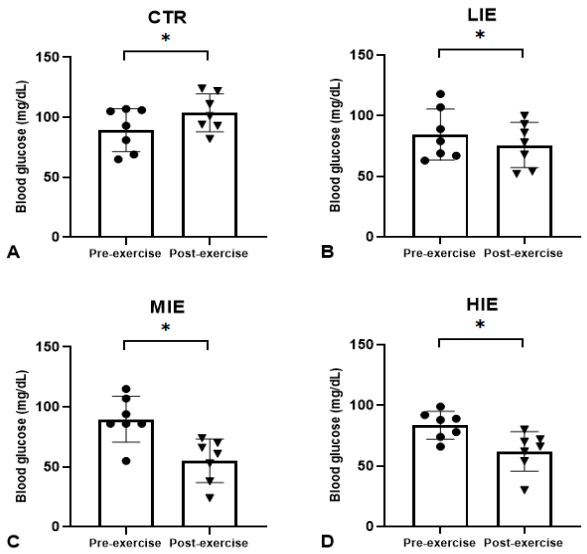

Figure 3. Mean of blood glucose between pre-exercise and post-exercise in each group. Description: (*) Significant at pre-exercise in each group ($p \leq 0.05$)

Table 2.
Differences in weight, Lee index, blood glucose, TNF-α, and adiponectin between groups (CTR vs LIE vs MIE vs HIE)

Parameters	Groups (n=7; mean±SD)				p-Value
	CTR	LIE	MIE	HIE	
Pre-weight (gr)	24.29±1.61	23.14±1.35	23.57±1.62	23.14±1.68	0.490$^{\$}$
Post-weight (gr)	34.57±4.58	31.86±1.35	28.86±2.19*	28.57±4.28*	0.009$^{\$}$
Δ-weight (gr)	10.29±3.31	8.72±1.38	5.29±1.98*	5.43±4.69*	0.013$^{\$}$
Pre-LI (gr/mm)	0.325±0.019	0.317±0.012	0.332±0.016	0.323±0.012	0.362$^{\$}$
Post-LI (gr/mm)	0.315±0.014	0.334±0.013	0.314±0.018	0.321±0.019	0.110$^{\$}$
Δ-LI (gr/mm)	-0.012±0.021†	0.019±0.010	-0.017±0.017†	-0.004±0.026	0.014$^{\$}$
Pre-blood glucose (mg/dL)	89.43±17.91	84.57±21.17	89.86±19.16	83.72±11.47	0.877$^{\$}$
Post-blood glucose (mg/dL)	103.86±15.74	75.86±18.68*	55.14±18.24*	62.00±16.29*	0.000$^{\$}$
Δ-blood glucose (mg/dL)	14.43±7.05	-8.71±8.75*	-34.72±20.27*†	-21.71±15.84*	0.000$^{\$}$
Post-TNF-α (ng/mL)	103.43±42.21	93.82±60.87	34.52±15.35*†^	68.14±26.14	0.004$^{\#}$
Post-Adiponectin (pg/mL)	27.39±7.48	66.74±7.81*	235.13±47.94*†^	147.92±19.46*	0.000$^{\$}$

Description: LI: Lee index. (*) Significant at CTR ($p \leq 0.05$). (†) Significant at LIE ($p \leq 0.05$). (^) Significant at HIE ($p \leq 0.05$). $^{\$}$One-way ANOVA test. $^{\#}$Kruskal Wallis Test.

Table 3.
Relationship between TNF-α and adiponectin with weight, Lee index, and blood glucose

Parameters	Post-TNF-α (ng/mL)		Post-Adiponectin (pg/mL)	
	r	*p*-Value	*r*	*p*-Value
Δ-weight (gr)	0.501**	$p \leq 0.001$	-0.541**	$p \leq 0.001$
Δ-LI (gr/mm)	0.147	$p \geq 0.05$	-0.264	$p \geq 0.05$
Δ-blood glucose (mg/dL)	0.651**	$p \leq 0.001$	-0.686**	$p \leq 0.001$

Description: (*) Significant with $p \leq 0.05$. (**) Significant with $p \leq 0.001$.

Discussion

Several studies have reported the positive impacts of exercise on weight gain, serum TNF-α, and adiponectin levels, but which intensity has have most beneficial effect is still unclear. This study aimed to prove how the effect of exercise intensity on body weight changes, Lee's index, and the correlation between blood glucose level with TNF-α and adiponectin as inflammation markers in mice due to administration of high fructose ad libitum in mice. Mice were used in this study because this species is a species that has been widely used in previous studies (Liu et al., 2017; McKie et al., 2019; da Costa Daniele et al., 2020; Raun et al., 2020). The use of mice in sports-related research is due to the similarity in metabolism between mice and humans (Raun et al., 2020). The genes of mice and humans also have homologous proximity so that mice can simulate physiological and pathological phenomena in humans (Guo et al., 2020).

Swimming was used in previous studies to see the effect of exercise on improving several functions in mice (Zhao et al., 2018; Bashiri et al., 2020; Li et al., 2018; Ludtke et al., 2020). All muscles and ligaments will be active when swimming, and it is an aerobic endurance exercise (Guo et al., 2020). The swimming duration used was 80% of the maximum duration to prevent mice from drowning, and the water temperature was maintained at 30±1°C. Mice were also adapted to this exercise before the intervention for 3-7 days (Guo et al., 2020). The duration of swimming used in this study was 8 weeks because, in previous studies, there had been a decrease in inflammatory mediators due to exercise intervention. In addition, to obtain a long-term effect, the study was carried

out for more than 6 weeks, while if the study was carried out for less than 6 weeks, it was classified as short-term (Guo et al., 2020). Weights are used to get the expected intensity, whereas low intensity uses 3% body weight, moderate 6% body weight, and 9% body weight (Prasetya et al., 2018). 30% fructose ad libitum given for 8 weeks can increase hepatic TNF-α levels (Kanuri et al., 2011), and stimulate metabolic changes in weight gain, basal glucose levels, and glucose tolerance tests (De Souza et al., 2021).

The study results showed that there is a significant difference between pre and post-intervention body weight which explains how the influence of each intervention. To see how the effect of giving fructose ad libitum is, it can be seen in CTR (fructose administration without swimming), where there is the highest increase in body weight. This is following previous studies (De Souza et al., 2021; Pereira et al., 2017; Wang et al., 2022), that fructose further increases body weight. Giving fructose more triggers obesity due to the specificity of its metabolism compared to glucose. Intake of fructose will be absorbed quickly by the intestinal epithelium and then released into the bloodstream. Fructose in the bloodstream will be absorbed by different tissues but not by the pancreas' beta cells, which causes fructose not to trigger insulin, like glucose did. In addition, fructose does not stimulate leptin release and does not suppress the release of ghrelin in starvation. These three peptide hormones control food intake and basal energy expenditure, acting on both the central nervous system and peripheral tissues. Ghrelin plays a role in increasing forkhead box protein 01 (FoxO1) which binds deoxyribonucleic acid (DNA), both insulin and leptin phosphorylate FoxO1 releasing it from DNA, thereby reducing hunger signaling and liver gluconeogenesis and contributing to increased energy expenditure (Pereira et al., 2017).

While the discussion has detailed the molecular mechanisms of fructose metabolism and its link to obesity, it is crucial to emphasize that weight gain is a multifactorial process (Herman & Birnbaum, 2021). Fructose consumption alone does not directly cause obesity or increase body weight unless it is accompanied by other determining factors, such as a sustained caloric surplus (Shi et al., 2021). The body's energy balance plays a fundamental role in weight regulation, where excess caloric intake, regardless of its source, is the primary driver of weight gain (Hall et al., 2022). Fructose may contribute to this process by promoting lipogenesis in the liver and influencing insulin resistance, but without a chronic excess in caloric intake, these effects may not lead to significant weight gain or obesity (Stanhope, 2016). Therefore, it is important to recognize that the relationship between fructose and obesity is complex and involves the interaction of multiple dietary and lifestyle factors, rather than fructose acting as an independent agent of weight gain.

In mice, the administration of fructose will directly affect the hypothalamus. Rats showed an increase in food intake, whereas glucose injection had the opposite effect (Basciano et al., 2005). This can explain the cause of the increased prevalence of obesity in individuals who consume fructose in the form of drinks or sweet foods. Increased levels of fructose-1P in cells activate other important energy pathways. SREBP1c initiates the transcription of the proteins fatty acyl-CoA synthase (FAS) and acetyl-CoA carboxylase (ACC). These mechanisms prepare the cell for increased fatty acid synthesis using carbon chains supplied by intracellular fructose. Fructose-1P will be converted into glyceraldehyde and dihydrosietonphosphate, assisted by the enzyme fructose-1P aldalose. Glyceraldehyde provides a carbon chain for pyruvate production, which later be reduced into Acetyl-CoA in the mitochondria. Acetyl-CoA is converted to citrate in the mitochondrial matrix through the Krebs cycle. Citrate migrates to the cytoplasm and is converted to malonyl-CoA by the ACC enzyme. If there is an excess of malonyl-CoA in the cytoplasm, the activity of the protein carnitine palmitoyl transferase 1 (CPT-1) will be inhibited, thereby blocking the transport of lipids to the mitochondria and stopping B-oxidation. Malonyl-CoA by the FAS enzyme (transcribed by increased SREBP1c activity) is converted to acyl-CoA. Acyl-CoA has three different targets in the cell. Acyl-CoA, which produces triglyceride molecules that accumulate in hepatocytes, will cause non-alcoholic fatty liver disease. Acyl-CoA that binds to apolipoprotein B (ApoB) will produce VLDL. At the same time, the other amount will diffuse in the form of free fatty acids into the bloodstream which will trigger hypercholesterolemia and dyslipidemia. Excessive lipids in the blood will reach white adipose tissue, resulting in white adipose tissue hypertrophy; skeletal muscle, which triggers insulin resistance; or pancreas, which inhibits insulin production and secretion. High levels of Acyl-CoA are converted to diacylglycerol (DAG) by diacylglycerol acyltransferase. DAG activates protein kinase C epsilon (PKCε), leading to activation of c-jun-N-terminal protein kinase-1 (JNK1). JNK1 causes hepatic insulin resistance by phosphorylation of IRS-1 at the Serine307 residue (IRS-1Ser307). The mechanism of insulin resistance will result in prolonged gluconeogenesis and lead to a marked increase in blood glucose, and is involved in weight gain. Thus there is no full signal that will cause a hyperenergetic state, and then adipose tissue will hypertrophy. This will make fructose and further increase the adipose mass in the body. It can be concluded that fructose may be a carbohydrate with greater obesogenic potential than other sugars (Pereira et al., 2017).

As shown in figure 1, we can see the effect of exercise intensity on preventing weight gain in mice. The suppression of weight gain in MIE was the largest, followed by LIE, and CTR. The suppression of weight gain in LIE, MIE and HIE occurs due to increased energy expenditure when swimming. When swimming, the skeletal muscles and ligaments will

contract (Guo et al., 2020). Skeletal muscles are organs responsible for energy expenditure and endurance exercise. The plasticity of adult skeletal muscle can be converted into various types of fibers in response to exercise training. Muscle conversion from glycolytic type II (fast fiber) to the more oxidative type I (slow fiber) is likely mediated by Ca^{2+} signaling mechanisms involving kinases. The kinase itself is dependent on calmodulin and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α). PGC1α plays a role in regulating gene expression involved in mitochondrial biogenesis, metabolic function, carbohydrate metabolism, and lipid metabolism. They are also closely related to the pathogenesis of obesity, diabetes, and cardiomyopathy. Downstream of PGC1α, uncoupling protein 1 (UCP1) is exclusively expressed in the inner mitochondrial membrane. UCP1 has a role in separating ATP synthesis from oxidative phosphorylation, thereby dissipating energy as heat (Li et al., 2018). Exercise significantly increased cAMP levels and protein kinase A (PKA) activity in brown adipose tissue, signalling increased lipolysis. In addition, exercise significantly activates gene programs of oxidative phosphorylation and fatty acid oxidation in skeletal muscle. These results suggest that exercise enhances the anti-obesity effect by increasing energy expenditure through activation of fat oxidation in skeletal muscle and lipolysis in brown adipose tissue (Ohyama et al., 2015).

A limitation of this study was the restricted blood sample collection, which allowed for the measurement of only two variables, TNF-α and adiponectin. Future research should expand the scope to include additional inflammatory markers such as IL-6, IL-10, and hs-CRP to provide a more comprehensive analysis of the inflammatory response. Another important limitation was the lack of precise monitoring of the daily fructose intake for each mouse. Recording the exact amount of fructose consumed by each mouse could help clarify the relationship between fructose intake and metabolic changes, ensuring that all subjects receive comparable doses. Additionally, a larger sample size is recommended for future studies to increase the statistical power and reliability of the findings. Moreover, future research could benefit from the use of oral gavage for fructose administration, which would ensure consistent dosing across all subjects. Extending the intervention period is also advisable to better understand the long-term effects of fructose consumption and exercise on inflammation and metabolism. Finally, providing a more detailed evaluation of the initial health status of the mice before starting the experiment could strengthen the selection criteria, helping to control for pre-existing conditions that might affect the results.

Conclusion

This findings prove that exercise intervention with three different types of intensity carried out 3x/week for 8 weeks increased adiponectin levels, while TNF-α levels were only found to decrease in the moderate-intensity exercise group. Therefore, moderate-intensity exercise can be used as a basis for recommendations for preventing the negative effects of a high-carbohydrate, high-calorie diet.

Conflict of interest

The authors declare no conflict of interest in this study.

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