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The Effect of Exercise Intensity on Pancreatic and Liver GLUT-2 Expression in High Fructose-Fed Mice

El efecto de la intensidad del ejercicio sobre la expresión de GLUT-2 pancreático y hepático en ratones alimentados con alto contenido de fructose

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Abstract. Excessive high fructose corn syrup (HFCS) leads to metabolic disorders characterized by decreased expression of pancreatic GLUT-2 and increased expression of liver GLUT-2. Exercise is reported to be a non-pharmacological therapy to improve metabolic disorders. This study aims to compare differences in exercise intensity to changes in pancreatic and liver GLUT-2 expression in mice induced by high fructose. A total of 36 male mice (Mus musculus), weighing 20-30 grams, 8-week-old, were randomly divided into 4 groups: C (Control; n = 9), G_1 (light intensity swimming exercises; n = 9), G_2 (moderate intensity swimming exercises; n = 9), and G_3 (heavy intensity swimming exercises; n = 9). All groups were given 30% fructose solution orally (oral ad libitum) for 8 weeks. Meanwhile, swimming exercise was given out 3×/week for 8 weeks with three different intensities. Pancreatic and liver GLUT-2 expression was measured using immunohistochemistry (IHC) and the results of pancreatic and liver GLUT-2 expression measurements were evaluated using the Immunoreactive Score (IRS). The analysis of body weight using one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) post hoc test indicates that G_2 significantly reduces weight compared to C, G_1, and G_3 ($p \leq 0.05$). Statistical analysis was done using the Kruskal-Wallis non-parametric test and followed up with the Mann-Whitney U Test with a significant level of 5%. The data showed that G_2 significantly increased expression of pancreatic GLUT-2 and decreased expression of liver GLUT-2 compared to C, G_1, and G_3 ($p \leq 0.05$). In conclusion, moderate-intensity exercise has the most optimal effect in increasing the expression of pancreatic GLUT-2 and decreasing the expression of liver GLUT-2.

Keywords: Obesity, Insulin resistance, GLUT-2 expression, Exercise, High fructose

Resumen. El exceso de jarabe de maíz con alto contenido de fructosa (JMAF) conduce a trastornos metabólicos caracterizados por una disminución de la expresión de GLUT-2 pancreático y una mayor expresión de GLUT-2 hepático. Se informa que el ejercicio es una terapia no farmacológica para mejorar los trastornos metabólicos. Este estudio tiene como objetivo comparar las diferencias en la intensidad del ejercicio con los cambios en la expresión de GLUT-2 pancreático y hepático en ratones inducidos por un alto contenido de fructosa. Un total de 36 ratones macho (Mus musculus), con un peso de 20-30 gramos, de 8 semanas de edad, se dividieron aleatoriamente en 4 grupos: C (Control; n = 9), G1 (ejercicios de natación de intensidad ligera; n = 9) , G2 (ejercicios de natación de intensidad moderada; n = 9) y G3 (ejercicios de natación de intensidad intensa; n = 9). Todos los grupos recibieron una solución de fructosa al 30% por vía oral (oral ad libitum) durante 8 semanas. Mientras tanto, se realizó ejercicio de natación 3 veces por semana durante 8 semanas con tres intensidades diferentes. La expresión de GLUT-2 pancreático y hepático se midió mediante inmunohistoquímica (IHC) y los resultados de las mediciones de expresión de GLUT-2 pancreático y hepático se evaluaron mediante la puntuación inmunorreactiva (IRS). El análisis del peso corporal mediante ANOVA unidireccional seguido de la prueba post hoc de diferencia significativa honesta (HSD) de Tukey indica que G2 reduce significativamente el peso en comparación con C, G1 y G3 ($p \leq 0,05$). El análisis estadístico se realizó mediante la prueba no paramétrica de Kruskal-Wallis y seguido con la prueba U de Mann-Whitney con un nivel de significancia del 5%. Los datos mostraron que G2 aumentó significativamente la expresión de GLUT-2 pancreático y disminuyó la expresión de GLUT-2 hepático en comparación con C, G1 y G3 ($p \leq 0,05$). En conclusión, el ejercicio de intensidad moderada tiene el efecto más óptimo para aumentar la expresión del GLUT-2 pancreático y disminuir la expresión del GLUT-2 hepático.

Palabras clave: Obesidad, Resistencia a la insulina, Expresión de GLUT-2, Ejercicio, Alto contenido de fructosa

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Introduction

The global dietary landscape has undergone significant transformations due to technological advancements and globalization, leading to sedentary lifestyles and a surge in the consumption of high-calorie foods (Susanti et al., 2019; Sheludiakova et al., 2012). Among these, high fructose corn syrup (HFCS) has emerged as a predominant sweetener in food and beverage production, raising concerns over its health implications (Stricker et al., 2021). It was reported in the United States that HFCS consumption experienced rapid development in 1970-2000 with total HFCS consumption increasing by almost 30% (Lancaster, 2020), while there was a 100-fold increase in HFCS consumption in Indonesia in 2005 – 2012. This indicates that Indonesia is a country with high fructose consumption (Ferraris et al., 2018). The increase in HFCS consumption is associated with the phenomenon of the "Western diet" which is currently popular in various countries (Maj et al., 2021). The Western diet consists of energy-dense, high-fat, and high-glucose foods and drinks (Meyers et al., 2017). This trend reflects a broader adoption of the "Western diet," characterized by energy-dense, high-fat, and high-glucose foods and beverages, which has become increasingly popular worldwide (Meyers et al., 2017; Min et al., 2016). The escalating intake of fructose is closely linked to the prevalence of obesity (Bocarsly et al., 2010; Ter Horst & Serlie, 2017), a condition that poses a significant threat to global health (Goran et al., 2013). Obesity is reported to be one of the causes of increased morbidity and mortality in both developed and developing countries (Hu et al., 2017). This is because obesity increases the risk of disease complications

such as hypertension (El Meouchy et al., 2022), chronic kidney disease (Stasi et al., 2022), osteoarthritis (King et al., 2013), cancer (Pati et al., 2023), dyslipidemia (Chan et al., 2016), cardiovascular disease (Chang et al., 2015), type 2 diabetes mellitus (Klein et al., 2022), nonalcoholic fatty liver disease (NAFLD) (Sarwar et al., 2018), and insulin resistance (Ter Horst & Serlie, 2017). Physiologically, obesity is reported to decrease pancreatic glucose transporter-2 (GLUT-2) expression (Gong & Muzumdar, 2012) and increase liver GLUT-2 expression (Douard & Ferraris, 2013). Previous studies reported that mice induced by fructose caused a decrease in pancreatic GLUT-2 expression (Hattori et al., 2021). Similar results were also found in mice given streptozotocin for 3 weeks showing decreased regulation and expression of pancreatic GLUT-2 (Hahn et al., 2020). A study on rat models of diabetes mellitus induced by streptozotocin also reported reduced expression of pancreatic GLUT-2 (Taskinen et al., 2019). Decreased GLUT2 expression has an impact on glucose homeostasis and insulin resistance (Berger & Zdzieblo, 2020). Moreover, previous studies reported that streptozotocin-induced diabetic rats had increased expression of GLUT-2 in the liver caused by high blood glucose and low insulin range, exacerbating metabolic dysfunction in the development of NAFLD (Karim et al, 2012; Teodhora et al., 2021).

Recent findings suggest that exercise, as a non-pharmacological intervention, can enhance insulin secretion (Leturque et al., 2009), improve insulin sensitivity (Conn et al., 2014), and stabilize glucose homeostasis (Slentz et al., 2016). The beneficial effects of exercise extend to the cellular level. Muscle contractions resulting from exercise increase oxygen supply which has a positive effect on the biogenesis of organs and cells in the body (Wang et al., 2021), by activating peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) thereby preventing oxidative stress (Prasun, 2020). Studies have shown that various intensities of physical exercise can modulate pancreatic GLUT-2 expression, with implications for hepatic glucose production and liver GLUT-2 expression. A previous study on mice given mild-intensity treadmill exercise for 40 weeks increased pancreatic GLUT-2 expression (Piao et al., 2017). A similar study also found that rats given moderate-intensity swimming exercise for 12 weeks increased pancreatic GLUT-2 expression (Király et al., 2008). This was supported by another study of mice given acute strenuous swimming exercise resulting in increased pancreatic GLUT-2 expression (Nonaka et al., 2020). This suggests that physical exercise with various intensities can increase pancreatic GLUT-2 expression (Sokhanvardastjerdi et al., 2020) and decrease liver GLUT-2 expression through decreased hepatic glucose production (Narasimhan et al., 2015). However, the optimal exercise intensity for regulating GLUT-2 expression remains unclear (Johnson, 2020). This study aims to elucidate the effects of different exercise intensities on pancreatic and liver GLUT-2 expression in fructose-induced mice, providing valuable insights into the management of fructose-related metabolic disorders.

Materials and Methods

Animal and experimental models

This study used a posttest-only control group design. The sample in this study were 36 male mice (Mus musculus), weighing 20-30 grams, 8 week-old. Mice were randomly divided into 4 groups: C (control group; n = 9), G_1 (light intensity swimming exercise (3% body weight load); n = 9), G_2 (moderate intensity swimming exercise (6% body weight load); n = 9), and G_3 (heavy intensity swimming exercise (9% body weight load); n = 9). All groups were given 30% fructose solution orally (oral ad libitum) for 8 weeks. Mice were placed in cages measuring 30 cm x 45 cm x 20 cm, made of acrylic material equipped with a feeding bowl, and each cage was filled with 4-5 mice. Food and drink were given at 07.00 a.m. at a dose of 20 grams/head/day. The room where the research was conducted has a temperature of 26 ± 2°C with a humidity level of 50-60% and the lighting was arranged with a light-dark cycle with a cycle of 12 hours of light and 12 hours of darkness (08:00–20:00) (Antoni et al., 2022; Rejeki et al., 2021). The study was conducted for 2 months (November-December 2022) at the Laboratory of the Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. All procedures applied in this study followed the experimental animal protocol welfare principles in experimental science published in the European Convention for the Protection of Vertebrate Animals and approved by the Ethics Committee of Airlangga University, Surabaya, Indonesia (No. 244/EC/KEPK/FKUA/2022).

Exercise procedure

Before the swimming intervention, the mice underwent acclimatization for 7 days without using any load (Antoni et al., 2022). Swimming exercise was carried out 3 times/week for 8 weeks at a light intensity with a load of 3% body weight (Sari et al., 2024), a moderate intensity with a load of 6% body weight (Antoni et al., 2022), and a heavy intensity with a load of 9% body weight (Amirazodi et al., 2022) with the exercise duration of each group being 80% of the maximum swimming time. Mice were incubated in a glass pond measuring 120 cm long, 80 cm wide, and 80 cm deep with a water temperature of 32°C. Swimming exercise was held every 3.00-5.00 p.m.

Histopathological evaluation

Organ sampling was done 24 hours after the intervention, where the mice were euthanized then pancreas and liver organs were taken and stored in a buffered formalin solution. After that, the organs were processed into paraffin blocks and sliced with a thickness of 5 µm, placed on a glass slide counted with poly-l-lysine for immunohistochemistry (IHC) staining. IHC staining was carried out by deparaffinized the preparation in xylene, rehydrating in graded alcohol, and rinsing in water. After that, endogenous peroxidase was performed and rinsed in a buffered saline solution

for 5 minutes. The next process was heating with a microwave heater for 20 minutes, after which the samples were cooled and washed for 20 minutes. Non-specific antigen binding was blocked by incubating the slides with a protein-blocking solution (Dakocytomation, Carpinteria, CA, USA) for 10 minutes. The slides were incubated for 30 minutes with the anti-GLUT2 polyclonal antibody at a 1:5000 dilution. The preparations were then washed for 5 minutes and incubated for 30 minutes with immunoglobulin at a dilution of 1:200. Color was developed over 30 minutes using ABC Elite solution (VECTASTAIN Elite ABC Kit Vector Laboratories, Inc, Burlingame, CA 94010 USA), and diaminobenzidine tetrahydrochloride dihydrate was applied as chromogen, counterstained using hematoxylin-eosin. After that, the examination was done using a microscope with 400x magnification (Pranoto et al., 2020).

Data collection

Mice weight were measured in all groups before and after the intervention using the Camry EK3250 toolbar (torsion balance) (0-5 kg). Pancreatic and liver GLUT2 expression was measured using immunohistochemistry (IHC) (Primer Antibody, Bioss Antibodies bs-0351R GLUT2 Polyclonal Antibody, Boston, Massachusetts, USA), with Nikon microscope reading (H600L, Tokyo, Japan), 400x magnification. The pancreatic and liver GLUT-2 expressions were assessed using the Immunoreactive Score (IRS) (Wróel et al., 2006). Immunoreactive Score (IRS) was employed to assess GLUT-2 expression due to its established efficacy in quantifying protein expression levels in histological samples (Zhou et al., 2024). The results represent the multiplication between the two parameters of the intensity of the color reaction and the percentage of cells and the resulting values range from 0 to 12 (Table 1).

$$\text{IRS} = \text{intensity of the color} \times \text{the percentage of cells}$$

Table 1.
Evaluation of the use of immunoreactive score (IRS)

Score	% positive cells	Score	Intensity of the reaction
0	Negative	0	Negative
1	<10%	1	Weak
2	10-50%	2	Moderate
3	51-80%	3	Strong
4	>80%		

Statistical analysis

Data were analyzed using a statistical software package for social science (SPSS) version 21 (Chicago, IL, USA). Images were created using GraphPad Prism version 5.0.0 for Windows software (GraphPad Software, San Diego, California USA). The normality test used was the Shapiro-Wilk test, while the homogeneity test was done using the Lavene Test, and the difference test using one-way ANOVA and Tukey's Honest Significant Difference (HSD) post hoc test. Abnormal data will be subjected to the Kruskal-Wallis non-parametric test and continued with the Mann–Whitney U Test. The relationship between the parameters was evaluated using Pearson's correlation coefficient test. All data were presented by mean ± standard deviation (SD) with a significant value of $p \leq 0.05$.

Results

The results of pre-post weight analysis are presented in Figure 1-2, while the results of analysis of pancreatic and liver GLUT-2 expression can be seen in Figure 3. Histological features of pancreatic and liver GLUT-2 expression are shown in Figure 4-5. Meanwhile, the results of the correlation analysis between weight with pancreatic and liver GLUT-2 expression are presented in Table 2.

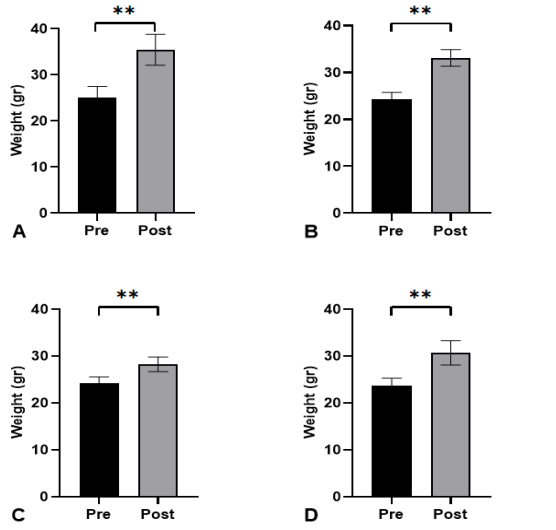

Figure 1. Changes in body weight (grams) between the pretest and posttest in (A) the control group, (B) the light intensity group, (C) the moderate intensity group, and (D) the heavy intensity group. Data are presented using mean ± SD. (**) Significant at pre ($p \leq 0.01$).

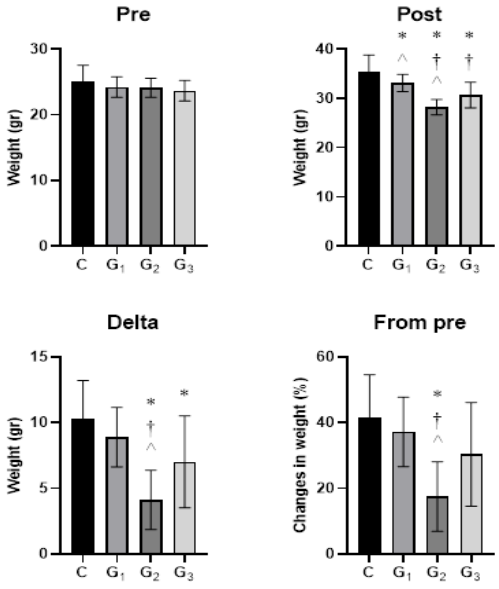

Figure 2. Differences in body weight (gr) between the pretest and posttest, delta, and change in the four groups. Data are presented using mean ± SD. (*) Significant at C ($p \leq 0.05$). (†) Significant at G_1 ($p \leq 0.05$). (^) Significant at G_3 ($p \leq 0.05$).

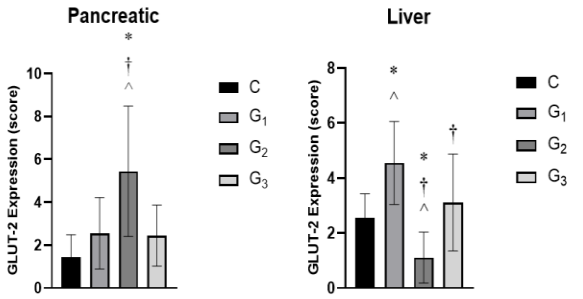

Figure 3. Pancreatic and liver GLUT-2 expression posttest in all four groups. Data are presented using mean ± SD. (*) significant at C ($p \leq 0.05$). (†) significant at G_1 ($p \leq 0.05$). (^) significant at G_3 ($p \leq 0.05$).

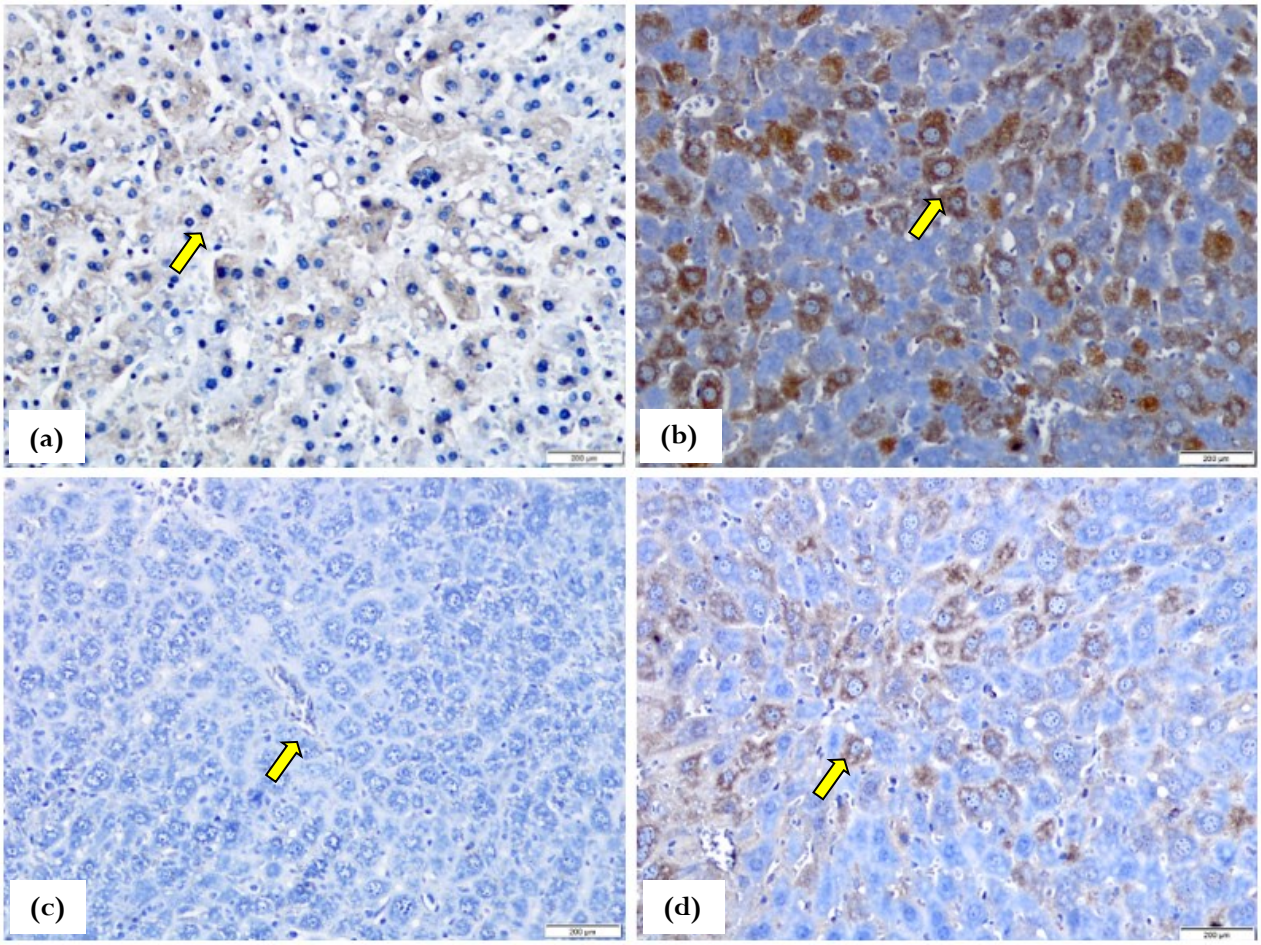

Figure 4. Liver GLUT-2 Expression Histopathology with 400x magnification. (a) Positive liver GLUT-2 expression in the hepatocyte membrane of the control group; (b) Positive liver GLUT-2 expression on hepatocyte membranes in the light-intensity swimming exercise group, (c) Negative liver GLUT-2 expression on hepatocyte membranes in the moderate intensity exercise group; (d) Positive liver GLUT-2 expression in hepatocyte membranes in the heavy intensity swimming exercise group.

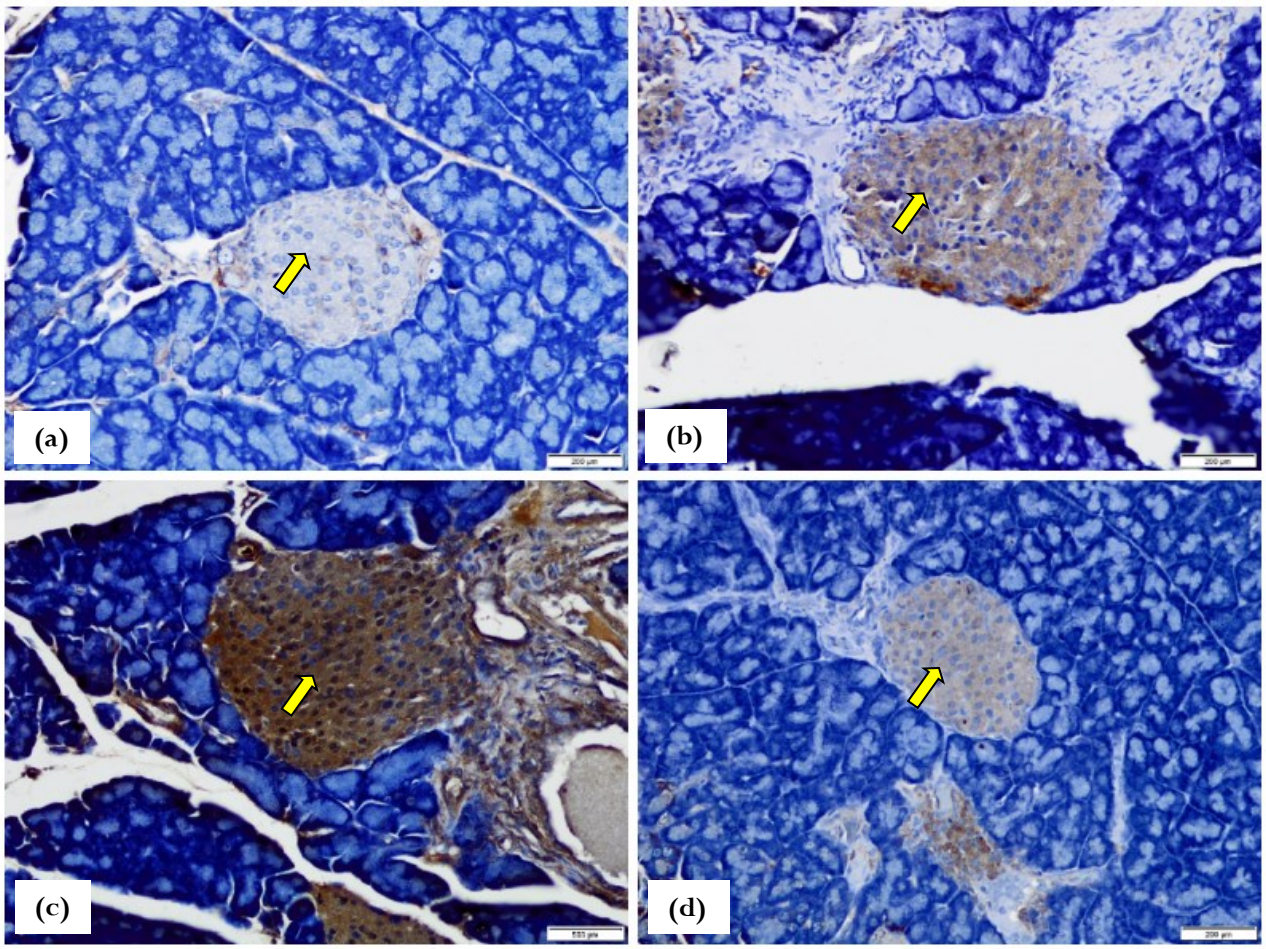

Figure 5. Pancreatic GLUT-2 Expression Hispathology with 400x magnification. (a) Negative GLUT-2 expression in the cytoplasm of pancreatic β cells of the control group; (b) Weak positive GLUT-2 expression in the cytoplasm of pancreatic β cells in the light-intensity swimming exercise group; (c) Strong positive GLUT-2 expression in the cytoplasm of pancreatic β cells in the moderate intensity swimming exercise group; (d) Weak positive GLUT-2 expression in the cytoplasm of pancreatic β cells in the heavy intensity swimming exercise group.

Table 2.
The correlation analysis between weight with pancreatic and liver GLUT-2 expression

Parameter	Expression of GLUT-2			
	Pancreatic		Liver	
	r	*p*-Value	*r*	*p*-Value
Post-Weight (gr)	-0.481**	$p \leq 0.001$	0.537**	$p \leq 0.001$
Delta-Weight (gr)	-0.510**	$p \leq 0.001$	0.500**	$p \leq 0.001$
Change-Weight (%)	-0.492**	$p \leq 0.001$	0.472**	$p \leq 0.001$

** Significant with $p \leq 0.001$.

Discussion

The findings of this study revealed a significant difference in the average body weight of mice across all groups before and after the intervention. Notably, while all groups exhibited weight gain, the control group experienced the highest increase. This aligns with prior research indicating that fructose administration can lead to significant weight gain in mice due to increased caloric intake surpassing energy expenditure (Munir et al., 2021). Weight gain occurs when the calories consumed exceed the calories expended. Consuming high fructose leads to excess substrates, resulting in an increase in triglycerides through lipogenesis (Stanhope, 2015). Triglycerides are insoluble in water and are packaged with proteins and phospholipids to form very low-density lipoprotein (VLDL), which is then released into the bloodstream towards adipose cells for storage (Ozougwu, 2017). This leads to an accumulation of fat storage, causing an increase in body weight.

Weight gain after intervention also occurred in the light-intensity exercise group, moderate-intensity exercise group, and high-intensity exercise group. These results are in line with previous research reporting that swimming exercises for 12 weeks could not prevent weight gain in mice with hyperlipidemia (Qian et al., 2020). Exercise does not always lead to weight loss; there are several factors causing weight gain after an exercise program. This is related to the increase in muscle mass and enhanced protein anabolism (Hall et al., 2020; Hargreaves & Spriet, 2020). Exercise can also influence blood circulation, gastrointestinal hormone response, stomach emptying, and brain activity, leading to an increase in appetite (Simões E Silva et al., 2020). The moderate-intensity exercise group experienced a significantly lower increase in body weight compared to the control group, light-intensity group, and high-intensity group. These results are supported by previous research indicating that mice given high fructose and moderate-intensity exercise for 4 weeks had a lower increase in body weight compared to the group without exercise (Hsu et al., 2021).

The strategic implementation of exercise protocols is

recognized for its beneficial impact on metabolism, a vital aspect of managing metabolic disorders (Beigrezaei, et al., 2021). In this study, the selection of varying exercise intensities—light, moderate, and high—was intentional, and aimed at investigating their distinct effects on metabolic processes. These varying intensities serve as proxies for different levels of physiological stress, providing a gradient to assess how exercise intensity correlates with metabolic adaptations (Liu, et al., 2019; Qi, et al., 2023).

Aerobic exercise, in particular, is known to enhance insulin sensitivity in skeletal muscles, which is pivotal for glucose uptake and utilization. This improvement in insulin sensitivity is not confined to muscles alone; it extends to other key metabolic tissues, including the liver, hypothalamus, and adipose tissue, thereby exerting a systemic effect (Pereira et al., 2017). The influence of aerobic exercise on the hypothalamus is noteworthy, as it plays a central role in energy homeostasis. Moderate-intensity exercise has been shown to modulate leptin levels, a hormone integral to satiety control, in animals subjected to a high-fructose diet (Pereira et al., 2017).

Moreover, the interplay between decreased calorie intake and exercise contributes to the prevention of triglyceride accumulation, thus mitigating fat deposition in adipose tissue. Exercise prompts the secretion of irisin from skeletal muscles, a myokine that induces the expression of Uncoupling Protein 1 (UCP1) genes. The upregulation of UCP1 is associated with increased energy expenditure and the browning of white adipose tissue (WAT), which promotes lipolysis and reduces lipid storage in adipocytes. This mechanism is corroborated by research that links elevated irisin levels to a reduction in visceral fat following consistent endurance exercise (Gonzalez-Gil & Elizondo-Montemayor, 2020).

The rationale for selecting specific exercise intensities was grounded in the hypothesis that each intensity level would elicit a unique metabolic response, particularly affecting GLUT-2 expression in the pancreas and liver. This nuanced approach allows for a comprehensive analysis of how exercise intensity can modulate metabolic regulation and adaptation.

Increased fructose in hepatocytes stimulates the expression of lipogenic enzymes which results in insulin resistance and increases intra-hepatic lipid supply through the formation of triglycerides thereby accelerating lipid accumulation in the liver (Chadt & Al-Hasani, 2020; Stanhope, 2016; Ter Horst & Serlie, 2017). Lipid accumulation in the liver contributes to the formation of lipotoxic species such as diacylglycerol (DAG) which contributes to the impaired performance of insulin in the liver. DAG accumulation can activate c-Jun protein N-terminal protein kinase-1 (JNK-1) after activation of Protein kinase C epsilon (PKCε). This protein causes insulin resistance in the liver through phosphorylation of insulin receptor substrate 1 (IRS-1) (Friedman et al., 2018; Pereira et al., 2017). The accumulation of lipids in the liver also triggers inflammasome activation due to the response to Damage-associated Molecular Patterns (DAMPs), subsequently leading to the secretion of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β), supporting apoptosis through caspase-1 activation (Friedman et al., 2018). Hepatocyte damage can induce the release of Reactive Oxygen Species (ROS). Increased ROS production can activate serine/threonine proteins such as c-Jun N-terminal kinase (JNK) and Inhibitor of Nuclear Factor Kappa-B Kinase (IKK). These proteins play a role in the occurrence of insulin resistance in the liver (Sodhi et al., 2015; Zhang et al., 2020). In conditions of insulin resistance, inhibition of insulin receptor substrate (IRS) phosphorylation occurs which results in increased binding of the GLUT-2 gene promoter to hepatocytes nuclear factor 3β (HNF3β) and hepatocytes nuclear factor 1α (HNF1α). This mechanism causes an increase in liver GLUT-2 expression. Previous studies on diabetic mice showed that the binding of the liver GLUT-2 gene promoter to HNF3β and HNF1α increased by more than 50% (David-Silva et al., 2013; Tomaz et al., 2016; Narasimhan et al., 2015).

This research indicates a significant difference in the expression of liver GLUT-2 in mice induced with high fructose in the moderate-intensity swimming exercise group compared to the control group. Moderate-intensity exercise significantly decreases the expression of liver GLUT-2 induced by high fructose in mice. These findings align with earlier research on mice subjected to moderate-intensity treadmill exercise for 8 weeks, showing lower GLUT-2 liver expression compared to the control group (Simões E Silva et al., 2020). Skeletal muscle contraction due to moderate-intensity exercise can trigger myokine secretion, such as interleukin-6 (IL-6), activated through mitogen-activated protein kinase (MAPK), acting as an anti-inflammatory mediator (Gonzalez-Gil & Elizondo-Montemayor, 2020). This myokine stimulates the production of anti-inflammatory cytokines and cytokine inhibitors such as IL-10 which play a role in inhibiting the synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-1β (Aryana et al., 2018). Exercise can also produce hepatokines such as fibroblast growth factor 21 (FGF21) which are secreted from the liver. These hepatokines support the enhancement of fatty acid oxidation by increasing peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), to prevent intrahepatic lipid accumulation. A study on mice has reported that exercise can affect the level and activity of FGF21 which has an impact on reducing lipid levels (Gonzalez-Gil & Elizondo-Montemayor, 2020). Meanwhile, the decrease in lipid accumulation through this mechanism affects the increase in insulin sensitivity (Guo et al., 2020). Insulin binding in the liver can lead to a decrease in liver GLUT-2 expression that is dependent and concomitant with a decrease in insulin-mediated hepatic glucose production. Increased insulin sensitivity also affects the decrease in the binding of the promoter of the liver GLUT-2 gene to HNF3β and HNF1α. Insulin receptor (IR) and Liver

GLUT-2 on the membrane of hepatocytes are interconnected and both are internalized into the endosomal compartment when insulin is bound so that GLUT-2 expression decreases in the hepatocyte membrane (Narasimhan et al., 2015)

The results of this study show that the expression of liver GLUT-2 in the light-intensity exercise group experienced the highest increase compared to the other groups. This finding is consistent with previous research on mice subjected to light-intensity exercise, showing a threefold increased risk of type 2 diabetes compared to high-intensity exercise, accompanied by a twofold increase in GLUT-2 liver expression (Simões E Silva et al., 2020; Thorens, 2015). It is known that PGC-1α can prevent intrahepatic lipid accumulation, impacting insulin sensitivity improvement. Light-intensity exercise produces PGC-1α in small amounts, thus not yet capable of generating a positive effect on insulin resistance. Previous research has proven that the expression of PGC-1α in the light-intensity exercise group is found to be minimal (Brandt et al., 2017).

The results of this study showed that there was a significant difference in pancreatic GLUT-2 expression of mice induced by high fructose in the moderate-intensity swimming exercise group compared to the control group. This study supports the findings of a previous study which found that mice given moderate-intensity exercise for 12 weeks increased pancreatic GLUT-2 expression (Király et al., 2008). Glucose is a source of energy needed by many organisms and is central to cell metabolism (Berger & Zdzieblo, 2020). However, hyperglycemia adversely affects the function and survival of pancreatic β cells (Tomita, 2016). Previous studies concluded that the effects of high glucose exposure lead to decreased expression of pancreatic GLUT-2 (Bocarsly et al., 2010; Hattori et al., 2021; Teodhora et al, 2021). Thus, metabolic regulation depends on mitochondria which have an important role in nutrients to produce energy balance (Barbieri & Sestili, 2012). Normally, pancreatic β cells secrete insulin after glucose uptake through GLUT2 expressed on the cell surface. GLUT2 plays a crucial role in pancreatic β cells for insulin secretion through an ionic pathway, specifically the K^+-ATP-dependent pathway. Fructose, via the tricarboxylic acid cycle, generates adenosine triphosphate (ATP). An increased ATP ratio in the cytoplasm leads to the closure of K^+-ATP channels, causing membrane depolarization that inhibits potassium efflux from the cell. This results in the opening of Ca^{2+} channels, allowing Ca^{2+} influx into the cell, triggering insulin granule exocytosis (Sun et al., 2023; Seino et al., 2010). Subsequently, insulin is released from the granules and secreted into the bloodstream (Khin et al., 2023). The condition of obesity and hyperglycemia is metabolic stress that can induce an increase in reactive oxygen species (ROS) (Fariss et al., 2005; Galicia-Garcia et al., 2020). Increased ROS decreases adenosin triphosphate (ATP) production causing damage to the death of pancreatic cells (Kim et al., 2008; Ma et al., 2012). The expression of pancreatic GLUT-2 is regulated by various factors, including mRNA, protein levels, and genetics. At the genetic level, pancreatic and duodenal homeobox (Pdx-1) regulates GLUT-2 in the pancreas (Sun et al., 2023; Zhou & Melton; Kaneto et al., 2008). Rad et al. demonstrated that hyperglycemia conditions inhibit Pdx-1 expression (Robertson et al., 2007), which can subsequently reduce pancreatic GLUT-2 expression (Hattori et al., 2021; Rad et al., 2022; Nahdi et al., 2017).

Exercise is considered a nonpharmacological therapy to prevent and treat metabolic stress (Wang et al., 2022). Exercise can have a proliferative effect and prevent pancreatic β cell death (Narasimhan et al., 2015; Curran et al., 2020). As demonstrated by previous research, male mice subjected to moderate-intensity exercise for 8 weeks increased pancreatic GLUT-2 expression (Simões E Silva et al., 2020). Exercise enhances Pdx-1 levels and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) (Behrestaq et al., 2018) for the biogenesis and maintenance of pancreatic β cell function through increased expression of the target genes nuclear factor erythroid2-related factor 2 (Nrf2), superoxide dismutase (SOD), and glutathione peroxidase (GPx), thereby suppressing reactive oxygen species (ROS) elevation (Radak et al., 2013), bolstering antioxidant defenses (Miyata et al., 2008), and increasing GLUT2 pancreatic expression (Sokhanvardastjerdi et al., 2020). The results of this study indicate that moderate-intensity swimming exercise significantly increases pancreatic GLUT-2 expression in mice induced with fructose.

Exercise has metabolic effects on peripheral tissues, such as muscles and pancreatic β cells. It stimulates glucose uptake in peripheral tissues, reduces insulin resistance, and lowers blood sugar levels, potentially alleviating metabolic stress on pancreatic beta cells (Lv et al., 2022). Moderate-intensity exercise induces an increase in reactive oxygen species (ROS), triggering antioxidant responses and preventing oxidative stress (Briones & Touyz, 2009). Moderate-intensity exercise has anti-inflammatory effects on pancreatic beta cells, reducing inflammation in animal studies (Sharif et al., 2018). Exercise can stimulate the expression and production of IL-6 (Leuchtmann et al., 2022; Narendran et al., 2015)), which functions in pancreatic β cells to suppress inflammatory responses (Lv et al., 2022). IL-6 exhibits anti-inflammatory properties, stimulating the release of interleukin-10 (IL-10) and interleukin-receptor antagonist (IL-Ra), and reducing the production of TNF-α and interleukin-1 (IL-1) (Petersen & Pedersen, 2005; Langlois et al., 2021). IL-6 secreted in muscles can stimulate L cells in the intestine to produce glucagon-like peptide-1 (GLP-1), protecting pancreatic β cells and enhancing insulin secretion (Fujiwara et al., 2019). Extracellular signal-regulated kinase1/2 (ERK1/2) and cAMP response element-binding protein (CREB) increase after exercise (Widegren et al., 2000; Zanjani et al., 2019; Curran et al., 2020). ERK1/2 and CREB are molecules that regulate the transcriptional survival of pancreatic beta cells. Increased ERK 1/2 and CREB stimulate Pdx-1 upregulation (Curran et al., 2020) and enhance GLUT-2 expression. This study also found that

moderate-intensity swimming exercise significantly increased the expression of pancreatic GLUT-2 in mice induced by fructose. Exercise increases the demand for adenosine diphosphate (ADP) causing adenosine monophosphate-activated protein kinase (AMPK) activation which then stimulates ATP formation. On the other hand, AMPK is correlated with increasing mitochondrial function through activating PGC-1α for pancreas biogenesis (Lee & Song, 2018). Moderate-intensity exercise results in optimal muscle contraction and ATP production. Whereas light intensity exercise is not able to provide an effect and cannot suppress the increase in ROS resulting from hyperglycemia. Then the muscle contraction resulting from heavy-intensity training can exceed the threshold (Nurdin et al., 2019) causing stress to be the cause of muscle damage (He et al., 2016; Huang et al., 2022; Lu et al., 2021; Stožer et al., 2020).

Excessive muscle contraction causes an increase in the stretch to break in the sarcomere resulting in a loss of muscle strength. This can burden other structures, such as the T-tubules. Excitation contraction coupling (ECC) occurs associated with loss of strength and damage to the sarcoplasmic reticulum (SR) membrane resulting in an uncontrolled release of Ca^{2+}. Opening of stretch-activated channels (SAC) and damage to the sarcolemma results in increased $[Na^{+}]$ and $[Ca^{2+}]$. Increased $[Ca^{2+}]$ contributes to cellular injury through the activation of calpain protease (CAL) which promotes further sarcolemma damage and high $[Ca^{2+}]$ also increases Ca^{2+} concentration in mitochondria thereby increasing ROS production (Stožer et al., 2020). Increased ROS production causes tissue damage and decreased expression of pancreatic GLUT-2. Furthermore, increased ROS can increase liver GLUT-2 expression through serine/threonine activation such as c-Jun N-terminal kinase (JNK) and inhibitor of nuclear factor kappa-B kinase (IKK). This protein plays a role in the phosphorylation of insulin receptor substrate (IRS), causing insulin resistance in the liver (Zhang et al., 2020).

Exercise is widely recognized for its protective role in preventing metabolic disorders. It contributes to the maintenance of organ homeostasis, including the regulation of GLUT-2 expression in the pancreas and liver. Through its ability to increase insulin secretion, enhance insulin sensitivity, maintain glucose homeostasis, and prevent inflammation, exercise emerges as a pivotal element in the preservation of metabolic health. Consequently, identifying the optimal intensity of exercise is essential for fostering the recovery of pancreatic and liver functions and mitigating oxidative stress in tissues. This study, however, is not without limitations. It did not incorporate other parameters such as insulin receptor substrate (IRS), insulin secretion, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), which are integral to understanding the full scope of liver GLUT-2 and pancreatic GLUT-2 expression. Additionally, the absence of a control group that was not administered fructose represents a missed opportunity to observe GLUT-2 expression in a baseline scenario. To build upon the findings of this study, future research should consider the effects of combined exercise modalities on liver and pancreatic GLUT-2 expression. Such investigations could provide a more nuanced understanding of the interplay between different exercise intensities and their impact on glucose control. It has been suggested that combined exercise may offer superior benefits in regulating glucose levels (Latif et al., 2017; Dotzert et al., 2018). Further studies could also explore the inclusion of additional metabolic parameters, such as IRS and HOMA-IR, to provide a more holistic view of the metabolic changes induced by exercise.

The practical implications of this study are significant for the development of exercise-based interventions tailored to improve metabolic health. By delineating the specific benefits of varying exercise intensities, healthcare providers can better guide patients in selecting the most appropriate exercise regimens to support their metabolic recovery. Moreover, the insights gained from this research can inform public health strategies aimed at combating metabolic disorders through lifestyle modifications.

In conclusion, while this study has contributed valuable knowledge to the field of exercise physiology and metabolic health, it also opens avenues for further research to enhance our understanding and application of exercise as a therapeutic tool. The comprehensive analysis of the study's results, their implications, and their relevance to the existing body of knowledge effectively bridges the gap between research findings and their practical implications, making valuable contributions to the scientific understanding of exercise physiology and metabolic health.

Conclusions

This study proves that moderate-intensity swimming exercise for 8 weeks increases pancreatic GLUT-2 expression and decreases liver GLUT-2 expression in high-fructose-induced mice. The use of exercise with moderate intensity has the most optimal effect compared to light and heavy intensity. Therefore, the results of this study can be used as the basis for recommendations for further research if developed in humans.

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Conflicts of Interest

The authors declare no conflict of interest.

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