Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2026;1 (19): 46-56

Research Article

The effect of a period of interval training on the expression of G6Pase and PEPCK genes in the liver tissue of obese type 2 diabetic rats

Asghar zolfaghari[1], Abdolali Banaeifar[2]*, Sajad Arshadi[3]

1. PHD student, Department of Exercise Physiology, ST.C., Islamic Azad University, Tehran, Iran.

2. Associated Professor, Department of Exercise Physiology, ST.C., Islamic Azad University, Tehran, Iran.

Received: 18 December 2025
Accepted: 25 February 2026

Abstract

Introduction: The present study was conducted to determine the effect of 10 weeks of interval training on the expression of G6Paseo PEPCK genes, which are related to gluconeogenesis in liver tissue in type 2 obese rats.

Methods: The statistical population of the present study consisted of obese type 2 diabetic rats. For this purpose, twenty-one 10-week-old Wistar rats were randomly divided into 3 groups (7 rats in each group): 1) healthy control 2) interval training 3) obese control group. At the beginning of the protocol, all rats were fed a high-fat diet for 8 weeks. Then, except for the obese control group, three other groups were made diabetic by intraperitoneal injection of STZ and then participated in a period of interval training. The intense interval training program was performed for 10 weeks, 5 sessions per week, in the form of treadmill running with intervals of 60 seconds of running and 120 seconds of rest, at a speed of 16 to 36 meters per minute. 48 hours after the last activity session, rats were anesthetized with a combination of xylazine and ketamine. After removing the tissue, the preparation steps were performed to examine the expression of the desired gene. The expression of the G6Pase and PEPCK genes was measured using the Realtime PCR method. Central and dispersion indices were used to describe the data statistically. The Shapiro-Wilk test was used to examine the data status and to examine the research hypotheses, the one-way analysis of variance (ANOVA) test was used and, if significant, the Tukey post hoc test was used to show the difference between the groups. The significance level was considered to be P≥0.05.

Results: The results of the present study showed that diabetes led to increased expression of G6Pase and PEPCK in the diabetic group compared to the healthy obese control group(p=.001). In addition, interval training was able to change these indices towards the values of the control group.

Conclusion: Based on the findings of the present study, improvement in the response of G6Pase and PEPCK to interval training as genes effective in the gluconeogenesis process leads to a decrease in the rate of gluconeogenesis and possibly an improvement in glycemic status in type 2 diabetic rats.

Keywords:
Interval training, Obese rats, Type 2 diabetes, Gene expression, G6Pase, PEPC

***Corresponding author:** Abdolali Banaeifar
Address: Associated Professor, Department of Exercise Physiology, ST.C., Islamic Azad University, Tehran, Iran.
Email: A.banaeifar@azad.ac.ir **Tell:**09122251779
A.B: 0000-0001-7271-3382

1. Introduction

Type 2 diabetes is the most common chronic metabolic disease that occurs due to glucose intolerance due to an imbalance between insulin stores and insulin requirements. Urban development and a sedentary lifestyle have caused this disease to increase rapidly. Many factors contribute to the occurrence of this disease, insulin resistance caused by obesity and increased blood glucose are known to be factors that accelerate this disease (1). Blood glucose regulation in diabetes is one of the important goals of controlling and treating this disease. One of the most important pathological mechanisms affecting diabetes is impaired hepatic glucose metabolism, which can lead to the development of the disease (2).

The liver is a key organ in maintaining glucose homeostasis and balance in the body, regulating glucose synthesis through pathways such as glycogenolysis and gluconeogenesis from non-carbohydrate precursors such as pyruvate, glycerol, lactate, and amino acids such as alanine (3). The rate of gluconeogenesis is controlled and regulated by the activity of enzymes such as PEPCK (phosphoenolpyruvate carboxykinase) and glucose-6-phosphatase (G-6-pase) (4).

A hallmark of type 2 diabetes is increased endogenous glucose production, primarily due to increased hepatic glucose production (HGP) (5). The two rate-limiting enzymes of hepatic glucose production are HGP, the catalytic subunit of glucose 6-phosphatase (encoded by G6PC) and phosphoenolpyruvate carboxykinase (encoded by PCK1 and PCK2). Because the expression of these enzymes is suppressed by insulin, it is widely believed that patients with type 2 diabetes (T2D) have increased expression of G6PC and PCK due to hepatic insulin resistance. However, correlating the expression of these enzymes with diabetes or glycemia in humans has been challenging (6).

PEPCK is one of the key enzymes involved in gluconeogenesis. The enzyme pyruvate carboxylase converts pyruvate to oxaloacetate in the mitochondria, and then the conversion of oxaloacetate to phosphoenolpyruvate is carried out by PEPCK. PEPCK expression is also controlled by insulin. After a meal, pancreatic beta cells produce and secrete insulin, which binds to its receptor and phosphorylates AKT, which inhibits its activity through PGC1a, and ultimately inhibits gluconeogenesis (7). The physiological significance of dysregulation of hepatic gluconeogenesis genes suggests that overexpression of PEPCK in the liver causes glucose intolerance. In a study by Pastik et al. (8), increased expression of PEPCK in the liver led to fasting hyperglycemia and peripheral insulin resistance. Increased glucose through gluconeogenesis or through overexpression of PEPCK is responsible for the development of metabolic disorders in type 2 diabetes (8).

Glucose-6-phosphatase is a rate-limiting enzyme in gluconeogenesis, which is mainly expressed in the liver and plays an important role in the production of glucose to other organs. The activity of this enzyme is stimulated by the cAMP signaling pathway and inhibited by insulin. In insulin-deficient diabetic model rat, G6P-ase activity is increased (9).

The importance of diabetes is such that many studies and interventions have been conducted in this regard with the aim of controlling or reducing the severity of the disease in affected individuals. One of these interventions is exercise (6.7 Shokrollahi). One of the approaches to studying the effect of exercise on enzymes affecting the gluconeogenesis process (10)

In the study of Shokrollahi et al. (2019), resistance training significantly reduced the expression of G6Pase in liver cells, which led to a decrease in the expression of G-6-pase and improved beta cell function (11). Jakob et al. (2015) also reported an increase in the expression of G6Pase in liver cells following one hour of running exercise in obese rats (12). In the study of Yarmohammadi et al. (2018), 12 weeks of aerobic exercise also led to a decrease in the expression of G6Pase in the liver tissue of diabetic rats (13). Also, in a 10-week study, aerobic exercise significantly reduced PEPCK expression in nicotinamide-streptozotocin-induced diabetic male rats (14).

In general, it can be said that understanding the molecular cellular regulators in the gluconeogenesis pathway can greatly help in the treatment of diabetes. Although some studies have been conducted in this field, there is less research evidence on changes in G6Pase and PEPCK in liver tissue. Therefore, the present study aims to investigate the role of resistance training on the expression of PEPCK, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase (G-6-pase) genes in the liver tissue of type 2 diabetic rats.

2. Materials and Methods

The statistical population of the present study consists of all male Wistar rats of the Pasteur Institute of Iran, from which 21 10-week-old rats weighing 220±20 grams (initially) were purchased and divided into healthy groups and after induction of type 2 diabetes, they were randomly divided into 2 diabetic control groups and an interval training group. The method of induction of type 2 diabetes was through 8 weeks of high-fat diet followed by intraperitoneal injection of STZ.

Exercise Protocol:

One week after familiarization with the laboratory environment, familiarization exercises were performed to climb a ladder for resistance training and a treadmill for rodents. After that, the animals were divided into four groups: healthy (7 animals), diabetic control (7 animals), resistance training (7 animals), and interval training (7 animals).Interval training was performed for 10 weeks, 5 sessions per week, in the form of treadmill running. Running speed increased from 16 m/min in the first week of training to 36 m/min in the tenth week of training. The activity time was 60 seconds and the rest time was 120 seconds, which was performed 10 times per sessionThe healthy and diabetic control groups did not participate in any training program (15).

Biochemical Analysis of Variables: 48 hours after the last training session and after a 12-hour fast, the rats were anesthetized with a combination of xylazine (3-5 mg/kg) and ketamine (30-50 mg/kg). Then, their liver tissue was removed, washed in physiological saline, and immediately frozen in liquid nitrogen and transferred to a -80 freezer for measuring variables. All ethical standards for working with laboratory animals were observed according to the instructions. PCR was used to measure the expression of the genes of interest, with GAPDH as the control gene. To determine the quality and quantity of the extracted RNAs, the OD of all samples was measured with a Nano Drop device. Then, cDNA synthesis was performed using a Takara kit (TAKARA cat NO. 6130) and according to the manufacturer's instructions. For RNA extraction, 50 mg of frozen mouse liver tissue was homogenized and,

according to the manufacturer's instructions, RNA solution was extracted from it and purified from any DNA contamination and RNA degrading enzymes with DNase enzyme. 2 µg of mRNA from each sample was used to synthesize the first strand of DNA. The relative expression of the studied genes in the liver was measured with the help of their specific primers (Table 1). Primers were designed using Oligo 7 software, and all primers were blasted on the NCBI website for specificity and accuracy. The absorbance ratio of 260 to 280 ng for all extracted samples was 1.8 to 2. To check the quality of extracted RNA, electrophoresis and 1% agarose gel were used. Quantitative analysis was performed using a real-time PCR device, Step One Plus. The average Ct values of replicates were measured for each sample, and the comparative Ct method was used to determine the relative expression level of target genes.

Table 1. Primer Sequence Specifications for Each Gene

Gene	Forward	Reverse
G6Pase	GGTTGGGATACTGGGCTGTG	TTGTAGATGCCCCGGATGTG
PEPC	TGCCCCAGGAAGTGAGGAAG	CAGTGAGAGCCAGCCAACAG

The Shapiro-Wilk test was used to determine the normality of the data, the one-way analysis of variance statistical model was used to determine the significance of the difference between the variables, and if significant, the Tukey post hoc test was used to determine the location of the difference. Statistical operations were performed at a significance level of $P \geq 0.05$ and using Spss software version 26.

3. Results

The results of one-way analysis of variance showed that the expression of G-6-Pase in the diabetic control group and the interval training group was significantly different from the obese control group (p=0.001). In other words, the values of this index increased significantly compared to the control group. Also, a significant difference was observed between the exercise group and the diabetic group (p=0.001). In such a way that the expression values of the G-6-Pase index in the exercise group were lower than the diabetic group.(figure1)

The results also showed that the expression of PEPCK in the diabetic control group and the interval training group was significantly different from the obese control group (p=0.001). In other words, the values of this index increased significantly compared to the control group. Also, a significant difference was observed between the exercise group and the diabetic group (p=0.001). In such a way that the expression values of the G-6-Pase index in the exercise group were lower than the diabetic group.(figure2)

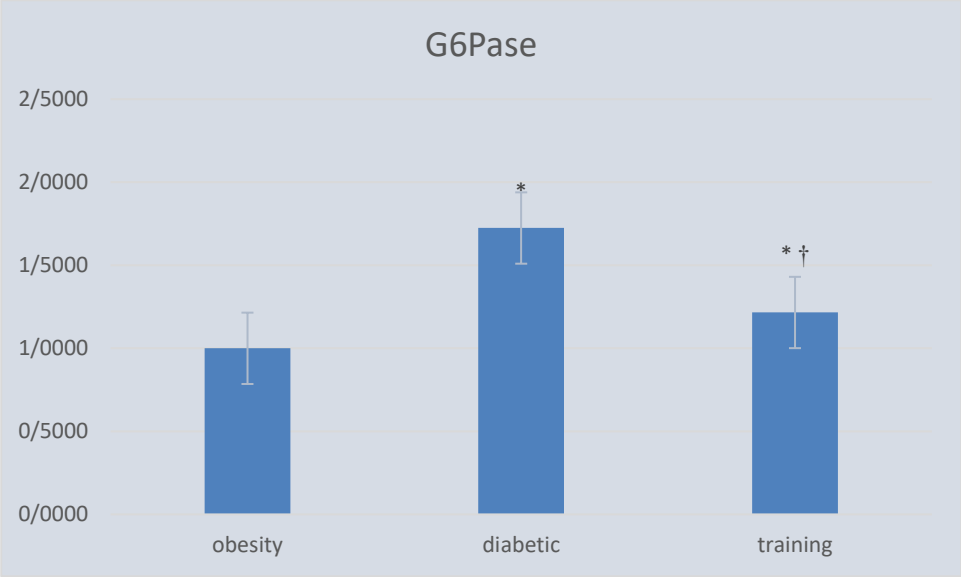

Figure 1: Mean G6Pase expression in study groups

*. Significant change compared to obese control group.

† Significant change compared to diabetic control

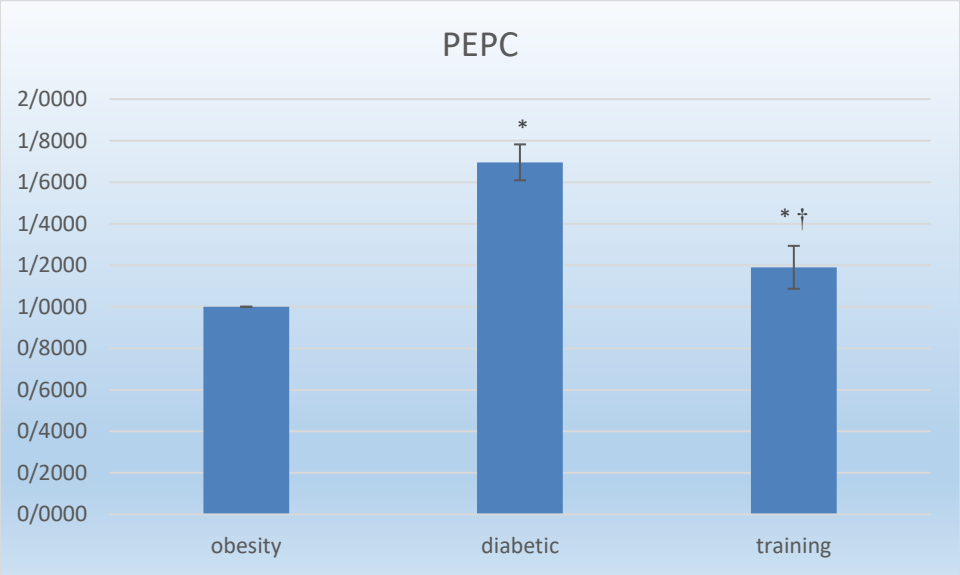

Figure 2: Mean PEPC expression in study groups

*. Significant change compared to obese control group.

† Significant change compared to diabetic control

4. Discussion

The results of the present study on the expression of PECK and G-6-pase genes showed that diabetes led to an increase in these genes in the diabetic group compared to the obese control group. Also, interval training led to a decrease in the expression of G-6-pase and PECK genes compared to the diabetic group.

In this regard, in line with the present study, in the study of Kazemi Nasab et al. (2019) the effect of eight weeks of aerobic training on the expression of PEPCK and G6Pase in the liver tissue of obese prediabetic rats showed that the relative expression of key genes in gluconeogenesis, including G6Pase and PEPCK, was significantly reduced in the liver of trained rats compared to untrained rats (16). Shokrollahi Ardakani also points out in his study that 12 weeks of resistance training significantly reduced glucose 6-phosphatase (G6Pase) expression in liver cells, as well as glucose levels and beta cell function in type 2 diabetic rats compared to the control group, leading to a significant decrease in glucose, beta cell function, and G6Pase expression in liver cells (11). Increased hepatic glucose release due to impaired function of enzymes effective in the hepatic gluconeogenesis process is one of the main characteristics of type 2 diabetes (16). The results of the present study seem to confirm the role of interval training in improving changes in glucose levels and beta cell function, as well as the expression of hepatic gluconeogenic genes compared to the control group.

Ghahramani et al. (2019) studied the effect of aerobic exercise on the expression of HNF-4α and G6Pase genes in the liver tissue of male rats with type 2 diabetes induced by nicotinamide-streptozotocin (17).

Changes in G6Pase protein expression between the two aerobic diabetic and diabetic control groups did not show a significant difference, which is not consistent with the results of the present study. Other inconsistent studies include the study by Nik Seresht et al. (2022), which aimed to determine the effect of 6 weeks of high-intensity interval training (HIIT) on G6Pase expression in liver tissue in obese type 2 diabetic rats and compared with the non-diabetic obese group. It was shown that interval training in the diabetic group did not cause a change in G6Pase expression compared to the diabetic control group (18). It is possible that the duration of the training period (6 weeks versus 10 weeks in the present study) is the reason for the difference in the results.

In the study by Shojaei et al. (2023), examining the effect of aerobic exercise on the function of the local renin-angiotensin system and the gluconeogenesis pathway in the liver tissue of type 2 diabetic rats, eight weeks of aerobic exercise significantly reduced the expression of PEPCK genes and blood glucose levels compared to the diabetic-non-exercising group (19). In the study by Ghahremani et al. (2019), the effect of 10 weeks of aerobic exercise significantly reduced the expression of PEPCK protein in the aerobic diabetic group compared to the control group. Aerobic exercise also reduced insulin resistance (14). The effects of endurance exercise on phosphoenolpyruvate carboxykinase (PEPCK) in obese Zucker rats by Chang also showed that exercise training significantly reduced PEPCK (20).

Studies show that insulin, by binding to hepatic receptors, regulates signaling cascades that activate enzymes involved in the uptake and release of glucose from the liver in the processes of gluconeogenesis, glycolysis, and glycogen metabolism (13). The presence of insulin in the liver stimulates GCK and reduces the expression of G6Pase, which leads to long-term changes in GCK and G6Pase proteins that facilitate the release and uptake of glucose. It has been shown that the genetic regulation of liver GCK and G6Pase changes rapidly in response to changes in hepatic insulin in dogs (21), and since changes in glucose levels in response to exercise are also dependent on other hormonal and genetic changes, PEPCK and G6Pase could be effective as other transcription factors in this context (20).

Clinical studies, especially in the last decade, have shown that type 2 diabetes (T2D) is not only the result of insulin resistance or impaired insulin signaling pathways in target tissues such as adipose tissue or muscle, but also involves defects in several mechanisms, the prevalence or severity of the disease (22). On the other hand, increased blood glucose levels are also the result of excessive hepatic glucose release. In this context, in addition to gluconeogenic enzymes such as fructose 1,6-diphosphatase (FDPase), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase), which regulate the rate of the G6Pase process (23), also strongly affect hepatic glucose release, which is primarily controlled and regulated by insulin (24).

The decreased expression of genes involved in hepatic gluconeogenesis can also be attributed to changes in the signaling pathways of other transcription factors. In the study by Lima et al. (2009), the researchers attributed the decreased expression of PEPCK to the decreased expression of TRB3 after a prolonged recovery period after prolonged exercise. Thus, in the aforementioned study, TRB3 expression was significantly reduced in the liver tissue of obese diabetic rats at 8 h after a prolonged swimming session compared to the control group, and the researchers noted that the decreased expression of TRB3 would lead to a decrease in hepatic glucose release due to the effect on protein levels and PEPCK expression. These researchers also suggested that the hepatic glucose production after a prolonged recovery period after prolonged exercise is rooted in the improvement of insulin signaling pathways (25).

Despite some contradictory findings mentioned above, since the increase in blood glucose or hyperglycemia in type 2 diabetes is due to various factors such as insulin secretion, membrane glucose uptake in target tissues, or hepatic glucose release, the decrease in glucose can be attributed to stimuli such as drug use, diet, and exercise (26, 27). In the present study, it seems that interval training, by reducing the expression of PECPCK and G6Pase as enzymes effective in the gluconeogenesis process, reduces the rate of gluconeogenesis and hepatic glucose release in type 2 diabetic rats.

۵. Conclusion

The present study aimed to determine the effect of 10 weeks of interval training on some gluconeogenic genes in liver tissue and diabetic obese rats, and showed that diabetes led to increased expression of G6p and PECK genes. In addition, interval training, as an effective factor on the gluconeogenesis process, was able to change these indices towards the values of the diabetic control group.

Acknowledgements

This research is the results of a part of a specialized doctoral thesis. The authors thank and appreciate all the people who participated in this research.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: A.Z, A.B, S.A ; Methodology: A.Z, A.B, S.A ; Software: A.Z, A.B, S.A ; Validation: A.Z, A.B, S.A ; Formal analysis: A.Z, A.B, S.A ; Investigation: A.Z, A.B, S.A ; Resources: A.Z, A.B, S.A ; Data curation: A.Z, A.B, S.A ; Writing - original draft: A.Z, A.B, S.A ; Writing - review & editing: A.Z, A.B, S.A ; Visualization: A.Z, A.B, S.A ; Supervision: A.Z, A.B, S.A ; Project administration: A.Z, A.B, S.A ; Funding acquisition: : A.Z, A.B, S.A .

References

1. Regufe, V. M., Pinto, C. M., & Perez, P. M. (2020). Metabolic syndrome in type 2 diabetic patients: A review of current evidence. *Porto biomedical journal*, *5*(6), e101.

2. Hameed, I., Masoodi, S. R., Mir, S. A., Nabi, M., Ghazanfar, K., & Ganai, B. A. (2015). Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. *World journal of diabetes*, *6*(4), 598.

3. Agius L. Hormonal and metabolite regulation of hepatic glucokinase. Annual review of nutrition. 2016 Jul 17;36(1):389-415.

4. Wang L, Guo Y, Pan M, Li X, Huang D, Liu Y, Wu C, Zhang W, Mai K. Functions of forkhead box O on glucose metabolism in abalone Haliotis discus hannai and its responses to high levels of dietary lipid. Genes. 2021 Feb 20;12(2):297.

5. Rizza, R. A. (2010). Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes*, *59*(11), 2697-2707.

6. Konopelska S, Kienitz T, Quinkler M. Downregulation of Hepatic Glucose-6-Phosphatase-α in Patients With Hepatic Steatosis. Obesity. 2011 Dec;19(12):2322-6.

7. Wu H, Deng X, Shi Y, Su Y, Wei J, Duan H. PGC-1α, glucose metabolism and type 2 diabetes mellitus. J endocrinol. 2016 Jun 1;229(3):R99-115.

8. Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes & metabolism. 2004 Nov 1;30(5):398-408.

9. Palsamy, P., & Subramanian, S. (2009). Modulatory effects of resveratrol on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin–nicotinamide-induced diabetic rats. *Chemico-biological interactions*, *179*(2-3), 356-362.

10. Naghizadeh, N., Riyahi Malayeri, S., Roozbahani, M., Khademi, A., Shirvani, H. The Effectiveness of Endurance Training and Nano Curcumin Supplementation on the Expression of Mir-21and P53 Genes in Brain Tumor Tissue in an Animal Model of Glioblastoma Multiform. Journal of Nutrition,Fasting and Health, 2024; 12(1): 26-35. doi: 10.22038/jnfh.2023.73964.1455Shokrollahi Ardakani Ahmad, Abednatanzi Hossein, Gholami Mandana, Shakeri Nader. The effect of 12 weeks of resistance training on the expression of glucose-6-phosphatase and phosphoenolpyruvate kinase in liver cells, glucose levels and beta cell function in type 2 diabetic rats.

11. Knudsen JG, Biensø RS, Hassing HA, Jakobsen AH, Pilegaard H. Exercise-induced regulation of key factors in substrate choice and gluconeogenesis in mouse liver. Molecular and cellular biochemistry. 2015 May;403(1):209-17.

12.Yarmohammadi, M., Behboudi, L. and Eizadi, M., 2019. Effect of Aerobic Training on Glucose-6-phosphatase Expression in the Liver Hepatocytes and Fasting Glucose in Type II Diabetic Rats. *Journal of Diabetes Nursing*, *6*(4), pp.618-630.

13. Ghahraman Darshegi Mehri, Benaifar Abdolali, Arshadi Sajjad, Soheili Shahram. The effect of aerobic exercise on the PGC-1α/PEPCK signaling pathway in liver cells of male rats induced with nicotinamide-streptozotocin diabetes.

14. Karimi M, Eizadi M. The effect of interval training on FOXO1 expression in pancreas tissue of diabetic rats with high fat diet and STZ. Razi J Med Sci. 2019;26(6):95-104

15. Kazemi Nasab F, Marandi SM, Shirkhani S, Sheikhanian Poor A, Ghaedi K. The Effect of 8 Weeks Aerobic Exercise on LXRa, PEPCK, and G6PC2 mRNA in Obese Prediabetic Mice. Sport Physiology. 2020 Dec 21;12(48):17-38.

16. Ghahraman Darshegi, Mehri, Benaifar, Arshadi, Soheili. The effect of aerobic exercise on the relative expression of HNF-4α and G6Pase genes in the liver tissue of male rats with type 2 diabetes induced by nicotinamide-streptozotocin. Daneshvar Pezgezi. 2020 Sep 26;28(2):14-27.

17. Nikseresht Fatemeh, Bahrami Mustafa, Rahmati Masoud. The effect of interval training on the expression of G6Pase in liver cells, glucose and insulin in obese type 2 diabetic rats..

18. Shojaei M, Kazeminasab F, Pourvaghar M. The effect of aerobic exercise on the function of the local renin-angiotensin system and gluconeogenesis pathway in the liver tissue of type 2 diabetic rats. Feyz Medical Sciences Journal. 2023 Aug 10;27(4):368-77.

19. Chang SP, Chen YH, Chang WC, Liu IM, Cheng JT. Merit of physical exercise to reverse the higher gene expression of hepatic phosphoenolpyruvate carboxykinase in obese Zucker rats. Life sciences. 2006 Jun 13;79(3):240-6.

20. Ramnanan CJ, Edgerton DS, Rivera N, Irimia-Dominguez J, Farmer B, Neal DW, Lautz M, Donahue EP, Meyer CM, Roach PJ, Cherrington AD. Molecular characterization of insulin-mediated suppression of hepatic glucose production in vivo. Diabetes. 2010 Jun 1;59(6):1302-11.

21. Mackenzie RW, Elliott BT. Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 diabetes. Diabetes, metabolic syndrome and obesity: targets and therapy. 2014 Feb 13:55-64.

22. Sakamaki JI, Daitoku H, Kaneko Y, Hagiwara A, Ueno K, Fukamizu A. GSK3β regulates gluconeogenic gene expression through HNF4α and FOXO1. Journal of Receptors and Signal Transduction. 2012 Apr 1;32(2):96-101.

23. Konopelska S, Kienitz T, Quinkler M. Downregulation of Hepatic Glucose-6-Phosphatase-α in Patients With Hepatic Steatosis. Obesity. 2011 Dec;19(12):2322-6.

24. Lima AF, Ropelle ER, Pauli JR, Cintra DE, Frederico MJ, Pinho RA, Velloso LA, De Souza CT. Acute exercise reduces insulin resistance-induced TRB3 expression and amelioration of the hepatic production of glucose in the liver of diabetic mice. Journal of cellular physiology. 2009 Oct;221(1):92-7.

25. Wang L, Guo Y, Pan M, Li X, Huang D, Liu Y, Wu C, Zhang W, Mai K. Functions of forkhead box O on glucose metabolism in abalone Haliotis discus hannai and its responses to high levels of dietary lipid. Genes. 2021 Feb 20;12(2):297.

26. Basu R, Barosa C, Jones J, Dube S, Carter R, Basu A, Rizza RA. Pathogenesis of prediabetes: role of the liver in isolated fasting hyperglycemia and combined fasting and postprandial hyperglycemia. The Journal of Clinical Endocrinology & Metabolism. 2013 Mar 1;98(3):E409-17.