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Effects of Eight Weeks of Swimming Training on Fetuin-A, FGF21 Levels, and Oxidative Stress in Liver Tissue of Mice with Type 2 Diabetes

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Abstract

Objective: The aim of the present study was to investigate the effect of eight weeks of swimming training on Fetuin-A, FGF21, and oxidative stress levels in the liver tissue of type 2 diabetic mice.

Materials and Methods: A total of 32 male BALB/c mice were randomly assigned to four groups: healthy control (HC), healthy training (HT), diabetic control (DC) and diabetic training (DT). Type 2 diabetes was induced using a high fat diet combined with streptozotocin. Swimming sessions were conducted five days/week over eight weeks with progressive intensity. Liver samples were harvested 48 hours after the final session. Fetuin-A and FGF21 gene expression were measured via real-time PCR, and ROS levels were quantified using a fluorescence assay. One-way ANOVA with Tukey's post hoc test was used for comparisons ($P \leq 0.05$).

Results: Diabetes induction significantly increased Fetuin-A, FGF21, and ROS levels in the DC group compared to the HC group ($P < 0.001$). The adaptation resulting from eight weeks of swimming training in the diabetic exercise group probably decreased Fetuin-A and ROS and increased FGF21 ($P < 0.001$). In the HT group, decreased Fetuin-A and increased FGF21 were also observed compared to the HC group ($P < 0.001$).

Conclusion: Swimming training modulates key hepatokines(decreasing Fetuin-A and increasing FGF21) and reduces oxidative stress, thereby creating a more favorable metabolic environment in the liver of diabetic mice. These findings in the studied group suggest that swimming training could probably be considered as a non-pharmacological strategy.

Keywords: Type 2 diabetes, Swimming training, Fetuin-A, FGF21, Oxidative stress, Liver

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Introduction

Type 2 diabetes (T2D) is currently recognized as a global health challenge, primarily driven by the growing prevalence of obesity and inactive lifestyles (1-3). This metabolic disease is characterized by persistent hyperglycemia, reduced insulin action, and impaired function of multiple organs, with the liver being a key target (3,4). As the principal organ governing glucose and lipid balance, the liver occupies a central position in the pathophysiology of T2D (5,6). Under diabetic conditions, hepatic dysfunction results in augmented glucose output, lipid deposition, and elevated oxidative stress, all of which facilitate the initiation and progression of diabetes related complications (7,8).

The liver produces various secretory proteins known as hepatokines, which are believed to regulate energy metabolism, insulin responsiveness, and glucose equilibrium through communication among different tissues (9,6). Among these hepatokines, Fetuin A and fibroblast growth factor 21 (FGF21) have drawn particular interest because of their significant and frequently opposing metabolic functions (10,11).

Fetuin A, which is mainly secreted by hepatocytes, acts as an endogenous blocker of the insulin receptor, thereby attenuating insulin sensitivity by interfering with receptor autophosphorylation in peripheral tissues (12,13). Elevated Fetuin A levels have been consistently observed in patients with T2D and obesity, and this increase is closely correlated with insulin resistance, inflammatory responses, and oxidative stress (14,11). Moreover, as a positive acute phase protein, Fetuin A can trigger systemic inflammation by engaging inflammatory cascades such as the TLR4 pathway (15).

In contrast, FGF21 is considered a metabolically beneficial hepatokine that exerts protective effects against metabolic disturbances through enhanced fatty acid oxidation, increased glucose uptake, and improved insulin sensitivity (16-18). It modulates energy homeostasis by stimulating signaling routes like AMPK and PGC 1α (19). Nevertheless, in diabetes and obesity, circulating FGF21 levels are frequently raised, a phenomenon that has been interpreted as a state of FGF21 resistance, akin to the resistance observed with insulin and leptin (18,19).

A prominent feature of T2D is the excessive generation of reactive oxygen species (ROS), leading to oxidative stress (20,21). In the diabetic state, elevated glucose and free fatty acids contribute to mitochondrial impairment and enhanced ROS formation in various organs, including the liver (21,22). This oxidative burden promotes the worsening of insulin resistance and diabetic complications by causing macromolecular injury, stimulating inflammatory pathways and disrupting insulin signal transduction (20,11).Recent investigations have uncovered a close interplay between oxidative stress and hepatokines, indicating that ROS can modulate the expression and release of hepatokines such as Fetuin A and FGF21 (11,22).

Physical exercise training is widely acknowledged as a safe and effective non pharmacological approach for the management of T2D (23,24). Considerable evidence indicates that sustained exercise training at different intensities can assist in glycemic control and attenuate diabetic complications by enhancing insulin sensitivity, curbing inflammation, and regulating oxidative balance (23,7). It is important to highlight that, unlike the favorable adaptations induced by chronic exercise, a single acute bout may transiently elevate oxidative stress and inflammation; however, regular training promotes antioxidant and anti inflammatory adaptations (25). Among various exercise modalities, swimming training, which imposes minimal joint stress, has been extensively employed in animal studies as a chronic intervention (26-28). Earlier reports have shown that regular swimming induced adaptations can ameliorate liver function by reducing inflammation and oxidative damage in the hepatic tissue of diabetic mice (29,21,26).

Given the advantageous influence of swimming training on hepatic function, several studies have explored its effects on hepatokines. Shams et al. (2024) demonstrated that cold water swimming influences FGF21 levels in male Wistar rats (17). Similarly, Dastah and Babaei (2021) reported that aquatic exercise reduces Fetuin A and elevates FGF21 in women with T2D (13). In a study by Saberi et al. (2024), exercise training alleviated renal injury in diabetic mice via reductions in Fetuin

A, oxidative stress, and inflammation (14). Additionally, Lima et al. (2015) showed that exercise training enhances hepatic antioxidant enzyme activity in diabetic rodents (21).

More contemporary findings have further corroborated the modulatory role of exercise on hepatokines. Ataeinosrat et al. (2025) reported that various exercise training modalities can adjust hepatokine concentrations, including Fetuin A and FGF21, in obese men (30). In a comprehensive review, Iglesias (2025) underscored the endocrine role of the liver and the significance of hepatokines in health and disease, with special emphasis on Fetuin A and FGF21 in metabolic disorders (10). A meta analysis by Filimidou et al. (2025) reinforced the link between FGF21 levels and non alcoholic fatty liver disease, a condition often associated with diabetes (18). Moreover, Shouman et al. (2025) stressed the contribution of hepatokines, including Fetuin A and FGF21, to the modulation of oxidative stress and inflammation (11). while Song et al. (2025) elucidated molecular mechanisms concerning hepatokines and oxidative stress (22).

Although existing evidence points to the independent impact of swimming training on certain hepatokines and oxidative stress markers, there is a paucity of information regarding the concurrent effects of swimming training on both Fetuin A and FGF21 and their association with oxidative stress in the liver tissue of T2D mice. Considering the pivotal roles of these two hepatokines in glucose metabolism and insulin resistance, along with the critical contribution of oxidative stress to diabetic pathophysiology, exploring the simultaneous influence of swimming training on these parameters could yield deeper insights into the mechanisms through which exercise improves hepatic function under diabetic conditions. Despite previous research that examined these hepatokines individually, the combined assessment of Fetuin A, FGF21 and oxidative stress in the liver of type 2 diabetic mice following swimming training remains unexplored. The novelty of this study lies in the simultaneous measurement of these three variables, potentially offering a more holistic view of exercise induced hepatic adaptations. Accordingly, the current investigation aimed to determine the effect of an eight week swimming training program on Fetuin A, FGF21 and oxidative stress levels in the liver tissue of type 2 diabetic mice.

Material and methods

This was an experimental study with a controlled laboratory design. Thirty-two adult male BALB/c mice, aged 8-10 weeks and weighing 26 (± 3.22) g, were used (31). Animals were housed under standard conditions (temperature 22 ± 2°C, humidity 55 ± 5%, 12:12 hour light-dark cycle) (32, 33). Standard pellet food and water were provided ad libitum throughout the study. A combination of a high-fat diet (HFD) and streptozotocin (STZ) injection was used to induce T2D [34, 32). Diabetic groups were fed an HFD (60% calories from fat, 26% from carbohydrate, 24% from protein) for 5 weeks (35, 33). After 5 weeks, following a 12-hour fast, mice received a single intraperitoneal injection of STZ at 20 mg/kg body weight in citrate buffer (pH 4.5) (36). Four days after injection, blood glucose levels were measured via tail vein puncture, and diabetic mice were divided into groups based on fasting blood glucose levels (37).

After diabetes confirmation, mice were randomly divided into 4 groups of 8: healthy control (HC), healthy training (HT), diabetic control (DC) and diabetic training (DT).

The swimming training protocol was performed for 8 weeks, 5 sessions per week (38-40). Training was conducted in rodent-specific tanks (80 × 45 × 80 cm) with a water depth of 50 cm and temperature of 35 ± 1°C (38,40). The first week was dedicated to habituation, during which swimming duration increased from 5 to 10 minutes (41,40). Weeks 2-4 consisted of baseline training without weight (15-25 minutes) (42). During weeks 5-8, training intensity was increased by adding weights (2-5% of body weight) for 30-45 minutes (43,44). All sessions were held at a

specified time of day, and water and ambient temperatures were controlled.(Table1).

Forty-eight hours after the last training session and following a 12-hour fast, mice were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) (17). Liver tissue was isolated, immediately frozen in liquid nitrogen, and stored at -80°C (28).

Total RNA was extracted using an RNA extraction kit (Pars Tous, Iran) according to the manufacturer's instructions. Gene expression measurement methods were performed based on a protocol similar to the study by BonDurant et al. (2017) (16). RNA concentration and purity were determined using a microspectrophotometer (Eppendorf, Germany) at 260 and 280 nm wavelengths, and samples with a 260/280 ratio above 1.8 were used for cDNA synthesis (21). cDNA was synthesized from 1 µg of total RNA using a cDNA synthesis kit (Pars Tous, Iran) according to the instructions, and the product was stored at -20°C until real-time PCR (8).

Gene expression levels of FGF21 and Fetuin-A were measured by real-time PCR using a Lava 96 DaanGene Co., Ltd (China) device (7). GAPDH was used as an internal control. The reaction was performed in a 12.5 µL volume containing 6.25 µL of 2X SYBR Green Master Mix (Pars Tous, Iran), 0.25 µL of each primer (10 µM), 2.75 µL of nuclease-free water, and 3 µL of cDNA. Thermal cycling conditions included 95°C for 3 minutes, followed by 40 cycles (95°C for 15 seconds and 60°C for 45 seconds).

ROS levels were measured using the DCFH-DA probe-based fluorescence method (20). One hundred mg of liver tissue was homogenized in 1 mL of phosphate-buffered saline (PBS, 0.1 M, pH 7.4). Then, 10 µL of the homogenate was mixed with 100 µL of PBS and 5 µL of DCFH-DA (10 µM) and incubated for 30 minutes at 37°C in the dark. Fluorescence intensity was read at excitation wavelength 485 nm and emission wavelength 530 nm. ROS concentration was calculated based on a hydrogen peroxide (H_2O_2) standard curve and reported as µmol/mg protein (45).

Total protein concentration for ROS data normalization was measured by the Bradford method (13). Bradford reagent was prepared by dissolving 10 mg of Coomassie Brilliant Blue G-250 in 5 mL of 95% ethanol and 10 mL of 85% phosphoric acid, with the final volume brought to 100 mL with distilled water. A standard curve was prepared with bovine serum albumin (Sigma, USA) concentrations of 0, 2, 4, 6, 10, 15 and 20 µg/mL. In a 96-well plate, 20 µL of sample was mixed with 40 µL of Bradford reagent and 140 µL of distilled water. After 5 minutes, absorbance was read at 595 nm using a plate reader (BioTek ELX800, USA). Protein concentration was calculated using the standard curve linear equation and reported as µg/mL (46).

Statistical analysis

Data were described using mean and standard deviation. Normality of data distribution was assessed by the Shapiro-Wilk test, and homogeneity of variances was assessed by Levene's test. One-way ANOVA was used for between-group comparisons, and Tukey's post hoc test was used when significance was indicated. Significance level was set at $P \leq 0.05$, and analyses were performed using SPSS version 26.

Ethical considerations

The Research Ethics Committee of Islamic Azad University ,Marvdasht Branch granted approval for this study under the ethics code IR.AJU.M.REC.1404.569.

Results

Descriptive data for FGF21 and Fetuin-A gene expression levels and ROS levels in liver tissue across experimental groups are presented in table 2.

The Shapiro-Wilk test showed that the distribution of all variables in different groups was normal, with significance levels greater than 0.05 for all cases ($P > 0.05$). Levene's test results indicated homogeneity of variances for

FGF21 (P= 0.401), Fetuin-A (P= 0.052) and ROS (P= 0.128) (Table 3).

The results of one-way analysis of variance (ANOVA) showed that the interventions had a significant effect on the levels of FGF21 (F= 530.94, df= 3, P< 0.001, η^2= 0.983), Fetuin-A (F= 1162.18, df= 3, P< 0.001, η^2= 0.992) and ROS (F= 3538.95, df= 3, P< 0.001, η^2= 0.997) in the liver tissue (Table 4).

To determine the exact location of between-group differences, Tukey's post hoc test was used. Results for study variables are presented in Table 4.

Diabetes induction led to a significant increase in FGF21 levels in the DC group compared to the HC group (P< 0.001). The HT group also showed a significant increase compared to HC (P< 0.001). Training intervention in the diabetic group (DT) caused a significant increase in FGF21 compared to the DC group (P< 0.001). The highest FGF21 level was observed in the DT group, which differed significantly from all other groups (P< 0.001).

Diabetes induction caused a significant increase in Fetuin-A levels in the DC group compared to the HC group (P< 0.001). The HT group showed a significant decrease compared to HC (P< 0.001). Training intervention in the diabetic group (DT) led to a significant decrease in Fetuin-A compared to the DC group (P< 0.001). The HT group showed the lowest Fetuin-A level among groups, which differed significantly from all groups (P< 0.001).

Discussion

The results of the present study showed that induction of type 2 diabetes using a high-fat diet combined with STZ injection led to a significant increase in Fetuin-A, FGF21, and ROS levels in the liver tissue of BALB/c mice. These observations are in line with previous studies that have reported increases in inflammatory hepatokines and oxidative stress under diabetes and obesity conditions (47,9-11). The rise in Fetuin-A observed in the diabetic control (DC) group compared to the healthy control (HC) group (3.59 vs. 1.05) can be attributed to the role of this protein as a natural insulin receptor inhibitor and an acute-phase inflammatory protein (12,15). Type 2 diabetes is accompanied by chronic hyperglycemia and elevated free fatty acids, which, through the activation of signaling pathways such as NF-κB and JNK, likely lead to increased Fetuin-A production in the liver (11,22). This finding is consistent with the study by Saberi et al. (2024), which demonstrated increased Fetuin-A in type 2 diabetic rats (14).

Furthermore, the increase in FGF21 levels in the DC group (2.25) compared to HC (1.10) in our study is consistent with the findings of Shams et al. (2024) and Filimidou et al. (2025) (17,18). The elevation of FGF21 in diabetic and obese states is commonly interpreted as a manifestation of FGF21 resistance (19,16). In this situation, elevated FGF21 levels are likely unable to exert their beneficial effects, including increased fatty acid oxidation and improved insulin sensitivity, due to reduced sensitivity of target tissues to this protective hepatokine (48,49). In other words, the rise in FGF21 represents an inefficient compensatory response to metabolic dysfunction in the studied group.

The marked increase in ROS levels in the DC group (3.91) compared to HC (0.98) further confirms the presence of systemic oxidative stress, particularly in the liver tissue under diabetic conditions in the studied group (20,45). Hyperglycemia promotes excessive ROS production via several mechanisms, including activation of the polyol pathway, formation of advanced glycation end products (AGEs), and mitochondrial dysfunction (50,4). Increased ROS can likely contribute to the exacerbation of insulin resistance and the progression of diabetic complications through activation of inflammatory pathways and disruption of insulin signaling (3,1). The study by Lima et al. (2015) also confirmed increased oxidative stress markers in the liver tissue of diabetic rats (21). The main and novel finding of the present study was the effect of eight weeks of swimming training on these variables in the studied group.

Table 1. Eight-week swimming training protocol

Training intensity	**Weight** (% body weight)	**Duration** (min)	Week
Habituation	0	10	1
Light training	0	15	2
Light training	0	20	3
Light training	0	25	4
Increasing intensity	2	30	5
Increasing intensity	2	35	6
High intensity	5	40	7
High intensity	5	45	8

Table 2. Descriptive indices of FGF21, Fetuin-A, and ROS levels in liver tissue (mean ± SD)

Group	FGF21	Fetuin-A	ROS
HC	1.10 (± 0.05)	1.06 (± 0.12)	0.98 (± 0.04)
HT	1.69 (± 0.10)	0.72 (± 0.03)	0.95 (± 0.05)
DC	2.25 (± 0.09)	3.59 (± 0.12)	3.91 (± 0.08)
DT	2.82 (± 0.09)	2.22 (± 0.11)	1.86 (± 0.07)

HC: healthy control, HT: healthy training, DC: diabetic control, DT: diabetic training

Table 3. One-way ANOVA results for study variables

Variable	Source of variation	Sum of squares	df	Mean square	F	*P*-value	η^2
	Between groups	12.972	3	4.324			
FGF21	Within groups	0.228	28	0.008	530.939	*P*< 0.001	0.983
	Total	13.200	31				
	Between groups	40.660	3	13.553			
Fetuin-A	Within groups	0.327	28	0.012	1162.178	*P*< 0.001	0.992
	Total	40.987	31				
	Between groups	46.070	3	15.357			
ROS	Within groups	0.122	28	0.004	3538.947	*P*< 0.001	0.997
	Total	46.191	31				

Df: degrees of freedom, F: F-statistic, η^2: eta squared (effect size);

Table 4. Tukey's post hoc test results for between-group comparisons

Variable	Group comparison	Mean difference	*P*-value
FGF21	HT > HC	0.586 *	*P*< 0.001
	DC > HC	1.144 *	*P*< 0.001
	DT > HC	1.713 *	*P*< 0.001
	DT > HT	1.126 *	*P*< 0.001
	DT > DC	0.569 *	*P*< 0.001
	DC > HT	0.557 *	*P*< 0.001
	HC > HT	0.340 *	*P*< 0.001
Fetuin-A	DC > HC	2.536 *	*P*< 0.001
	DC > HT	2.876 *	*P*< 0.001
	DC > DT	1.369 *	*P*< 0.001
	DT > HC	1.168 *	*P*< 0.001
	DT > HT	1.508 *	*P*< 0.001
ROS	DC > HC	2.923 *	*P*< 0.001
	DC > HT	2.953 *	*P*< 0.001
	DC > DT	2.045 *	*P*< 0.001
	DT > HC	0.878 *	*P*< 0.001
	DT > HT	0.908 *	*P*< 0.001
	HC ≈ HT	0.030	*P*= 0.799

HC: healthy control, HT: healthy training, DC: diabetic control, DT: diabetic training
*Mean difference is significant at the *P*< 0.05 level."≈" indicates no significant difference between groups.

The results revealed that swimming training in the diabetic exercise (DT) group resulted in a significant reduction in Fetuin-A (2.23) and ROS (1.86) and a significant increase in FGF21

(2.82) compared to the DC group in the studied group.

In the studied group, the decline in Fetuin-A with swimming training can likely be explained from several aspects. First, adaptations resulting from endurance training are associated with improved insulin sensitivity and reduced hyperglycemia and hyperlipidemia (26,23,51). Decreased glucose and free fatty acid levels reduce the primary stimulus for Fetuin-A production in the liver. Second, swimming training, through modulation of inflammatory pathways, likely reduces Fetuin-A gene expression in the liver by reducing NF-κB activation (27,29). Our findings are consistent with the results of Dastah and Babaei (2021), who showed a decrease in Fetuin-A following water training in diabetic women (13). Furthermore, the study by Ataeinosrat et al. (2025) confirms that different exercise training models can modulate Fetuin-A levels in obese men (30). The study by Saberi et al. (2024) also reported a decrease in Fetuin-A following exercise training in diabetic rats (14). The results of our study regarding the decrease in Fetuin-A are completely consistent with this evidence.

The increase in FGF21 in the DT group was another finding of this study in the studied group. This increase can likely be attributed to several mechanisms. First, adaptations resulting from exercise training induce FGF21 expression and secretion through activation of the AMPK and PGC-1α pathways in the liver and skeletal muscle (28,7,8,46). Fuentes-Barría et al. (2025) emphasize in their review article that activation of AMPK and PGC-1α in response to muscle contractions plays a key role in the secretion of myokines and hepatokines such as FGF21 (52). Second, with exercise-induced improvement in insulin sensitivity, FGF21 resistance is likely reduced, and despite its increased levels, its beneficial effects may be exerted (16,24). The study by Zare Karizak (2024) also showed an increase in FGF21 in the heart tissue of diabetic rats following endurance and resistance training (53). The study by Ma et al. (2025) also confirmed the role of resistance training in increasing FGF21 and activating the PI3K/Akt pathway in the skeletal muscle of diabetic mice (54). However, the study by Khalafi et al. (2021) in a meta-analysis showed that the FGF21 response to acute exercise may depend on the intensity and duration of exercise (55). The present study, by applying an 8-week progressive swimming protocol, produced a significant increase in FGF21, which is consistent with long-term interventional studies.

The decrease in ROS in the DT group in this study likely indicates the antioxidant effect of swimming training in the studied group. Adaptations resulting from regular endurance training increase antioxidant defense capacity by increasing the expression and activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in various tissues, including the liver (21,38,42). The study by Lima et al. (2015) explicitly showed improvements in liver antioxidant enzymes in trained diabetic rats (21). Studies by Lima et al. (2013) and Xu et al. (2018) also confirm antioxidant adaptations induced by swimming training (38, 56). Furthermore, the reduction in ROS production is likely due to improved mitochondrial function and reduced electron leakage from the respiratory chain under diabetic conditions (51,57). The study by Basereh et al. (2025) also investigated the relationship between oxidative stress and exercise response in diabetic conditions (58).

Interestingly, in the healthy exercise (HT) group in the studied group, Fetuin-A (0.72) and ROS (0.95) levels decreased compared to the HC group, while FGF21 (1.69) increased. These findings confirm the beneficial effects of swimming training even in non-diabetic conditions in the studied group. The decrease in Fetuin-A in healthy individuals can likely contribute to further improvement in insulin sensitivity (13). The lack of significant change in ROS between HC and HT groups suggests that swimming training does not cause additional oxidative stress under healthy

conditions, but rather maintains oxidative homeostasis by strengthening the antioxidant defense system (38). The increase in FGF21 in the HT group also indicates the physiological response of this hepatokine to exercise for improving energy metabolism in the studied group (17,59).

Taken together, the findings of this study in the studied group suggest that swimming training, possibly through the modulation of key hepatokines (decreasing Fetuin-A and increasing FGF21) and reducing oxidative stress, may contribute to improving the metabolic environment and liver function in type 2 diabetes conditions. It seems that activation of the AMPK/PGC-1α pathway plays a central role in these beneficial adaptations in the studied group (52,43,37).

Despite the valuable findings, this study had some limitations. These include the lack of measurement of phosphorylated AMPK protein levels and direct investigation of signaling pathways. Additionally, the study was conducted on an animal model, and generalization of the results to humans requires caution. It is suggested that future studies investigate the simultaneous effects of swimming training with other interventions such as antioxidant supplements on these indicators. Furthermore, conducting clinical studies to confirm these findings in type 2 diabetic patients is essential.

Conclusion

The present study in the studied group showed that eight weeks of swimming training in type 2 diabetic mice likely leads to a significant decrease in the levels of the inflammatory hepatokine Fetuin-A and the oxidative stress marker ROS, and a significant increase in the levels of the protective hepatokine FGF21 in the liver tissue. These changes indicate the modulating effects of swimming training on the hepato-inflammatory and oxidative axis under diabetic conditions in the studied group. It seems that these beneficial adaptations are created through the activation of energy-dependent signaling pathways such as AMPK and improvement of mitochondrial function. The findings of this study in the studied group suggest the therapeutic potential of swimming training as an effective non-pharmacological strategy in improving metabolic disorders and reducing complications caused by type 2 diabetes. With caution, it can be suggested that swimming training may possibly be considered as a complementary strategy alongside drug treatments for diabetic patients. Conducting future clinical trials to confirm these findings in the human population seems necessary.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' contributions

M.Z: Conceptualization, Investigation, Writing- Original Draft, Formal analysis.
S.F: Methodology, Validation, Supervision.
A.Sh: Conceptualization, Project administration, Writing- Review & Editing, Data Curation, Supervision.

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