

Original Article



Exercise Training and Taurine Supplementation, Alone or in Combination, Reduce Hepatic TLR4 and NFKB1 mRNA Expression in Streptozotocin-Induced Diabetic Rats

Mohammad Babaei¹, Ameneh Pourrahim^{1*}, Reza Farzizadeh¹, Abolfazl Bayrami²

¹Department of Exercise Physiology, Faculty of Educational Sciences and Psychology, University of Mohaghegh Ardabili, Ardabil, Iran

²Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran

*Corresponding Author: Ameneh Pourrahim, Email: amenehpourrahim@yahoo.com

Abstract

Introduction: Chronic hyperglycemia drives hepatic inflammation in diabetes largely via the toll-like receptor 4 (TLR4)/nuclear factor kappa B1 (NFKB1) axis. Although exercise and taurine each possess anti-inflammatory properties, their combined effects on this pathway are unknown. This study investigated whether an 8-week combined exercise and taurine intervention, alone and in combination, downregulates hepatic TLR4 and NFKB1 mRNA expression in streptozotocin (STZ)-induced diabetic rats.

Methods: Fifty male Wistar rats (8 weeks old) were randomly and equally assigned to healthy control (HC), diabetic control (DC), diabetic + taurine (DT; 100 mg/kg/day, i.p.), diabetic + exercise (DE), and DE + taurine (DET) groups. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg). Following the 8-week intervention, hepatic TLR4 and NFKB1 mRNA expression levels were quantified by real-time polymerase chain reaction.

Results: One-way ANOVA revealed significant group effects for TLR4 ($\eta^2=0.90$, $P<0.001$) and NFKB1 ($\eta^2=0.85$, $P<0.001$). All intervention groups demonstrated significantly lower TLR4 expression compared with the DC group. The DET group exhibited lower TLR4 expression than either intervention alone. For NFKB1, DT alone outperformed exercise alone ($P=0.002$). Regarding NFKB1 expression, taurine supplementation alone produced the lowest values among the intervention groups. Finally, the addition of exercise to taurine conferred no further reduction compared with DT alone ($P=0.007$).

Conclusion: Combined exercise training and taurine supplementation were associated with lower hepatic TLR4 expression, whereas taurine supplementation alone produced the greatest reduction in NFKB1 expression, suggesting that both interventions may contribute to attenuating diabetes-associated hepatic inflammation.

Keywords: Exercise, Taurine, Toll-like receptor 4, Diabetes mellitus experimental, Liver

Received: May 8, 2026, Revised: July 18, 2026, Accepted: July 19, 2026, ePublished: October 10, 2026

Introduction

Diabetes mellitus represents a major non-communicable disease and a significant global health burden, accounting for roughly 96% of all diagnosed diabetes cases worldwide. It is a multifaceted and progressive metabolic condition in which pancreatic β -cells fail to secrete sufficient insulin, a defect that is not autoimmune in origin and typically develops on a foundation of insulin resistance and the metabolic syndrome (1). Sustained high blood glucose is the central driver of both microvascular (e.g., retinopathy, nephropathy, and neuropathy) and macrovascular (e.g., cardiovascular, cerebrovascular, and peripheral arterial diseases) complications, culminating in irreversible target-organ damage (2).

The pathogenesis and progression of streptozotocin (STZ)-induced experimental diabetes are governed by a tightly coupled, bidirectional interplay among hyperglycemia, oxidative stress, and inflammation. Hyperglycemia drives the excessive generation of reactive oxygen species (ROS), which not only damage cellular

components but also promote the release of inflammatory mediators. These mediators further stimulate ROS production, thereby establishing a self-reinforcing vicious cycle (3). Central to this inflammatory response is the toll-like receptor 4 (TLR4)/nuclear factor kappa B 1 (NF- κ B) signaling axis. In diabetes, chronic hyperglycemia and elevated ROS promote the release of damage-associated molecular patterns (e.g., HMGB1 and HSP70) and increase circulating free fatty acids, which act as ligands that bind and activate TLR4 (4). Upon activation, TLR4 recruits adaptor proteins (e.g., MyD88 and TRIF), leading to the activation of IRAK and TRAF6 signaling cascades. Ultimately, these pathways stimulate the I κ B kinase (IKK) complex, resulting in the phosphorylation and degradation of I κ B proteins. Consequently, NF- κ B transcription factors translocate to the nucleus and induce the expression of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin 1 beta (IL-1 β), and IL-6. Through MyD88-dependent and TRIF-dependent pathways, TLR4 activates the transcription factor NF- κ B.

Furthermore, the accumulation of advanced glycation end-products (AGEs) and the activation of their receptor independently upregulate NF- κ B expression (5). In addition, aberrant activation of NF- κ B orchestrates the transcription of a wide array of pro-inflammatory cytokines (e.g., TNF- α , IL-6, and IL-1 β), which directly impair insulin signaling and promote apoptosis, playing a pivotal role in cellular damage and diabetic complications (6).

Robust evidence supports the beneficial effects of exercise training and targeted nutritional interventions in managing experimental diabetes, partly through their anti-inflammatory properties (6). Generally, exercise is proposed to mitigate metabolic disease by reducing the production of pro-inflammatory cytokines. Preclinical studies have demonstrated that six weeks of aerobic exercise significantly downregulates *TLR4* gene expression and pro-inflammatory cytokines in the spinal cord of diabetic mice (7), and eight weeks of training can inhibit NF- κ B nuclear translocation by reducing I κ B α phosphorylation. Taurine, a sulfonate-containing beta-amino acid abundant in excitable tissues, is a promising complementary nutraceutical (8). Owing to its potent antioxidant and anti-inflammatory effects, taurine impedes the TLR4/NF- κ B pathway. Moreover, it directly scavenges free radicals, enhances endogenous antioxidant enzymes, and reduces AGE formation, thereby preventing the activation of TLR4 by inflammatory ligands and subsequently suppressing NF- κ B-driven gene expression (9).

Despite these documented benefits, the anti-inflammatory efficacy of exercise is not unequivocal. According to several studies, exercise has no significant effect on inflammatory markers. For instance, high-intensity interval training did not alter TLR4 levels in rats fed a high-fat diet (10); more precisely, 8 weeks of intense interval training failed to change NF- κ B protein expression in the heart of STZ-induced diabetic rats (11), and a single bout of high-intensity exercise actually increased *TLR4* gene expression in young men (12). These inconsistencies highlight the need for more effective combinatorial strategies.

To our knowledge, no study has so far investigated the effects of combined exercise training and taurine supplementation on the hepatic TLR4 and NFKB1 messenger ribonucleic acid (mRNA) expression in a diabetic model. Therefore, the present study has been designed to test the hypothesis that eight weeks of combined exercise training and taurine supplementation downregulate the gene expression of *TLR4* and *NFKB1* in the liver tissue of STZ-induced diabetic Wistar rats.

Materials and Methods

Animals and Housing

A total of 50 male Wistar rats (age: 8 weeks, average body mass: 230.86 ± 13.11 g) were supplied by the Rodent Research Center (Tabriz, Iran). The animals were maintained in polycarbonate cages (four per cage) under tightly regulated environmental settings (a 12-hour light/12-hour dark cycle, ambient temperature

of $22 \pm 2^\circ\text{C}$, and relative humidity of $45 \pm 5\%$). Further, standard rodent chow and drinking water were available without restriction. After a 7-day acclimation period, the rats were randomly allocated to five groups of ten following a previously described randomization method: (1) healthy control (HC), (2) diabetic control (DC), (3) diabetic + taurine (DT), (4) diabetic + exercise (DE), and (5) DE + taurine (DET). The sample size was determined a priori using G*Power (version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany). Given that no previous study has directly measured hepatic TLR4 and NFKB1 mRNA expression in this specific STZ-induced diabetic rat model, the effect size was estimated based on the study by Sedaghat et al (13); they employed an identical five-group design (control, DM, DM + taurine, DM + combined exercise, DM + taurine + combined exercise; $n = 8/\text{group}$) in STZ-induced diabetic rats treated with taurine and combined exercise training. Using the most conservative outcome reported in that study (blood glucose response; $F = 17.39$, $df = 4,35$), the corresponding effect size was partial $\eta^2 = 0.665$ (Cohen's $f = 1.41$), indicating that as few as 3 animals per group would provide 80% power for a one-way analysis of variance (ANOVA) with five groups at $\alpha = 0.05$. Since this reference effect size was derived from a physiological rather than a molecular outcome, and gene expression differences may be more variable than those observed for blood glucose, the calculated minimum alone was considered insufficient; instead, a sample size of 8 animals per group (13) was adopted as a more conservative, empirically validated benchmark for this experimental model. It was further increased to 10 animals per group in order to account for anticipated mortality during the 8-week STZ-induced diabetes protocol, reported to reach up to 20% in comparable models (13). The final sample size ($n = 10$ per group), therefore, provided a substantial margin above the calculated statistical minimum.

Induction of Diabetes

After a 12-hour fasting period, diabetes was induced in the designated groups by a single intraperitoneal injection of STZ (Sigma S0130, USA), freshly dissolved in 0.1 M citrate buffer (pH: 4.5), at a dose of 55 mg/kg body weight. The injection volume was adjusted according to body weight and administered at 1 mL/kg. In addition, HC animals received an equal volume of the citrate buffer vehicle alone. Seventy-two hours after STZ administration, fasting blood glucose was measured from tail-vein blood samples using a glucometer, and rats with fasting blood glucose concentrations ≥ 250 mg/dL were considered diabetic and included in the study (13). To minimize acute hypoglycemic mortality following STZ administration, animals had free access to 5% glucose solution during the first 24 hours after injection. Ultimately, body weight and fasting blood glucose were monitored throughout the study in order to verify maintenance of the diabetic state.

Combined Exercise Training Protocol

Animals in the exercise groups underwent an 8-week

combined (resistance and endurance) training program, 5 days per week.

Resistance Training: The protocol involved climbing a 1-m ladder inclined at 85° with a housing chamber at the top. Progression included weeks 1–3. Rats performed 8, 10, and 12 unweighted climbs per session, respectively. From weeks 4–8, they performed 15 climbs per session with a weight equivalent to 3% of their body weight secured to the tail (13).

Endurance Training: Following a 5-minute rest period, the animals ran on a motorized rodent treadmill at a 0° incline. The protocol was progressed as weeks 1–3. Speed and duration increased from 15 to 17 m/min and from 15 to 40 minutes, respectively. From weeks 4–8, these two parameters were held constant at 20 m/min for 40 minutes. Additionally, gentle manual stimulation with a sponge was used to encourage running when necessary (13). Finally, endurance exercise was performed on a five-lane motorized rodent treadmill (Model DSI-580, Danesh Salar Iranian Company, Tehran, Iran) specifically designed for laboratory rats (Table 1).

Taurine Supplementation Protocol

Rats in the taurine-supplemented groups received a daily intraperitoneal (i.p.) injection of taurine (Myprotein, UK; purity > 99%, no additives or flavoring), dissolved in 0.9% normal saline, at a dose of 100 mg/kg body weight for 8 weeks (5 days/week) (14). The selected taurine dose was based on previous research that successfully employed this dosage in STZ-induced diabetic rats and reported beneficial metabolic and anti-inflammatory effects (14). The intraperitoneal route was chosen to ensure accurate dose delivery and minimize inter-animal variability in taurine intake that may occur when supplementation is provided through drinking water. In addition, this administration route has been used in previous experimental studies investigating the metabolic and anti-inflammatory effects of taurine in the rodent models of diabetes. Taurine was administered approximately after the exercise session throughout the intervention period (13).

Tissue and Blood Collection

Frothy-eight hours after the last training bout and after an overnight fast (12 hours), the animals were rendered

unconscious by an intraperitoneal administration of ketamine (Rotex Medica 10%, Germany) combined with xylazine (Alfasan 2%, Netherlands). Once deep anesthesia was achieved, the chest cavity was opened, and whole blood was collected directly from the left ventricle by cardiac puncture. Then, the blood was immediately spun at 3,000 rpm for 15 minutes in order to separate serum, which was then promptly transferred to a –80°C freezer. Further, liver tissue was harvested next, with the sequence of dissection alternating across the different experimental groups to counteract any time-dependent effects on biochemical parameters. The excised liver specimens were flash-frozen in liquid nitrogen and stored at –80°C until further processing. Eventually, all downstream biochemical and molecular assays were performed at the central laboratory of Ardabil University of Medical Sciences.

Serum Insulin and Insulin Resistance Assessment

Insulin concentrations in the serum were measured with a commercial enzyme-linked immunosorbent assay kit (ABCLONL, Catalog No. RK09278, USA), strictly following the manufacturer's instructions. Prior to the assay, all reagents and specimens were brought to ambient temperature. Absorbance was measured using a microplate reader (BioTek ELx800, BioTek Instruments, Winooski, VT, USA) at 450 nm with reference correction at 630 nm. Furthermore, a standard curve was fitted using a four-parameter or five-parameter logistic model to convert absorbance values to insulin concentrations. Subsequently, insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) equation (15):

$$\text{HOMA-IR} = [\text{Fasting Insulin } (\mu\text{U/mL}) \times \text{Fasting Glucose } (\text{mg/dL})] / 405$$

Hepatic Gene Expression Analysis (TLR4 and NFKB1)

Liver tissue was processed for total RNA isolation using a commercial extraction kit (Yekta Tajhiz, Iran), which employs a guanidine thiocyanate-based lysis buffer. Chloroform was added to achieve phase separation, and RNA was precipitated from the upper aqueous layer with ethanol. Moreover, the RNA pellet was rinsed with 70% ethanol, air-dried, and dissolved in diethyl pyrocarbonate-treated water. In addition, RNA purity and concentration were spectrophotometrically assessed, and an A260/A280 ratio of approximately 2.0 confirmed high purity. Residual genomic DNA was removed by DNase I treatment. Afterward, 1 µg of total RNA was reverse-transcribed into complementary DNA using a commercial kit (Yekta Tajhiz, Iran) containing oligo-dT and random hexamer primers, following standard conditions, including incubation at 42–50°C for 30–60 minutes, followed by enzyme inactivation at 70°C for 5–15 minutes (Table 2). Further, quantitative real-time polymerase chain reaction (RT-PCR) was performed on an Applied Biosystems StepOnePlus instrument (Thermo Fisher Scientific, USA) using SYBR Green master mix. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 5–10 minutes, then 40–45

Table 1. Combined Training Protocol

Training Weeks		Number of Climbs	Load (% Body Weight)		Duration (Minutes)	Speed (m/min)
1		8	–		15	15
2		10	–		15	15
3		12	–		15	15
4	Resistance training	15	3	Endurance training	40	17
5		15	3		40	20
6		15	3		40	20
7		15	3		40	20
8		15	3		40	20

cycles of denaturation at 95°C (15–30 seconds), annealing at 55–60°C (30–60 seconds), and extension at 72°C (30–60 seconds). A post-amplification melting curve analysis verified the specificity of each PCR product. Furthermore, relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method, normalizing to glyceraldehyde-3-phosphate dehydrogenase as the internal reference gene and using the HC group as the calibrator. All catalog numbers for internal control and target gene primers are listed in Table 2.

Statistical Analysis

The Shapiro–Wilk test and Levene’s test were applied to assess the normality of the data distribution and verify the homogeneity of variances, respectively. Between-group differences were analyzed using one-way ANOVA, followed by Tukey’s honestly significant difference post-hoc test for pairwise comparisons. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corporation, Armonk, NY, USA), whereas graphs were generated using GraphPad Prism, version 8.4.3 (GraphPad Software, San Diego, CA, USA).

Blinding

The researcher performing quantitative PCR analysis and gene expression quantification was blinded to group allocation until data analysis was complete.

Results

Data Normality and Descriptive Statistics

The Shapiro–Wilk test confirmed that all variables were normally distributed ($P > 0.05$). Descriptive statistics,

including means $\pm$ standard deviations (SD), for all outcomes across the five groups are presented in Table 3.

Data are expressed as means $\pm$ SD. Statistical analyses were conducted using one-way ANOVA followed by Tukey’s post-hoc test. Superscript letters indicate significant differences ($P < 0.05$; a = significantly different from HC; b = significantly different from DC; c = significantly different from DT; d = significantly different from DE). Pre-test food intake was measured after diabetes induction and before the start of the interventions. Therefore, the higher food intake observed in diabetic groups reflects STZ-induced hyperphagia.

Between-Group Comparisons

One-way ANOVA revealed significant overall between-group differences for both hepatic TLR4 mRNA expression ($F = 111.00$, $P < 0.001$, $\eta^2 = 0.90$) and NFKB1 mRNA expression ($F = 64.61$, $P < 0.001$, $\eta^2 = 0.85$). Among the intervention groups, the lowest NFKB1 expression was observed in the taurine-treated group, whereas the combined intervention group showed intermediate values between taurine alone and exercise alone.

Post-Hoc Analyses for TLR4 Gene Expression

Post-hoc comparisons using Tukey’s honestly significant difference test demonstrated that, similar to the DC group, hepatic TLR4 expression was significantly lower in each intervention group: DT ($P < 0.001$), DE ($P < 0.001$), and DET ($P < 0.001$). No significant difference was observed between DT and DE ($P = 0.59$). Based on the results, hepatic TLR4 expression was substantially lower in DET than in either DT ($P = 0.043$) or DE ($P = 0.007$), indicating that the combined intervention was associated with the lowest TLR4 expression among the diabetic groups (Figure 1).

Post-Hoc Analyses for NFKB1 Messenger Ribonucleic Acid Expression

Similarly, NFKB1 mRNA expression was remarkably lower in all intervention groups compared with DC (DT: $P < 0.001$; DE: $P < 0.001$; DET: $P < 0.001$). Likewise, taurine supplementation alone (DT) reduced NFKB1 expression significantly more than exercise (DE) alone ($P = 0.002$). However, the combined intervention (DET)

Table 2. Primers Used in This Study

Gene	Primer Direction	Primer Sequence (5'–3')
Toll-like receptor 4	Forward	AGCTTTGGTCAGTTGGCTCT
	Reverse	CAGGATGACACCATTGAAGC
Nuclear factor kappa B subunit 1	Forward	TTACGGGAGATGTGAAGATG
	Reverse	ATGATGGCTAAGTGTAGGAC
Glyceraldehyde-3-phosphate dehydrogenase	Forward	GCTGGTCATCAACGGGAAA
	Reverse	CGCCAGTAGACTCCACGACAT

Table 3. Means $\pm$ Standard Deviations of the Research Variables

Variable	Time	HC (n = 10)	DC (n = 10)	DT (n = 10)	DE (n = 10)	DET (n = 10)	P-Value
Body weight (g)	Pre	254.00 $\pm$ 31.54	225.40 $\pm$ 47.79	227.40 $\pm$ 27.06	221.50 $\pm$ 20.49	226.00 $\pm$ 26.30	0.171
	Post	345.00 $\pm$ 40.74	186.70 $\pm$ 30.81 ^a	229.70 $\pm$ 55.22 ^a	242.50 $\pm$ 31.30 ^a	266.60 $\pm$ 57.70 ^{ab}	<0.001
Food intake (g/rat/week)	Pre	234.43 $\pm$ 41.27	381.14 $\pm$ 30.55 ^a	355.71 $\pm$ 38.76 ^a	339.71 $\pm$ 59.35 ^a	329.86 $\pm$ 36.52 ^a	<0.001
	Post	242.57 $\pm$ 25.32	296.28 $\pm$ 6.05 ^a	355.28 $\pm$ 30.99 ^{abd}	333.71 $\pm$ 6.05 ^{abd}	300.43 $\pm$ 6.80 ^{acd}	<0.001
Serum insulin (μ U/mL)	Post	2.58 $\pm$ 0.27	1.54 $\pm$ 0.19 ^a	2.06 $\pm$ 0.11 ^{ab}	2.05 $\pm$ 0.08 ^{ab}	2.11 $\pm$ 0.15 ^{ab}	<0.001
Fasting blood glucose (mg/dL)	Post	87.30 $\pm$ 8.69	386.50 $\pm$ 48.06 ^a	246.30 $\pm$ 10.61 ^{ab}	276.10 $\pm$ 8.84 ^{ab}	191.90 $\pm$ 18.60 ^{abcd}	<0.001
HOMA-IR	Post	0.81 $\pm$ 0.30	4.98 $\pm$ 0.74 ^a	1.94 $\pm$ 0.74 ^{abd}	2.74 $\pm$ 0.18 ^{abc}	1.57 $\pm$ 0.22 ^{abd}	<0.001
TLR4 mRNA expression	Post	1.06 $\pm$ 0.17	4.06 $\pm$ 0.44 ^a	2.41 $\pm$ 0.44 ^{ab}	2.62 $\pm$ 0.35 ^{ab}	1.98 $\pm$ 0.22 ^{abd}	<0.001
NFKB1 mRNA expression	Post	0.89 $\pm$ 0.48	2.93 $\pm$ 0.29 ^a	1.29 $\pm$ 0.19 ^{abd}	1.92 $\pm$ 0.24 ^{abc}	1.44 $\pm$ 0.23 ^{abd}	<0.001

Note. SD: Standard deviation; HC: Healthy control; DC: Diabetic control; DT: Diabetic + taurine; DE: Diabetic + exercise; DET: Diabetic + exercise + taurine; HOMA-IR: Homeostasis model assessment of insulin resistance; TLR4: Toll-Like Receptor 4; mRNA: Messenger ribonucleic acid; NFKB1: Nuclear factor kappa B1

did not produce any additional reduction compared with taurine supplementation alone ($P=0.82$), although NFKB1 expression remained considerably lower than in DE ($P=0.007$) (Figure 2). Among the intervention groups, the lowest NFKB1 expression was observed in the taurine-treated group.

Discussion

The findings of this present study revealed that eight weeks of exercise training and taurine supplementation, administered either alone or in combination, significantly reduced hepatic TLR4 and NFKB1 mRNA expression in STZ-induced diabetic rats, representing a potential downregulatory effect on the TLR4/ NFKB1 axis at the transcriptional level. All intervention groups exhibited lower expression of these inflammatory markers compared with the DC group. Notably, the combined intervention led to the lowest TLR4 expression, whereas taurine supplementation alone was associated with the lowest NFKB1 expression. Overall, these transcriptional changes suggest that both exercise training and taurine supplementation may contribute to modulating hepatic TLR4 and NFKB1 mRNA expression in a manner consistent with a potential anti-inflammatory effect;

however, in the absence of downstream inflammatory protein or histological markers, this interpretation remains inferential rather than directly demonstrated. Considering that inflammatory mediators and signaling molecules, such as IL-10, irisin, glucocorticoids, activate adenosine monophosphate-activated protein kinase (AMPK), Sirtuin-1 (Sirt1), and p38 mitogen-activated protein kinase (MAPK), were not directly measured in the present study, the proposed mechanisms should be considered speculative and based on evidence from previous investigations.

The significant decrease in *TLR4* gene expression across all intervention groups is in strong agreement with prior reports, indicating that exercise training downregulates TLR4 in various diabetic models (16). Although not measured in this study, one plausible but untested mechanism reported elsewhere involves the exercise-induced elevation of anti-inflammatory cytokines such as IL-4 and IL-10 (17). Moreover, one possible mechanism may involve exercise-induced alterations in glucocorticoid signaling; however, corticosterone concentrations were not computed in this study. Therefore, this explanation remains speculative and should be interpreted with caution. Glucocorticoids can suppress pro-inflammatory signaling through the inhibition of the p38 MAPK pathway,

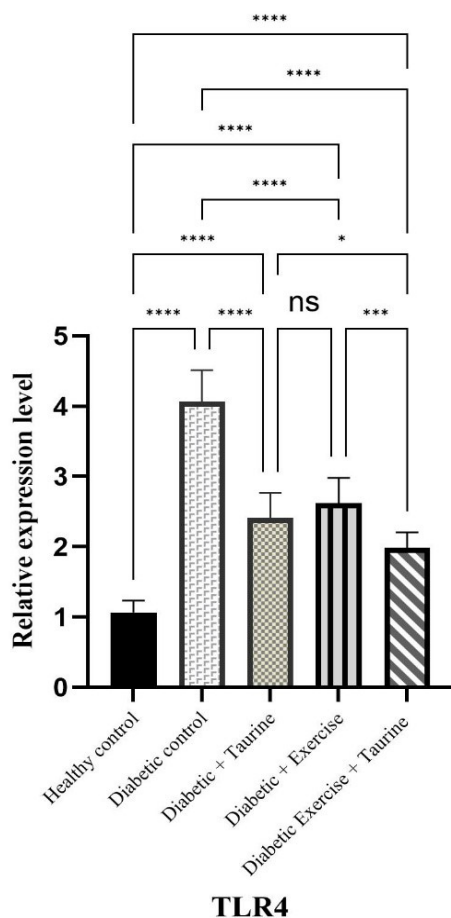


Figure 1. Relative Hepatic TLR4 mRNA Expression Across Experimental Groups: Healthy Control, Diabetic Control, Diabetic + Taurine, Diabetic + Exercise, and Diabetic + Exercise + Taurine

Note. TLR4: Toll-like receptor 4; mRNA: Messenger ribonucleic acid. Values are expressed as means±standard deviation (n=10 per group). *** $P<0.001$

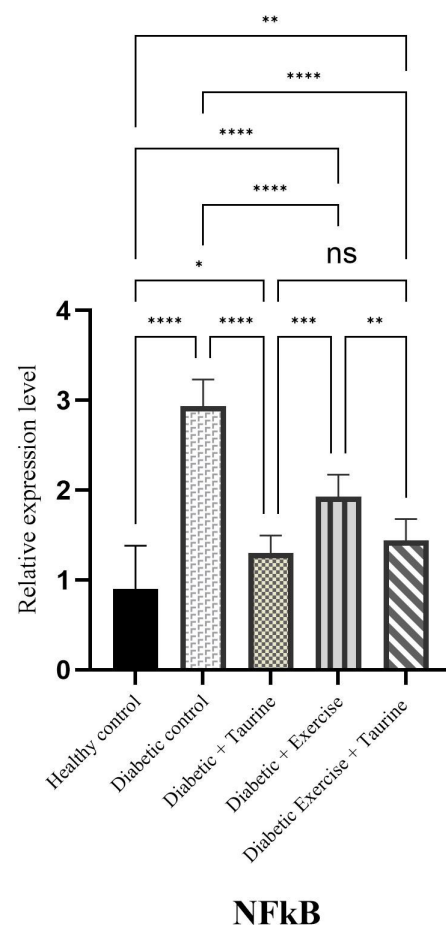


Figure 2. Relative Hepatic NFKB1 mRNA Expression in Healthy Control, Diabetic Control (DC), Diabetic + Taurine, Diabetic + Exercise, and Diabetic + Exercise + Taurine Groups

Note. NFKB1: Nuclear factor kappa B1; mRNA: Messenger ribonucleic acid

thereby attenuating TLR4-driven responses (18). Previous studies suggest that taurine may inhibit TLR4. Taurine supplementation independently exerts a modulatory effect on TLR4, which is consistent with evidence that taurine inhibits the MyD88-dependent TLR4 signaling cascade and directly suppresses the receptor's expression, thereby reducing inflammatory cytokine secretion (16).

Contrary to these findings, Sayyad et al reported that eight weeks of high-intensity interval training (HIIT) did not alter hepatic TLR4 protein levels in high-fat diet-fed rats (10). This discrepancy is plausibly attributable to the applied divergent exercise modalities. HIIT can induce substantial tissue microdamage and provoke an acute inflammatory response that mimics clinical overreaching, partly by elevating circulating lipopolysaccharide levels and upregulating TLR2, TLR4, and NFKB1. Additionally, the combination of a high-fat diet with extreme exercise may overwhelm mitochondrial function and amplify adipose tissue macrophage infiltration, resulting in a sustained pro-inflammatory environment that masks the anti-inflammatory benefits of exercise (19). In our study, the use of a moderate-intensity combined training protocol likely avoided such excessive physiological stress, thus allowing the anti-inflammatory adaptations to manifest.

The downregulation of NFKB1 mRNA expression observed in this study is consistent with a body of literature highlighting the inhibitory effect of regular exercise on this central inflammatory transcription factor (20). Exercise is known to reduce the production of ROS and the accumulation of AGEs in diabetic conditions (21), both of which are potent upstream activators of NF- κ B (22). In addition, exercise training preserves the expression of Sirt-1, a key metabolic sensor that deacetylates and inactivates the NF- κ B complex, thereby dampening inflammatory gene transcription. Another integrative mechanism involves the myokine irisin, which is released during exercise and activates AMPK, leading to the inhibition of NF- κ B activity and a subsequent reduction in downstream inflammatory mediators such as cyclooxygenase-2, IL-1 β , IL-6, and TNF- α (23). Likewise, our results align with the findings that exercise reduces IKK β phosphorylation in metabolic tissues, thus preventing the phosphorylation and degradation of I κ B and blocking NF- κ B nuclear translocation (24).

Other research has shown that taurine's capacity to inhibit NF- κ B signaling is well-supported and multifaceted. It has been reported to (1) directly bind to TLR4, impeding the TLR4/NF- κ B pathway, (2) block the nuclear translocation of NF- κ B, and (3) scavenge hypochlorous acid, forming the less toxic taurine chloramine, which neutralizes TNF-induced NF- κ B activation. It should be noted that these mechanisms were reported at the protein/signaling level in other studies and were not directly assessed in the present investigation, which was limited to TLR4 and NFKB1 mRNA expression (25). Additionally, taurine can suppress NFKB1 while concurrently upregulating the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 axis and glucose transporter type 1, creating a coordinated

anti-inflammatory and cytoprotective response (25). Importantly, a differential pattern was observed between the two genes. The combined intervention resulted in lower TLR4 expression compared with either intervention alone. However, no similar pattern was found for NFKB1 expression, where taurine supplementation alone produced the lowest expression values. Therefore, the present findings do not provide direct evidence of an interaction between the combined exercise and taurine interventions. Interestingly, the combined intervention failed to enhance the inhibitory effect of taurine on NFKB1 expression. The DT group displayed the lowest mean NFKB1 expression among the intervention groups, whereas the DET group depicted slightly higher values. Although the difference between DT and DET was not statistically significant, these findings indicated that adding exercise training did not further suppress NFKB1 expression beyond the effect caused by taurine supplementation alone under the present experimental conditions. One possible explanation is that taurine supplementation had already induced a substantial reduction in NFKB1 expression, thereby limiting the potential for additional decreases following exercise training. Alternatively, the molecular adaptations elicited by exercise and taurine may not be completely additive with respect to this specific inflammatory pathway. Given that intermediary signaling molecules were not determined in this study, the mechanisms underlying this observation remain speculative, warranting further investigation. This nuanced finding underscores that while taurine and exercise share converging downstream pathways, their combinatorial efficacy may be target-specific and requires further mechanistic exploration.

The lack of NF- κ B modulation reported by Rami et al (26) following HIIT is likely due to the excessive oxidative stress generated by extreme-intensity exercise. Supramaximal efforts can overactivate nicotinamide adenine dinucleotide phosphate oxidase and promote ischemic-reperfusion-like calcium dyshomeostasis, leading to a burst of ROS production that sustains NF- κ B activation (27), counteracting any potential anti-inflammatory adaptation.

The present study had several limitations. Gene expression was exclusively assessed at the mRNA level by RT-PCR. Moreover, protein-level validation (e.g., Western blot for TLR4 and total and phospho-p65 NFKB1) or immunohistochemical localization was not performed in this study. Considering that mRNA abundance does not always correlate directly with protein expression or activity, the functional relevance of the observed transcriptional changes remains to be confirmed.

A further major limitation was the use of HOMA-IR as an index of insulin resistance in an STZ-induced diabetic model. HOMA-IR, originally validated in the context of preserved endogenous insulin secretion, assumes a functioning β -cell mass. Given that STZ selectively destroys pancreatic β -cells, circulating insulin concentrations in the diabetic groups were markedly reduced, and under conditions of near-absolute insulin deficiency, HOMA-

IR values may not accurately reflect true insulin resistance and should be interpreted with caution accordingly. Further, HOMA-IR was primarily reported for consistency with prior literature in this model in this study. Thus, future studies should incorporate direct measures such as hyperinsulinemic-euglycemic clamp techniques. In addition, due to financial constraints, it was impossible to measure key intermediary molecules involved in inflammatory signaling, including p38 MAPK, Sirt-1, IL-10, corticosterone, antioxidant enzyme activities, or components of the irisin/AMPK axis. Therefore, the mechanistic explanations proposed in this study are based on biological plausibility and previous literature rather than direct experimental evidence from the present investigation. Hence, future studies should incorporate these analyses to better elucidate the molecular pathways underlying the observed changes in hepatic TLR4 and NFKB1 expression.

Finally, an additional limitation lies in the housing conditions; rats were group-housed (four per cage), which prevented the accurate measurement of individual food intake. As a result, the potential confounding effect of differential caloric consumption on inflammatory markers cannot be entirely excluded, highlighting the necessity of employing individually housed animals or pair-feeding protocols to address this limitation in future investigations.

Conclusion

Our findings demonstrated that eight weeks of exercise training and taurine supplementation, administered either alone or in combination, could reduce hepatic TLR4 and NFKB1 mRNA expression in STZ-induced diabetic rats, suggesting that both interventions may contribute to the attenuation of diabetes-associated hepatic inflammation. Translationally, it can be proposed that patients with diabetes, under appropriate supervision from a physician and an exercise physiologist, may derive significant benefits from incorporating combined training and taurine supplementation into their management plan.

Acknowledgments

This article has been derived from a doctoral dissertation. We extend our sincere gratitude to all those who supported and facilitated this study.

Authors' Contribution

Conceptualization: Ameneh Pourrahim, Reza Farzizadeh
Data Curation: Mohammad Babaei
Investigation: Mohammad Babaei, Ameneh Pourrahim, Reza Farzizadeh, Abolfazl Bayrami
Methodology: Ameneh Pourrahim, Reza Farzizadeh, Abolfazl Bayrami
Supervision: Ameneh Pourrahim, Reza Farzizadeh
Writing—Original Draft: Mohammad Babaei, Ameneh Pourrahim
Writing—Review & Editing: Mohammad Babaei, Ameneh Pourrahim, Reza Farzizadeh

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

All procedures involving animals were conducted in accordance

with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and complied with institutional ethical guidelines approved by the Research Ethics Committee of the University of Mohaghegh Ardabili (IR.UMA.REC.1404.025).

Funding

None.

Intelligence Use Disclosure

During the preparation of this manuscript, the authors exclusively used a large language model (ChatGPT) to assist with language editing, paraphrasing, and improvement of the clarity and readability of the text. Moreover, all AI-generated suggestions were critically reviewed and revised by the authors. The authors take full responsibility for the accuracy, originality, and scientific integrity of the final manuscript.

References

- Ahmadi P, Ghahramani M. A review of apoptosis in diabetic patients with the approach of exercise activity. *EBNESINA* 2024;26(3):86-99. doi:10.22034/26.3.86
- Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: cause or consequence? *J Hepatol* 2018;68(2):335-52. doi:10.1016/j.jhep.2017.09.021
- Papachristoforou E, Lambadiari V, Maratou E, Makrilakis K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. *J Diabetes Res* 2020;2020:7489795. doi:10.1155/2020/7489795
- Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 8th ed. Elsevier Health Sciences; 2014.
- Lee J, Yun JS, Ko SH. Advanced glycation end products and their effect on vascular complications in type 2 diabetes mellitus. *Nutrients* 2022;14(15):3086. doi:10.3390/nu14153086
- Behera S, Lamare AA, Rattan R, Patnaik B, Das S. Association of NFKB1 gene polymorphism with inflammatory markers in patients of type 2 diabetes mellitus with or without renal involvement in eastern India. *J Diabetes Mellitus* 2020;10(3):169-81. doi:10.4236/jdm.2020.103014
- Shamsipour R, Shariati M. Effect of aerobic training and melatonin consumption on TNF- α expression in the spinal cord tissue of male rats with type 1 diabetes. *Jentashapir J Cell Mol Biol* 2023;14(2): e132170. doi:10.5812/jcmb-132170
- Rais N, Ved A, Shadab M, Ahmad R, Shahid M. Taurine, a non-proteinous essential amino acid for human body systems: an overview. *Arab Gulf J Sci Res* 2023;41(1):48-66. doi:10.1108/agjsr-04-2022-0019
- Ahmed K, Choi HN, Yim JE. The impact of taurine on obesity-induced diabetes mellitus: mechanisms underlying its effect. *Endocrinol Metab (Seoul)* 2023;38(5):482-92. doi:10.3803/EnM.2023.1776
- Sayyad M, Sheikhan H, Moqaddasi M, Jowhari A. The effect of 8 weeks of high-intensity interval water training on liver TLR-4 protein and serum TNF- α in male rats with non-alcoholic steatohepatitis by high-fat diet. *J Appl Health Stud Sport Physiol* 2022;9(1):125-35.
- Ghardashi Afousi A, Gaeini A, Rakhshan K, Naderi N, Darbandi Azar A, Aboutaleb N. Targeting necroptotic cell death pathway by high-intensity interval training (HIIT) decreases development of post-ischemic adverse remodelling after myocardial ischemia / reperfusion injury. *J Cell Commun Signal* 2019;13(2):255-67. doi:10.1007/s12079-018-0481-3
- Khakroo Abkenar I, Rahmani-Nia F, Lombardi G. The effect of different intensities of acute and chronic aerobic activity on the signaling pathway of the inflammasome NLRP3 complex and TLR4 and some inflammatory cytokines in young men. *J Pract Stud Biosci Sport* 2020;8(16):116-30. doi:10.22077/

- jpsbs.2019.1982.1454
13. Sedaghat M, Choobineh S, Ravasi AA. Taurine with combined aerobic and resistance exercise training alleviates myocardium apoptosis in STZ-induced diabetes rats via Akt signaling pathway. *Life Sci* 2020;258:118225. doi:10.1016/j.lfs.2020.118225
 14. Nasimi R, Tadibi V. Acute effects of different doses of taurine on physical and cognitive functions in elderly men. *Sport Physiol* 2025;17(65):113-97. doi:10.22089/spj.2025.14573.2241
 15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9. doi:10.1007/bf00280883
 16. Ouyang G, Wang N, Tong J, Sun W, Yang J, Wu G. Alleviation of taurine on liver injury of type 2 diabetic rats by improving antioxidant and anti-inflammatory capacity. *Heliyon* 2024;10(7): e28400. doi:10.1016/j.heliyon.2024.e28400
 17. Nagai M, Kaji H. Thermal effect on heat shock protein 70 family to prevent atherosclerotic cardiovascular disease. *Biomolecules* 2023;13(5):867. doi:10.3390/biom13050867
 18. Padmalayam I. The heat shock response: its role in pathogenesis of type 2 diabetes and its complications, and implications for therapeutic intervention. *Discov Med* 2014;18(97):29-39.
 19. Song M, Bai Y, Song F. High-fat diet and neuroinflammation: the role of mitochondria. *Pharmacol Res* 2025;212:107615. doi:10.1016/j.phrs.2025.107615
 20. Hou G, Tang S, Li Q, Li W, Xi X. Exercise combined with metformin ameliorates diabetic kidney disease by increasing renal autophagy and reducing oxidative stress in rats with high-fat diet and streptozotocin induced diabetes. *Biochem Biophys Res Commun* 2025;752:151373. doi:10.1016/j.bbrc.2025.151373
 21. Boor P, Celec P, Behuliak M, Grancic P, Kebis A, Kukan M, et al. Regular moderate exercise reduces advanced glycation and ameliorates early diabetic nephropathy in obese Zucker rats. *Metabolism* 2009;58(11):1669-77. doi:10.1016/j.metabol.2009.05.025
 22. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014;18(1):1-14. doi:10.4196/kjpp.2014.18.1.1
 23. Jiang X, Hu Y, Zhou Y, Chen J, Sun C, Chen Z, et al. Irisin protects female mice with LPS-induced endometritis through the AMPK/NF- κ B pathway. *Iran J Basic Med Sci* 2021;24(9):1247-53. doi:10.22038/ijbms.2021.56781.12678
 24. Oliveira AG, Carvalho BM, Tobar N, Ropelle ER, Pauli JR, Bagarolli RA, et al. Physical exercise reduces circulating lipopolysaccharide and TLR4 activation and improves insulin signaling in tissues of DIO rats. *Diabetes* 2011;60(3):784-96. doi:10.2337/db09-1907
 25. Barbiera A, Sorrentino S, Lepore E, Carfi A, Sica G, Dobrowolny G, et al. Taurine attenuates catabolic processes related to the onset of sarcopenia. *Int J Mol Sci* 2020;21(22):8865. doi:10.3390/ijms21228865
 26. Rami M, Azimpour M, Khoramipour K. The effect of 8 weeks of high intensity interval training on the levels of Wnt and NF- κ B proteins in the heart tissue of male Wistar rats with type 2 diabetes. *J Sport Exerc Physiol* 2022;15(4):19-30. doi:10.52547/joeppa.15.4.19
 27. Liu HW, Chang SJ. Moderate exercise suppresses NF- κ B signaling and activates the SIRT1-AMPK-PGC1 α axis to attenuate muscle loss in diabetic db/db mice. *Front Physiol* 2018;9:636. doi:10.3389/fphys.2018.00636