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

Running title: Exercise and Taurine on Hepatic TLR4 and NFKB1

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Abstract

Background and aims: 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. We investigated whether an 8-week combined exercise and taurine intervention, alone and in combination, downregulates hepatic TLR4 and NFKB1 mRNA expression in streptozotocin-induced diabetic rats.

Methods: Fifty male Wistar rats (8 weeks old) were randomly assigned to five groups (n=10/group): healthy control (HC), diabetic control (DC), diabetic + taurine (DT; 100 mg/kg/day, i.p.), diabetic + exercise (DE), and diabetic + exercise + taurine (DET). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (55 mg/kg). Following the 8-week intervention, hepatic TLR4 and NFKB1 mRNA expression levels were quantified by real-time PCR.

Results: One-way ANOVA revealed significant group effects for TLR4 (η^2=0.90, P<0.001) and NFKB1 (η^2=0.85, P<0.001). All intervention groups showed significantly lower TLR4 expression compared with the DC group. The DET group exhibited lower TLR4 expression than either intervention alone. For NFKB1, taurine alone (DT) outperformed exercise alone (DE) (P=0.002).

For NFKB1 expression, taurine supplementation alone produced the lowest values among the intervention groups. The addition of exercise to taurine did not confer any further reduction compared with taurine 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. These findings suggest that both interventions may contribute to attenuating diabetes-associated hepatic inflammation.

Keywords: Exercise, Taurine, Toll-Like Receptor 4, Diabetes Mellitus Experimental, Liver

1. Introduction

Accounting for roughly 96% of all diagnosed diabetes cases worldwide, Diabetes mellitus represents a major non-communicable disease and a significant global health burden. 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 usually develops on a foundation of insulin resistance and the metabolic syndrome (1). Sustained high blood glucose is the central driver of both microvascular complications, such as retinopathy, nephropathy, and neuropathy, and macrovascular complications, including cardiovascular, cerebrovascular, and peripheral arterial disease, which together culminate in irreversible target-organ damage (2).

The pathogenesis and progression of streptozotocin-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, in turn, further stimulate ROS production, 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-κB) signaling axis. In diabetes, chronic hyperglycemia and elevated ROS promote the release of damage-associated molecular patterns (DAMPs), such as HMGB1 and HSP70, and increase circulating free fatty acids. These act as ligands that bind and activate TLR4 (4). Upon activation, TLR4 recruits adaptor proteins such as MyD88 and TRIF, leading to activation of IRAK and TRAF6 signaling cascades. These pathways ultimately stimulate the IκB kinase (IKK) complex, resulting in phosphorylation and degradation of IκB proteins. Consequently, NF-κB transcription factors translocate to the nucleus and induce the expression of pro-inflammatory cytokines, including Tumor Necrosis Factor-Alpha (TNF-α), Interleukin 1 beta (IL-1β), and Interleukin 6 (IL-6). Through MyD88-dependent and TRIF-dependent pathways, TLR4 activates the transcription factor NF-κB. Furthermore, the accumulation of advanced glycation end-products (AGEs) and the activation of their receptor (RAGE) independently upregulate NF-κB expression (5). Aberrant activation of NF-κB orchestrates the transcription of a wide array of pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β), 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). Exercise is proposed to limit metabolic disease by reducing pro-inflammatory cytokine production. 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-κB nuclear translocation by reducing IκBα 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-κB pathway. 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-κB-driven gene expression (9).

Despite these documented benefits, the anti-inflammatory efficacy of exercise is not unequivocal. Several studies report no significant effect of exercise on inflammatory markers. For instance, high-intensity interval training did not alter TLR4 levels in rats fed a high-fat diet (10), 8 weeks of intense interval training failed to change NF-κB 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 investigated the effects of combined exercise training and taurine supplementation on the hepatic TLR4 and NFKB1 mRNA expression in a diabetic model. Therefore, the present study was 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 streptozotocin-induced diabetic Wistar rats.

2. Materials and Methods

2.1 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 ± 2 °C, and relative humidity of 45 ± 5%. Standard rodent chow and drinking water were available without restriction. After a 7-day acclimation period, the rats were

randomly allocated into 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) Diabetic + Exercise + Taurine (DET). The sample size was determined a priori using G*Power (version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Germany). Because 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 from Sedaghat et al. (13), who employed an identical five-group design (control, DM, DM+taurine, DM+combined exercise, DM+taurine+combined exercise; n = 8/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 $f = 1.41$), which indicated that as few as 3 animals per group would provide 80% power for a one-way ANOVA with five groups at $\alpha = 0.05$. Because 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, we did not rely on the calculated minimum alone. Instead, we adopted the sample size of 8 animals per group used by Sedaghat et al. (13) as a more conservative, empirically validated benchmark for this experimental model, and further increased it to 10 animals per group 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/group) therefore provided a substantial margin above the calculated statistical minimum.

2.2 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. The healthy control 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. Body weight and fasting blood glucose were monitored throughout the study to verify maintenance of the diabetic state.

2.3 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-meter ladder inclined at 85° with a housing chamber at the top. Progression was as follows: 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, rats ran on a motorized rodent treadmill at a 0° incline. The protocol was progressed as follows: weeks 1-3, speed increased from 15 to 17 m/min and duration from 15 to 40 min. From weeks 4-8, the speed and duration were held constant at 20 m/min for 40 minutes. Gentle manual stimulation with a sponge was used to encourage running when necessary (13). Endurance exercise was performed on a five-lane motorized rodent treadmill (Model DSI-580, Danesh Salar Iranian Co., Tehran, Iran) specifically designed for laboratory rats. (Table 1).

Table 1. Combined Training Protocol

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

2.4 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 studies 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 to minimize inter-animal variability in taurine intake that may occur when supplementation is provided through drinking water. This administration route has also been used in previous experimental studies investigating the metabolic and anti-inflammatory effects of taurine in rodent models of diabetes. Taurine was administered approximately after the exercise session throughout the intervention period (13).

2.5 Tissue and Blood Collection

At 48 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. The blood was immediately spun at 3000 rpm for 15 minutes to separate serum, which was then promptly transferred to a −80°C freezer. 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. All downstream biochemical and molecular assays were performed at the central laboratory of Ardabil University of Medical Sciences.

2.6 Serum Insulin and Insulin Resistance Assessment

Insulin concentrations in serum were measured with a commercial ELISA kit (Abclonal, 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. A standard curve was fitted using a four- or five-parameter logistic model to convert absorbance values to insulin concentrations. Insulin resistance was subsequently estimated by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) equation (15):

HOMA-IR = [Fasting Insulin (μU/mL) × Fasting Glucose (mg/dL)] / 405

2.7 Hepatic Gene Expression Analysis (TLR4 and NFKB1)

Liver tissue was processed for total RNA isolation using a commercial extraction kit (Yekta Tajhiz, Iran) that 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. The RNA pellet was rinsed with 70% ethanol, air-dried, and dissolved in DEPC-treated water. RNA purity and concentration were assessed spectrophotometrically; an A260/A280 ratio of approximately 2.0 confirmed high purity. Residual genomic DNA was removed by DNase I treatment. Subsequently, 1 μg of total RNA was reverse-transcribed into cDNA using a commercial kit (Yekta Tajhiz, Iran) containing oligo-dT and random hexamer primers, following standard conditions: incubation at 42–50°C for 30–60 min, followed by enzyme inactivation at 70°C for 5–15 min (Table 2). Quantitative real-time 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 min, then 40–45 cycles of denaturation at 95°C (15–30 s), annealing at 55–60°C (30–60 s), and extension at 72°C (30–60 s). A post-amplification melting curve analysis verified the specificity of each PCR product. Relative gene expression was calculated with the 2^(-ΔΔCt) method, normalizing to GAPDH as the internal reference gene and using the healthy control group as the calibrator. All catalog numbers for internal control and target gene primers are listed in Table 2.

Table 2. Primers Used

Gene	Primer Direction	Primer Sequence (5′–3′)
Toll-Like Receptor 4 (TLR4)	Forward	AGCTTTGGTCAGTTGGCTCT
	Reverse	CAGGATGACACCATTGAAGC
	Forward	TTACGGGAGATGTGAAGATG

Nuclear Factor Kappa B Subunit 1 (NFKB1)	Reverse	ATGATGGCTAAGTGTAGGAC
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	Forward	GCTGGTCATCAACGGGAAA
	Reverse	CGCCAGTAGACTCCACGACAT

2.8 Statistical Analysis

The Shapiro–Wilk test was used to assess the normality of the data distribution, and Levene's test was applied to verify the homogeneity of variances. Between-group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) 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 Corp., Armonk, NY, USA), whereas graphs were generated using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA, USA).

2.9 Blinding

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

3. Results

3.1 Data Normality and Descriptive Statistics

The Shapiro-Wilk test confirmed that all variables were normally distributed ($P > 0.05$). Descriptive statistics (mean ± SD) for all outcomes across the five groups are presented in Table 3.

Table 3. Mean ± SD 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 ± 31.54	225.40 ± 47.79	227.40 ± 27.06	221.50 ± 20.49	226.00 ± 26.30	0.171
	Post	345.00 ± 40.74	186.70 ± 30.81[a]	229.70 ± 55.22[a]	242.50 ± 31.30[a]	266.60 ± 57.70[ab]	<0.001
Food Intake (g/rat/week)	Pre	234.43 ± 41.27	381.14 ± 30.55[a]	355.71 ± 38.76[a]	339.71 ± 59.35[a]	329.86 ± 36.52[a]	<0.001
	Post	242.57 ± 25.32	296.28 ± 6.05[a]	355.28 ± 30.99[abd]	333.71 ± 6.05[abd]	300.43 ± 6.80[acd]	<0.001
Serum Insulin (µU/mL)	Post	2.58 ± 0.27	1.54 ± 0.19[a]	2.06 ± 0.11[ab]	2.05 ± 0.08[ab]	2.11 ± 0.15[ab]	<0.001
Fasting Blood Glucose (mg/dL)	Post	87.30 ± 8.69	386.50 ± 48.06[a]	246.30 ± 10.61[ab]	276.10 ± 8.84[ab]	191.90 ± 18.60[abcd]	<0.001
HOMA-IR	Post	0.81 ± 0.30	4.98 ± 0.74[a]	1.94 ± 0.74[abd]	2.74 ± 0.18[abc]	1.57 ± 0.22[abd]	<0.001
TLR4 mRNA Expression	Post	1.06 ± 0.17	4.06 ± 0.44[a]	2.41 ± 0.44[ab]	2.62 ± 0.35[ab]	1.98 ± 0.22[abd]	<0.001

NFKB1 mRNA Expression	Post	0.89 ± 0.48	2.93 ± 0.29[a]	1.29 ± 0.19[abd]	1.92 ± 0.24[abc]	1.44 ± 0.23[abd]	<0.001

Data are presented as mean ± SD. Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Superscript letters indicate significant differences ($P < 0.05$): a = significantly different from Healthy Control (HC); b = significantly different from Diabetic Control (DC); c = significantly different from Diabetic + Taurine (DT); d = significantly different from Diabetic + Exercise (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.

3.2 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.

3.3 Post-Hoc Analyses for TLR4 Gene Expression

Post-hoc comparisons using Tukey's HSD test revealed that, relative to the Diabetic Control (DC) group, hepatic TLR4 expression was significantly lower in each intervention group: Diabetic + Taurine (DT, $P < 0.001$), Diabetic + Exercise (DE, $P < 0.001$), and Diabetic + Exercise + Taurine (DET, $P < 0.001$). No significant difference was observed between DT and DE ($P = 0.59$). Hepatic TLR4 expression was significantly 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).

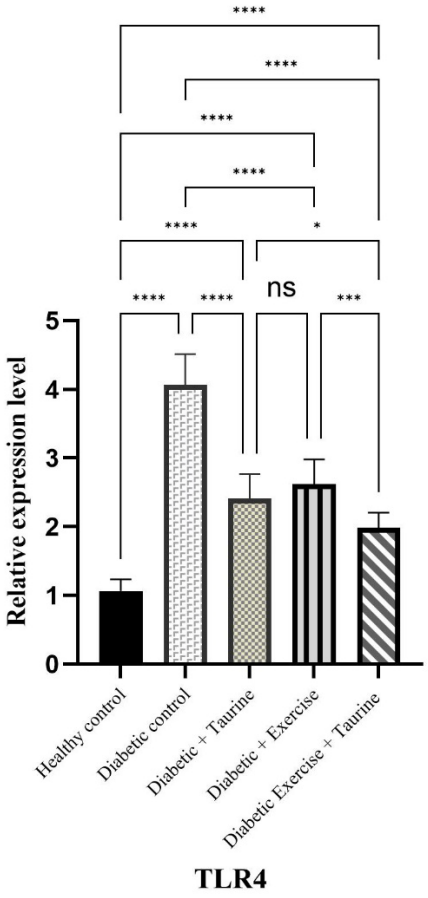

Figure 1. Relative hepatic TLR4 mRNA expression across experimental groups: Healthy Control (HC), Diabetic Control (DC), Diabetic + Taurine (DT), Diabetic + Exercise (DE), and Diabetic + Exercise + Taurine (DET). Values are mean ± SD (n=10). ***P < 0.001

3.4 Post-Hoc Analyses for NFKB1 mRNA expression

Similarly, NFKB1 mRNA expression was significantly lower in all intervention groups compared with DC (DT: P < 0.001; DE: P < 0.001; DET: P < 0.001). Taurine supplementation alone (DT) reduced NFKB1 expression significantly more than exercise alone (DE) (P = 0.002). However, the combined intervention (DET) did not produce any additional reduction compared with taurine

supplementation alone (P = 0.82), although NFKB1 expression remained significantly lower than in DE (P = 0.007) (Figure 2). Among the intervention groups, the lowest NFKB1 expression was observed in the taurine-treated group.

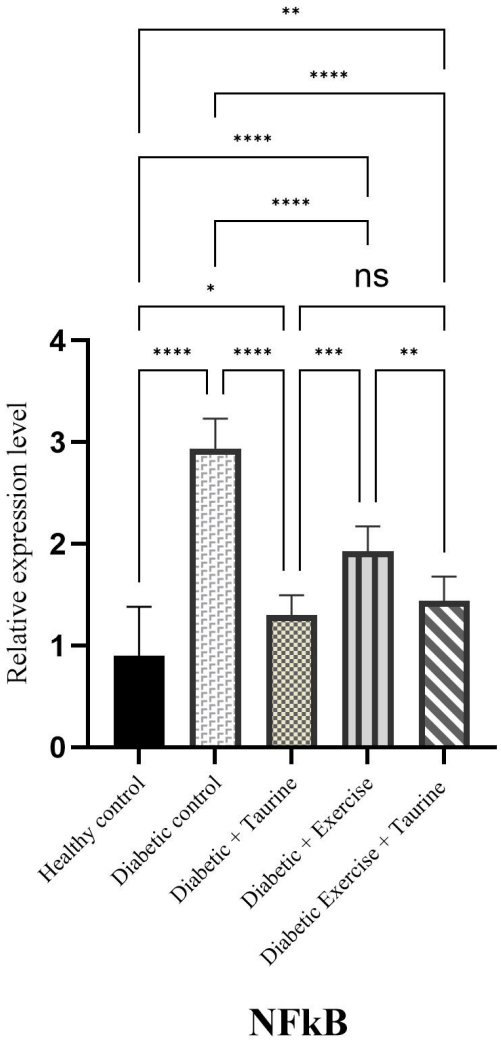

Figure 2. Relative hepatic NFKB1 mRNA expression in Healthy Control (HC), Diabetic Control (DC), Diabetic + Taurine (DT), Diabetic + Exercise (DE), and Diabetic + Exercise + Taurine (DET) groups.

4. Discussion

The present study demonstrates that eight weeks of exercise training and taurine supplementation, administered either alone or in combination, significantly reduced hepatic TLR4 and NFKB1 mRNA expression in streptozotocin-induced diabetic rats, suggesting 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 diabetic control group. Notably, the combined intervention produced the lowest TLR4 expression, whereas taurine supplementation alone was associated with the lowest NFKB1 expression. 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. Because inflammatory mediators and signaling molecules such as IL-10, irisin, glucocorticoids, AMPK, Sirt1, and p38 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 demonstrating that exercise training downregulates TLR4 in various diabetic models (16). Although not measured in this study, one plausible but untested mechanism reported elsewhere involves exercise-induced elevation of anti-inflammatory cytokines such as IL-4 and Interleukin -10 (IL-10) (17). One possible mechanism may involve exercise-induced alterations in glucocorticoid signaling; however, corticosterone concentrations were not measured in the present study. Therefore, this explanation remains speculative and should be interpreted with caution. Glucocorticoids can suppress pro-inflammatory signaling through inhibition of the p38

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

Contrary to these findings, Sayyad et al. (2022) 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 divergent exercise modalities employed. HIIT can induce substantial tissue microdamage and provoke an acute inflammatory response that mimics clinical overreaching, partly by elevating circulating lipopolysaccharide (LPS) 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). Our use of a moderate-intensity combined training protocol likely avoided such excessive physiological stress, allowing the anti-inflammatory adaptations to manifest.

The downregulation of NFKB1 mRNA expression observed here 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 reactive oxygen species (ROS) and the accumulation of advanced glycation end-products (AGEs) in diabetic conditions (21), both of which are potent upstream activators of NF-κB (22). Additionally, exercise training preserves the expression of Sirtuin-1 (Sirt-1), a key metabolic sensor that deacetylates and inactivates the NF-κB 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-κB activity and a subsequent reduction in downstream inflammatory mediators such as COX-2, IL-1β, IL-6, and TNF-α (23). Our results also align with the finding that exercise reduces IKKβ phosphorylation in metabolic tissues, thus preventing the phosphorylation and degradation of IκB and blocking NF-κB nuclear translocation (24).

Other research has shown that taurine's capacity to inhibit NF-κB signaling is well-supported and multifaceted. It has been reported to directly bind to TLR4, impeding the TLR4/NF-κB pathway; to block the nuclear translocation of NF-κB; and to scavenge hypochlorous acid (HOCl), forming the less toxic taurine chloramine (Tau-Cl), which neutralizes TNF-induced NF-κB 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 Nrf2/HO-1 axis and GLUT1, creating a coordinated anti-inflammatory and cytoprotective response (25). Importantly, we observed a differential pattern between the two genes. The combined intervention resulted in lower TLR4 expression compared with either intervention alone. However, a similar pattern was not observed for NFKB1 expression, where taurine supplementation alone produced the lowest expression values. Therefore, the present findings do not provide direct evidence for a combined intervention interaction between exercise and taurine. Interestingly, the combined intervention did not enhance the inhibitory effect of taurine on NFKB1 expression. In fact, the DT group exhibited the lowest mean NFKB1 expression among the intervention groups, whereas the DET group showed slightly higher values. Although the difference between DT and DET was not statistically significant, these findings indicate that the addition of exercise training did not provide further suppression of NFKB1 expression beyond

that achieved 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 fully additive with respect to this specific inflammatory pathway. Because intermediary signaling molecules were not measured, the mechanisms underlying this observation remain speculative and warrant further investigation. This nuanced finding underscores that while taurine and exercise share converging downstream pathways, their combinatorial efficacy may be target-specific and warrants further mechanistic exploration.

The lack of NF-κB modulation reported by Rami et al. (2022) (26) following HIIT is likely due to the excessive oxidative stress generated by extreme-intensity exercise. Supramaximal efforts can overactivate NADPH oxidase and promote ischemic-reperfusion-like calcium dyshomeostasis, leading to a burst of ROS production that sustains NF-κB activation (27), counteracting any potential anti-inflammatory adaptation.

Several limitations of the present study should be acknowledged. Perhaps the most critical limitation is that gene expression was assessed exclusively at the mRNA level by real-time PCR. Protein-level validation (e.g., Western blot for TLR4, total and phospho-p65 NFKB1) or immunohistochemical localization was not performed. Because 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 is the use of HOMA-IR as an index of insulin resistance in an STZ-induced diabetic model. HOMA-IR was originally validated in the context of preserved endogenous insulin secretion and assumes a functioning β-cell mass. Because 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 therefore be interpreted with caution. HOMA-IR was reported here primarily for consistency with prior literature in this model, and future studies should incorporate direct measures such as hyperinsulinemic-euglycemic clamp techniques. In addition, due to financial constraints, we were unable 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. 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. Therefore, the potential confounding effect of differential caloric consumption on inflammatory markers cannot be entirely excluded. Future investigations should employ individually housed animals or pair-feeding protocols to address this limitation

5. Conclusion

Our findings demonstrate that eight weeks of exercise training and taurine supplementation, administered either alone or in combination, reduced hepatic TLR4 and NFKB1 mRNA expression in STZ-induced diabetic rats. These findings suggest that both interventions may contribute to the attenuation of diabetes-associated hepatic inflammation. Translationally, we propose that patients with diabetes, under appropriate supervision from a physician and an exercise physiologist, may

derive significant benefit from incorporating combined training and taurine supplementation into their management plan.

Conflict of Interest Disclosure

The authors declare no conflicts of interest.

Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, the authors used a large language model (ChatGPT) exclusively to assist with language editing, paraphrasing, and improving the clarity and readability of the text. 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.

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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).

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