Research Article

High-intensity interval training and ketone ester supplementation attenuate hepatic oxidative stress and inflammation through modulation of the Keap1/Nrf2/NF-κB axis in Western diet-fed mice

Maryam Sarsangi Aliabad[1], Mousa Khalafi[*1], Aref Habibi Maleki[2]

Abstract

Western diet (WD) consumption promotes oxidative stress and inflammation and plays a central role in the development of fatty liver disease. This study investigated the effects of high-intensity interval training (HIIT), ketone ester supplementation, and their combination on hepatic Keap1, Nrf2, and NF-κB protein levels in WD-fed mice. Thirty male C57BL/6J mice were randomly assigned to five groups (n=6): normal diet (ND), WD, WD plus ketone ester (WD+KE), WD plus HIIT (WD+HIIT), and WD plus HIIT combined with ketone ester supplementation (WD+HIIT+KE). Except for the ND group, all animals consumed a WD for eight weeks. HIIT was performed for four weeks (three sessions/week), and ketone ester was administered daily by oral gavage. Hepatic protein levels of Keap1, Nrf2, and NF-κB were determined using Western blotting. WD feeding significantly increased hepatic Keap1 and NF-κB protein levels and reduced Nrf2 levels compared with the ND group. HIIT and ketone ester, both independently and in combination, significantly decreased NF-κB and increased Nrf2 protein levels compared with the WD group. In addition, HIIT alone and in combination with ketone ester supplementation significantly reduced hepatic Keap1 levels ($p<0.05$). Overall, both interventions alleviated WD-induced alterations in hepatic oxidative stress and inflammatory signaling. The combined intervention elicited the most pronounced molecular responses, suggesting potential synergistic effects of HIIT and ketone ester supplementation in attenuating Western diet-induced oxidative stress and inflammation.

Key Words: Western diet; Non-alcoholic fatty liver disease; High-intensity interval training; Ketone ester; Oxidative stress; Inflammation

Introduction

For decades, the global prevalence of overweight, obesity, and their associated metabolic complications has escalated dramatically, emerging as one of the most critical public health challenges of the 21st century (Blüher, 2019; Williams, Mesidor, Winters, Dubbert, & Wyatt, 2015). This trend is largely driven by maladaptive lifestyle changes, including physical inactivity and increased consumption of hypercaloric diets rich in fats and refined sugars (Kerr, Anderson, & Lippman, 2017). Central to this pattern is the Western Diet (WD), characterized by high intakes of saturated fats, simple sugars, and processed foods, along with low fiber content. This dietary pattern plays a pivotal role in the development of obesity and metabolic syndrome (Bettiga et al., 2019; Clemente-Suárez, Beltrán-Velasco, Redondo-Flórez, Martín-Rodríguez, & Tornero-Aguilera, 2023). Accumulating evidence indicates that chronic consumption of a Western diet promotes low-grade systemic inflammation, mitochondrial dysfunction, and excessive production of reactive oxygen species (ROS), thereby creating a pro-pathogenic environment for chronic disease development (Adolph & Tilg, 2024; Aslan, 2023; Kaliszewska, Allison, Martini, & Arias, 2021).

A major metabolic consequence of Western diet consumption is non-alcoholic fatty liver disease (NAFLD) (Romualdo et al., 2022; Salehi-Sahlabadi et al., 2021). NAFLD is the most prevalent chronic liver disorder worldwide and encompasses a spectrum ranging from simple steatosis to inflammation, fibrosis, and advanced non-alcoholic steatohepatitis (NASH) (Engin, 2017; Q. Liu, Bengmark, & Qu, 2010). Although its exact mechanisms are not fully understood, oxidative stress and chronic inflammation are considered central drivers of disease progression (Arroyave-Ospina, Wu, Geng, & Moshage, 2021; Delli Bovi et al., 2021; Farzanegi, Dana, Ebrahimpoor, Asadi, & Azarbayjani, 2019). Excess hepatic lipid accumulation impairs mitochondrial function and increases reactive oxygen species

1. Department of Sport Sciences, Faculty of Humanities, University of Kashan, Kashan, Iran.
2. Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran.

*Author for correspondence: mousa.khalafi@kashanu.ac.ir

M S A: 0009-0004-5263-0941, M Kh: 0000-0001-6465-1281, A H M: 0000-0001-7715-0734

Received 2 June 2026; Accepted 11 August 2026

production, ultimately leading to cellular injury and activation of inflammatory pathways (J. Gong, Tu, Liu, & Tian, 2023; Masarone et al., 2018).

Among the molecular pathways governing the oxidative stress response, the Keap1/Nrf2 signaling axis is of paramount importance. Nrf2 (Nuclear factor erythroid 2-related factor 2) is a master transcription factor that regulates the cellular antioxidant defense system; its activation induces the expression of cytoprotective genes that mitigate oxidative injury (Jaiswal, 2004). Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1 (Kelch-like ECH-associated protein 1) and targeted for proteasomal degradation. However, under oxidative stress, this interaction is disrupted, allowing Nrf2 to translocate into the nucleus and upregulate antioxidant gene expression (Kaspar, Niture, & Jaiswal, 2009). Conversely, Nuclear Factor-kappa B (NF-κB) serves as a primary regulator of inflammatory responses; its activation is associated with the upregulation of pro-inflammatory cytokines and the exacerbation of hepatic injury (Ali, El-Tawil, Al-Mokaddem, & Abd El-Rahman, 2021).

Given the critical roles of oxidative stress and inflammation in NAFLD progression, non-pharmacological interventions—including exercise training and nutritional strategies—have garnered significant research interest. High-Intensity Interval Training (HIIT) has emerged as a particularly effective modality due to its time-efficiency and superior capacity to enhance cardiorespiratory fitness, metabolic health, and antioxidant capacity while reducing fat mass (Atakan, Li, KoŞar, Turnagöl, & Yan, 2021; Khalafi et al., 2025; Reljic, 2025). Evidence indicates that HIIT exerts robust hepatoprotective effects by optimizing mitochondrial function and modulating inflammation and oxidative stress-related signaling pathways (Heiat & Shojaei Fard, 2026; Y. Zhang, Wei, Liu, & Guo, 2025). In tandem with exercise, ketone ester (KE) supplementation has recently surfaced as a novel nutritional intervention designed to induce nutritional ketosis without the need for stringent carbohydrate restriction (Falkenhain, Islam, & Little, 2023; J.-L. Liu & Xia, 2026; Saris & Timmers, 2022). Ketone bodies, particularly beta-hydroxybutyrate (βHB), serve not only as alternative metabolic substrates during periods of low glucose availability but also as potent signaling molecules that regulate cellular metabolism (Veech, Chance, Kashiwaya, Lardy, & Cahill Jr, 2001). Growing evidence suggests that ketone bodies can enhance cellular metabolic efficiency and attenuate oxidative stress by suppressing mitochondrial ROS production and improving bioenergetics (Chu, Zhang, & Xie, 2021; Kesl et al., 2016; Li et al., 2025; Shahtaghi, Soni, Bakrey, Bigdelitabar, & Jain, 2024).

Furthermore, studies have demonstrated that exogenous ketone supplementation can elevate circulating ketone levels and induce nutritional ketosis without deleterious effects on lipid profiles, making it a more practical and safer alternative to ketogenic diets (Clarke, Tchabanenko, Pawlosky, Carter, King, et al., a2012; Clarke, Tchabanenko, Pawlosky, Carter, Knight, et al., b2012). Collectively, these findings suggest that ketone supplementation may offer significant metabolic and cellular protection through the modulation of oxidative stress, inflammation, and mitochondrial function.

Despite the documented benefits of HIIT and ketone supplementation individually, limited information is available regarding the combined effects of these interventions on hepatic oxidative stress- and inflammation-related signaling pathways in the context of Western diet consumption. Therefore, the present study aimed to investigate the effects of high-intensity interval training (HIIT), ketone ester supplementation (KE), and their combination on hepatic Keap1, Nrf2, and NF-κB protein levels in mice fed a Western diet.

Materials and Methods

Study design and ethical considerations

This study employed an experimental design, and all ethical considerations related to the use of laboratory animals were observed in accordance with the approved institutional ethical guidelines throughout the research process. Thirty male C57BL/6J mice, 8 weeks of age and weighing approximately 20 ±3 g, were obtained from the Research Center of the Pasteur Institute of Iran (Karaj) and transferred to the Exercise Physiology Laboratory, Faculty of Humanities, University of Kashan.

The mice were housed in polycarbonate cages under controlled environmental conditions, including a temperature of 22±2°C, relative humidity of approximately 55%, and a 12:12-h light/dark cycle in the animal facility. During this period, the animals were maintained on a standard chow diet. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Faculty of Sports Sciences, University of Tehran (IR.UT.SPORT.REC.1403.112).

Animal grouping

Two weeks after acclimatization to the new laboratory environment, the mice were randomly allocated into five groups of six animals each:

1) Standard diet group (ND)

2) Western diet group (WD)

3) Western diet plus ketone ester supplementation group (WD+KE)

4) Western diet plus exercise training group (WD+HIIT)

5) Western diet plus combined exercise training and ketone ester supplementation group (WD+HIIT+KE)

In the first phase of the intervention, all groups except the ND group were fed a Western diet for eight weeks. In the second phase, mice in the WD+HIIT and WD+HIIT+KE groups underwent a 4-week exercise training program. In addition, mice in the WD+KE and WD+HIIT+KE groups received ketone ester supplementation for 4 weeks. To ensure uniformity, control mice received the same volume of vehicle solution without the active compound.

Standard and Western diets

The standard diet (ND) consisted of 10% of calories from fat, 67% from carbohydrates, and 23% from protein. The Western diet (WD) consisted of 45% of calories from fat, 35% from carbohydrates, and 20% from protein. In the WD, the primary fat sources were lard and soybean oil, whereas carbohydrates were mainly provided by maltodextrin and sucrose. In the ND, carbohydrates were primarily derived from corn starch, maltodextrin, and sucrose, while soybean oil served as the principal fat source. To further enhance the metabolic characteristics associated with the Western diet, the mice's drinking water was supplemented with 50 g/L carbohydrate, composed of 55% fructose and 45% sucrose (Hafstad et al., 2013). After 8 weeks of feeding with this diet, animals assigned to the exercise, supplementation, or combined intervention groups entered the second phase, which lasted 4 weeks.

Exercise training protocol

Following the initial acclimatization period, mice in the WD+HIIT and WD+HIIT+KE groups underwent a 1-week familiarization period on the treadmill before beginning the 4-week training intervention. Thereafter, a high-intensity interval training (HIIT) protocol was implemented for 4 weeks, with three sessions per week at a 5-degree incline.

Each training session consisted of 10 bouts of 2-minute running at 85–90% of maximal speed, interspersed with 2-minute active recovery periods at 50% of maximal speed. Running speed was progressively increased over the course of the training period, beginning at 19 m/min in the first week and reaching 22 m/min in the fourth week. Likewise, active recovery speed increased from 8 m/min in week 1 to 11 m/min in week 4. At the beginning of each session, a 10-minute warm-up at 6 m/min was performed, followed by a 5-minute cool-down at 5 m/min at the end of the session.

To determine maximal running speed, a graded treadmill running test was performed. In this test, after a low-speed warm-up, trea--dmill speed was increased by 2 m/min every 2 minutes. This process continued until the mice were no longer able to continue running. The highest running speed achieved before exhaustion was considered the maximal running speed (Al-Atwan, Khalafi, & Habibi Maleki, 2025; Habibi Maleki, Tolouei Azar, Razi, & Tofighi, 2024). Exhaustion was defined as the inability of the animal to resume running despite repeated gentle tactile stimulation. This protocol was designed to preserve the principles of HIIT while also allowing the laboratory animals to adapt gradually to the exercise load (Habibi Maleki et al., 2024).

Ketone ester supplementation protocol

To assess the effects of ketone ester supplementation on physiological and metabolic parameters, Oxford Ketone Ester (ΔG) was used. The supplement was administered daily by gavage at a dose of 5 g/kg body weight and the administered dose was adjusted according to each mouse's weekly changes in body weight. The selected dose was based on previously published rodent studies demonstrating that ketone ester administration at this level effectively induces nutritional ketosis and produces measurable metabolic effects without reported toxicity (Kesl et al., 2016). Animals in the control groups received an equivalent volume of the vehicle solution without the active ingredient.

Tissue sampling and laboratory analyses

Forty-eight hours after the final exercise session, the mice were anesthetized via ketamine and xylazine injection, and liver tissue was harvested. Liver tissue was collected from all animals. For Western blot analyses, samples from five randomly selected mice per group were analyzed. The liver samples were washed with cold saline solution and weighed. Thereafter, the samples were stored at −70°C.

Western blotting was used to assess the protein levels of Keap1, Nrf2, and NF-κB. Briefly, liver tissues were homogenized in RIPA lysis buffer containing protease and phosphatase inhibitors. The samples were then centrifuged at 12,000 g for 15 min at 4°C, and the supernatant containing total protein was collected. Total protein concentration was determined using the Bradford assay. To examine the expression of Keap1, Nrf2, and NF-κB proteins, equal amounts of protein (30–50 µg) were mixed with Laemmli sample buffer and denatured for 5 min at 95°C. The samples were then separated by SDS-PAGE on polyacrylamide gels, including a 4% stacking gel and a 2–12% resolving gel. After electrophoresis, proteins were transferred to PVDF membranes using a wet transfer system at 100 V for 90 min at 4°C.

To prevent nonspecific binding, the membranes were blocked for 1 h at room temperature in TBS-T containing 5% nonfat milk. They were then incubated overnight at 4°C with the following primary antibodies:

• Keap1 (Santa Cruz Biotechnology, sc-74446, 1:500)

• Nrf2 (Santa Cruz Biotechnology, sc-36524, 1:500)

• NF-κB p65 (Santa Cruz Biotechnology, sc-37218, 1:500)

After washing with TBS-T, the membranes were incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, 1: 5,000) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit, and images were captured using a gel documentation system. Band intensities were quantified using ImageJ software, and the resulting values were normalized to β-actin as the loading control.

Statistical analysis

The Shapiro–Wilk test was used to assess the normality of the data distribution. An independent t-test was applied to compare differences between the ND and WD groups. For comparisons among all five experimental groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test to determine pairwise group differences. All data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 26, and $p<0.05$ was considered statistically significant. Figures were prepared using GraphPad Prism 10.

Results

Exercise training and combined exercise training plus ketone ester supplementation reduced hepatic Keap1 protein levels

Hepatic Keap1 protein levels were significantly higher in the WD group than in the ND group (t=4.85, p=0.001). Compared with the WD group, Keap1 protein levels were significantly reduced in both the WD+HIIT group (p=0.04) and the WD+HIIT+KE group (p =0.001). No significant difference was observed between the WD+KE and WD groups (p=0.21). However, Keap1 protein levels were significantly lower in the WD+HIIT+KE group than in the WD+KE group (p=0.04) (Figure 1).

Exercise training, ketone ester supplementation, and their combination increased hepatic Nrf2 protein levels

The WD group exhibited significantly lower hepatic Nrf2 protein levels than the ND group (t = −7.00, p = 0.001). Nrf2 protein levels were significantly increased in the WD+HIIT, WD+KE, and WD+HIIT+KE groups compared with the WD group (all p = 0.001).No significant difference was found between the WD+HIIT

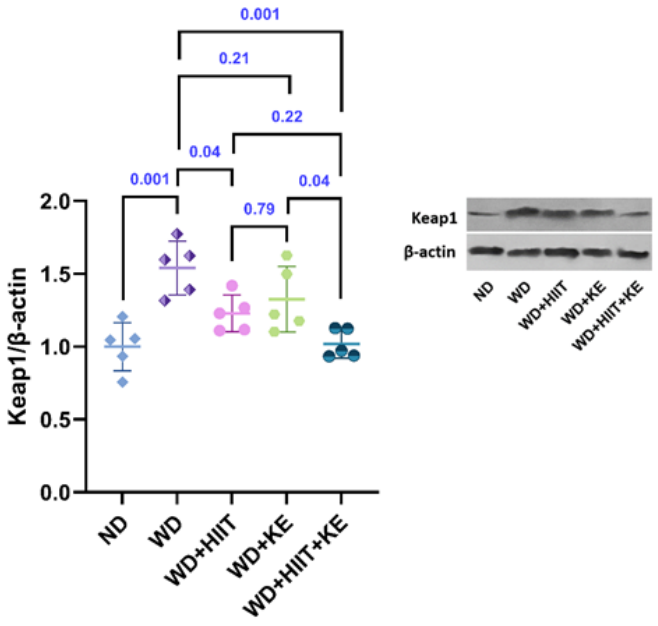

Figure 1. Hepatic Keap1 protein levels in response to high-intensity interval training and ketone ester supplementation. Keap1 protein expression in liver tissue of mice across experimental groups. Individual data points represent five randomly selected animals per group used for Western blot analysis (n = 5), and horizontal bars indicate group means. P-values for multiple comparisons are indicated above the brackets. Abbreviations: ND, normal diet; WD, Western diet; HIIT, high-intensity interval training; KE, ketone ester.

+KE and WD+HIIT groups (p=0.08). In contrast, Nrf2 protein levels were significantly higher in the WD+HIIT+KE group than in the WD+KE group (p=0.001). A significant difference was also observed between the WD+HIIT and WD+KE groups (p=0.03) (Figure 2).

Exercise training, ketone ester supplementation, and their combination reduced hepatic NF-κB protein levels

Hepatic NF-κB protein levels were significantly higher in the WD group than in the ND group (t=8.96, p=0.001). Compared with the WD group, NF-κB protein levels were significantly reduced in the WD+HIIT, WD+KE, and WD+HIIT+KE groups (all p=0.001). No significant differences were observed among the three intervention groups (all p>0.05) (Figure 3).

Discussion

The present study aimed to investigate the effects of high-intensity interval training (HIIT), ketone ester (KE) supplementation, and their combination (KE+HIIT) on key molecular markers of oxidative stress and inflammation (Keap1, Nrf2, and NF-κB) in the liver tissue of mice fed a Western diet (WD). Our primary findings demonstrate that a Western diet significantly increases hepatic Keap1 and NF-κB protein levels while concomitantly reducing Nrf2 levels. Conversely, HIIT intervention successfully reversed these trends by decreasing

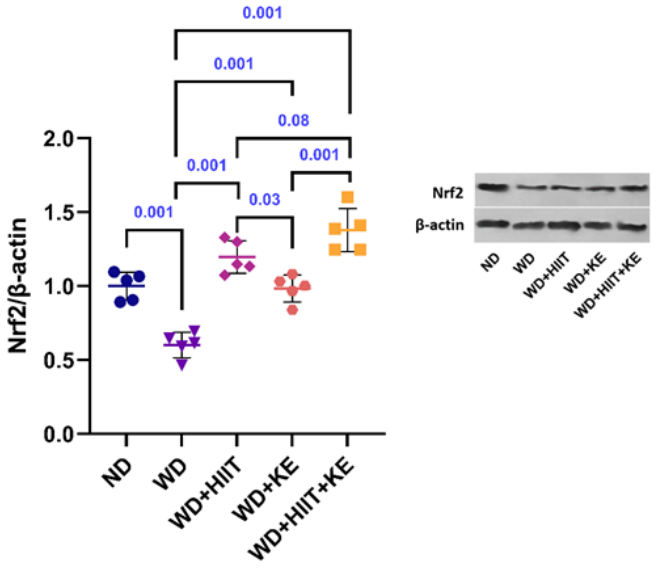

Figure 2. Hepatic Nrf2 protein levels in response to high-intensity interval training and ketone ester supplementation. Nrf2 protein expression in liver tissue of mice across experimental groups. Individual data points represent five randomly selected animals per group used for Western blot analysis (n = 5), and horizontal bars indicate group means. P-values for multiple comparisons are indicated above the brackets. Abbreviations: ND, normal diet; WD, Western diet; HIIT, high-intensity interval training; KE, ketone ester.

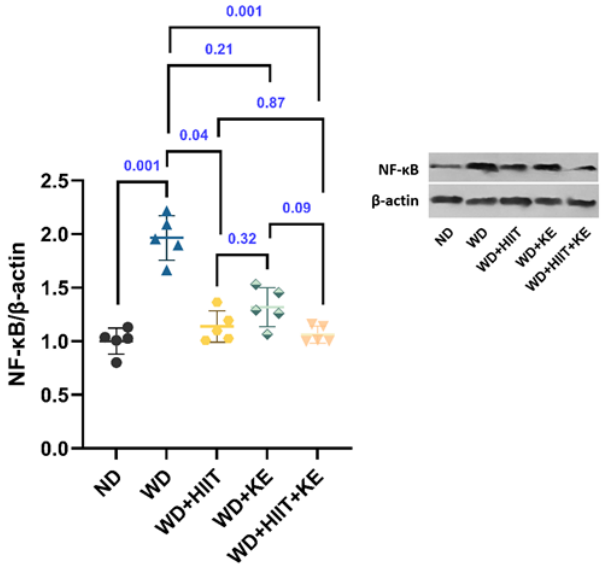

Figure 3. Hepatic NF-κB protein levels in response to high-intensity interval training and ketone ester supplementation. NF-κB protein expression in liver tissue of mice across experimental groups. Individual data points represent five randomly selected animals per group used for Western blot analysis (n = 5), and horizontal bars indicate group means. P-values for multiple comparisons are indicated above the brackets. Abbreviations: ND, normal diet; WD, Western diet; HIIT, high-intensity interval training; KE, ketone ester.

Keap1 and NF-κB and upregulating Nrf2. Furthermore, while KE supplementation enhanced Nrf2 and suppressed NF-κB, it did not significantly alter Keap1 levels. Notably, the combined intervention (HIIT+KE) yielded substantial improvements across all assessed markers. Collectively, these results suggest that exercise training and ketone supplementation exert hepatoprotective effects under WD conditions, likely through the modulation of redox-sensitive and inflammatory signaling pathways. The observed elevation of Keap1 and NF-κB, alongside the suppression of Nrf2 in the WD group, aligns with extensive evidence indicating that high-fat, high-carbohydrate diets disrupt cellular redox homeostasis, fostering chronic inflammation and hepatic injury (Liao et al., 2025; Nanizawa et al., 2022). The excessive accumulation of fatty acids in hepatocytes is a hallmark of NAFLD and can lead to incomplete beta-oxidation and mitochondrial dysfunction. This ultimately results in increased reactive oxygen species (ROS) production (Begriche, Igoudjil, Pessayre, & Fromenty, 2006; Lee, Park, & Roh, 2019).

Elevated ROS levels directly trigger inflammatory cascades through activation of NF-κB, a master transcription factor regulating pro-inflammatory cytokine expression. Under such conditions, increased Keap1 expression may sequester Nrf2 and prevent activation of endogenous antioxidant defense systems (Cásedas et al., 2026). The resulting decline in Nrf2 activity leads to downregulation of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and heme oxygen-nase-1 (HO-1). This impairs the cellular capacity to counteract oxidative stress. Collectively, this imbalance between oxidative and antioxidant systems represents a central mechanism in NAFLD progression (H.-M. Zhou et al., 2025). This imbalance between oxidative and antioxidant systems represents a fundamental mechanism in the progression of NAFLD. Our findings further demonstrate that HIIT effectively reduces Keap1 and NF-κB while increasing Nrf2 levels. These results are consistent with studies reporting the protective effects of exercise on liver tissue and oxidative stress pathways (C.-K. Zhang, Wang, & Li, 2025). Exercise modalities, particularly HIIT, enhance mitochondrial efficiency, metabolic crosstalk, aerobic capacity, and antioxidant defenses (Han, Lu, & Yan, 2022; Skelly, Bailleul, & Gillen, 2021; Wei, Ahmad, Chen, Fatima, & Shah, 2025).

A key mechanism underlying these adaptations is activation of the Nrf2 signaling pathway in response to exercise-induced physiological stress. Nrf2 activation upregulates antioxidant gene expression and maintains cellular redox balance (Goodfellow et al., 2020). Additionally, HIIT reduces metabolic pressure on hepatocytes by decreasing hepatic lipid accumulation, improving insulin sensitivity, and enhancing fatty acid oxidation (Abassi et al., 2025; Khoramipour et al., 2025). Consequently, reduced hepatic lipid load limits ROS production and inhibits activation of inflammatory pathways such as NF-κB. Furthermore, exercise improves electron transport chain function and reduces free radical production through increased PGC-1α expression and mi-

-tochondrial biogenesis (Nakao, Maeda, & Iemitsu, 2026).

These adaptations may be partly related to exercise-induced systemic signaling between skeletal muscle and the liver. Contracting skeletal muscle releases myokines and metabolic mediators that have been suggested to influence hepatic lipid metabolism, inflammation, and oxidative stress regulation. However, these mediators were not directly measured in the present study; therefore, any involvement of muscle–liver crosstalk in the observed adaptations remains speculative and should be interpreted with caution.

Regarding nutritional intervention, KE supplementation significantly increased Nrf2 and decreased NF-κB protein levels. These observations are consistent with previous reports on the antioxidant and anti-inflammatory properties of ketone bodies (Y. Gong, Wei, Liu, & Du, 2026; Pinto, Bonucci, Maggi, Corsi, & Businaro, 2018; Rojas-Morales, Pedraza-Chaverri, & Tapia, 2020). Beta-hydroxybutyrate (βHB), the primary circulating ketone body, functions not only as an energy substrate but also as a signaling molecule that modulates multiple cellular pathways.

One proposed mechanism for βHB's protective effects is the inhibition of Class I histone deacetylases (HDACs), which enhances the expression of antioxidant genes and fortifies cellular defenses against oxidative stress (Xiang, Wang, Lan, Zhang, & Wei, 2023; T. Zhou et al., 2022). Moreover, ketone bodies have been shown to improve mitochondrial metabolic efficiency and suppress ROS generation (Frey et al., 2017). βHB can also inhibit NF-κB signaling by preventing the activation of the NLRP3 inflammasome and reducing the production of pro-inflammatory cytokines (Kong et al., 2021). Interestingly, the lack of significant change in Keap1 levels within the KE group suggests that the supplement's effects may be mediated through downstream activation of antioxidant pathways or modulation of inflammatory signals rather than direct alterations in Keap1 protein abundance.

Furthermore, the combined HIIT and KE intervention (HIIT+KE) resulted in improvements in all markers, including reduced NF-κB and Keap1 and increased Nrf2. These effects likely reflect complementary mechanisms of exercise-induced metabolic adaptation and ketone-mediated signaling. While exercise enhances energy expenditure and mitochondrial function, exogenous ketones provide an efficient fuel source and reduce ROS production by optimizing electron transport chain efficiency (Kesl et al., 2016). However, the absence of a significant difference between the HIIT-only and combined (HIIT+KE) groups suggests that exercise may be the primary driver of these adaptations in this model.

Strengths and limitations

This study has several notable strengths. First, it simultaneously evaluated the effects of HIIT and ketone ester supplementation on key hepatic oxidative stress and inflammatory pathways in a Western diet model, providing novel mechanistic insights into their individual and combined effects. Second, the direct quantification of Keap1, Nrf2, and NF-κB protein levels allows for a precise assessment of molecular shifts.

Nonetheless, certain limitations should be acknowledged. We focused solely on total protein levels; however, the biological activity of Nrf2 and NF-κB is largely dependent on their nuclear translocation and subcellular localization. Therefore, future studies should incorporate nuclear and cytoplasmic fractionation approaches, as well as assessments of transcriptional activity, to provide a more comprehensive evaluation of pathway activation. In addition, direct measurements of oxidative stress biomarkers (e.g., ROS, MDA, GSH, SOD, and catalase), inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β), and systemic metabolic parameters were not performed. Therefore, although the observed alterations in Keap1/Nrf2/NF-κB protein expression suggest modulation of oxidative stress and inflammatory signaling, mechanistic interpretations should be made with appropriate caution. Furthermore, the absence of a normal diet plus ketone ester (ND+KE) group precludes evaluation of the independent effects of ketone ester supplementation under physiological conditions. Future studies should include such a group to determine whether the observed responses are specific to Western diet-induced metabolic disturbances. Finally, the use of a single dose of ketone ester and the relatively short intervention period limit the ability to determine dose-dependent responses and the long-term sustainability of the observed effects. Future studies employing longer intervention durations are needed to evaluate the durability, potential cumulative effects, and translational relevance of these findings.

Conclusion

In conclusion, Western diet consumption disrupted hepatic redox homeostasis and promoted inflammatory signaling, as evidenced by increased Keap1 and NF-κB levels and reduced Nrf2 expression. HIIT and ketone ester supplementation attenuated these alterations, with the combined intervention producing the most consistent improvements across the evaluated molecular markers. These findings support the potential utility of exercise training and ketone ester supplementation as complementary non-pharmacological strategies for mitigating diet-induced hepatic oxidative stress and inflammation.

What is already known on this subject?

• Western diet consumption is strongly associated with metabolic disorders, including the non-alcoholic fatty liver disease (NAFLD),

primarily through the induction of oxidative stress and chronic low-grade inflammation.

• Disruption of hepatic redox homeostasis and activation of inflammatory signaling pathways, particularly NF-κB, are key mechanisms involved in NAFLD progression.

• The Keap1/Nrf2 signaling pathway is a central regulator of cellular antioxidant defense, and its dysregulation contributes to increased susceptibility to oxidative damage in metabolic diseases.

• High-intensity interval training (HIIT) improves metabolic health, enhances mitochondrial function, and reduces oxidative stress and inflammation in both clinical and preclinical models.

• Ketone ester supplementation has emerged as a metabolic intervention that improves mitochondrial efficiency and modulates oxidative and inflammatory signaling pathways.

• Although exercise-based and nutritional strategies have independently shown protective effects against diet-induced metabolic disturbances, their combined impact on hepatic redox regulation remains insufficiently understood.

What this study adds?

• This study provides new evidence that high-intensity interval training (HIIT) and ketone ester supplementation modulate hepatic redox and inflammatory signaling pathways in a Western diet-induced mouse model.

• HIIT, ketone ester supplementation, and their combination significantly improved the Keap1/Nrf2/NF-κB signaling axis, with HIIT showing particularly strong effects on Keap1 regulation.

• Ketone ester supplementation enhanced Nrf2 activation and reduced NF-κB protein levels, supporting its antioxidant and anti-inflammatory potential.

• The combined intervention did not show clear additive effects across all markers, but resulted in more consistent improvements in hepatic redox homeostasis.

Organ Cross-Talk Tips:

- HIIT may promote hepatic redox adaptation through systemic signals released from contracting skeletal muscle, although myokines were not directly measured in this study.
- Chronic WD feeding promotes hepatic lipid accumulation and oxidative stress, likely reflecting altered adipocyte–liver communication and systemic metabolic dysfunction.

Acknowledgements

This study is derived from a Master's thesis in Exercise Physiology at the University of Kashan. We sincerely thank the faculty members of the Department of Sports Sciences at the University of Kashan and the Physiology Research Center of the Iran University of Medical Sciences for their invaluable support.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in the present research.

Ethical approval All experimental procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Faculty of Sports Sciences, University of Tehran (Approval No. IR.UT.SPORT.REC.1403.112; approved on 04.01.2025).

Informed consent Animal study.

Author contributions

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

References

Abassi, W., Ouerghi, N., Hammami, M. B., Jebabli, N., Feki, M., Bouassida, A., . . . Knechtle, B. (2025). High-Intensity Interval Training Reduces Liver Enzyme Levels and Improves MASLD-Related Biomarkers in Overweight/Obese Girls. Nutrients, 17(1). doi: https://doi.org/10.3390/nu17010164

Adolph, T. E., & Tilg, H. (2024). Western diets and chronic diseases. Nature Medicine, 30(8), 2133-2147. DOI: https://doi.org/10.1038/s41591-024-03165-6

Al-Atwan, M. A. A., Khalafi, M., & Habibi Maleki, A. (2025). Effect of High-Intensity Interval Training and Alternate-Day Fasting on Angiogenic Protein Expression in Visceral Adipose Tissue of Rats Fed a High-Fat, High-Fructose Diet. Journal of Mazandaran University of Medical Sciences, 35(244), 27-41. http://jmums.mazums.ac.ir/article-1-21365-en.html

Ali, A. M., El-Tawil, O. S., Al-Mokaddem, A. K., & Abd El-Rahman, S. S. (2021). Promoted inhibition of TLR4/miR-155/NFkB p65 signaling by cannabinoid receptor 2 agonist (AM1241), aborts inflammation and progress of hepatic fibrosis induced by thioacetamide. Chemico-Biological Interactions, 336, 109398.DOI: https://doi.org/10.1016/j.cbi.2021.109398

Arroyave-Ospina, J. C., Wu, Z., Geng, Y., & Moshage, H. (2021). Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy. Antioxidants, 10(2), 174.DOI: https://doi.org/10.3390/antiox10020174

Aslan, B. (2023). Western diet and cognitive impairment: links to potential mechanisms: a review. International Journal of Nutrition Sciences, 8(3), 131-143. DOI: https://doi.org/10.30476/ijns.2023.98726.1234

Atakan, M. M., Li, Y., Koşar, Ş. N., Turnagöl, H. H., & Yan, X. (2021). Evidence-based effects of high-intensity interval training on exercise capacity and health: A review with historical perspective. International journal of environmental research and public health, 18(13), 7201. doi: https://doi.org/10.3390/ijerph18137201

Begriche, K., Igoudjil, A., Pessayre, D., & Fromenty, B. (2006). Mitochondrial dysfunction in NASH: Causes, consequences and possible means to prevent it. Mitochondrion, 6(1), 1-28. doi: https://doi.org/10.1016/j.mito.2005.10.004

Bettiga, A., Fiorio, F., Di Marco, F., Trevisani, F., Romani, A., Porrini, E., . . . Vago, R. (2019). The modern western diet rich in advanced glycation end-products (AGEs): An overview of its impact on obesity and early progression of renal pathology. Nutrients, 11(8), 1748.DOI: https://doi.org/10.3390/nu11081748

Blüher, M. (2019). Obesity: global epidemiology and pathogenesis. Nature reviews endocrinology, 15(5), 288-298.DOI: https://doi.org/10.1038/s41574-019-0176-8

Cásedas, G., Rojas-Márquez, H., Ventura, L., Moliner, C., Maggi, F., Rubio-Castellanos, A., & López, V. (2026). Involvement of Keap1/Nrf2 and the antioxidant defence in cytoprotective effects induced by cannabis polyphenols in SH-SY5Y neuronal cells. Biomedicine & Pharmacotherapy, 196, 119048. doi: https://doi.org/10.1016/j.biopha.2026.119048

Chu, Y., Zhang, C., & Xie, M. (2021). Beta-hydroxybutyrate, friend or foe for stressed hearts. Frontiers in aging, 2, 681513.doi: https://doi.org/10.3389/fragi.2021.681513

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., King, M. T., Musa-Veloso, K., . . . VanItallie, T. B. (a2012). Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. Regulatory Toxicology and Pharmacology, 63(3), 401-408.DOI: https://doi.org/10.1016/j.yrtph.2012.04.008

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Knight, N. S., Murray, A. J., . . . Roberts, A. (b2012). Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate. Regulatory Toxicology and Pharmacology, 63(2), 196-208.DOI: https://doi.org/10.1016/j.yrtph.2012.04.001

Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global impacts of western diet and its effects on metabolism and health: a narrative review. Nutrients, 15(12), 2749.DOI: https://doi.org/10.3390/nu15122749

Delli Bovi, A. P., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., & Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. Frontiers in medicine, 8, 595371.DOI: https://doi.org/10.3389/fmed.2021.595371

Engin, A. (2017). Non-alcoholic fatty liver disease. Obesity and Lipotoxicity, 443-467.DOI: https://doi.org/10.1007/978-3-319-48382-5_19

Falkenhain, K., Islam, H., & Little, J. P. (2023). Exogenous ketone supplementation: an emerging tool for physiologists with potential as a metabolic therapy. Experimental physiology, 108(2), 177-187.DOI: https://doi.org/10.1113/EP090430

Farzanegi, P., Dana, A., Ebrahimpoor, Z., Asadi, M., & Azarbayjani, M. A. (2019). Mechanisms of beneficial effects of exercise training on non-alcoholic fatty liver disease (NAFLD): Roles of oxidative stress and inflammation. European journal of sport science, 19(7), 994-1003.DOI: https://doi.org/10.1080/17461391.2019.1571114

Frey, S., Geffroy, G., Desquiret-Dumas, V., Gueguen, N., Bris, C., Belal, S., . . . Procaccio, V. (2017). The addition of ketone bodies alleviates mitochondrial dysfunction by restoring complex I assembly in a MELAS cellular model. Biochim Biophys Acta Mol Basis Dis, 1863(1), 284-291. doi: https://doi.org/10.1016/j.bbadis.2016.10.028

Gong, J., Tu, W., Liu, J., & Tian, D. (2023). Hepatocytes: A key role in liver inflammation. Frontiers in immunology, 13, 1083780.doi: https://doi.org/10.3389/fimmu.2022.1083780

Gong, Y., Wei, Y., Liu, Y., & Du, T. (2026). The value of targeting ketone body metabolism in inflammatory and autoimmune diseases. Journal of Translational Medicine, 24(1), 558. doi:10.1186/s12967-026-07884-x. DOI: https://doi.org/10.1186/s12967-026-07884-x

Goodfellow, M. J., Borcar, A., Proctor, J. L., Greco, T., Rosenthal, R. E., & Fiskum, G. (2020). Transcriptional activation of antioxidant gene expression by Nrf2 protects against mitochondrial dysfunction and neuronal death associated with acute and chronic neurodegeneration. Exp Neurol, 328, 113247. doi: https://doi.org/10.1016/j.expneurol.2020.113247

Habibi Maleki, A., Tolouei Azar, J., Razi, M., & Tofighi, A. (2024). The Effect of Different Exercise Modalities on Sertoli-germ Cells Metabolic Interactions in High-fat Diet-induced Obesity Rat Models: Implication on Glucose and Lactate Transport, Igf1, and Igf1R-dependent Pathways. Reprod Sci, 31(8), 2246-2260. doi: https://doi.org/10.1007/s43032-024-01533-8

Hafstad, A. D., Lund, J., Hadler-Olsen, E., Höper, A. C., Larsen, T. S., & Aasum, E. (2013). High-and moderate-intensity training normalizes ventricular function and mechanoenergetics in mice with diet-induced obesity. Diabetes, 62(7), 2287-2294.DOI: https://doi.org/10.2337/db12-1580

Han, C., Lu, P., & Yan, S. Z. (2022). Effects of high-intensity interval training on mitochondrial supercomplex assembly and biogenesis, mitophagy, and the AMP-activated protein kinase pathway in the soleus muscle of aged female rats. Exp Gerontol, 158, 111648. doi: https://doi.org/10.1016/j.exger.2021.111648

Heiat, F., & Shojaei Fard, M. (2026). Effects of High-Intensity Interval Training and Moderate-Intensity Continuous Training on PGC-1α, SIRT3, and Non-Alcoholic Fatty Liver Disease: A Narrative Review. Journal of Advanced Biomedical Sciences, 16(1), 3-16. DOI: https://doi.org/10.18502/jabs.v16i1.20130

Jaiswal, A. K. (2004). Nrf2 signaling in coordinated activation of antioxidant gene expression. Free Radical Biology and Medicine, 36(10), 1199-1207. DOI: https://doi.org/10.1016/j.freeradbiomed.2004.02.074

Kaliszewska, A., Allison, J., Martini, M., & Arias, N. (2021). The interaction of diet and mitochondrial dysfunction in aging and cognition. International Journal of Molecular Sciences, 22(7), 3574. doi: https://doi.org/10.3390/ijms22073574

Kaspar, J. W., Niture, S. K., & Jaiswal, A. K. (2009). Nrf2: INrf2 (Keap1) signaling in oxidative stress. Free Radical Biology and Medicine, 47(9), 1304-1309.DOI: https://doi.org/10.1016/j.freeradbiomed.2009.07.035

Kerr, J., Anderson, C., & Lippman, S. M. (2017). Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. The Lancet Oncology, 18(8), e457-e471.DOI: https://doi.org/10.1016/S1470-2045(17)30411-4

Kesl, S. L., Poff, A. M., Ward, N. P., Fiorelli, T. N., Ari, C., Van Putten, A. J., . . . D'Agostino, D. P. (2016). Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats. Nutrition & metabolism, 13(1), 9.DOI: https://doi.org/10.1186/s12986-016-0069-y

Khalafi, M., Habibi Maleki, A., Symonds, M. E., Azali Alamdari, K., Ehsanifar, M., & Rosenkranz, S. K. (2025). Comparative Efficacy of Different Exercise Modes on Inflammatory Markers in Patients With Type 2 Diabetes Mellitus: A Systematic Review With Pairwise and Network Meta‐Analyses. Obesity Reviews, 26(10), e13954.DOI: https://doi.org/10.1111/obr.13954

Khoramipour, K., Hosseini, N. S., Hill, J. W., Khoramipour, K., Khoramipour, K., Izquierdo, S. M., . . . Saheli, M. (2025). High intensity interval training attenuate insulin resistance in diabetic rats accompanied by improvements in liver metabolism and spexin signaling. Sci Rep, 15(1), 30682. doi: https://doi.org/10.1038/s41598-025-15432-8

Kong, G., Liu, J., Li, R., Lin, J., Huang, Z., Yang, Z., . . . Wu, X. (2021). Ketone Metabolite β-Hydroxybutyrate Ameliorates Inflammation After Spinal Cord Injury by Inhibiting the NLRP3 Inflammasome. Neurochem Res, 46(2), 213-229. doi: https://doi.org/10.1007/s11064-020-03156-2

Lee, J., Park, J.-S., & Roh, Y. S. (2019). Molecular insights into the role of mitochondria in non-alcoholic fatty liver disease. Archives of Pharmacal Research, 42(11), 935-946. doi: https://doi.org/10.1007/s12272-019-01178-1

Li, J., He, W., Wu, Q., Qin, Y., Luo, C., Dai, Z., . . . Cao, L. (2025). Ketogenic diets and β-hydroxybutyrate in the prevention and treatment of diabetic kidney disease: current progress and future perspectives. BMC nephrology, 26(1), 127.DOI: https://doi.org/10.1186/s12882-025-04019-0

Liao, Z., He, X., Gu, X., Ye, T., Chen, A., Guo, Y., . . . Niu, J. (2025). Mechanisms of high-glucose-induced mitochondrial damage and glycolipid accumulation in largemouth bass. Journal of Animal Science and Biotechnology, 16(1), 132. doi: https://doi.org/10.1186/s40104-025-01261-2

Liu, J.-L., & Xia, L. (2026). Ketone Bodies in Metabolism and Signaling: Revisiting the Paradigm from Biochemistry to Pancreatic β-Cell Regulation. Metabolism and Diseases, 100005. https://doi.org/10.1016/j.metdis.2026.100005

Liu, Q., Bengmark, S., & Qu, S. (2010). The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). Lipids in health and disease, 9(1), 42.doi: https://doi.org/10.1186/1476-511X-9-42

Masarone, M., Rosato, V., Dallio, M., Gravina, A. G., Aglitti, A., Loguercio, C., . . . Persico, M. (2018). Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. Oxidative medicine and cellular longevity, 2018(1), 9547613.DOI: https://doi.org/10.1155/2018/9547613

Nakao, K., Maeda, S., & Iemitsu, M. (2026). Exercise training improves mitochondrial oxidative energy metabolism through PGC-1α-dependent transcriptional pathway in the aged rat heart. Experimental Gerontology, 217, 113100. doi: https://doi.org/10.1016/j.exger.2026.113100

Nanizawa, E., Otsuka, S., Hatayama, N., Tamaki, Y., Hayashi, Y., Ishikawa, T., . . . Naito, M. (2022). Short-term high-fat and high-carbohydrate diets increase susceptibility to liver injury by inducing hepatic procoagulant and proinflammatory conditions with different balances. Nutrition, 101, 111710. doi: https://doi.org/10.1016/j.nut.2022.111710

Pinto, A., Bonucci, A., Maggi, E., Corsi, M., & Businaro, R. (2018). Anti-Oxidant and Anti-Inflammatory Activity of Ketogenic Diet: New Perspectives for Neuroprotection in Alzheimer's Disease. Antioxidants (Basel), 7(5). doi: https://doi.org/10.3390/antiox7050063

Reljic, D. (2025). High-Intensity Interval Training as Redox Medicine: Targeting Oxidative Stress and Antioxidant Adaptations in Cardiometabolic Disease Cohorts. Antioxidants, 14(8), 937.DOI: https://doi.org/10.3390/antiox14080937

Rojas-Morales, P., Pedraza-Chaverri, J., & Tapia, E. (2020). Ketone bodies, stress response, and redox homeostasis. Redox Biol, 29, 101395. doi: https://doi.org/10.1016/j.redox.2019.101395

Romualdo, G. R., Valente, L. C., Sprocatti, A. C., Bacil, G. P., de Souza, I. P., Rodrigues, J., . . . Barbisan, L. F. (2022). Western diet–induced mouse model of non-alcoholic fatty liver disease associated with metabolic outcomes: Features of gut microbiome-liver-adipose tissue axis. Nutrition, 103, 111836.DOI: https://doi.org/10.1016/j.nut.2022.111836

Salehi-Sahlabadi, A., Sadat, S., Beigrezaei, S., Pourmasomi, M., Feizi, A., Ghiasvand, R., . . . Miraghajani, M. (2021). Dietary patterns and risk of non-alcoholic fatty liver disease. BMC gastroenterology, 21(1), 41.DOI: https://doi.org/10.1186/s12876-021-01612-z

Saris, C. G., & Timmers, S. (2022). Ketogenic diets and Ketone suplementation: A strategy for therapeutic intervention. Frontiers in nutrition, 9, 947567.DOI: https://doi.org/10.3389/fnut.2022.947567

Shahtaghi, N. R., Soni, B., Bakrey, H., Bigdelitabar, S., & Jain, S. K. (2024). Beta-Hydroxybutyrate: A supplemental molecule for various diseases. Current Drug Targets, 25(14), 919-933 .DOI: https://doi.org/10.2174/0113894501312168240821082224

Skelly, L. E., Bailleul, C., & Gillen, J. B. (2021). Physiological Responses to Low-Volume Interval Training in Women. Sports Medicine - Open, 7(1), 99. doi: https://doi.org/10.1186/s40798-021-00390-y

Veech, R. L., Chance, B., Kashiwaya, Y., Lardy, H. A., & Cahill Jr, G. F. (2001). Ketone bodies, potential therapeutic uses. IUBMB life, 51(4), 241-247.DOI: 10.1080/152165401753311780

Wei, Z., Ahmad, M., Chen, R., Fatima, S., & Shah, S. (2025). High-intensity interval training improves mitochondrial function and attenuates cardiomyocytes damage in ischemia-reperfusion. Int J Cardiol Heart Vasc, 60, 101756. doi: https://doi.org/10.1016/j.ijcha.2025.101756

Williams, E. P., Mesidor, M., Winters, K., Dubbert, P. M., & Wyatt, S. B. (2015). Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Current obesity reports, 4(3), 363-370.DOI: https://doi.org/10.1007/s13679-015-0169-4

Xiang, Y., Wang, Q.-Q., Lan, X.-Q., Zhang, H.-J., & Wei, D.-X. (2023). Function and treatment strategies of β-hydroxybutyrate in aging. Smart Materials in

Medicine, 4, 160-172. doi: https://doi.org/10.1016/j.smaim.2022.09.003

Zhang, C.-K., Wang, Z.-Z., & Li, F.-H. (2025). Long-term aerobic exercise enhances liver health: miRNA regulation and oxidative stress alleviation. Biochemical and Biophysical Research Communications, 759, 151677. doi: https://doi.org/10.1016/j.bbrc.2025.151677

Zhang, Y., Wei, Y., Liu, H., & Guo, Y. (2025). Protective effect of exercise on metabolic dysfunction-associated fatty liver disease: Potential epigenetic mechanisms. International Journal of Molecular Medicine, 56(4), 146.DOI: https://doi.org/10.3892/ijmm.2025.5587

Zhou, H.-M., Chai, Y., Mao, X., Rao, J.-X., Zhang, S., Zhou, G.-S., . . . Duan, J.-A. (2025). Regression of oxidative stress by targeting Nrf2/HO-1 signaling: The potential therapeutic drugs for cerebral ischemia-reperfusion injury. Biomedicine & Pharmacotherapy, 193, 118809. doi: https://doi.org/10.1016/j.biopha.2025.118809

Zhou, T., Cheng, X., He, Y., Xie, Y., Xu, F., Xu, Y., & Huang, W. (2022). Function and mechanism of histone β-hydroxybutyrylation in health and disease. Front Immunol, 13, 981285. doi: https://doi.org/10.3389/fimmu.2022.981285