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Effect of Exercise Training and L-Carnitine on Mitochondrial Dynamics and Antioxidant Status in the Liver of Male Wistar Rats

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Abstract

Introduction: The liver is a very important organ, which is involved in processes such as gluconeogenesis, protein synthesis, glycogen storage, and detoxification. Mitochondrial dynamics, governed by proteins involved in fusion and fission, play a crucial role in maintaining liver functionality. Impaired mitochondrial dynamics and elevated oxidative stress contribute to hepatic pathology and metabolic imbalance.

Methods: This study investigated the independent and combined effects of intraperitoneal L-carnitine supplementation and performing high-intensity interval training (HIIT) on hepatic gene expression related to mitochondrial dynamics and antioxidant defense in male Wistar rats. A total of 32 rats were assigned to four groups: Control, L-carnitine, HIIT, and HIIT-L-carnitine combination. Following the intervention period, liver tissues were collected to assess the expression levels of key genes involved in mitochondrial dynamics and oxidative stress regulation.

Results: It was found that both HIIT and L-carnitine supplementation independently enhanced mitochondrial fusion in the liver, with significant increases in OPA1 expression ($P < 0.001$ for HIIT vs. control and $P = 0.007$ for L-carnitine vs. control). The combined intervention further amplified this effect, with OPA1 expression significantly upregulated compared with the L-carnitine group ($P = 0.044$). The combination of HIIT – L-carnitine significantly reduced both FIS1 and DRP1 compared with controls ($P < 0.001$). Additionally, antioxidant-related genes NRF2 ($P < 0.01$) and HO-1 were upregulated by each intervention, with the combined group showing additive effects ($P < 0.001$).

Conclusion: These findings highlight the potential of integrating exercise and nutritional supplementation as non-pharmacological strategies to optimize mitochondrial dynamics and enhance oxidative resilience, even in non-pathological conditions.

Keywords: Mitochondria, Carnitine, High-intensity interval training, Mitofusins, Dynamin-related protein 1

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Introduction

The liver, a critical organ in maintaining metabolic homeostasis, performs a myriad of essential functions, such as gluconeogenesis, protein synthesis, glycogen storage, cholesterol metabolism, and detoxification (1-3). At the heart of these processes lie mitochondria, dynamic organelles whose structural and functional integrity relies on two opposing yet complementary processes: Mitochondrial fusion and fission. These processes, collectively termed mitochondrial dynamics, enable mitochondria to adapt to fluctuating metabolic demands and cellular stressors. Mitofusin 1 (MFN1) and optic atrophy 1 (OPA1), key regulators of mitochondrial fusion, contribute to the preservation of mitochondrial integrity, membrane potential, energy efficiency, and network connectivity, effectively reducing metabolic and oxidative dysfunctions (4,5). Conversely, mitochondrial fission, predominantly governed by dynamin-related protein 1 (DRP1) and fission protein 1 (FIS1), ensures the segregation of damaged mitochondria for autophagic clearance. Excessive fission, however, leads to mitochondrial fragmentation and dysfunction, triggering apoptosis and metabolic disturbances (3-6). Dysregulated mitochondrial dynamics have been implicated in metabolic and hepatic pathologies (2-5).

In addition to mitochondrial dynamics, the modulation of reactive oxygen species (ROS) is pivotal for liver health. As unavoidable derivatives of oxidative phosphorylation, ROS function as signaling entities when maintained at physiological levels (2,7,8). However, excessive ROS production results in oxidative stress, impairing mitochondrial and cellular homeostasis. Cellular antioxidant defenses, coordinated by transcriptional regulators like nuclear factor erythroid 2-related factor 2

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(NRF2), combat oxidative stress by upregulating genes like heme oxygenase-1 (HO-1) (7,9). HO-1 plays a protective role against ROS-induced cellular damage by catalyzing the breakdown of pro-oxidant heme into biliverdin, carbon monoxide, and free iron. Biliverdin is then enzymatically converted into bilirubin, a potent endogenous antioxidant that contributes to redox balance (8,10).

High-intensity interval training (HIIT) has gained recognition as a non-pharmacological intervention capable of influencing mitochondrial dynamics and oxidative stress (6,11,12). Defined by alternating cycles of vigorous exertion and active recovery, HIIT enhances mitochondrial biogenesis, enzymatic activity, and oxidative capacity in various tissues, including the liver (11,13). Mechanistically, transient ROS bursts induced by HIIT activate the NRF2 pathway, promoting antioxidant defenses and adaptive mitochondrial responses (9,12). Furthermore, HIIT has been shown to counteract metabolic derangements in conditions such as obesity and diabetes by restoring mitochondrial integrity (9,10,14,15). This evidence highlights its therapeutic and preventive potential in metabolic health.

Parallel to the exercise-induced adaptations, L-carnitine supports mitochondrial function by enabling the translocation of long-chain fatty acids into the mitochondrial matrix, where they undergo β-oxidation (16,17). Its supplementation has demonstrated efficacy in improving mitochondrial efficiency, enhancing fatty acid oxidation, and reducing ROS generation, thereby reducing oxidative stress (18-20). Additionally, L-carnitine promotes autophagy, ensuring the clearance of damaged mitochondria and supporting mitochondrial turnover (18). Importantly, it complements exercise-induced adaptations by reducing the oxidative stress that may arise during physical activity (17,21).

The synergistic effects of combining exercise modalities with L-carnitine supplementation have gained increasing interest. Studies have shown that resistance training combined with L-carnitine upregulates mitochondrial fusion while downregulating fission, thereby improving mitochondrial integrity (12). Similarly, endurance training paired with L-carnitine has been reported to enhance fatty acid oxidation and mitochondrial biogenesis, further supporting metabolic resilience and oxidative balance (13). While these findings highlight the potential benefits of such combinations, the specific mechanisms by which HIIT and L-carnitine together modulate mitochondrial dynamics and oxidative defenses in the liver remain underexplored.

Given the increasing prevalence of oxidative stress and metabolic dysfunctions, investigating preventive strategies in non-diseased models is crucial. Unlike most studies focusing on pathological conditions, this study employs healthy male Wistar rats to explore baseline mitochondrial responses to HIIT and L-carnitine. By elucidating the interplay between exercise and supplementation in non-pathological conditions, this research aimed to provide foundational insights into optimizing mitochondrial function and metabolic resilience in the general population.

Thus, the present study investigates the independent and combined effects of HIIT and L-carnitine supplementation on the expression of mitochondrial fusion (OPA1, MFN1) and fission (DRP1, FIS1) genes, as well as antioxidant defense genes (NRF2, HO-1), in the livers of healthy rats. The research investigates how exercise and nutrient-based interventions converge to support mitochondrial health and redox balance, offering a foundation for integrative therapeutic strategies.

Methods

Materials

L-carnitine (L-carnitine hydrochloride, Sigma, Cat. No.: C0283), Total RNA isolation kit (Bio Basic, BS1361), cDNA synthesis kit (Parstous, A101162), and SYBR green high-ROX (Ampliqon, A325402) were utilized in this study.

Animals and Experimental Design

The animal procedure was approved by the guidelines of the Animal Care Committee of the Ethics Committee of Kerman University of Medical Sciences (Ethical code: IR.KMU.AEC.1403.115). All methods were performed in accordance with the ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) guidelines 2.0. Thirty-two healthy male Wistar rats (Aged 8 weeks and weighing 210 ± 10 g) were selected to investigate the baseline effects of intraperitoneal administration of L-carnitine along with performing HIIT on the mitochondrial dynamics and antioxidant responses. While studies often focus on diseased models to assess therapeutic efficacy, our approach seeks to elucidate the preventive and adaptive mechanisms in non-pathological conditions, thereby providing insights into the foundational benefits of these interventions.

The rats were supplied by the Physiology Research Center in Kerman. The rats were housed under standard laboratory conditions with a controlled temperature of 22 ± 2°C and a 12:12-hour light-dark cycle. Water and food were provided ad libitum. The animals were acclimatized to the laboratory environment to ensure their health and adaptation before the experiments began. The rats were randomly divided into four groups (n = 8 per group): 1. Control group: No intervention; 2. L-carnitine group: Received intraperitoneal injections of L-carnitine at a dose of 200 mg/kg body weight for 4 weeks; 3. Train group: Subjected to HIIT for 4 weeks; 4. Combined group (L-carnitine-Train): Subjected to HIIT and received L-carnitine supplementation (17,22).

Exercise training protocol

The High-Intensity Interval Training (HIIT) protocol employed in this study was meticulously structured to ensure optimal intensity and measurable adaptations in mitochondrial dynamics and antioxidant gene expression. The training protocol spanned a duration of 4 weeks, with sessions conducted 5 days per week. Each session consisted of alternating phases of intense exercise and active recovery on a motorized treadmill. The high-intensity phase involved running at 80% of the rats' maximal speed for 2 minutes, progressively increasing to 95% of maximal speed

over the course of the program. This phase was followed by 2 minutes of active recovery at 50% of maximal speed, ensuring a balanced cycle of exertion and recovery. The total duration of exercise per session began at 14 minutes in the initial week, gradually extending to 30 minutes as the rats adapted to the protocol. Blood lactate levels exceeding 6 mmol/L served as confirmation of high-intensity exercise achieved during the intervals. To minimize stress and promote adaptation, a familiarization phase lasting 2 weeks preceded the training protocol, allowing the rats to acclimatize to the treadmill environment. A graded exercise test was performed during this phase to determine the maximal running speed for each rat and assess their physiological response to incremental workload increases. For consistency and stress control, the non-training control group was subjected to stationary sessions on the treadmill, devoid of active running, to isolate the effects of HIIT intervention (17,23).

L-Carnitine Supplementation

L-carnitine was administered intraperitoneally at a dose of 200 mg/kg body weight once daily. The supplementation was initiated simultaneously with the HIIT protocol and continued throughout the 4-week study duration (24).

Tissue Collection and RNA Extraction

Forty-eight hours after the final exercise session, the animals were anesthetized using ketamine and xylazine (80/5 mg/kg), and liver tissues were dissected. The tissues were immediately frozen in liquid nitrogen and stored at -80°C for molecular analysis. Total RNA was extracted from approximately 20 mg of liver tissue using the EZ-10 Spin Column-based Total RNA extraction Kit (BioBasic), according to the kit's protocol (17, 24, 25).

cDNA Synthesis and Real-Time PCR

First-strand cDNA was synthesized from 500 ng of total RNA using the cDNA Synthesis Kit (Parstous). Gene expression fold change was measured using SYBR Green-based real-time PCR (ABI Step One Plus, Applied Biosystems). The reaction mixture (20 µL) included 10 µL of SYBR Premix 2X-high ROX (Ampliqon), 1 µL each of forward and reverse primers, 1 µL of cDNA, and 7 µL of DNase- and RNase-free water. The thermal cycling program consisted of an initial enzyme activation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 20 seconds (denaturation) and 45 seconds at the annealing temperature. Gene expression levels were normalized to the housekeeping gene 18S using the $2^{\wedge-\Delta\Delta CT}$ method (20). Primer sequences for the targeted genes are provided in Table 1 (17, 24, 25).

Statistical Analysis

Quantitative data are presented as mean ± Standard Deviation (SD). Statistical comparisons between experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple pairwise comparisons. Body weight was measured on Day 1 and Day 30, and Body Weight Gain (BWG) was calculated as the difference between these two time points. Each of these three variables, Day 1 weight, Day 30 weight, and BWG, was analyzed independently across the four groups using one-way ANOVA. No longitudinal or repeated measures analysis was applied, as no within-subject comparisons over time were performed. A P value less than 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS software (Version 21.0).

Results

Body Weight Analysis

Body weight was measured on Day 1 and Day 30, and Body Weight Gain (BWG) was calculated as the difference between these two time points (Table 2). Each of these three variables, Day 1 weight, Day 30 weight, and BWG, was analyzed independently across the four groups using one-way ANOVA. Rats showed no significant differences in initial body weight, which was used as the baseline. Finally, the L-Carnitine-Train combination led to significant weight loss versus control ($P=0.018$) and Train ($P=0.015$). BWG, calculated as Day 30 minus Day 1, was significantly lower in L-carnitine supplementation ($P=0.009$) and L-Carnitine-Train ($P<0.0001$) groups compared to control (Table 2).

The combination of L-carnitine supplementation plus HIIT significantly reduced FIS1 and DRP1 expression compared with the control group ($P<0.001$) (Figures 1 and 2). Moreover, both interventions independently reduced the expression of the DRP1 gene associated with mitochondrial fission in the liver ($P=0.005$ and $P=0.042$, respectively, for HIIT and L-carnitine vs. the control group) (Figure 2).

Our findings demonstrated that the independent

Table 1. Primer Sequences for Target Gene Expression Analysis

Gene name	Forward primer	Reverse primer
MFN1	TGGGGAGGTGCTGTCTCGGA	ACCAATCCCGCTGGGGAGGA
OPA1	CAGCTGGCAGAAGATCTCAAG	CATGAGCAGGATTTTGACACC
FIS1	CTGTTACAGACTGAGCCCCA	TGAGGCCTGTCACCTTTCTT
DRP1	GCAGCCGTAGTCCTCAAAGA	CTCCACCTTTTGAAGCCAGG
NRF2	AGAACGAGAGGACACCTGGTCA	GCTTCTGGGATGCTGGAAACGT
HO-1	CACTCTGGAGATGACACCTGAG	GTGTTCCTCTGTCAGCATCACC
18S	CTTAGAGGGACAAGTGGCG	ACGCTGAGCCAGTCAGTGTA

Table 2. Body weight measurements and weight gain after 30 days of treatment across experimental groups

Group	Baseline Day 1 (g)	Final dayDay 30 (g)	Body Weight Gain (BWG) (g)
Control	165.9 ± 9.6	194.6 ± 8.7	29.0 ± 3.8
L-carnitine	169.0 ± 16.5	181.8 ± 14.3	12.8 ± 7.9 **
Train	169.4 ± 10.1	191.0 ± 8.5	21.6 ± 4.6
L-Carnitine-Train	168.7 ± 8.6	168.9 ± 5.4 *‡	0.22 ± 7.5 ***###‡‡

Data are expressed as Mean ± SD (n = 8 per group). Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post-hoc test. Body weight was measured on Day 1 and Day 30, and Body Weight Gain (BWG) was calculated as the difference between these two time points. Group comparisons were conducted independently for Day 1, Day 30, and BWG, each analyzed separately using one-way ANOVA. $P < 0.05$ was considered significant. *Statistically significant compared to the Control group, # statistically significant compared to the L-Carnitine group, ‡ statistically significant compared to the Train group. *, #, ‡ $P < 0.05$; **, ##, ‡‡ $P < 0.01$; ***, ###, ‡‡‡ $P < 0.001$.

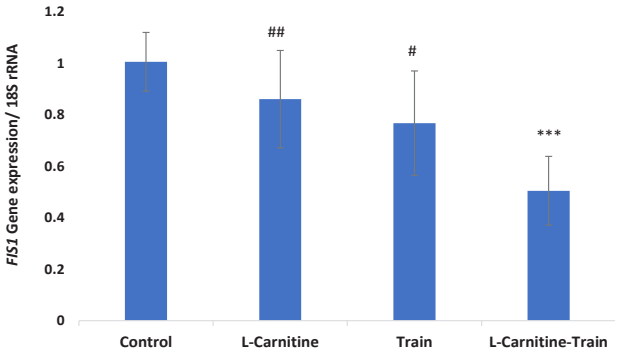


Figure 1. *FIS1* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD; $P < 0.05$ was considered significant; n = 8. * Statistically significant compared to the Control group; # statistically significant compared to the L-Carnitine-Train group. *, # $P < 0.05$; **, ## $P < 0.01$; ***, ### $P < 0.001$

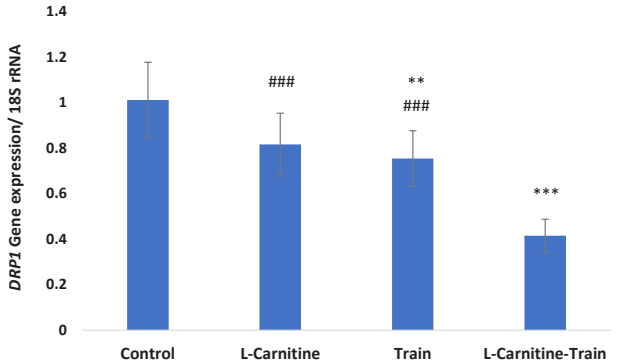
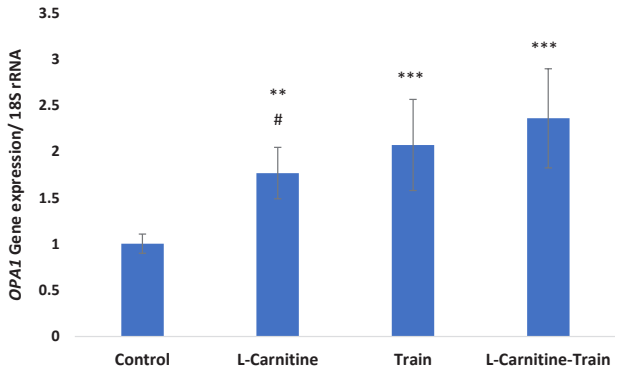


Figure 2. *DRP1* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD, $P<0.05$ was considered as significant, n=8. * Statistically significant compared to Control group, # statistically significant compared to L-Carnitine-Train group. *, # $P < 0.05$; **, ## $P < 0.01$; ***, ### $P < 0.001$



igure 3. *OPA1* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD, $P<0.05$ was considered as significant, n=8. * Statistically significant compared to Control group, # statistically significant compared to L-Carnitine-Train group. *, # $P < 0.05$; **, ## $P < 0.01$; ***, ### $P < 0.001$

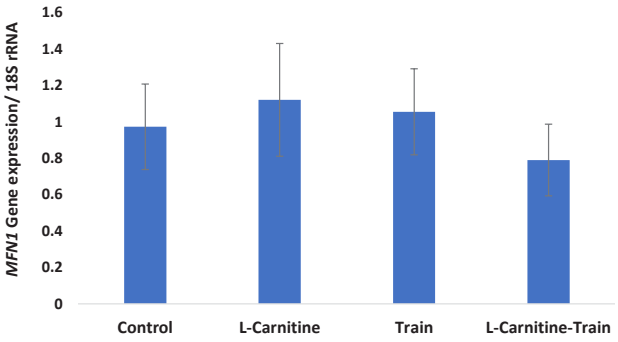


Figure 4. *MFN1* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD, $P<0.05$ was considered as significant, n=8. * Statistically significant compared to Control group, # statistically significant compared to L-Carnitine-Train group

administration of either HIIT or oral L-carnitine supplementation increased mitochondrial fusion (indicated by elevated expression of OPA1) in the liver ($P < 0.001$ and $P = 0.007$, respectively, for HIIT and L-carnitine vs. the control group). Combination of L-carnitine plus HIIT also significantly increased OPA1 gene expression compared to the L-carnitine supplementation group ($P = 0.044$). (Figure 3). MFN1 expression was not significantly changed by our interventions (Figure 4). Additionally, the expression of genes involved in regulating the antioxidant response, such as NRF2 ($P < 0.01$) and heme oxygenase-1 (HO-1), was upregulated by each intervention (Figures 5 and 6).

Discussion

The present study demonstrates the independent and synergistic effects of high-intensity interval training (HIIT) and L-carnitine supplementation on mitochondrial dynamics and antioxidant responses in the liver of healthy male Wistar rats. By targeting mitochondrial fusion and fission mechanisms, as well as oxidative resilience, this research offers foundational insights into

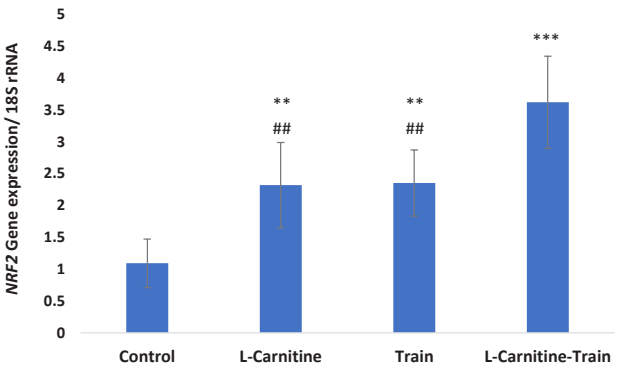


Figure 5. *NRF2* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD, $P<0.05$ was considered as significant, n=8. * Statistically significant compared to Control group, # statistically significant compared to L-Carnitine-Train group. *, # $P < 0.05$; **, ## $P < 0.01$; ***, ### $P < 0.001$

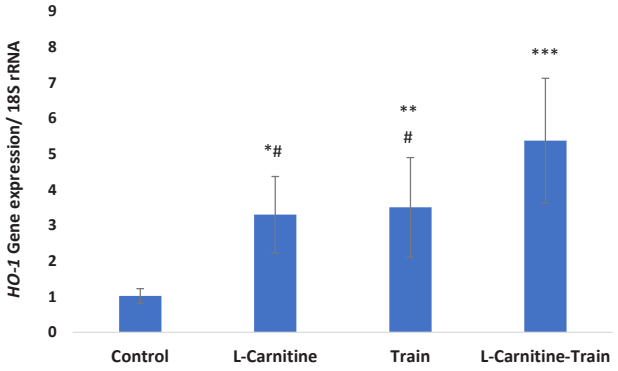


Figure 6. *HO-1* gene expression quantified by the real-time PCR method in the liver. The study groups were as follows: Control; L-Carnitine group, which received 200 mg/kg L-carnitine; Train group, which performed high-intensity interval training (HIIT); and L-Carnitine – Train group. Data are expressed as Mean ± SD, $P<0.05$ was considered as significant, n=8. * Statistically significant compared to Control group, # statistically significant compared to L-Carnitine-Train group. *, # $P < 0.05$; **, ## $P < 0.01$; ***, ### $P < 0.001$

non-pharmacological strategies for optimizing hepatic health and reducing oxidative stress in non-pathological conditions.

Mitochondrial fusion plays a critical role in maintaining mitochondrial integrity, energy efficiency, and adaptability to metabolic stress. Factors such as OPA1 and MFN1 regulate fusion processes that ensure the formation of elongated mitochondrial networks, which facilitate substrate distribution and minimize damage accumulation (4). In the present study, both HIIT and L-carnitine supplementation independently increased OPA1 expression, indicating improved mitochondrial fusion. The combined intervention further amplified OPA1 expression compared to L-carnitine alone. These findings align with prior research suggesting that exercise and dietary supplementation enhance mitochondrial dynamics by promoting mitochondrial fusion (12).

Interestingly, MFN1 expression remained unchanged across all experimental groups. While MFN1 is important in mitochondrial tethering during fusion, its regulation may be context-dependent and influenced by tissue-specific signaling pathways or basal levels of activity. This observation warrants further investigation into the differential roles of fusion-related proteins in hepatic mitochondrial adaptation.

Mitochondrial fission facilitates the segregation and removal of dysfunctional mitochondria through autophagy, supporting cellular homeostasis. However, excessive fission, mediated by fission factors such as DRP1 and FIS1, leads to mitochondrial fragmentation, impaired energy production, and activation of apoptotic pathways (2). Our findings reveal that both HIIT and L-carnitine supplementation independently reduced DRP1 expression, suggesting a shift towards decreased mitochondrial fragmentation. The combination of these interventions further reduced both DRP1 and FIS1 expression, highlighting the additive benefits of integrating exercise and supplementation in promoting mitochondrial stability. The absence of significant changes in FIS1 expression following independent interventions may suggest that fission-related adaptations primarily hinge on DRP1 modulation in the liver under normal physiological conditions. Nonetheless, the combined intervention's ability to downregulate FIS1 underscores the importance of complementary mechanisms in achieving optimal mitochondrial dynamics.

The liver is highly susceptible to oxidative damage due to its central role in metabolic processes, which inherently generate ROS. Antioxidant responses, regulated by transcription factors such as NRF2 and HO-1, are essential for maintaining redox balance and protecting hepatic tissue from ROS-induced harm (7). In the present study, NRF2 and HO-1 expression were significantly upregulated by HIIT and L-carnitine independently, with the combined intervention eliciting additive effects. These results corroborate previous findings indicating that both interventions enhance antioxidant capacity through distinct yet overlapping mechanisms (8,20).

HIIT-induced ROS production appears to serve as a signaling mechanism that activates NRF2-mediated pathways, promoting the transcription of antioxidant genes like HO-1. This adaptive response attenuates oxidative damage while enhancing hepatic resilience to metabolic disturbances (12). Concurrently, L-carnitine supplementation has been shown to reduce ROS generation and induce autophagy, mechanisms that collectively support redox homeostasis and mitochondrial turnover (18). The observed additive effects of HIIT and L-carnitine suggest complementary roles in modulating oxidative stress and antioxidant defenses.

The synergistic benefits observed in the combined intervention group are likely attributable to distinct yet interconnected pathways activated by HIIT and L-carnitine. HIIT enhances mitochondrial biogenesis and antioxidant responses through exercise-specific mechanisms involving ROS signaling and NRF2 activation (10). Meanwhile, L-carnitine facilitates fatty acid oxidation, improves mitochondrial quality control, and activates autophagy pathways mediated by AMPK (16,20). The integration

of these pathways likely amplifies mitochondrial fusion, reduces fission, and enhances antioxidant defenses, resulting in improved hepatic mitochondrial function and resilience.

The findings of this study have important implications for developing preventive strategies aimed at optimizing liver health and reducing oxidative stress. By targeting mitochondrial dynamics and antioxidant defenses, interventions such as HIIT and L-carnitine supplementation may offer non-pharmacological approaches to reducing the risk of metabolic and hepatic disorders. Importantly, the study underscores the potential benefits of these interventions in non-pathological conditions, paving the way for broader applications in health promotion and disease prevention.

Several limitations must be acknowledged when interpreting these findings. First, the use of male Wistar rats in a controlled laboratory setting may limit the generalizability of the results to other species, genders, or age groups. Future studies should expand the scope to include diverse populations, including females and older animals, to better understand the broader impacts of HIIT and L-carnitine supplementation. Second, while gene expression analyses provide valuable insights into molecular adaptations, direct assessment of protein levels and enzymatic activity is needed to confirm the functional outcomes of these interventions. Incorporating proteomic and metabolic analyses will offer a more comprehensive understanding of mitochondrial and antioxidant responses.

Although this study was conducted in healthy animal models, future investigations into the effects of HIIT and L-carnitine supplementation under pathological conditions, such as obesity, diabetes, or non-alcoholic fatty liver disease (NAFLD), could offer deeper insight into their therapeutic relevance. Moreover, clarifying the dose-dependent responses and evaluating the long-term consequences of these interventions remain essential directions for subsequent research.

Conclusion

In summary, the study demonstrates the independent and synergistic effects of HIIT and L-carnitine supplementation on mitochondrial dynamics and antioxidant responses in the liver of healthy male Wistar rats. By enhancing mitochondrial fusion, reducing fission, and upregulating key antioxidant genes, these interventions hold promise as non-pharmacological strategies for optimizing hepatic health and reducing oxidative stress. The observed additive effects highlight the potential of integrative approaches in promoting mitochondrial functionality and metabolic resilience. Future studies should address the limitations outlined and expand the scope to pathological models to further elucidate the therapeutic potential of HIIT and L-carnitine.

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Authors' Contribution

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Formal Analysis: Soheil Aminizadeh
Funding Acquisition: Beydolah Shahouzehi
Investigation: Beydolah Shahouzehi, Soheil Aminizadeh
Methodology: Beydolah Shahouzehi, Soheil Aminizadeh
Project Administration: Beydolah Shahouzehi, Soheil Aminizadeh
Supervision: Beydolah Shahouzehi, Soheil Aminizadeh
Writing–Original Draft: Beydolah Shahouzehi

Competing Interests

All the authors declare that they have no conflict of interest.

Data Availability Statement

All necessary data will be available from the corresponding author upon reasonable request.

Ethical Approval

The animal procedure was approved in accordance with the guidelines of the Animal Care Committee of the Ethics Committee of Kerman University of Medical Sciences (Ethical code: IR.KMU.AEC.1403.115). All methods were performed in accordance with the ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) guidelines 2.0.

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