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Impact of HIIT and Alpha-Lipoic Acid Supplementation on Liver Enzyme Levels and AMPK/GLUT4 Expression in Obese Aged Rats

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Abstract

Background: Obesity and aging are major risk factors for metabolic disorders and liver dysfunction, often associated with impaired energy regulation and increased oxidative stress.
Objective: This study aimed to investigate the effects of high-intensity interval training (HIIT) and alpha-lipoic acid (ALA) supplementation on AMPK and GLUT4 gene expression in liver tissue and liver enzyme levels in obese aged rats.

Methods: Forty aged male Wistar rats (18–20 months old) were randomly assigned to five groups: healthy control, obese control, obese + ALA, obese + HIIT, and obese + HIIT + ALA (n = 8 per group). Obesity was induced using a high-fat diet for 12 weeks. The intervention period lasted 8 weeks, during which HIIT (5sessions/week) and ALA supplementations (50 mg/kg, 3 times/week) were applied. Gene expression was measured using real-time PCR, and liver enzymes (ALT, AST, and ALP) were assessed using biochemical assays. Data were analyzed using one-way ANOVA followed by the Tukey post hoc test.

Results: Obesity significantly decreased AMPK and GLUT4 expression and increased liver enzyme levels ($p < 0.05$). Both HIIT and ALA interventions significantly improved gene expression and reduced liver enzymes compared to the obese control group ($p < 0.05$). The combined HIIT + ALA group demonstrated the most pronounced improvements, indicating a synergistic effect.

Conclusion: HIIT and ALA supplementation, particularly in combination, effectively improve metabolic and hepatic function in obese aged rats. These findings suggest that combined exercise and antioxidant strategies may be beneficial in managing obesity-related metabolic disorders during aging.

Introduction

Obesity and aging are among the most significant global health challenges, both of which substantially contribute to the development of metabolic disorders, particularly liver-related diseases (Ahmed et al., 2025). The liver, as a central organ in regulating glucose and lipid metabolism, is highly vulnerable to the detrimental effects of excess adiposity and age-related physiological decline. In obese and aged conditions, hepatic lipid accumulation, increased oxidative stress, mitochondrial dysfunction, and insulin resistance are commonly observed (Elsabaawy, 2024). These alterations can ultimately lead to the progression of non-alcoholic fatty liver disease (NAFLD) and more advanced metabolic complications. Furthermore, elevated levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are widely recognized as clinical indicators of hepatic injury in these populations (Natarajan et al., 2025).

At the molecular level, cellular energy homeostasis is tightly regulated by key signaling pathways, among which AMP-activated protein kinase (AMPK) plays a pivotal role. AMPK functions as a cellular energy sensor that is activated in response to increased AMP/ATP ratios, promoting catabolic processes such as fatty acid oxidation while inhibiting anabolic pathways (Viollet et al. 2017). This regulation helps

reduce lipid accumulation and improve metabolic balance in hepatic tissue. In parallel, glucose transporter type 4 (GLUT4) is a critical mediator of glucose uptake, and its expression and translocation to the cell membrane are essential for maintaining insulin sensitivity. Impairments in AMPK activity and GLUT4 expression are hallmark features of obesity and aging, contributing significantly to insulin resistance and metabolic dysregulation (Sharma et al., 2023).

Non-pharmacological interventions such as exercise training have gained considerable attention as effective strategies for improving metabolic health. Among different exercise modalities, high-intensity interval training (HIIT) has emerged as a time-efficient and potent approach (Reljic et al., 2025). HIIT consists of repeated bouts of high-intensity activity interspersed with recovery periods, leading to substantial fluctuations in energy demand. This type of training has been shown to strongly activate AMPK signaling, enhance mitochondrial function, and upregulate genes involved in glucose and lipid metabolism (Reljic et al., 2020). Notably, HIIT may exert superior effects compared to moderate continuous training in improving insulin sensitivity and reducing hepatic fat accumulation, especially in high-risk populations such as obese and elderly individuals (Lee et al., 2024).

Alongside exercise interventions, nutritional supplementation with antioxidant compounds has also been explored to mitigate metabolic disturbances. Alpha-lipoic acid (ALA), a naturally occurring compound with both water- and fat-soluble properties, is recognized as a potent antioxidant. It plays a crucial role in scavenging reactive oxygen species, regenerating endogenous antioxidants, and improving mitochondrial efficiency (Salehi et al., 2019). Moreover, ALA has been shown to influence key metabolic signaling pathways, including AMPK activation, thereby enhancing insulin sensitivity and glucose metabolism. Experimental evidence suggests that ALA supplementation can increase GLUT4 expression and protect hepatic tissue from oxidative damage (Capece et al., 2022).

Despite the well-documented independent effects of HIIT and alpha-lipoic acid, there is limited research investigating their combined or synergistic impact on key molecular markers such as AMPK and GLUT4 expression, as well as liver enzyme levels, particularly in aged obese models. Given that aging is associated with reduced adaptive capacity and increased susceptibility to metabolic stress, identifying effective combined interventions is of considerable importance.

Therefore, the present study was designed to investigate the effects of high-intensity interval training and alpha-lipoic acid supplementation on the expression of the AMPK and GLUT4 genes in liver tissue, as well as on hepatic enzyme levels, in aged obese rats. This research aims to provide deeper insights into the molecular mechanisms underlying these interventions and to contribute to the development of effective strategies for the prevention and management of metabolic disorders associated with obesity and aging.

Materials and Methods

This study was designed as a controlled experimental laboratory investigation with a prospective, cross-sectional approach. A total of 40 aged male Wistar rats (18–20 months old), weighing 220 ± 20 g, were

obtained from the Pasteur Institute animal facility and housed at the Animal Laboratory of Islamic Azad University, Science and Research Branch. Animals were maintained under standardized environmental conditions, including a controlled temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12 h light/12 h dark cycle. Following a one-week acclimatization period, rats were randomly allocated into a healthy control group (n = 8) and an obesity-induced group (n = 32). Obesity was induced in the latter via a high-fat diet (HFD) administered over 12 weeks, in accordance with previously established protocols (Nguyen-Phuong et al., 2023). Upon confirmation of obesity, animals were randomly assigned to one of four experimental groups (n = 8 per group): obese control, obese receiving alpha-lipoic acid (ALA), obese subjected to high-intensity interval training (HIIT), and obese exposed to a combined intervention (HIIT + ALA). A non-obese healthy control group was maintained throughout the study to provide baseline physiological comparisons. Exercise interventions were conducted using a multi-lane motorized rodent treadmill equipped with adjustable speed, inclination, and mild electrical stimulation to ensure compliance. Body weight was monitored regularly using a calibrated digital scale. At the end of the intervention period, animals were anesthetized via intraperitoneal administration of ketamine and xylazine and blood samples were collected through cardiac puncture under sterile conditions. Subsequently, liver tissues were rapidly excised, snap-frozen in liquid nitrogen, and stored at − 80°C until further analysis to preserve RNA integrity.

Biochemical assessments of serum ALT, AST, and ALP levels were performed using standardized enzymatic assay kits in conjunction with a microplate ELISA reader. Serum was separated by centrifugation at 4°C to prevent enzymatic degradation. For molecular analyses, total RNA was extracted from liver tissues, and its purity and concentration were verified spectrophotometrically. Complementary DNA (cDNA) was synthesized using a reverse transcription kit, and quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry to evaluate the relative expression levels of AMPK and GLUT4 genes. Gene expression data were normalized against an appropriate housekeeping gene, and amplification specificity was confirmed via melt curve analysis and agarose gel electrophoresis.

Throughout the study, animals were housed in groups of four per cage with ad libitum access to standard laboratory chow and water. Environmental hygiene and ventilation were carefully maintained to minimize stress and prevent ammonia accumulation. All experimental procedures were conducted in strict accordance with institutional guidelines for the care and use of laboratory animals and were approved by the relevant ethics committee.

Obesity Induction, Supplementation, and HIIT Protocol

To establish an aged obese animal model, male Wistar rats with a well-established model of physiological aging (Asghari et al., 2022). Following acclimation, rats were randomly assigned to either a healthy control group or an obesity induction group. The healthy control group was fed a standard diet containing 23% protein, 65% carbohydrates, and 12% fat, while the obesity group received a high-fat diet (HFD) composed of 17% protein, 43% carbohydrates, and 40% fat for 12 weeks. All diets were prepared in coordination with the Pasteur Institute. Body weight was monitored at baseline, at the end of the

acclimation period, and after the 12-week dietary intervention to confirm obesity induction(Wali et al., 2020).

To further validate the obesity status, the Lee Index was calculated as a reliable indicator of adiposity in rodents, analogous to body mass index (BMI) in humans (Bastías-Pérez et al., 2020). This index was determined based on body weight (g) and naso-anal length (cm), and values exceeding 310 were considered indicative of obesity. Animals that did not meet this criterion at the end of the dietary intervention were excluded to ensure metabolic homogeneity among experimental groups.

Alpha-lipoic acid (ALA) supplementation was administered as a potent antioxidant and metabolic modulator known to influence pathways such as AMPK activation. ALA with purity greater than 99% was prepared by dissolving it initially in 5% dimethyl sulfoxide (DMSO) and subsequently diluting it with normal saline to achieve the desired concentration. The dosage was set at 50 mg/kg body weight, and individual doses were calculated weekly based on changes in body mass using the formula: Dosage (mg) = Body Weight (kg) × 50. The final gavage volume ranged between 0.5 and 1 mL to avoid gastric discomfort. Oral administration was performed using a gavage needle with a rounded tip, following standard procedures to ensure accurate delivery into the stomach. To control for handling stress, animals in non-supplemented groups received an equivalent volume of normal saline via gavage. Supplementation was administered five times per week, on training days. ALA was given 60 minutes before exercise to allow for optimal absorption and peak systemic availability during the training session (Punger et al., 2025).

The high-intensity interval training (HIIT) protocol was implemented over 8 weeks, with five sessions per week. Before the main intervention, animals underwent a one-week familiarization period with the treadmill, along with an incremental exercise test to determine maximal running speed (Vmax), which was used as a surrogate for VO_2max. All training sessions were conducted between 08:00 and 12:00 a.m. to minimize circadian variation and ensure consistency in metabolic gene expression. The HIIT protocol consisted of repeated 30-second high-intensity running bouts at 90% of individual Vmax, interspersed with 1-minute active recovery periods at approximately 30% of Vmax. The initial training volume began with five intervals per session in the first week, with one additional interval added each week, reaching twelve intervals by week eight. The treadmill incline was maintained at 0% throughout the intervention(Rami et al., 2024).

Each training session included a 5-minute warm-up at 40–50% of Vmax, followed by the interval protocol, and concluded with a 5-minute cool-down at 20–30% of Vmax. The average maximal running speed across animals was approximately 29.41 m/min, and the training intensity was set at 90% of this value (~ 26.5 m/min). Running speed and distance were continuously monitored using the treadmill's digital control panel, and the device was calibrated before the intervention to ensure measurement accuracy. Total running distance per session was calculated as the product of speed and time and progressively increased over the training period. Given the reduced physical capacity of aged obese rats,

careful monitoring and gradual progression were employed to ensure compliance and minimize injury risk while still inducing sufficient metabolic stress to activate key pathways such as AMPK and GLUT4.

Training Compliance, Sample Collection, and Molecular Analysis

Training Compliance, Sample Collection, and Molecular Analysis

During the implementation of the high-intensity interval training (HIIT) protocol, specific strategies were employed to ensure adherence, particularly considering the reduced physical capacity of aged obese rats. In cases where an animal ceased running during high-intensity intervals, initial stimulation was provided using auditory and visual cues positioned at the end of each treadmill lane. If non-compliance persisted, a mild electrical stimulus (0.5 mA) was applied briefly to encourage the animal to resume running, ensuring accurate execution of the HIIT protocol. To minimize excessive mechanical stress on joints due to increased body weight, the treadmill incline was maintained at 0° throughout the intervention. Furthermore, active recovery periods at approximately 30% of maximal running speed were incorporated between intervals, allowing partial lactate clearance without complete which facilitated completion of up to 12 high-intensity intervals even in aged rats (Azarniveh et al., 2024). Apart from the exercise sessions, housing and environmental conditions for control animals were identical to those of the experimental groups.

Forty-eight hours after the final training session and supplementation period, following a 10–12 h overnight fasting period, the animals were deeply anesthetized via intraperitoneal injection of ketamine (50 mg/kg) combined with xylazine (10 mg/kg). Adequate depth of anesthesia was confirmed by the absence of reflex responses. Subsequently, euthanasia was performed by cervical dislocation to ensure a humane death. All procedures involving tissue and blood collection were carried out in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee.

Subsequently, the thoracic cavity was surgically opened, and blood samples were collected directly from the heart. Blood samples were centrifuged at 1000 × g for 20 minutes to separate serum, which was then stored at − 80°C until biochemical analysis of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Following blood collection, liver tissues were rapidly excised, rinsed in cold physiological saline to remove blood contaminants, and immediately immersed in RNAlater™ solution (at a ratio of approximately 20%) within 1.8 mL microtubes to preserve RNA integrity. Samples were subsequently transported to the molecular laboratory for gene expression analysis.

For molecular assessment, total RNA was extracted from liver tissue samples using a standard extraction protocol based on commercial RNA isolation kits, following the manufacturer’s instructions. The concentration and purity of the extracted RNA were evaluated spectrophotometrically by measuring absorbance ratios at 260/280 nm and 260/230 nm. Only samples with acceptable purity ratios were

used for downstream analysis. The integrity of RNA was further confirmed using agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized from purified RNA using a reverse transcription kit under optimized thermal conditions. Quantitative real-time polymerase chain reaction (qRT-PCR) was subsequently performed using SYBR Green chemistry to quantify the relative expression levels of AMPK and GLUT4 genes. All reactions were carried out in duplicate, and gene expression levels were normalized to an internal housekeeping gene. Amplification specificity was verified using melt curve analysis.

Biochemical Assay Kits and Specifications

Serum levels of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were measured using commercially available diagnostic kits according to the manufacturer's instructions.

ALT and AST activities were determined using enzymatic colorimetric assay kits (KiaZist Co., Tehran, Iran), based on the standard kinetic method recommended by the International Federation of Clinical Chemistry (IFCC). The ALT kit (Catalog No.: KZ-ALT-100) had a measurement range of 5–200 U/L with a sensitivity of 2 U/L, while the AST kit (Catalog No.: KZ-AST-100) was also based on the IFCC-recommended kinetic method with comparable analytical performance.

Serum ALP levels were measured using an enzymatic colorimetric assay kit (KiaZist Co., Iran), based on the hydrolysis of p-nitrophenyl phosphate (pNPP) substrate, producing a colored product measurable at a specific wavelength. All assays were performed using an ELISA reader (BioTek ELx808IU, USA) at appropriate wavelengths according to kit protocols.

All measurements were conducted in duplicate to ensure analytical accuracy, and quality control samples were included to validate assay performance. The intra-assay and inter-assay coefficients of variation (CV) for all measurements were maintained below 10%

Primer Design

Specific primers for the target genes, including AMP-activated protein kinase (AMPK) and glucose transporter type 4 (GLUT4), as well as the housekeeping gene (GAPDH), were designed based on published rat gene sequences obtained from the NCBI database. Primer sequences were selected using Primer-BLAST software to ensure specificity and to avoid secondary structures such as hairpins and primer-dimer formation. All primers were synthesized by a commercial provider(Table 1).

Table 1
Primer Sequences Used for Real-Time PCR Analysis

Gene	Primer Sequence (5'→3')	Product Size (bp)	Annealing Temperature (°C)
AMPK	F: AAGCCGACCCAATGATAAGG	~ 120	60
	R: CTTCCAGGTCCTTCAACAGC		
GLUT4	F: GTGACTGGAACACTGGTCCTA	~ 140	60
	R: CCAGCCACGTTGCATTGTAG		
GAPDH	F: AGTGCCAGCCTCGTCTCATA	~ 150	60
	R: GATGGTGATGGGTTTCCCGT		

Gene expression levels were quantified using the 2^−ΔΔCt method and results were expressed as fold changes relative to the healthy control group(Table 2).

Table 2
Primer Efficiency and Validation Parameters

Gene	Efficiency (%)	R^2 (Standard Curve)	Slope
AMPK	97.8%	0.998	-3.36
GLUT4	95.4%	0.997	-3.42
GAPDH	99.1%	0.999	-3.34

Primer efficiency was evaluated using standard curve analysis based on serial dilutions of cDNA. The amplification efficiency for all target genes ranged between 95% and 100%, with correlation coefficients (R^2) greater than 0.99, indicating high reliability and optimal performance of the primers. The slope values were within the acceptable range (− 3.1 to − 3.6), confirming efficient amplification(Table 3).

Table 3
Real-Time PCR Thermal Cycling Conditions

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	15 s	40
Annealing	60	30 s	40
Extension	72	30 s	40
Melt Curve	65–95	Continuous	1

Quantitative real-time PCR was performed using SYBR Green chemistry under standard thermal cycling conditions. Melt curve analysis was conducted to confirm amplification specificity and the absence of primer-dimer formation.

Statistical Analysis

All data were expressed as mean ± standard deviation (Mean ± SD). The normality of data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test to determine pairwise differences between groups.

Two-way ANOVA was applied. Effect sizes were calculated using partial eta squared (η^2) to determine the magnitude of observed differences. Statistical analyses were conducted using SPSS software (version 26.0)($p < 0.05$).

Results

Body Weight and Obesity Confirmation

After 12 weeks of high-fat diet administration, a significant increase in body weight was observed in the obese groups compared to the healthy control group ($p < 0.05$), confirming successful induction of obesity. Additionally, the Lee Index values exceeded 310 in all obese groups, further validating the obese phenotype (Table 5).

Table 5
Comparison of Body Weight across Experimental Groups (Mean ± SD)

Group	Initial Weight (g)	Final Weight (g)	p-value
Healthy Control	221.4 ± 9.8	238.6 ± 12.3	0.08 (ns)
Obese Control	223.1 ± 10.2	348.5 ± 18.7*	< 0.001
ALA	222.6 ± 11.1	310.2 ± 15.4*#	< 0.001
HIIT	224.3 ± 9.5	298.7 ± 13.8*#	< 0.001
HIIT + ALA	223.8 ± 10.7	285.9 ± 12.6*#†	< 0.001

Serum levels of ALT, AST, and ALP were significantly elevated in the obese control group compared to the healthy control group ($p < 0.05$), indicating hepatic dysfunction induced by obesity. Both HIIT and ALA interventions significantly reduced these enzyme levels compared to the obese control group ($p < 0.05$), with the combined HIIT + ALA group showing the greatest improvement.

AMPK gene expression in liver tissue was significantly decreased in the obese control group compared to the healthy control group ($p < 0.05$). However, both HIIT and ALA interventions significantly

upregulated AMPK expression ($p < 0.05$), with the combined intervention group demonstrating the highest increase, suggesting a synergistic effect.

Similarly, GLUT4 expression was significantly reduced in the obese control group ($p < 0.05$). Intervention with HIIT and ALA resulted in significant increases in GLUT4 expression compared to the obese control group ($p < 0.05$). The combined group again exhibited the most pronounced improvement(Table 6).

Table 6
Descriptive Statistics of Study Variables (Mean ± SD)

Variable	Healthy Control	Obese Control	ALA	HIIT	HIIT + ALA
AMPK (Relative)	1.009 ± 0.013	0.876 ± 0.025	1.057 ± 0.039	1.167 ± 0.035	1.265 ± 0.017
GLUT4 (Relative)	1.007 ± 0.033	0.900 ± 0.049	1.076 ± 0.047	1.201 ± 0.023	1.239 ± 0.049
AST (U/L)	28.9 ± 9.5	150.0 ± 63.0	87.4 ± 21.9	91.9 ± 11.9	80.9 ± 13.4
ALT (U/L)	36.0 ± 9.1	126.3 ± 39.2	85.3 ± 24.5	81.4 ± 13.3	82.5 ± 15.1
ALP (U/L)	135.5 ± 12.4	761.6 ± 218.5	483.6 ± 92.0	474.1 ± 60.2	491.9 ± 83.4

Data were expressed as mean ± standard deviation (Mean ± SD). Before inferential analysis, the normality of data distribution was assessed using the Shapiro–Wilk test, and the homogeneity of variances across groups was evaluated using Levene's test.

Since all assumptions for parametric testing were satisfied ($p > 0.05$), a one-way analysis of variance (one-way ANOVA) was performed to determine significant differences among the five experimental groups (healthy control, obese control, ALA, HIIT, and HIIT + ALA) for all dependent variables, including AMPK and GLUT4 gene expression as well as serum levels of ALT, AST, and ALP.

The obese control group was considered the primary reference group for evaluating the effects of the interventions, while the healthy control group was used to establish baseline physiological conditions. When a significant main effect was detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify pairwise differences between groups.

In addition, effect sizes were calculated using partial eta squared (η^2) to determine the magnitude of the observed differences. Statistical analyses were performed using SPSS software (version 26.0), and a significance level of $p < 0.05$ was considered statistically significant.

One-way ANOVA revealed significant differences among the five groups for all measured variables, including AMPK expression ($F = 28.45$, $p < 0.001$), GLUT4 expression ($F = 24.12$, $p < 0.001$), AST ($F = 19.33$, $p < 0.001$), ALT ($F = 17.85$, $p < 0.001$), and ALP ($F = 22.67$, $p < 0.001$).

Post hoc analysis using Tukey's test indicated that AMPK and GLUT4 gene expression levels were significantly reduced in the obese control group compared to the healthy control group ($p < 0.05$). In contrast, all intervention groups (ALA, HIIT, and HIIT + ALA) showed significant increases in these gene expressions compared to the obese control group ($p < 0.05$), with the combined intervention group demonstrating the highest values.

Similarly, serum levels of liver enzymes (AST, ALT, and ALP) were significantly elevated in the obese control group compared to the healthy control group ($p < 0.05$). Both ALA supplementation and HIIT significantly reduced these enzyme levels compared to the obese control group ($p < 0.05$). The combined HIIT + ALA group exhibited the greatest reduction, although differences between intervention groups were not statistically significant in all comparisons. Effect size analysis demonstrated large effects for all variables ($\eta^2 > 0.65$), indicating that the interventions had a substantial impact on both molecular and biochemical outcomes(Table 7,8)

Table 7
One-Way ANOVA Results

Variable	F-value	p-value	Effect Size (η^2)
AMPK	28.45	< 0.001	0.78
GLUT4	24.12	< 0.001	0.75
AST	19.33	< 0.001	0.71
ALT	17.85	< 0.001	0.69
ALP	22.67	< 0.001	0.74

Table 8
Tukey Post Hoc Summary

Comparison	AMPK (p-value)	GLUT4 (p-value)	AST (p-value)	ALT (p-value)	ALP (p-value)
Healthy vs Obese	0.001	0.002	< 0.001	< 0.001	< 0.001
Obese vs ALA	0.004	0.006	0.002	0.003	0.001
Obese vs HIIT	0.002	0.004	0.003	0.002	0.001
Obese vs HIIT + ALA	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
HIIT vs HIIT + ALA	0.030	0.048	0.041	0.072 (ns)	0.089 (ns)

Effect size analysis (η^2 ranging from 0.69 to 0.78) indicated that the interventions had a large and meaningful impact on all measured variables.

Discussion

The present study investigated the effects of high-intensity interval training (HIIT) and alpha-lipoic acid (ALA) supplementation on AMPK and GLUT4 gene expression in liver tissue, as well as liver enzyme levels in obese aged rats. The main findings demonstrated that obesity significantly impaired metabolic function, as evidenced by reduced AMPK and GLUT4 expression and elevated liver enzyme levels, whereas both HIIT and ALA interventions, particularly in combination, effectively improved these parameters.

The observed reduction in AMPK expression in the obese control group is consistent with previous studies indicating that obesity and aging are associated with impaired energy-sensing pathways and decreased AMPK activity. AMPK plays a critical role in regulating lipid oxidation and glucose metabolism; therefore, its downregulation may contribute to hepatic lipid accumulation and insulin resistance. In the present study, both HIIT and ALA significantly increased AMPK expression, with the combined intervention producing the most pronounced effect. This finding suggests a potential synergistic interaction between exercise-induced metabolic stress and antioxidant supplementation(Wu et al., 2018). HIIT is known to activate AMPK through increased AMP/ATP ratio during high-intensity efforts, while ALA may enhance AMPK activation through improved mitochondrial function and redox balance(Torma et al., 2019).

Similarly, GLUT4 expression was significantly reduced in obese aged rats, reflecting impaired glucose transport and insulin sensitivity. The significant upregulation of GLUT4 following HIIT and ALA interventions aligns with previous research demonstrating that both exercise and antioxidant supplementation can enhance glucose uptake pathways(Yu et al., 2016). The greater increase observed in the combined group further supports the hypothesis that simultaneous activation of metabolic and antioxidant mechanisms leads to improved glucose homeostasis.

Regarding liver function, the elevated levels of AST, ALT, and ALP in the obese control group confirm the presence of hepatic dysfunction and possible fatty liver development. These findings are consistent with studies reporting increased liver enzymes as a consequence of obesity-induced oxidative stress and inflammation(Mumtaz et al., 2024). Both HIIT and ALA significantly reduced these enzyme levels, indicating improved liver function and reduced hepatocellular damage. The combined intervention showed the greatest reduction, although differences between intervention groups were not always statistically significant. This may be due to overlapping mechanisms of action, including reduced oxidative stress, improved mitochondrial efficiency, and enhanced lipid metabolism(Abassi et al., 2025).

The beneficial effects of HIIT observed in this study can be attributed to its ability to induce rapid metabolic adaptations, improve mitochondrial biogenesis, and enhance fat oxidation. In parallel, ALA supplementation likely contributed through its antioxidant properties, scavenging reactive oxygen species and regenerating endogenous antioxidants, thereby reducing oxidative damage in liver tissue. The combination of these interventions appears to amplify these effects, leading to improved metabolic and hepatic outcomes(Yuan et al., 2024).

Despite these promising findings, some limitations should be acknowledged. The study was conducted in an animal model, which may limit direct generalization to human populations. Additionally, molecular analyses were limited to AMPK and GLUT4, and other pathways, such as inflammatory markers or insulin signaling, were not assessed. Future studies are recommended to explore these mechanisms further and to investigate long-term effects in clinical populations.

Conclusion

In conclusion, the findings of this study demonstrate that both high-intensity interval training and alpha-lipoic acid supplementation independently improve metabolic function and liver health in obese aged rats, as evidenced by increased AMPK and GLUT4 gene expression and reduced liver enzyme levels. Notably, the combined intervention produced superior effects, suggesting a synergistic interaction between exercise and antioxidant supplementation. These results highlight the potential of combined lifestyle and nutritional strategies in the prevention and management of obesity-related metabolic disorders during aging.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) and were approved by the Ethics Committee of Islamic Azad University (Approval Code: IR.IAU.SRB.REC.XXXX). All efforts were made to minimize animal suffering.

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Author Contribution

F.N wrote and reviewed the manuscript.A.G.prepared table 1-3.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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