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Short communication

Investigation of the genes expression involved in fatty acid (*Cpt1β* and *Mcd*) and glucose metabolism (*Hk2*, *Pdk4*, and *Glut4*) in cardiac tissue following Maca supplementation and aerobic training

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Abstract

Objective: Cardiac metabolic flexibility is critical for maintaining cardiac function. Aerobic training converts this flexibility into adaptations over time. Maca (*Lepidium meyenii walp*.) as plant with phytoestrogens could be cardioprotective and improve metabolic health. This study aimed to investigate the effects of aerobic training and Maca root extract and their combination on the expression of key cardiac metabolic genes in Wistar rats.

Materials and methods: Twenty-four rats were randomly assigned to four groups (n=6): control (CON), maca (MAC), aerobic training (AT), and maca+ aerobic training (MAC+AT). The AT and MAC+AT groups performed an aerobic training on a treadmill for 8 weeks at a speed of 28 m/min. The MAC and MAC+AT groups received maca root powder orally (100 mg/kg/day). Gene expression of *Cpt1β*, *Mcd*, *Hk2*, *Glut4*, and *Pdk4* was assessed in cardiac tissue using qPCR.

Results: The expression of *Cpt1β* was upregulated in the MAC, AT, and MAC+AT groups compared to the CON. *Glut4* expression was increased in the MAC+AT compared to CON group. *Hk2* gene expression was upregulated in the MAC group compared to the AT group. No significant differences were observed between groups in the expression of *Mcd* and *Pdk4* gene.

Conclusion: The finding suggests that aerobic training and maca may independently improve the gene expression involved in cardiac fatty acid and glucose metabolism. These findings highlight the potential of non-pharmacological interventions to support cardiac metabolic health. Further studies are needed to determine optimal dosages, duration, and potential long-term or interactive effects.

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Introduction

As a metabolically active tissue, the heart requires a continuous supply of energy for its proper function. The primary energy sources in cardiac cells are the oxidation of fatty acids and glucose. Under normal conditions, about 60-70% of the energy required by the heart is derived from the oxidation of fatty acids, with the remainder coming from the oxidation of

carbohydrates(Stanley et al. 2005). There is growing evidence that changes in energy metabolism in the heart may be one of the mechanisms involved in the development of cardiovascular adaptations under various conditions such as exercise, starvation, and heart failure(Lopaschuk et al. 2021). Proper regulation of fatty acid and glucose metabolism in the heart plays a critical role in maintaining cardiac function and preventing heart failure, ischemia, and other cardiovascular diseases.

Beta-oxidation and glycolysis are two fundamental metabolic pathways that supply energy to cardiac cells. Beta-oxidation is the primary catabolic process for fatty acid metabolism, occurring within mitochondria where long-chain fatty acids are broken down into acetyl-CoA units to fuel the tricarboxylic acid (TCA) cycle and oxidative phosphorylation(Schooneman et al. 2013). The heart's performance is directly dependent on the activity of specific enzymes and transporters that control metabolic pathways. Key proteins involved in this process include carnitine palmitoyl transferase 1 (*Cpt1β*) which facilitates the entry of fatty acids into mitochondria and malonyl-CoA decarboxylase (*Mcd*) which breaks down malonyl-CoA, an inhibitor of *Cpt1β*.

In contrast, glycolysis is the cytosolic process that breaks down glucose into pyruvate, generating ATP and NADH. It serves as a rapid but less efficient energy source compared to beta-oxidation. The rate of glycolysis in cardiac cells is regulated by glucose transporter 4 (*Glut4*) which mediates glucose uptake, and hexokinase (*HK*), which phosphorylates glucose to glucose-6-phosphate, committing it to glycolysis (Abel 2004) and pyruvate dehydrogenase kinase *(Pdk4*), all of which play important roles in glucose transport into the cell and regulation of the citric acid cycle(Lopaschuk et al. 2010). Pyruvate, the end product of glycolysis, can either be converted to lactate under anaerobic conditions or enter mitochondria for further oxidation via the pyruvate dehydrogenase (*PDH*) complex, whose activity is modulated by pyruvate dehydrogenase kinase (*Pdk4*)(Stanley et al. 2005).

In recent years, the role of exercise in improving cardiac metabolism has garnered widespread attention. Aerobic exercise increases the efficiency of substrate utilization, enhances mitochondrial oxidative capacity, and regulates the expression of key metabolic genes in cardiac muscle(Holloszy and Coyle 1984). Long-term endurance training can increase the expression of genes related to fatty acid metabolism, such as *Cpt1β,* in the heart tissue, which in turn improves oxidative capacity and cardiac performance(Greene et al. 2012).

On the other hand, there is a growing interest in natural supplements as adjuncts to improve cardiovascular health. Among these, the maca (*Lepidium meyenii walp*.), commonly known as maca, has drawn attention due to its antioxidant, metabolic-modulating, and anti-inflammatory properties(Melnikovova et al. 2015). Reduced oxidative stress may indirectly enhance the expression and activity of the cardiac isoform of *Cpt1β* through activation of AMPK- and PPARα-dependent regulatory pathways (Dong et al. 2024).

Active compounds in maca such as polyphenols, flavonoids, and specific alkaloids, can influence metabolic pathways that affect cardiac function(Melnikovova et al. 2015). Some studies have shown that maca ethanol extract, especially maca root, is rich in glocosinolates which can improve glucose metabolism and insulin sensitivity, contributing to metabolic health(Meissner et al. 2006). Maca may enhance mitochondrial function through increased antioxidant response and mitochondrial biogenesis pathways, indirectly supporting heart health(Sahin et al. 2021).

However, the effects of maca supplementation on the expression of metabolic genes in cardiac tissue are not fully understood. Given the heart's ability to

flexibly alter its fuel preference in response to various physiological conditions, combining aerobic training with bioactive supplements like maca may have synergistic effects on improving cardiac metabolism. This highlights the importance of studying the combined effects of these two interventions.

Previous studies have separately investigated the effects of exercise or natural supplements on the metabolism of muscle or the liver. However, there is very limited information on the combined effects of aerobic training and maca supplementation on metabolic gene expression in cardiac tissue. Due to the lack of specific studies in this area, further research is needed to explore how the combination of aerobic exercise and maca supplementation influences the expression of key metabolic genes such as *Cpt1β, Mcd, HK, Glut4*, and *Pdk4* in cardiac tissue. Understanding these interactions could open new pathways for improving cardiac metabolic function in both healthy and diseased states. Therefore, the present study was designed to investigate the effects of aerobic exercise combined with maca supplementation on the expression of key energy metabolism genes in the cardiac tissue of adult male rats.

Materials and Methods

Animals and housing conditions

In this study, 24 male Wistar rats (weighing: 250 ± 25 g, age: 4–6 weeks) were obtained from the Pasteur Institute (Amol, Iran). The animals were housed under controlled environmental conditions in four groups in polyethylene cages, including a temperature of 22 ± 1.4°C, relative humidity of 55–60%, and a 12-hr light/dark cycle. Throughout the study, the rats had free access (*ad libitum*) to standard rat chow (Behparvar Co., Iran) and water. A one-week acclimatization period was provided to allow the animals to adapt to the laboratory environment. Following weight matching, the rats were randomly assigned to four groups (n=6 per group): control (CON), maca supplementation (MAC), aerobic training (AT), and combined maca supplementation and aerobic training (MAC+AT).

Body weight was recorded at baseline (week 0) and subsequently every two weeks (weeks 2, 4, 6, and 8) throughout the experimental period using a digital balance.

Aerobic exercise protocol

The training groups (AT and MAC+AT) underwent an eight-week aerobic training program on a treadmill. The initial protocol consisted of running at 15 m/min for 10 min, followed by 20 m/min for 8 min. Over the first three weeks, the speed was progressively increased to 28 m/min for 60 min, which was maintained until the end of the study (Ghanbari-Niaki 2010; Nazemi et al. 2023)

Maca supplementation

Maca root powder (BulkSupplements Co, USA) was freshly suspended in normal saline and administered orally by gavage at a dose of 100 mg/kg body weight once daily, two hours after exercise, to the MAC and MAC+AT groups, as previously described (Lentz et al. 2007; Mohamed et al. 2024). The CON group received an equivalent volume of normal saline by oral gavage throughout the experimental period.

Ethical considerations

All experimental procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals and approved by the Ethics Committee of the University of Mazandaran, Babolsar, Iran (Ethics Code: IR.UMZ.REC.1403.089). The study adhered to the principles outlined in the Guide for the Care and Use of Laboratory Animals. The animal species was selected based on its suitability for the research objectives, and the minimum number of animals required for statistical validity was used. Efforts were made to minimize stress, pain, and discomfort to the animals.

Euthanasia was performed in accordance with ethical standards to ensure minimal suffering. Housing, nutrition, and disposal of animal remains were managed in compliance with ethical and regulatory standards.

Tissue collection

Forty-eight hours after the final training session and maca administration, all rats were fasted and anesthetized with an intraperitoneal injection of ketamine (30–50 mg/kg body weight) and xylazine (3–5 mg/kg body weight). The heart tissue was collected and immediately stored at −80°C for subsequent molecular assays.

Gene expression analysis

Heart tissues (100 mg) were crushed on a sterile plate, homogenized with liquid nitrogen, and total RNA was extracted using a Denazist kit (Mashhad, Iran) according to the company's protocol. To eliminate genomic DNA contamination, extracted RNA was treated with DNase I enzyme. Complementary DNA (cDNA) was synthesized using the Yekta Tajhiz Azma cDNA synthesis kit (Tehran, Iran) with Oligo-dT primers. Reverse transcription was performed by incubation at 42°C for 60 min, followed by enzyme inactivation at 70°C. The expression levels of *Cpt1β*, *Mcd*, *Hk2*, *Pdk4*, and *Glut4* relative to the reference gene β-Actin were measured using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) in Rotor Gene 6000 (Corbett Research, Australia). Briefly, a 15 µl reaction volume containing 1.5 µl of cDNA (50 ng/µl), 7 µl of Real Q Plus 2x Master Mix Green, 0.5 µl of each of primers (10 pmol) and 5.5 µl of Ribonuclease-free water were applied to amplify gene fragments. The thermal cycling conditions were followed with 95°C for 15 min, 40 cycles at 95°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec. The expression level of each gene was normalized against the expression of β-actin as reference gene. Relative gene expression was calculated using the $2^{\wedge-\Delta\Delta CT}$ method(Livak and Schmittgen 2001). The specific primer sequences used in this study are provided in Table 1. Also, melt curve analyses were performed to determine optimal annealing temperatures using temperature gradient function (55°C-95°C). PCR efficiencies were measured from the slope of standard curve. To perform a qPCR standard curve, 50 ng of cDNA was serially diluted (five-fold). Then, plotting Ct values (Y-axis) against the log of template amount or dilution (X-axis). The amount of target and reference in the samples using their CT values and the corresponding standard curve was calculated. The PCR efficiency formula was used to calculate the amplification efficiency (E) for each target gene in a PCR experiment (E = 10^(–1/S) – 1 (where S = slope of the standard curve)).

Table 1. Characterization of specific primers used in the qPCR process

Gene	*Forward primer (5'-…-3')*	*Reverse primer (5'-…-3')*	*Accession number*	*Product size(bp)*
MCD	***CTCGGCACCTTCCTCATAAA***	***GCTCGTTCCTCCCATACTCCT***	***NM_053477.2***	***154***
Hk2	***CTGTTTGAGAAGATGATTAGCGG***	***AAGGAGTTCTGGGCTGAGTTT***	***NM_012735.2***	***114***
Pdk4	***ATCAAGATTTCTGACCGAGGAG***	***CGTAACCAAAACCAGCCAAA***	***NM_053551.2***	***133***
Glut4	***CAACTGGACCTGTAACTTCATTGT***	***ACGGCAAATAGAAGGAAGACGTA***	***NM_012751.2***	***87***
CPT-1β	***ACACCAAAACATGTACCGCCTA***	***CCAGGAAAGGAGACCTAACCC***	***NM_013200.2***	***107***
β-Actin	***GTGTGACGTTGACATCCGTAAAGAC***	***TGCTAGGAGCCAGGGCAGTAAT***	***NM_031144.3***	***119***

Statistical analysis

The normality of the data was evaluated using the Kolmogorov-Smirnov test. Two-way analysis of variance was used to examine the separate and interaction effects of aerobic training and maca consumption. All results are reported as mean ± standard deviation (SD). Data analysis was conducted using SPSS software (version 21) and GraphPad (version 8) with a significance level set at $p \leq 0.05$.

Results

Effect of maca and aerobic training on body weight

The results showed that the body weight of rats in the MAC group in week 4 ($p \leq 0.01$) and week 8 ($p \leq 0.05$) were significantly higher compared to the CON group. Also, rats in the MAC group exhibited significantly higher body weight (332.7±40.2 g) compared to the AT group (292.5±22.26 g, p=0.02) and the MAC+AT group (282.2 ± 15.65 g, p=0.005). Furthermore, the body weight in the MAC group (330.2 ± 27.3 g) was significantly increased compared to the MAC+AT group (299.2± 18.7 g, p=0.03) in the fourth week. No significant differences were observed among the remaining groups at the corresponding time points.

The trend of weight changes is illustrated in Figure 1.

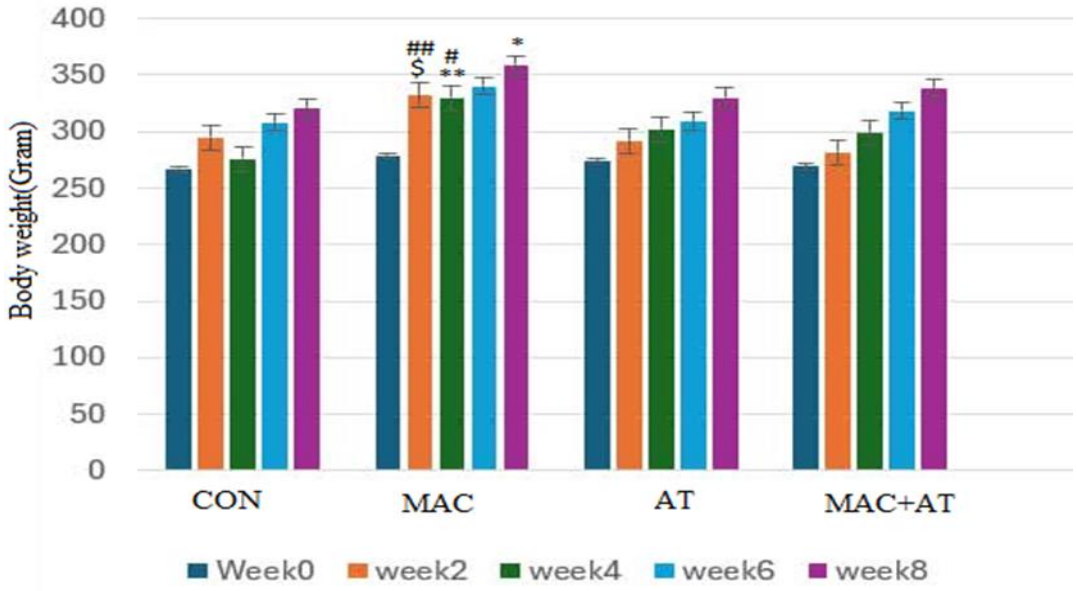

Figure 1. Body weight of rats in different experimental groups during the 8-week intervention. Data are presented as mean ± SD (n = 6). * ($p \leq 0.05$) and ** ($p \leq 0.01$) significant difference compared to the CON, $ ($p \leq 0.05$) significant difference compared to the AT and # ($p \leq 0.05$) and ## ($p \leq 0.01$) significant difference compared to the AT+MAC.

Gene expression analysis

The results of two-way ANOVA of *Cpt1β* gene expression indicated that the effect of MAC (p=0.016) and interaction effect (p=0.024) were statistically significant. There were significant differences in *Cpt1β* gene expression among the groups. One-way ANOVA demonstrated that the expression of the *Cpt1β* gene was significantly upregulated in the MAC (p= 0.003), AT (p= 0.03), and MAC + AT (p= 0.023) groups compared to the control group. However, no significant differences were observed among the other groups in the expression of *Cpt1β*. The results showed that the fold change of the *Cpt1β* gene expression nearly increased sixfold in the MAC group compared to the control group (Figure2).

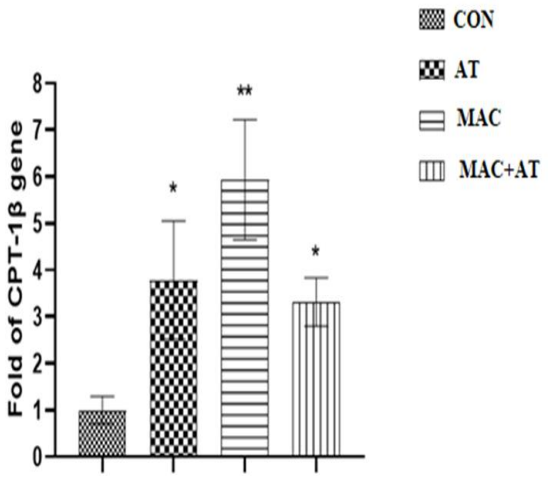

Figure 2. Relative expression of *Cpt1b* gene in cardiac tissue of different experimental groups. Data are presented as mean ± SEM (n = 6). *($p \leq 0.05$) **($p \leq 0.01$) significant difference compared to the CON group.

The results of two-way ANOVA of the *Mcd* gene expression indicated that the effects of MAC (p=0.79), AT (p=0.63) and interaction effect (p=0.71) were not statistically significant. These findings indicated that there were no significant changes among the groups in the expression of the *Mcd* gene (Figure 3).

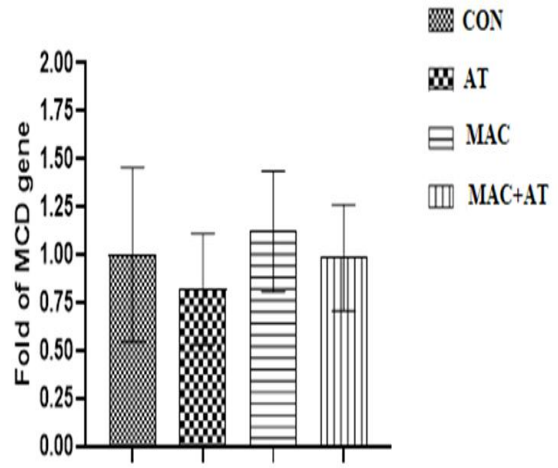

Figure 3. Fold change of the *Mcd* gene expression in different groups. Results are shown as means ± SEM (n = 6).

The results of two-way ANOVA of *Hk*2 gene expression indicated that the effect AT (p=0.03) was statistically significant. But the effect of MAC (p=0.13) and interaction effect (p=0.55) were not statistically significant. One-way ANOVA demonstrated that the expression of the *Hk2* gene significantly upregulated in the MAC group compared to the AT group (p= 0.013). However, no significant differences were observed among the other groups in the expression of *Hk2* (Figure 4).

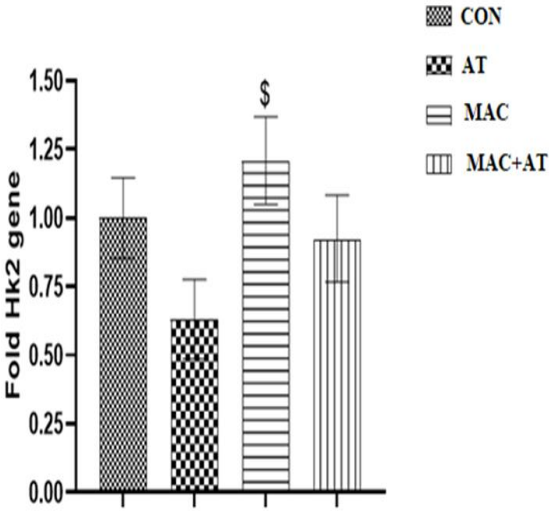

Figure 4. Relative expression of *Hk2* gene in cardiac tissue of experimental groups. Data are presented as mean ± SEM (n = 6). $^{\$}$($p \leq 0.05$) significant difference compared to the AT group

The results of two-way ANOVA of *Glut4* gene expression indicated that the effect of MAC (p=0.031) was statistically significant; but the effect of AT (p=0.25) and interaction effect (p=0.71) were not statistically significant. One-way ANOVA demonstrated that The expression of the *Glut4* gene was significantly upregulated in the MAC+AT group compared to the control group (p=0.027). Although, the expression of the Glut gene was increased in other groups compared to the control group, it was not statistically significant (Figure 5).

The results of two-way ANOVA of the *Pdk4* gene expression indicated that the effect of MAC (p=0.83), AT (p= 0.075) and interaction effect (p=0.96) were not statistically significant. These findings indicated that there were no significant changes between groups in the expression of the *Pdk4* gene (Figure 6).

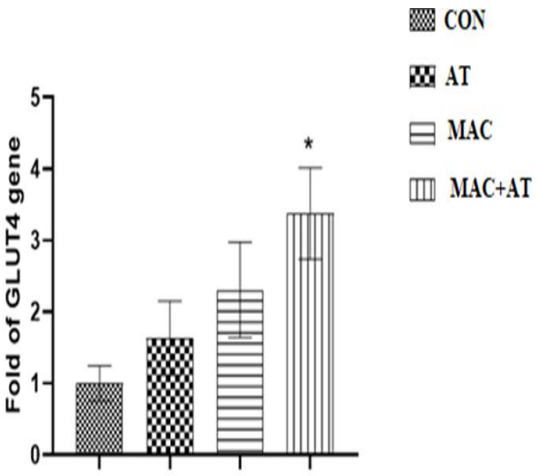

Figure 5. Fold change of the *Glut4* gene expression in different groups. Results are shown as means ± SEM (n = 6). *(p≤0.05) indicates a significant difference compared to the CON group.

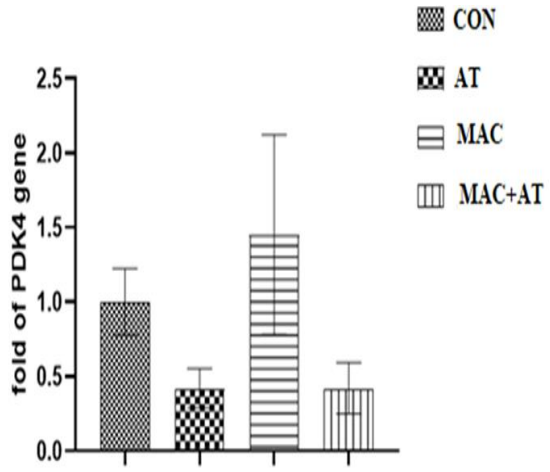

Figure 6. Fold change of the *Pdk4* gene expression in different groups. Results are shown as means ± SEM (n = 6).

The correlation between body weight and expression of genes involved in fatty acid and glucose metabolism

As illustrated in Figure 1, body weight increased progressively across all groups during the 8-week intervention, with a significant elevation observed in the MAC group compared with the CON, AT, and MAC+AT groups at week 8. To determine whether this difference could have influenced metabolic gene expression, correlation analyses were performed between final body weight and cardiac mRNA levels of *Cpt1β*, *Mcd*, *Pdk4*, *Hk2*, and *Glut4* genes. Pearson's correlation analysis revealed no significant associations between body weight and any of the examined genes ($p>0.05$).

These findings suggest that the observed upregulations of *Cpt1β* in the MAC, AT, and MAC+AT groups, as well as increased *Hk2* and *Glut4* expression in the MAC and MAC+AT groups, are not correlated to body weight.

Discussion

Beta-oxidation is the primary catabolic process by which, fatty acids are broken down in the mitochondria to generate acetyl-CoA which enters the citric acid cycle (Krebs cycle) to produce ATP, the cell's primary energy currency. In cardiac tissue, beta-oxidation is a critical pathway because the heart relies heavily on fatty acids as its primary energy source, contributing approximately 60–90% of its ATP under normal physiological conditions (Lopaschuk et al. 2010). The process begins with the transport of long-chain fatty acids into the mitochondria, facilitated by the enzyme carnitine palmitoyl transferase 1β (*Cpt1β)* which is a rate-limiting step in beta-oxidation.

In this study, the expression of the *Cpt1β* gene was significantly increased in the Maca-treated (MAC), aerobic training (AT), and combined Maca and aerobic training (MAC+AT) groups compared to the control (CON) group. This upregulation suggests an enhanced capacity for fatty acid transport into mitochondria, thereby increasing beta-oxidation and energy production in cardiac tissue. The observed effect of Maca on *Cpt1β* expression aligns with its reported antioxidant properties which may reduce oxidative stress and enhance mitochondrial function. For instance, Zhu et al. reported that maca improves energy metabolism by mitigating oxidative stress, which could indirectly support the activity of enzymes like *Cpt1β* involved in beta-oxidation(Zhu et al. 2022). Additionally, Choi et al demonstrated that maca activates signaling pathways associated with lipid metabolism,

potentially contributing to the observed increase in *Cpt1β* expression(Choi et al. 2012).

Inhibition of oxidative stress can increase the expression and activity of the cardiac isoform of the *Cpt1β* enzyme, but this effect is indirect and is mediated through PPAR- and AMPK-dependent regulatory pathways(Lopaschuk et al. 2021).

In addition, a review study showed that ginseng and maca, both of which are in the same family, can play an effective role in modulating energy systems in heart tissue. It seems that metabolic changes in heart tissue are one of the factors involved in the occurrence of some diseases including heart failure. A recent study demonstrated that ginseng can play an effective role in inhibiting or delaying heart failure by modulating effective cellular mechanisms including metabolic changes, inhibiting inflammation and oxidative damage(Parmar et al. 2024).

The pronounced upregulation of *Cpt1β* observed in this study, indicates an enhanced capacity for mitochondrial fatty acid transport in cardiac tissue. Although *Mcd* expression, which regulates the degradation of malonyl-CoA (a potent inhibitor of *Cpt1β*), remained unchanged, the increased *Cpt1β* level suggests that fatty acid oxidation was promoted through direct activation of the AMPK–PPARα signaling pathway rather than via alterations in malonyl-CoA turnover. This interpretation is consistent with previous findings showing that *Cpt1β* transcription can be upregulated independently of *Mcd* during exercise-induced metabolic adaptation(Angelini et al. 2021). Moreover, post-hoc power analysis indicated high statistical power for *Cpt1β* but relatively low power for *Mcd* and *Pdk4*, suggesting that subtle changes in these genes may have remained undetected. Collectively, these data imply that maca supplementation and aerobic training enhance fatty acid oxidation primarily through transcriptional activation of *Cpt1β*, while other related enzymes may be regulated post-transcriptionally under the present experimental conditions.

The increased beta-oxidation capacity in the MAC and MAC+AT groups may also be linked to maca's bioactive compounds, such as macamides and alkaloids which have been shown to improve energy metabolism(Lee et al. 2023). These compounds may enhance mitochondrial efficiency, allowing for greater fatty acid utilization. Furthermore, aerobic exercise is known to upregulate beta-oxidation by increasing the expression of genes involved in fatty acid metabolism, as reported by Egan and Zierath who highlighted the role of exercise in enhancing mitochondrial biogenesis and lipid oxidation in cardiac and skeletal muscle (Egan and Zierath 2013).

Glycolysis is the metabolic pathway that converts glucose into pyruvate, generating ATP and NADH in the cytoplasm. In the heart, glycolysis plays a complementary role to beta-oxidation, contributing approximately 10–40% of ATP production, particularly under conditions of high energy demand or reduced oxygen availability(Stanley et al. 2005). The process is initiated by hexokinase (*Hk2*) which phosphorylates glucose to glucose-6-phosphate, and is further regulated by the transport of glucose into cells via glucose transporter 4 (*Glut4*).

In this study, the expression of the *Hk2* gene was significantly increased in the MAC group compared to the AT group, indicating a positive effect of maca on cardiac glucose metabolism. This increase suggests that maca supplementation enhances the initial step of glycolysis, potentially increasing the availability of glucose-6-phosphate for energy production. This finding is consistent with Sahin et al. who reported that maca supplementation improves nutrient digestibility and enhances the expression of nutrient transporters, potentially supporting increased glycolytic activity in cardiac tissue(Sahin et al. 2021).

The selective upregulation of *Hk2* only in the MAC group compared with AT may reflect a maca-specific response rather than a general exercise effect. This pattern suggests that maca bioactive constituents might sensitize cardiac tissue to glucose utilization through pathways such as PI3K/Akt or AMPK signaling, even without the additional stimulus of endurance training(Sahin et al. 2021). The absence of a similar increase in the MAC+AT group could imply that concurrent aerobic training shifts energy reliance toward fatty acid oxidation, thereby attenuating the glycolytic drive induced by maca alone. This complementary regulation between β-oxidation and glycolysis supports the concept of improved metabolic flexibility rather than a single-pathway dominance.

Additionally, the expression of the *Glut4* gene was significantly increased in the combined maca and aerobic training (MAC+AT) group. *Glut4* facilitates glucose uptake into cardiac cells, and its upregulation is associated with improved insulin sensitivity and glucose metabolism. Previous evidence suggests that aerobic exercise enhances *GLUT4* expression and improves glucose metabolism through exercise-induced molecular adaptations (Egan and Zierath 2013), which is consistent with the findings observed in the MAC+AT group.

The synergistic effect of maca and exercise on *Glut4* expression may be mediated by the PI3K/Akt signaling pathway, which regulates glucose transporter translocation to the cell membrane (Shiojima and Walsh 2006). The lack of a significant increase in *Glut4* expression in the MAC group alone may be attributed to the relatively low dose of maca or the short intervention period (eight weeks).

The heart's ability to switch between beta-oxidation and glycolysis, known as metabolic flexibility, is essential for maintaining energy homeostasis under varying physiological conditions. The upregulation of *Cpt1β* and beta-oxidation in the MAC, AT, and MAC+AT groups, alongside increased *Hk2* and *Glut4* expression in the MAC and MAC+AT groups, suggests that maca and aerobic exercise may enhance the heart's metabolic flexibility. This dual enhancement of lipid and glucose metabolism could improve overall energy efficiency in cardiac tissue, particularly under conditions of increased energy demand, such as during exercise.

The antioxidant properties of Maca, as reported by Choi et al., may further support this metabolic flexibility by reducing oxidative stress, which can impair mitochondrial function and glycolytic enzyme activity. Additionally, the anabolic effects of maca, as reported by Sahin et al., may contribute to increased muscle mass and protein metabolism through enhanced mitochondrial biogenesis and antioxidant responses, which could indirectly influence the expression of metabolic genes like *Cpt1β*, *Glut4*, and *Hk2* in cardiac tissue (Choi et al. 2012; Sahin et al. 2021).

The study's findings on beta-oxidation and glycolysis are promising but have limitations. The use of male rats limits the generalizability of the results to female rats or humans. Although the sample size was sufficient to detect large and moderate effect sizes, we acknowledge that the study may have been underpowered to detect smaller effects, particularly for genes such as *Mcd* and *Pdk4*. Moreover, the eight-week intervention period may have been insufficient to elicit measurable changes in these genes. Both *Mcd* and *Pdk4* play critical roles in coordinating the metabolic switch between fatty acid oxidation and glucose utilization. Their lack of significant response in our study may suggest that a longer duration or higher dosage of maca extract is necessary to modulate their expression effectively (Coven et al. 2003).

Future research should explore the dose-dependent effects of maca on beta-oxidation and glycolysis, as well as its long-term impact on cardiac metabolism. Investigating the role of molecular

pathways, such as AMPK and PI3K/Akt, could provide deeper insights into the mechanisms underlying maca effects. Moreover, studies in human models and evaluations of cardiovascular function could help determine the clinical relevance of maca supplementation for metabolic health.

The overall findings indicate that both aerobic training and maca supplementation each appear to modulate the expression of key cardiac metabolic genes, suggesting potential independent contributions to cardiac energy regulation. These findings highlight the value of non-pharmacological strategies for improving cardiac metabolism, while future studies are warranted to examine potential interactive or long-term effects.

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Conflicts of interest

The authors report no conflicts of interest.

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Ethical Considerations

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by University of Mazandaran. Efforts were made to minimize animal suffering and reduce the number of animals used.

Code of Ethics

IR.UMZ.REC.1403.089

Authors' Contributions

The authors' accountabilities were as follows: Study design: A.A, Kh. N, Z.F; Data gathering: S.R, Kh. N, A. A; Statistical analysis: S.R, Kh. N, A. A; Drafting the manuscript: S.R; critically revised the manuscript: S. R, A. A, Kh. N. All authors have read and approved the final manuscript before submission.

Abbreviations

Carnitine palmitoyl transferase 1 β (*Cpt1β*); Malonyl-CoA decarboxylase (*Mcd*); Hexokinase (*HK);* Glucose transporter 4 (*Glut4*); pyruvate dehydrogenase kinase *(Pdk4*).

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