Research Article

Effects of resistance, endurance, and concurrent exercise on carnitine palmitoyltransferases 1, 2 and fatigue index in obese diabetic rats

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Abstract

The objective of this study was to evaluate the effects of endurance, resistance, and Concurrent endurance and resistance training on fat oxidation and fatigue levels in obese diabetic rats. A total of forty overweight rats (average weight of 380 ± 50 g) were randomly assigned to five distinct groups. Diabetes was induced through peritoneal administration of Streptozotocin at a dosage of 55 mg/kg body weight. Endurance training was conducted via moderate-intensity treadmill running (50%-70% of maximum running capacity) for a duration of eight weeks, with sessions lasting one hour per day, five days a week. Resistance training involved ladder climbing at 50-70% of maximum load, also for eight weeks, with five sessions per week, comprising 15 climbs per session and a one-minute rest interval between climbs. The Concurrent training regimen alternated between treadmill and ladder exercises. Levels of CPT1 and CPT2 were quantified using rat CPT1 and CPT2 ELISA kits, both prior to and 48 hours following the training period. Fatigue indices were assessed as well. All three training modalities significantly influenced CPT1 levels ($F=93.35$, $P=0.001$) and led to a notable increase in CPT2 levels ($F=26.76$, $P=0.001$). Additionally, significant alterations in fatigue indices were observed ($F=26.12$, $P=0.001$). The findings suggest that aerobic, resistance, and Concurrent training regimens can enhance metabolic function and prolong the duration until fatigue in elderly diabetic rats. Nonetheless, the extent of these improvements is somewhat contingent upon the specific type of exercise performed, with aerobic and Concurrent training demonstrating a more pronounced effect compared to resistance training.

Key Words: **CPT1, CPT2, Training, Diabetes, Fatigue**

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Introduction

Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus or late-onset diabetes, is a metabolic disorder characterized by elevated blood sugar levels due to decreased response to insulin or reduced insulin secretion (Padhi et al., 2020). This condition can lead to various complications such as cardiovascular diseases, kidney failure, stroke, and diabetic retinopathy (Saed et al., 2019). Obesity is a significant risk factor for the development of metabolic disorders like insulin resistance and type 2 diabetes. Insulin resistance and type 2 diabetes are associated with hyperglycemia, hyperinsulinemia, elevated serum free fatty acid levels, impaired lipid oxidation, and lipid accumulation in cells (Himanshu et al., 2020). The accumulation of lipids is closely linked to insulin resistance, with mitochondrial dysfunction being a key factor in this process (Nagu et al., 2019, Kordi et al., 2024). Carnitine palmitoyltransferases 1 and 2 (CPT1 and CPT2) are mitochondrial enzymes responsible for converting fat stores into energy and transporting long-chain fatty acids into mitochondria (Wang et al., 2020), respectively. The regulation of CPT2 activity, whether by malonyl-CoA or other metabolic pathways, remains unclear (Joshi et al., 2020). Wang et al. (2022) reported that CPT 1 and 2 facilitated import of long-chain fatty acids into mitochondria. Shirkhani et al. (2022) observed CPT1 reduction after a 10-week endurance training program in prediabetic rats. Engoti et al. (2019) also studied the effects of a 6-week endurance training program on mitochondrial enzymes in the hippocampus tissues of diabetic rats and reported that the training program increased CPT2 (Angouti et al, 2020).

Several studies examining lifestyles have indicated the potential for embracing aging as a natural process and potentially preventing premature aging. It has been demonstrated through research that engaging in physical exercise on rest days and even during recovery can lead to temporary changes in body systems. Prior research has proposed that sustained physical activity can serve as a safe intervention method for averting the aging of body systems and enhancing metabolism (Jahan Mihan et al 2022). Studies have revealed that heightened levels

of pain in individuals with diabetes are linked to clinical symptoms such as anxiety, depression, insomnia, and diminished mental and physical performance (Gholami et al., 2020). Consequently, pain resulting from peripheral diabetic neuropathy may manifest in symptoms associated with depression, anxiety, and chronic fatigue (Gholami et al., 2020). Individuals affected by peripheral diabetic neuropathy may experience fatigue due to their avoidance of physical activity and reduced cardiovascular fitness stemming from the complex effects of diabetes, obesity, lower limb pain, and numbness. According to existing evidence, fatigue may be a prevalent symptom among individuals with diabetes. Additionally, a study has indicated that reducing fatigue was correlated with enhanced mood and quality of life in individuals affected by diabetes (Clouding et al., 2014).

Muscle performance is influenced by the ability to align fatty acid and glucose oxidation with the energy requirements of the organism. The process of fatty acid β-oxidation, primarily responsible for muscle energy during rest and endurance activities, is regulated by CPT1, which converts long-chain fatty Acid-Acyl-CoA into acylcarnitine for mitochondrial transport and subsequent β-oxidation. Malonyl CoA, a product of lipogenesis synthesized by acetyl CoA carboxylase (ACC), inhibits CPT1, establishing it as a critical regulatory point in lipid oxidation. - Peroxisome proliferator-activated receptors (PPARs) function as nuclear transcription factors that modulate the expression of genes in the liver, skeletal muscles, and heart, which are essential for the uptake and oxidation of mitochondrial fatty acids, including those regulated by CPT1 and CPT2. This underscores the significant connection between carnitine palmitoyltransferases and muscle functionality, particularly in relation to fatigue. Research indicates that engaging in physical exercise can mitigate certain age-related muscular changes, highlighting the role of fatty acid oxidation in managing skeletal muscle fatigue (Henique et al., 2015). - The American College of Sports Medicine and the American Heart Association advocate for a minimum of 30 minutes of moderate-intensity physical activity on five days each week, alongside 20 minutes of endurance exercises three times weekly, to enhance overall health. The decline in physical fitness components—such as strength, endurance, agility, and flexibility—in older adults is attributed to decreased daily activity levels (Samadi et al., 2019), leading to challenges in daily functioning. Consequently, it is recommended that older individuals participate in regular moderate-intensity exercises, including both resistance and endurance training, to prevent, delay, or even reverse age-related issues (Safari et al., 2022; Asjodi et al., 2018).

Furthermore, acknowledging the significance of enhancing lifestyle choices and engaging in physical activity to prevent and manage chronic conditions like diabetes, the role of exercise becomes particularly crucial for the patient population. Physical activity serves as a vital intervention for improving metabolic function in individuals with diabetes.

Given the variability in research methodologies and the numerous inquiries regarding the appropriate types and intensities of exercise for older adults, it is noteworthy that most existing studies have primarily focused on aerobic, resistance, and concurrent training modalities, while the exploration of concurrent endurance and resistance training has received comparatively less attention. Additionally, the rising prevalence of chronic diseases, including diabetes, poses a substantial challenge for individuals and families alike. Therefore, this study aimed to explore the potential relationship between CPT1, CPT2, and levels of muscular fatigue. As a result, it examined the impacts of endurance, resistance, and concurrent endurance and resistance training on CPT1, CPT2, and fatigue metrics in obese diabetic rat concurrent.

Materials and Methods

Animals

In this applied experimental study, 40 rats (14 months old) with excess body weight (mean weight 380±50 g) were obtained from Pasteur Institute of Iran and transferred to the Animal House at Kermanshah University of Medical Sciences. The rats underwent a one-week acclimation period to minimize the potential impact of their relocation to a new environment on the research outcomes. Subsequently, they were moved to the laboratory setting and housed in transparent polycarbonate cages, with four rats per cage, at controlled temperature (20-24℃), relative humidity (45-55%), and a 12:12h light-dark cycle. Throughout the study, the rats were provided with free access to food (pellets from Behparvar Company) and water (in 500 mL bottles designed for laboratory animals). The rats in the control and chem groups

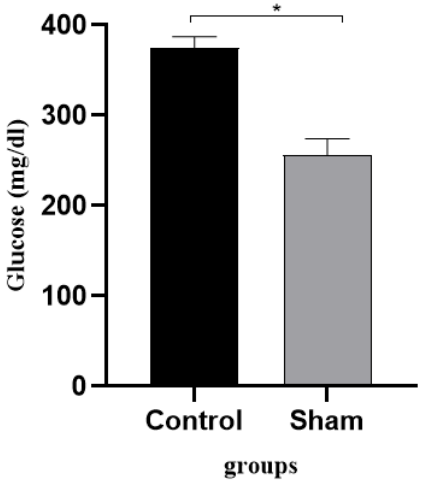

Figure 1. Serum glucose levels in the control and sham groups. The sign * indicates a significant change between the groups.

were used to induce diabetes, with streptozotocin (STZ) and nicotinamide injections, and to diagnose diabetes based on serum glucose levels. The rats classified as diabetic in the control group exhibited serum glucose levels exceeding 300 mg/dl. The graphical representation in Figure 1 illustrates the disparity in serum glucose levels between the control and chem groups.

Training protocol

The rats were familiarized with the treadmill by walking on it for five days at a pace of 10 meters per day with a speed of 5 meters per minute before the training began. The training intensity was determined by conducting an initial maximal running test on the treadmill, where the rats ran at 5 meters per minute for 3 minutes, with the speed increasing by 5 meters per minute every 3 minutes until exhaustion (Kordi et al., 2024).

For endurance training, the rats voluntarily trained on the treadmill without electrical stimulus, with free access to food and water, at a moderate intensity of running at 50-70% of their maximum capacity for 8 weeks, 5 days a week, for 1 hour each day (Santos et al., 2022).

In the resistance training protocol, the rats voluntarily trained on a suitable ladder without electrical stimulus and no food or water restriction. They performed the maximal load test three times in the first, fourth, and eighth weeks, where they carried loads equal to 75% of their body weight, with the load increasing by 15%, 25%, and 40% of body weight in the 1st, 4th, and 8th week tests, respectively. The resistance training intensity was determined based on the maximal load test, with the same intensity as the endurance training protocol (50-70% of body weight) for 8 weeks, 5 days a week, with 15 climbs per session and a 1-minute rest between climbs. Small metal loads were attached to the tails of the rats (Santos et al., 2023).

The concurrent training was conducted for 8 weeks, 5 days a week, at moderate intensity (50-70% of maximum load) on alternate days, with one day on the treadmill and one day on the ladder (Santos et al., 2023).

Fatigue indices

The fatigue indices were determined by having the training groups run on the treadmill starting at a speed of 10 m/min. Every 2 minutes, the speed was increased by 3 m/min until the rats reached exhaustion. The analysis was based on the distance co-vered on the treadmill and the speed as indicators of fatigue and athletic performance (Fasihi and Khaledi, 2020).

Measurement of the variables

Considering the groups that the rats were in, they were anesthetized using ketamine and xylazine in the ratio of 5:2 (48 hours after the last training session). They were then fixed on a surgical table and their chests were opened to separate the livers from the umbilici. The tissue was quickly put in liquid nitrogen and the liver tissue was powdered in liquid nitrogen and homogenized by hand in PBS buffer (1 mL of the buffer for each 100 mg of the tissue). The homogenized tissue was centrifuged for 10 min at 4000-6000 rpm. The amounts of the CPT1 and CPY2 proteins were measured using ELISA kits (made in Germany by ZellBio GmbH) with the sensitivity of 0.2 ng/mL and a BIOTEK ELISA reader (made in the US).

Statistical analysis

Statistical analysis included expressing descriptive data as mean ± SD, testing for normal distribution with the Shapiro-Wilk test, and using one-way ANOVA for between-group variance. Tukey's post hoc test was then applied for within-group variance. Statistical analyses were conducted in SPSS 26 at a significance level of 0.05, and graphical representations were created using GraphPad Prism 8.

Results

In order to examine the effects of endurance, resistance, and concurrent training programs on CPT1, CPT2, and fatigue indices in rats with excess body weight, the normality of the data was initially assessed using the Shapiro-Wilk test. The results indicated a normal distribution ($P<0.05$), leading to the utilization of one-way ANOVA for statistical analysis.

The mean and standard deviation of the rats' weight, CPT1, CPT2 variables, and fatigue index were displayed by groups in Table 1. Additionally, the outcomes of the one-way ANOVA test demonstrated no significant variance between the groups in terms of the weight variable ($F=1.01$, $P=0.403$).

The one-way ANOVA results for the CPT1 variable revealed significant effects of the training programs on CPT1 ($F=93.35$, $P=0.001$). Tukey's post hoc test further demonstrated significant differences between the control groups and the endurance training group (mean difference = -2.96, $P=0.001$), control groups

Table 1. Weight of rats, variables CPT1, CPT2 and fatigue index (mean ± standard deviation) by groups.

groups	Control	Endurance training	Resistance training	Concurrent training
Wight (g)	375/00 ± 16/34	353/75 ± 33/69	364/88 ± 26/21	357/75 ± 25/73
Carnitine palmitoyl transferase 1 (unit/liter)	4/49 ± 0/38	7/45 ± 0/12	5/92 ± 0/10	7/09 ± 0/66
Carnitine palmitoyl transferase 2 (unit/liter)	3/77 ± 0/52	4/78 ± 0/20	4/73 ± 0/29	5/19 ± 0/13
Fatigue (minutes)	15/44 ± 3/82	27/76 ± 2/76	23/31 ± 2/33	24/31 ± 2/33

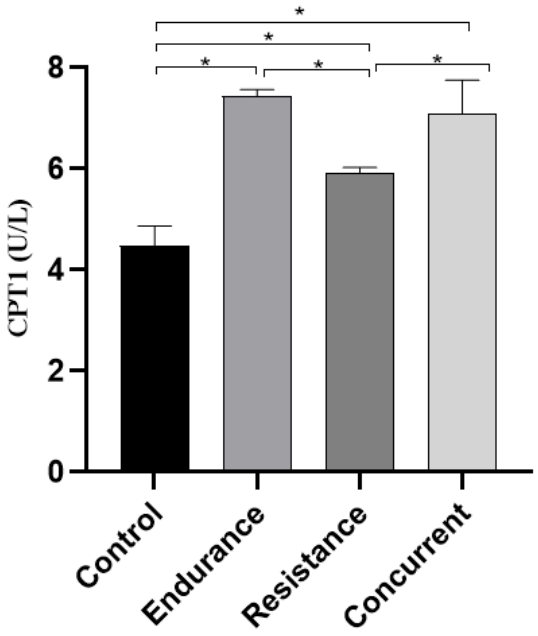

Figure 2. Mean ±SD for CPT1 in the groups. The sign * indicates a significant change between the groups.

and the endurance training group (mean difference=-2.96, P=0.001), control groups and resistance training group (mean difference=-1.43, P=0.001), control groups and concurrent training group (mean difference=-2.60, P=0.001), resistance training and endurance training groups (mean difference =1.53, P=0.001), and concurrent training and resistance training group (mean difference =1.16, P=0.001). Nevertheless, no significant distinction was observed between the concurrent and endurance training groups (mean difference=0.36, P=0.430) (Fig 2).

The results demonstrated a significant increase in CPT2 due to the training programs (F=26.76, P=0.001). Noteworthy differences were observed between the control and the endurance training groups (mean difference = -1.02, P=0.001), between the control and the resistance training groups (mean difference = -0.95, P=0.001), between the control and the concurrent training groups (mean difference = -1.41, P=0.001), and between the concurrent and the resistance training groups (mean difference = 0.46, P=0.054). However, no significant differences were found between the resistance and endurance training groups (mean difference = 0.04, P= 0.999), and between the concurrent and the endurance training groups (mean difference = - 0.41, P=0.105) (Fig 3).

The training interventions led to a substantial increase in the duration of athletic performance by reducing fatigue (F=26.12, P=0.001). This enhancement was statistically significant when comparing the control group with the endurance training group (mean difference = -12.32, P=0.001), the control group with the resistance training group (mean difference = -7.86, P=0.001), and the control group with the concurrent training group (mean difference = -8.86, P=0.001). Additionally, a significant difference was observed between the endurance and resistance training groups (mean difference = 4.45, P=0.001). However, no significant variances were found between the concurrent training and resistance training groups (mean difference = -1.00, P=0.999), as well as between the concurrent training and endurance training groups (mean difference = -3.45, P=0.140) (Fig 4).

Discussion

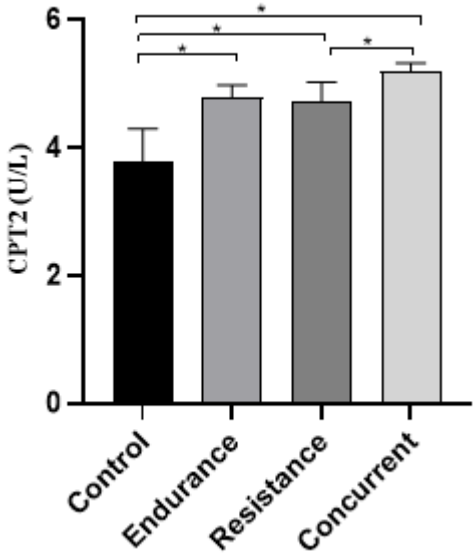

Figure 3. Mean ±SD for CPT2 in the groups. The sign * indicates a significant change between the groups.

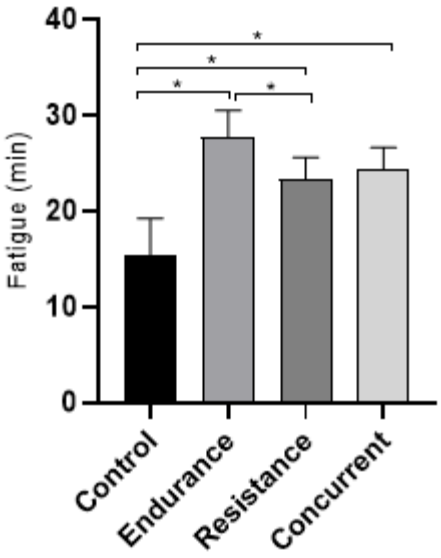

Figure 4. Mean ±SD for fatigue indices in the groups. The sign * indicates a significant change between the groups.

The study revealed that different types of training programs, including endurance, resistance, and concurrent training, had a significant impact on the levels of CPT1, CPT2, and fatigue indices. Specifically, it was found that endurance training was more effective in altering the transport of long-chain fatty acids compared to resistance and concurrent endurance and resistance training.

The carnitine palmitoyltransferase (CPT) system is comprised of two key enzymes, CPT1 and CPT2, which play a crucial role in the translocation of long-chain fatty acids into mitochondrial compartments. These enzymes are situated in the outer mitochondrial membrane (CPT1) and the inner mitochondrial membrane (CPT2) (Bonnefont et al., 2004). Research conducted by Angotti et al. (2020) demonstrated that a six-week aerobic exercise regimen led to an increase in CPT2 levels within the mitochondria of hippocampal tissue in diabetic rats. Supporting these findings, Aminizadeh et al. (2018) reported that a four-week endurance training program significantly elevated the expression of CPT1 in the skeletal muscles of male rats compared to a control group. In contrast, Bassami et al. (2013) found that circuit resistance training did not influence lipid metabolism, which diverges from the outcomes of the studies. Additionally, the physiological implications of malonyl-CoA's inhibition of CPT2 remain unclear; however, it is likely that variations in CPT2 levels are influenced by malonyl-CoA concentrations, given CPT2's heightened sensitivity to malonyl-CoA inhibition (Angouti et al., 2020).

CPT1 is predominantly present in the liver, kidney, spleen, lung, intestine, pancreas, lymphocytes, and ovary. Malonyl CoA serves as an intermediate in lipogenesis and acts as an inhibitor of CPT1. Elevated levels of malonyl CoA result in the inhibition of β-oxidation of fatty acids within the mitochondria. A recent study conducted on insulin-resistant muscles and type 2 diabetic subjects revealed heightened levels of malonyl-CoA and reduced fatty acid oxidation (Ahmed et al., 2021). Adequate duration and intensity of aerobic exercises have been shown to have positive effects on enhancing insulin sensitivity and increasing GLUT4 mRNA content. This increase in GLUT4 mRNA content contributes to improved glucose utilization and decreased insulin resistance, consequently reducing the influx of lipids into the liver. The increased presence of CPT2 in hippocampus tissue suggests that endurance training not only has no adverse effects on lipid metabolism but also has the potential to enhance cellular performance in lipid consumption. However, further research is necessary to substantiate this hypothesis (Angouti et al., 2020).

Carnitine palmitoyltransferase 1 (CPT1), carnitine palmitoyltransferase 2 (CPT2), and carnitine-acylcarnitine translocase (CACT) are integral elements of the fatty acid esterification transport mechanism located within the mitochondrial membrane. CPT1 functions as a transmembrane protein on the outer mitochondrial membrane, whereas CPT2 is positioned on the inner mitochondrial membrane. This transport system is vital for lipogenesis and the regulation of fatty acid metabolic homeostasis. Specifically, CPT1A and CPT1B, found on the outer mitochondrial membrane, initiate lipid metabolism by catalyzing the conversion of long-chain Acyl-CoA and carnitine into long-chain acylcarnitine and CoA. Subsequently, acylcarnitines are moved from the cytosol to the intermembrane space, where CPT2 on the inner membrane facilitates the conversion of the remaining acyl group back to CoA, thus making it available for β-oxidation. The carnitine released during this process is then transported back to the intermembrane space by CACT, enabling the re-transport of fatty acids. Although CPT1C does not significantly contribute to fatty acid oxidation, it influences neuronal oxidative metabolism, energy balance, and cellular senescence. The peroxisome proliferator-activated receptor (PPAR) family serves as a key transcription factor in the regulation of fatty acid oxidation, with evidence suggesting that PPAR activation modulates intracellular free fatty acid concentrations. The activation of PPAR enhances the expression and functionality of the CPT system, thereby affecting fatty acid metabolism (Wang et al., 2021). Studies have shown that mice deficient in hepatic Cpt2, which is essential for converting acyl-carnitine to Acyl-CoA in the mitochondrial matrix for β-oxidation, exhibit reduced hepatic gluconeogenesis, a condition that can alleviate obesity and related glucose intolerance. Additionally, it has been established that deficiencies in CPT1A and CPT2 result in distinct mechanisms that influence liver metabolism, which will be the subject of forthcoming research. Brown et al. found that murine white adipocytes display variances from other species, such as humans and mice, in relation to the main enzyme responsible for fatty acid metabolism, despite all three rodent species mainly expressing CPT1B. Furthermore, it is crucial to emphasize that, despite their comparable biochemical functions, the physiological impacts of CPT1 deficiency differ depending on the depot. Specifically, skeletal muscle-specific Cpt1b encourages an adaptive remodeling of muscle metabolism that is associated with resistance against obesity and insulin resistance (Demaugre et al., 1988).

The findings further indicated that training programs have the potential to extend exercise duration while simultaneously reducing fatigue levels. Specifically, the type of training employed revealed that endurance training was superior in mitigating fatigue when compared to resistance and concurrent training modalities. Supporting these findings, Ahmadi et al. (2019) noted a reduction in both central and peripheral fatigue following swimming training. Moreover, Bahati et al. (2023) found that after a six-week interval training regimen, male rats exhibited increased exercise duration alongside decreased fatigue. It has been established that elevated levels of free radicals in skeletal muscles contribute to fatigue, primarily due to a reduction in myo-

-filament Ca+2 sensitivity induced by hydrogen peroxide (Kano et al., 2024). As a known oxidant, hydrogen peroxide facilitates the release of calcium ions from sarcoplasmic reticulum vesicles and promotes the repair of Ca+2 channels within lipid bilayer membranes, which ultimately diminishes myofilament Ca+2 sensitivity and leads to fatigue (Supruniuk et al., 2023). Furthermore, research indicates that aerobic exercise enhances oxygen consumption in tissues such as muscles, which is associated with increased oxidative stress and a concurrent rise in the production of free radicals, particularly reactive oxygen species (ROS), within mitochondria (Pingitore et al., 2015). The ROS generated during physical activity is responsible for oxidative damage to proteins in muscle cells and cell membranes, significantly contributing to the onset of muscle fatigue (Wu et al., 2019).

Enhanced serum lactate dehydrogenase (LDH) activity, stemming from the enzyme's release during liver and muscle cell membrane damage, can serve as an indicator of fatigue. An alternative explanation could be the heightened glycolysis process post-exercise to fuel energy production, leading to increased lactate generation. Lactate dehydrogenase isoenzymes convert lactate molecules into pyruvate molecules, which then enter the tricarboxylic acid cycle to generate more ATP (Agan et al., 2013). Another potential factor contributing to reduced fatigue could be the enhancement of aerobic capacity following an 8-week training regimen, leading to improved lactate removal post-exercise due to elevated levels of lactate transporters in muscles (Agan et al., 2013). Summermatter et al. (2013) highlighted that interval training boosts lactate and monocarboxylate transporter (MCT4, MCT1) levels. MCT4, predominant in fast glycolytic muscle fibers, aids in lactate removal from these fibers, while MCT1, dominant in oxidative fibers, facilitates lactate uptake and removal (Nalbandian et al., 2016).

The heightened activity of CPTs coincides with increased muscle glycogen reserves, potentially indicating reduced glucose consumption due to lipid oxidation inhibition. Notably, increased muscle lipid oxidation leads to significant muscle regeneration, contributing to fatigue. These discoveries yield two physiological implications (Henique et al., 2015). One aspect to consider is that, beyond its role in energy supply, the rate of lipid oxidation may function as a metabolic sensor within skeletal muscles, thereby aiding their adaptation to changes in the environment. Another point is that this sensory function could elucidate the pronounced regulation of this metabolic pathway in skeletal muscles, as evidenced by a 30- to 100-fold increase in the sensitivity of CPT1 to malonyl CoA inhibition in skeletal muscles compared to the CPT1A isoform. The physiological significance of such rigorous regulation of CPT1 activity by malonyl CoA remains unclear. Consequently, the current study indicates that the direct stimulation of lipid oxidation may partially mitigate the decline in muscle function associated with aging (Henique et al., 2015).

The hypothesis gains further support from the results of a prior investigation carried out by Maes et al. (2005). Their research focused on the endogenous levels of fatty acids in 22 individuals diagnosed with chronic fatigue syndrome and 12 healthy individuals. The study revealed elevated levels of omega-6 polyunsaturated fatty acids and monounsaturated fatty acids in patients with chronic fatigue syndrome. When juxtaposed with our own findings, those of Maes et al. (2005) indicate that the ratio of free fatty acid to acylcarnitine for these acyl groups is roughly 2- to 3-fold higher in individuals with chronic fatigue syndrome compared to healthy controls, pointing towards a significant disruption in fatty acid/carnitine homeostasis (Maes et al., 2005). This disruption could be attributed to either (i) a decrease in the activity of AcylCoA synthase, which is essential for the conversion of free fatty acid to AcylCoA, or (ii) diminished activity of CPT-I. Considering that CPT-I serves as the key enzyme in mitochondrial fatty acid oxidation, it is postulated, based on these findings, that alterations in CPT-I activity play a role in the manifestation of fatigue symptoms (Reuter & Evans, 2011).

Conclusion

The findings of this study suggest that an 8-week endurance, resistance, and concurrent training regimen can enhance lipid metabolism by improving factors associated with the transportation of long-chain fatty acids. Additionally, this training regimen can also extend the duration of exercise in male diabetic elderly rats with excessive body weight. Overall, the results indicate that endurance training, followed by concurrent training, resulted in more significant changes compared to resistance training. Therefore, these training regimens may be beneficial in managing diabetes by improving insulin sensitivity and reducing excess body weight in diabetic patients.

Limitations of the research: The research faced certain limitations, notably the absence of measurements for blood glucose levels and the insulin resistance index. Additionally, incorporating assessments of muscle fatigue indicators, such as lactate dehydrogenase (LDH) and creatine kinase (CK), would have enhanced the study's comprehensiveness.

What is already known on this subject?

It is not uncommon for individuals to develop certain conditions like diabetes as they grow older. Moreover, it is widely recognized that diabetes has an impact on lipid metabolism. Previous studies on lifestyle have indicated that engaging in physical activities could potentially help maintain overall health and slow down the natural aging process, ultimately preventing premature aging.

What this study adds?

This investigation demonstrated significant correlations between fatigue and CPT1 and CPT2. Therefore, participating in endurance, resistance, or a combination of exercises may be an effective strategy to prevent age-related muscle loss and enhance metabolism, leading to improved fatigue levels. Furthermore, the study established connections between CPT1, CPT2 levels, athletic performance, and fatigue.

Organ Cross-Talk Tips:

- The association between substrate metabolism and fatigue is robust, and exercise serves to reinforce this relationship. The concurrent training regimen could serve as a viable approach to mitigating the effects of aging.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research was approved by the Medical Ethics Committee of Kermanshah Branch of Islamic Azad University with the code IR.IAU.HSH.REC.1402.142. Ethical principles were completely observed with respect to feeding the rats, providing living conditions for them, taking care of them, and disposing of their carcasses.

Informed consent Not applicable

Author contributions

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

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