Original Article

Effects of Endurance Training and Chlorogenic Acid on Oxidative and Nitrosative Stress Markers in Prediabetic Male Mice

Sahar Ghasemi Pour, Sayyed M. Marandi

Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran

Abstract

Background: Many studies have investigated the effects of exercise and chlorogenic acid in controlling and improving diabetes and reducing inflammation. This study aimed to investigate the effects of endurance exercise and chlorogenic acid on oxidative and nitrosative stress indicators in skeletal muscle tissue of male C57BL/6 mice. **Methods:** A total of 35 mice were randomly divided into two groups: a high-fat diet group to induce pre-diabetes and a normal diet group. After inducing pre-diabetes, mice in the high-fat diet group were further divided into control, chlorogenic acid, endurance training, and endurance training + chlorogenic acid groups (n = 7). The exercise protocol was performed incrementally (speed 15–23 m/min) for 10 weeks (three sessions per week for 45 min) on a treadmill. Chlorogenic acid was administered at a rate of 110 mg/kg of body weight three times a week via gavage. Next, 24 hours after the last intervention, tissue samples were taken from the quadriceps femoris muscles and analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Data were analyzed using one-way analysis of variance and Dunnett's post-hoc test at a significance level of $P < 0.05$. **Results:** Endurance training significantly decreased glutathione (P = 0.01) and glutathione peroxidase (P = 0.026). Chlorogenic acid consumption also significantly decreased glutathione peroxidase (P = 0.007) and significantly increased the total antioxidant capacity (P = 0.011). **Conclusion:** Endurance training and chlorogenic acid supplementation may be used as therapeutic strategies to improve antioxidant capacity and prevent or reduce diabetes-related complications.

Keywords: *Chlorogenic acid, endurance training, oxidative stress, nitrosative stress, pre-diabetes*

Introduction

The term prediabetes describes blood glucose levels above the normal range but below the diabetes diagnostic threshold.[1] According to the recent guidelines from The American Diabetes Association (ADA), prediabetes is the stage of intermediate hyperglycemia characterized by particular parameters, including impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and specific scope of hemoglobin A1c (HbA1c).[2] Prediabetes is important due to its high prevalence and significant risk of progression to overt type 2 diabetes among affected individuals with the condition.[3] Prediabetes serves as a crucial juncture for intervention, offering the opportunity to reverse the condition and forestall the onset of type 2 diabetes.[4] Diabetes remains an important epidemic in contemporary times.[5] Approximately 382 million people worldwide live with diabetes, which constitutes about 3/8% of the world's total population.[6] Prediabetes (impaired glucose tolerance) is recognized as an important factor in the risk of developing type 2 diabetes, defined by blood sugar levels higher than normal but lower than the threshold for diabetes.[6] According to the experts from the American Diabetes Association, nearly 70% of prediabetic individuals eventually develop diabetes.[6] It is predicted that over 470 million people worldwide will be diagnosed with prediabetes by 2030.[7] Understanding prediabetes is essential for reducing the incidence of type 2 diabetes.[8] Identifying factors associated with it and implementing interventions to combat it reduce healthcare costs and prevent chronic diseases.[9] The risk of developing prediabetes increases with overweight, sedentary lifestyle, age, and family history of diabetes. For every kilogram of body fat lost, the incidence of diabetes decreases by approximately 16%.[10] The International Diabetes Federation (IDF) estimates that 537 million

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Address for correspondence:
Prof. Sayyed M. Marandi, Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran.
E-mail: s.m.marandi@spr.ui.ac.ir

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adults aged 20--79 years will be living with diabetes in 2021, and this number is expected to increase to 783 million by 2045.[11] Furthermore, 541 million adults will have impaired glucose tolerance (IGT) and are at risk of developing diabetes.[11] The human body has various mechanisms to deal with oxidative–nitrosative stress by producing antioxidants. Shifting the balance between oxidants and antioxidants in favor of oxidants is known as oxidative–nitrosative stress.[12] Oxidative–nitrosative stress plays an important role in the pathogenesis and complications of diabetes.[13] In Aimaretti *et al*.'s[14] (2023) study on C57BL/6 mice, after 12 weeks of feeding with a high-fat diet, researchers examined the quadriceps muscles of these mice. They concluded that the level of oxidative stress increased in parallel with the progress of metabolic alterations induced by the high-fat diet. Additionally, the study found that oxidative stress caused by hyperglycemia activated various signaling pathways that contribute to insulin resistance. The researchers observed tissue-specific differential responses to the high-fat diet, including adaptations in the heart and tibialis anterior skeletal muscle. It is important to note that the study did not specifically examine the quadriceps muscle, but rather focused on other tissues. However, the findings regarding oxidative stress and insulin resistance are consistent with the general understanding of how high-fat diets affect metabolic health in mice models. The study of Denies *et al*.[15] (2014) involved C57BL/6 mice that were fed a high-fat diet for 12 weeks. Researchers examined the quadriceps muscle of these mice. They concluded that the level of oxidative stress increased in parallel with the progression of metabolic alterations induced by the high-fat diet. This study demonstrated the relationship between high-fat diets, oxidative stress, and metabolic changes in the skeletal muscles. Its findings emphasize the importance of healthy eating and controlling body fat for maintaining muscle health and preventing metabolic diseases. In various conditions of cardiovascular diseases and diabetes, total oxidative capacity (TAC) has emerged as a promising biomarker for prediction and diagnosis. TAC reflects the overall ability of cells to counteract oxidative stress, making it a valuable tool in assessing disease severity and monitoring treatment efficacy.[16] Physical activity is strongly linked to the incidence of diabetes, metabolic syndrome, and mortality. Generally, higher physical fitness is associated with lower rates of prediabetes, as evidenced by fasting blood sugar (FBS) or oral glucose tolerance test (OGTT) results or future diabetes risk.[17] Aerobic exercise has been shown to enhance insulin sensitivity and promote mitochondrial biogenesis. It also increases fat oxidation in adults with prediabetes or type 2 diabetes, both during rest and during exercise.[18] Furthermore, regular physical activity is believed to boost antioxidant defenses, reduce oxidative damage, and lower lipid peroxidation. These effects contribute to the prevention of cardiovascular diseases in individuals who are obese or overweight. Yanai *et al*.[19] (2018) believe that exercise therapy plays a crucial role in achieving blood sugar control in type 2 diabetes, particularly in male Wistar rats. Research has demonstrated that moderate-intensity endurance training can significantly impact the antioxidant enzyme activities in diabetic animals. A study from Bolboli and Khajehlandi *et al*[20] (2020) involving male mice revealed that after 6 weeks of moderate-intensity endurance training, the average activity of glutathione peroxidase (GPX) and superoxide dismutase (SOD) in the diabetic group increased compared to the healthy group. This finding underscores the potential of exercise to enhance antioxidant defenses in diabetic subjects. Glutathione peroxidase (GPX) is an enzyme with peroxidase activity that plays a crucial role in protecting organisms from oxidative damage. As part of the endogenous enzymatic defense system, GPX works in conjunction with other enzymes to safeguard cells against reactive oxygen species (ROS) damage.[21] Nitric oxide (NO) is a small, lipophilic molecule with a short half-life that participates in numerous biological processes. Serving as both an intracellular and intercellular messenger, NO plays a vital role in maintaining the vascular tone and is involved in various physiological processes.[22] In the context of cardiovascular diseases and diabetes, the TAC emerges as a promising biomarker for prediction and diagnosis. Alongside GPX, other antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) collectively protect blood vessels from ROS and maintain vascular function.[23] Superoxide dismutase (SOD) serves as the first line of defense against ROS, particularly during viral infections when ROS production is heightened. SOD, being a metal-containing antioxidant enzyme, plays a crucial role in cellular defense mechanisms. Nitric oxide (NO) is a gaseous molecule secreted by the endothelium, functioning as a key regulator of endothelial function. With extensive metabolic, vascular, and cellular effects, NO promotes vasodilation and plays a pivotal role in various biological processes and disorder regulations.[24] Due to its short half-life, NO values are typically low. However, its significance in cellular signaling pathways makes it a crucial molecule in tissue regulation across various organs.[25] Green coffee contains two metabolically active substances: chlorogenic acid and caffeine. Among these, chlorogenic acid (CGA) is the most prominent polyphenol in coffee, composed of caffeic acid and quinic acid.[26] CGA in green coffee reduces fat absorption in food by approximately 45% and stimulates metabolism by activating the AMPK signaling pathway.[27] Most prediabetic individuals will ultimately develop type 2 diabetes; however, this complication can be prevented through lifestyle modifications. Given the pervasive nature of diabetes in contemporary society and its associated economic and health ramifications, coupled with the scarcity of consistent research on the efficacy of endurance

training and chlorogenic acid, the present investigation aims to explore the impact of endurance training and chlorogenic acid supplementation on oxidative–nitrosative stress markers. Considering the widespread prevalence of diabetes in the present era and its economic and health consequences, what is the potential impact of endurance training and chlorogenic acid supplementation on oxidative–nitrosative stress indicators?

Methods and Materials

This study was approved by the Ethics Committee of the University of Isfahan (IR.UI.REC.1400.034) and conducted in accordance with the principles outlined in the Declaration of Helsinki. The experimental design consisted of six groups, was carried out as a pre-test (only for measuring blood sugar level) and post-test for all variables. In all, 35, 4-week-old C57BL/6 male mice were purchased from Royan Research Institute of Isfahan and kept under standard conditions with wood shavings as bedding material and maintained under controlled environmental conditions: 12-h light/dark cycle, relative humidity of 55 ± 5%, and ambient temperature of 22 ± 2°C in animal cages of Royan Institute of Biotechnology, Isfahan. Mice had free access to food and water. First, the mice were randomly divided into two groups; one group (including seven mice) received natural food from the beginning to the end, and the other group (28 mice) received a high-fat diet (including 60% fats, 20% carbohydrates, and 20% proteins) for 12 weeks to induce prediabetes.[1] After that, the pre-diabetic group was randomly divided into four groups. These groups include the prediabetes group (control group) with 10 weeks without intervention, the pre-diabetes group with 10 weeks of endurance training, the pre-diabetes group with 10 weeks of chlorogenic acid consumption, and finally, the pre-diabetic group with 10 weeks of simultaneous intervention of endurance training and chlorogenic acid consumption. Endurance training was performed on a motorized treadmill set at speeds ranging from 15 to 23 m/min for 45 min, three times per week for 10 consecutive weeks. Chlorogenic acid was administered orally via gavage at a dose of 110 mg/kg body weight, three times per week, at 8:00 AM daily. A familiarization exercise was performed at a speed of 7 m/min on a flat treadmill for 15 min. The initial training sessions commenced at this speed for 15 min per day, 5 days a week. The main endurance training protocol then began with a speed of 7 m/min on a flat treadmill for 15 min per day, 5 days a week. The intensity was progressively increased by 2 m/min every 2 weeks until reaching the maximum speed of 23 m/min. The duration of activity was gradually increased to 45 min per day over 1 week [Table 1].[28] The training protocol was incremental, with the intensity increasing by 2 m/min every 2 weeks. Supplements were administered 3 h before each training session to ensure peak absorption during exercise. Treadmill activity was performed 3 h after taking the supplement. Groups that did not undergo training intervention were placed on a stationary treadmill for 15 min, five times per week for 10 weeks to control for handling and environmental exposure. Tissue samples were collected from the quadriceps femoris muscles 24 h after the final intervention session.

Fasting blood sugar (FBS) and oral glucose tolerance test (OGTT) variables were measured in weeks 12 and 23 by collecting blood samples from the tail vein of mice and analyzing them using an Alpha TRAK glucometer from Zoetis. Blood glucose levels were measured at 0, 30, 60, 90, and 120 min after glucose injection. After completing the interventions, the mice were anesthetized with an intraperitoneal injection of ketamine and xylazine. The quadriceps muscles were excised by making an incision in front of the thigh. The tissue's total weight was recorded before being transferred to liquid nitrogen and stored in a freezer at −80°C. Enzymatic activity assays were conducted using commercially available kits: SOD (NS-15033 kit with a sensitivity of 0.2 units per mL), GSH (NS-15087 kit with a sensitivity of 1 μM), GPx (NS-15083 kit with a sensitivity of 0.5), TAC (NS-15013 kit with a sensitivity of 2 micromoles of Fe (2+)), and NO (NS-15043 kit with a sensitivity of 2.5 μM/mL) from Navand Salamat Company in Iran. Optical absorption was performed using a Synergy HTX Biotech multimode reader device made in America. Data analysis was performed using the SPSS version 25 software. The Kruskal–Wallis test was used to examine the significance of differences between groups. One-way analysis of variance (ANOVA) parametric test and Dunnett's post-hoc test were applied to compare the mean values among the experimental groups. All statistical calculations were performed at a significance level of $P < 0.05$.

Results

Table 2 presents a comprehensive overview of the final weight data, including both the total body weight and quadriceps muscle weight of mice after 23 weeks of intervention. The table provides mean values, standard deviations, and statistical significance levels for each group to facilitate a comparison between experimental conditions.

Table 1: Endurance training protocol

Weeks	Week 1	Weeks 2	Weeks 3 and 4	Weeks 5 and 6	Weeks 7 and 8	Weeks 9 and 10
Speed (m/min)	15	15	17	19	21	23
Sessions in a week	5 days	5 days	5 days	5 days	5 days	5 days
Minutes in every session (min)	45	45	45	45	45	45

Table 3 show Mean and standard deviation of factors in groups. The total body weight was measured immediately before euthanasia, whereas quadriceps muscle weight was determined post-mortem by carefully dissecting the muscles from each mouse.

Figure 1 illustrates the changes in blood sugar levels of animals after 12 weeks of being fed a high-fat diet and after 23 weeks of exercise and supplement intervention. Blood sugar levels were measured at 0, 30, 60, 90, and 120 min after glucose injection using an Alpha TRAK glucometer from Zoetis. After the initial intervention to induce pre-diabetes with a high-fat diet, a significant difference was observed in blood sugar levels between the normal and pre-diabetes diet groups ($P < 0.0006$), with an increase in blood sugar levels in the high-fat diet group. Following this period and the implementation of the main intervention (exercise and supplement), blood sugar levels in intervention groups were significantly lower than in the pre-diabetes group ($P < 0.012$). Additionally, blood sugar levels of the pre-diabetic group were significantly different from those of the normal diet group ($P < 0.05$). Statistical analysis revealed significant differences in blood sugar levels between intervention groups compared to the pre-diabetes group ($P < 0.012$). Chlorogenic acid supplementation resulted in a significant decrease in blood sugar levels ($P < 0.0002$) compared to the pre-diabetes group. Endurance exercise intervention led to a substantial reduction in blood sugar levels ($P < 0.0001$) compared to the pre-diabetes group. Furthermore, the combined effect of endurance exercise and chlorogenic acid supplementation also demonstrated a significant impact on reducing blood sugar levels ($P < 0.0002$) compared to the pre-diabetes group.

In the case of GSH, the activity of this protein decreased significantly after endurance training versus the normal state ($P = 0.010$). However, there was no significant change in the combined effect of endurance training + chlorogenic acid intake ($P = 0.097$) or chlorogenic acid intake ($P = 0.300$). The results of post-hoc analysis showed a significant decrease in this variable in the pre-diabetes state compared to the normal state ($P = 0.04$).

The GPx variable showed a significant decrease in the pre-diabetic group compared to normal ($P = 0.046$). This enzyme decreased significantly after endurance training versus the normal state ($P = 0.026$) and chlorogenic acid intake ($P = 0.007$), but there was no significant change in the combined effect of endurance training + chlorogenic acid intake ($P = 0.208$). However, the results of post-hoc analysis showed a significant decrease in this variable in the pre-diabetic state compared to the normal state ($P = 0.04$).

The NO variable showed a significant decrease in the pre-diabetic group compared to the normal group ($P = 0.046$). This enzyme did not change significantly after

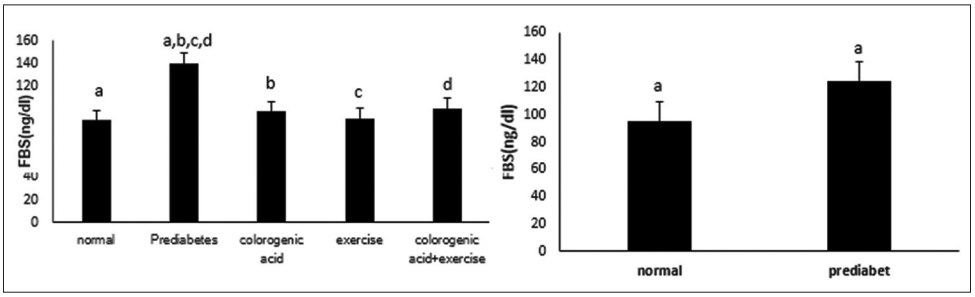

Figure 1: Changes in blood sugar levels of mice after 12 and 23 weeks of intervention. Groups with the same letters indicate significant changes at the $P < 0.05$ level

Table 2: Mean values, standard deviations, and statistical significance levels for final weight data, including both the total body weight and quadriceps muscle weight of mice after 23 weeks of intervention

Groups	Normal diet	SD	High-fat diet	SD	Endurance training	SD	Chlorogenic acid	SD	Endurance training + chlorogenic acid	SD
Quadriceps muscle weight (g)	22/0	0/03	0/23	0/03	0/13	0/02	0/19	0/03	0/21	0/03
Total weight of the mouse (g)	31/10	2/15	29/5	1/26	30/20	2/07	31/6	0/96	30/6	0/78

Table 3: Mean and standard deviation of factors in groups

Groups	NO		TAC		GSH		GPx	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chlorogenic acid	2.286	2.056	0.305	0.260	0.569	0.187	130.407	18.080
Exercise	0.803	0.211	0.326	0.033	0.346	0.010	137.181	13.083
Exercise + chlorogenic acid	1.254	0.175	0.375	0.246	0.349	0.128	223.306	28.519
Prediabetes	1.313	0.586	0.423	0.070	0.377	0.166	186.150	27.932

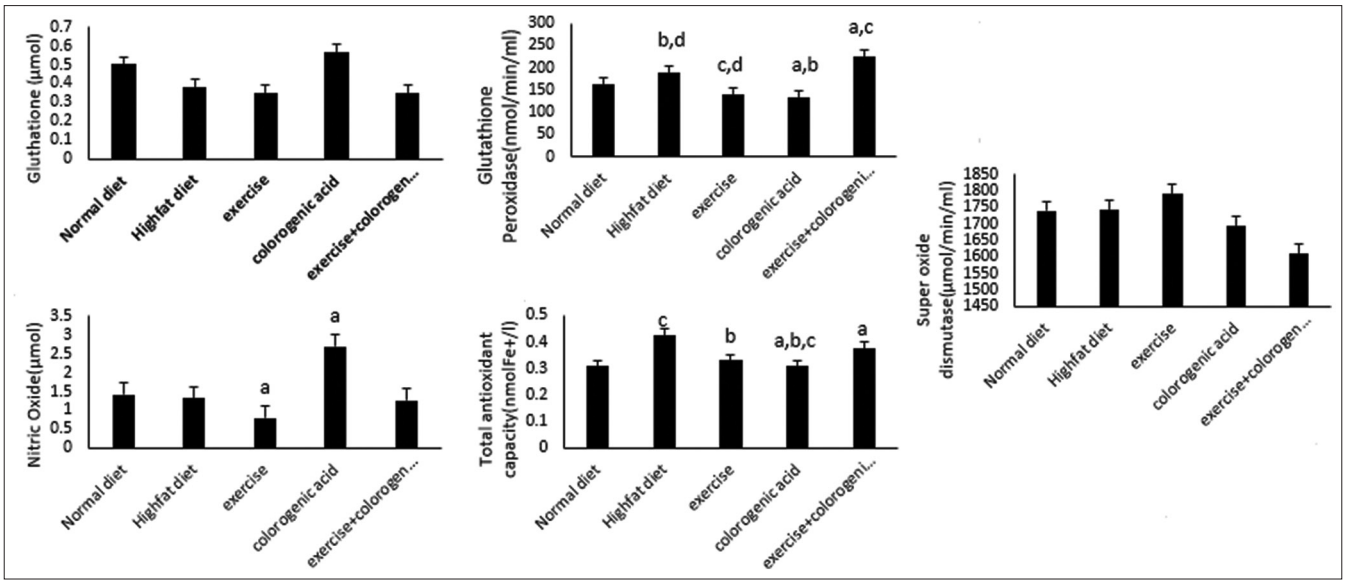

Figure 2: Changes in the levels of various variables. SOD enzyme activity did not change significantly after endurance training (*P* = 0.070), chlorogenic acid consumption (*P* = 0.094), or their simultaneous intervention (*P* = 0.558). According to the results of Dunnett's post-hoc test, there was a significant difference in the SOD enzyme activity (*P* = 1.00), but no difference between the pre-diabetes group and the normal diet group

endurance training versus the normal state (P = 0.082), chlorogenic acid intake (P = 0.211), or the combined effect of endurance training + chlorogenic acid intake (P = 0.100). However, the results of post-hoc analysis showed a significant decrease in this variable in the pre-diabetes state compared to the normal state (P = 0.04).

Finally, TAC capacity showed a significant increase in pre-diabetic conditions relative to normal conditions (P = 0.006). Chlorogenic acid consumption significantly increased the TAC (P = 0.011), but these values did not change significantly during training conditions (P = 0.063) or the combined effect of exercise training and chlorogenic acid intake (P = 0.072) compared to the pre-diabetic group. Figure 2 shows Changes in the levels of various variables (SOD, GSH, GPX, NO, TAC).

Discussion

In this study, it was shown that endurance training decreased glutathione levels and that glutathione peroxidase activity decreased significantly after endurance training and chlorogenic acid consumption. Additionally, the TAC increased significantly after chlorogenic acid intake. Research by Amar *et al.* (2020), titled "Investigation and Comparison of the Effects of Aerobic, Anaerobic, or Combined Exercises on Oxidative Stress and Antioxidant Activities in Men," aligns with the present study. The exercise protocol consisted of three random training periods (30 s Wingate anaerobic test, 30 min aerobic exercise at 60% maximum aerobic capacity, and combined aerobic and anaerobic exercises) in healthy men who had not previously exercised. Venous blood samples were collected at minutes 0, 5, 10, and 20 after each session. The levels of MDA, antioxidant activities (such as glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione reductase (GR)), and total antioxidant status (TAC) were evaluated. Regardless of the type of exercise, plasma levels of SOD, GPX, MDA, and GR increased above baseline values. In comparison to combined exercise, aerobic and anaerobic training produced faster responses for most parameters. Only plasma TAC content increased after aerobic exercise. The highest MDA response was recorded under anaerobic conditions. The highest GPX and SOD responses were recorded under both anaerobic and aerobic conditions. Statistics *et al.* concluded that aerobic, anaerobic, or combined exercises have the ability to significantly increase oxidative stress and antioxidant activities.[29] Yanai *et al*.[19] (2018) conducted a study titled "Therapeutic Exercise for Type 2 Diabetic Patients," which aligns with the present study. Male Wistar rats were divided into six groups. It was shown that aerobic exercise effectively controlled blood sugar levels in mice. The findings indicate that muscles activated by aerobic exercise extract energy from adenosine triphosphate (ATP) derived from amino acids, carbohydrates, and fatty acids through aerobic metabolism. Consequently, exercise can be utilized as a therapeutic approach to achieve blood sugar control in patients with type 2 diabetes. Bolboli and Khajehlandi (2019)[20] conducted a research titled "Comparative Study of the Effect of Endurance Training on Glutathione Peroxidase and Superoxide Dismutase Enzyme Activity in Heart Tissue of Healthy and Diabetic Rats," which aligns with the present study. Twenty-four male desert rats were placed in four groups of six. These groups included diabetic (DT), control (DC), trained healthy (HT), and healthy control (HC).

The rats performed moderate-intensity endurance training for 6 weeks, and their heart tissue was extracted 24 h after the last training session. The activity of glutathione

peroxidase and superoxide dismutase enzymes was measured. After 6 weeks of endurance training, there was a significant difference in the average concentration of glutathione peroxidase between the diabetic group and the healthy group. Additionally, there was a significant increase in the average concentration of superoxide dismutase in the diabetic training group compared to the control diabetic group. Moderate-intensity endurance training demonstrated significant effects on the cardiac antioxidant system in diabetic rats, indicating that physical training could be beneficial in preventing cardiovascular complications resulting from diabetes (92).

The research by Zavagari *et al.* (2018)[30] titled "Investigating the Impact of Endurance Training on Preventing the Adverse Effects of One Session of Exhaustive Exercise in Desert Rats" is also relevant to this study. It shows that exhaustive exercise increases oxidative damage, whereas endurance training can reduce the adverse effects of these exercises and sudden stress. In this study, 40 male Wistar rats were placed in two training and control groups. The training group performed endurance training at 75–60% of maximal oxygen consumption for 5 days a week for 8 weeks. After 8 weeks, both training and control groups were divided into two subgroups, and one subgroup from each group performed exhaustive exercise. In this research, malondialdehyde activity and the TAC were measured. Malondialdehyde activity showed only a significant increase in the exhaustive exercise group. Furthermore, there was a significant difference in the TAC between the endurance training plus exhaustive exercise group, the control plus exhaustive exercise group, and the control group without exhaustive exercise (88).

It is likely that after adaptations due to regular training, the transcription factor peroxisome-activated receptor gamma (PGC1α) is activated. PGC1α is a key regulator in mitochondrial biogenesis and can activate a group of transcription factors that lead to increased biogenesis. PGC1α also plays a role in skeletal muscle angiogenesis. With the increase in PGC1α caused by regular exercise, the mitochondrial content in skeletal muscles and the rate of muscle metabolism increase, resulting in an increased need for blood flow in the muscles. This leads to improved oxygen delivery and nutrient uptake during exercise. Exercise may increase insulin sensitivity in skeletal muscles and regulate glucose homeostasis through the reduction of inflammatory markers and an increase in the mRNA content of glucose transporter protein (GLUT).[31] The possible mechanism for physical activity involves the activation of PGC-1 and AMP in the skeletal muscles.[32] Physical activity improves the release and transport of glucose and oxidation of fatty acids through mitochondrial biogenesis and an increase in beta-oxidation enzymes. Physical activity also affects fat tissue and can increase body metabolism and proteolysis. It is possible that physical activity affects mitochondrial biogenesis, cell stress, and apoptosis. Physical activity not only affects visceral fat tissue but can also affect subcutaneous fat tissue. Therefore, physical activity has an effect on weight loss by affecting the body's fat reserves. Physical activity may also affect oxidative stress by increasing ATP consumption, creating adaptation in the body, and increasing the activity of oxidative enzymes. It is also likely that physical activity affects oxidative stress by increasing ATP consumption, creating adaptation in the body, and increasing the activity of oxidative enzymes.[33] In this study, it was shown that endurance training, chlorogenic acid consumption, and their simultaneous effect had no effect on SOD levels. The possible reason for an increase in SOD during exercise is that a reduction in the antioxidant defense system accelerates the production of new free radicals. Oxidative stress damages the structure and function of beta cells.[34] Several metabolic pathways contribute to diabetes complications, including the polyol pathway. Normally, the polyol pathway has minimal activity, but under hyperglycemic conditions caused by diabetes,[35] it becomes overactive. This leads to increased production of other types of reduced sugars such as glucose-6-phosphate and fructose through this pathway and glycolysis, ultimately resulting in elevated SOD levels. In this study, SOD levels did not show a significant change, whereas in other research, an increase was observed. The discrepancy between these findings may be attributed to differences in the training intensity, training protocol, subject type, and tissue examined. In contrast, C57BL6 mice in another study ran on a treadmill for 4 weeks, 3 times a week at low intensity. Nitric oxide is an unstable messenger molecule and a vasorelaxant agent derived from endothelium. It is synthesized in the body from the amino acid L-arginine under the influence of the enzyme nitric oxide synthase (NOS).

During exercise, blood pressure decreases due to the reduced catecholamine activity and decreased peripheral resistance to blood flow.[36] Physical activity may accelerate sodium excretion from the kidneys, leading to a reduction in the fluid volume and subsequently lower blood pressure.[37] Adaptations caused by physical activity include increased capillary density in active skeletal muscles,[38] enhanced cardiac output,[39] reduced vascular resistance due to increased distensibility, decreased resistance to blood flow, lowered environmental resistance, and heart rate during rest and activity, improved nervous regulation of blood vessels, and enhanced dilation of blood vessels. These adaptations result in an increased transverse surface area of cavities and improved blood vessel dilation, facilitating better removal of waste products during exercise and more effective blood pressure control. Nitric oxide plays a crucial role in the expression of the inducible nitric oxide synthase gene (iNOS) in response to inflammation.[40] It may activate NF-κB in mononuclear cells, leading to an increased production of nitric oxide from L-arginine.[41]

Conclusions

This study demonstrated that endurance training and chlorogenic acid intake affect GSH, GPx, and TAC levels in pre-diabetic mice. The findings suggest that combining endurance training and chlorogenic acid supplementation could potentially serve as a dual approach to prevent and manage diabetes, mitigate economic and health-related issues, inhibit the progression of oxidative damage, and enhance oxidative capacity. These potential benefits underscore the promising role of lifestyle interventions and dietary supplements in addressing diabetes-related challenges. Further research is warranted to fully elucidate the mechanisms underlying these effects and to explore the long-term implications of this combined approach for diabetes management and prevention.

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Conflicts of interest

There are no conflicts of interest.

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