

Effects of High Intensity Interval Training and Curcumin Supplement on Glutathione Peroxidase (GPX) Activity and Malondialdehyde (MDA) Concentration of the Liver in STZ Induced Diabetic Rats

Abdol-Majid Emami^{1*}, Hasan Matin Homae², Mohammad Ali Azarbayjani³

1. PhD student of Biochemistry and Exercise Metabolism, Islamic Azad University, Central Tehran branch, Tehran, Iran.
2. Assistant Professor, Islamic Azad University, Central Tehran branch, Tehran, Iran.
3. Professor, Islamic Azad University, Central Tehran branch, Tehran, Iran.

*Correspondence:

Abdol-Majid Emami, Biochemistry and Exercise Metabolism, Islamic Azad University, Central Tehran branch, Tehran, Iran.

Tel: (98) 913 357 9764

Email: majid.emami2@yahoo.com

Received: 22 March 2017

Accepted: 04 June 2017

Published in July 2017

Abstract

Objective: The purpose of the present study was investigation of eight weeks high intensity interval (HIIT) along with curcumin supplementation on liver Glutathione Peroxidase (GPX) Activity & Malondialdehyde (MDA) Concentration among diabetic male rats.

Materials and Methods: Thirty-two male mice were divided to four groups of control-diabetic, exercise-diabetic, supplementary-diabetic, and exercise-supplementary-diabetic. Eight weeks' aerobic training schedule was five weekly sessions for the two exercise groups, which each session executed on animal treadmill with speed of 24 (m/min), steep of 0°, and entire span of 18 min. The two supplementary groups were being fed daily amount of 50 (mg/kg) curcumin supplementary, by using the gavage technique. The rats were killed and liver tissues were separated, after the eight weeks' schedule. Thereafter, in order to study variations of GPX and MDA enzymes, Kits of Zelbio Co. were implemented.

Results: Activity levels of GPX enzyme and MDA concentration of the three groups of exercise-diabetic, supplementary-diabetic, and exercise-supplementary-diabetic in order increased and decreased than those of the control-diabetic one, significantly ($P \leq 0.05$).

Discussion: both exercise and curcumin can be recommended as effective interventions on improvement of Glutathione Peroxidase (GPX) Activity & Malondialdehyde (MDA) Concentration of the liver, in diabetic condition.

Keywords: GPX, MDA, Curcumin, HIIT, Diabetic male rats

Introduction

Diabetes prevalence is increasing throughout the world. According to the World Health Organization (WHO), the number of diabetic patients will reach to at least 366,000,000 persons, until 2030 (1). Diabetes prevalence is growing in both developing and developed countries (1).

The high concentration of glucose causes adverse endothelial effects among diabetic patients. Indeed, production of free-radicals and oxidative stress induce adverse endothelial performance that is relevant to hyperglycemia (2). The oxidative stress due to reactive oxygen species (ROS), which is created by

hyperglycemia, is one of the major focuses of the recent diabetic studies. There are various natural antioxidants that neutralize oxygen free-radicals and protect different parts of the cell from oxidative damages. Some of those antioxidants are enzyme (intracellular) ones, which include superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). The attitude of "new antioxidant" includes the restriction of free-radical's production and the enhancement of intracellular antioxidant protection (2), which is a different concept from the previous one that has only counted antioxidant action as cleaning-up free-radicals, before their production (2). According to the recent studies, the new antioxidants have been recognized as an effective issue on oxidative stress, especially on the manner of their production, through generating the equilibrium between free-radical's production and antioxidant defense (2). Although, the benefits of regular exercise on the health have been well demonstrated, few people follow those advices to perform physical activity. "Lack of time" is often mentioned, as one of the common problems versus performing regular exercise (3). Therefore, aerobic trainings were designed as high intensity interval trainings (HIIT). The recent studies showed the advantages of HIIT trainings with low volume in comparison with continuous and moderate intensity aerobic trainings.

Taking new medicine or protective compounds, especially natural antioxidants derived from plants, has a substantial weight. The Curcumin is extracted from turmeric. This herb is in the family of zingiberaceae (ginger). The protective properties for the liver were recognized (4).

Curcumin (or named as diferuloylmethane; (1E,6E)-1, 7-bis (4-hydroxy- 3-methoxyphenyl) -1, 6- heptadiene-3,5-dione) is yellow bright powder and it is the principal active ingredient of turmeric, which is considered as a high consuming Indian spice. The curcumin and other organic based compounds can prevent the occurrence of insulin resistance, through

various mechanisms. Consequently, curcumin can prevent from development of diabetes and complications of that disease, among people at the risk of developing diabetes (5). Fujiwara et al have investigated glucose generation in hepatic cells. After 120 min curcumin exposing, gluconeogenesis and glycogenolysis of isolated hepatocytes and also activity level of gluconeogenesis enzymes was measured. It has been cleared that the glucose generation of those cells would decrease the amount of 25 (μ molar), in the presence of curcumin. The reason of that issue was activation of AMP kinase, inhibition activity of glucose 6-phosphatase (G6Pase) and Phosphoenolpyruvate carboxykinase (PEPCK) enzymes (6).

Osawa has surveyed the effects of the above mentioned compound on hyperglycemia consequences. Some related problems of hyperglycemia are glucose autoxidation, glycation of proteins, and activating the polyol metabolic pathway. Those changes can lead to increase in ROS production and chemical variations of oxidative lipid, DNA, and proteins. Oxidative stress is important in development of diabetes complications such as nephropathy and neuropathy. Curcumin and tetrahydrocurcumin of turmeric would lead to ROS deduction and increase in glutathione concentration (as a fighting compound against oxidative stress) (7).

Another group of researchers have studied diabetic mice treated with curcumin, and concluded that curcumin would cause increment of the amount of insulin, increase in glucokinase activity of the liver, and decrease in activities of G6Pase and PEPCK. Those researchers have noted that curcumin would not lead to hypoglycemia and antioxidant, among non-diabetic mice (8).

The purpose of the present study was investigation of eight weeks high intensity interval (HIIT) along with curcumin supplementation on liver Glutathione Peroxidase (GPX) Activity & Malondialdehyde (MDA) Concentration among diabetic male rats.

Materials and Methods

This clinical trial was conducted in the faculty of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran (International Branch), 32 adult Wistar mice between 302-382 gr were studied. Diabetes mellitus induced by intraperitoneal injection of a single dose streptozotocin (STZ), dissolved in normal saline (55 mg per kg weight of rats). In order to confirm diabetes, fasting glucose levels were measured, four days after the injections. Blood sample were taken from mice-tails and glucose levels were measured by glucometer. Glucose level of 14 (mmol/l) was set as diabetic index. The mice were randomly divided to four groups of control (placebo), exercise, supplementary and exercise-supplementary. The rats were maintained in separated cages, in a manner of 12 hrs light/12 hrs darkness, at temperature domain of 22 ± 3 °C, and at relative humidity range of 40-50 %. The rats were fed by standard mice food packages (containing chewing calcium & phosphor seeds) and water free access. The exercise and exercise-supplementary groups were participated in eight weeks HIIT (five weekly sessions), which were consisted of running with intensity of 90 to 95 percentage of VO_2 Max on 10 lines animal treadmill with slope of 0° (3 min warm-up with speed of 5 (m/min), within entire session span of 18 min. Before the eight weeks' trainings period and in order to make the exercise mice familiar with the treadmill, those rats run on the treadmill in some orientation sessions, with span of 10 min, speed of 5 (m/min), and slope of 0°, during one week. Those treadmill orientation sessions had gradually turned to those of with 10 (m/min) speed, 0° slope, and 15 min span, toward the ending of the orientation period. The two supplementary groups took daily 50 mg curcumin supplementary per kg weight of

rats (daily consumption range of 302-382 gr, according to average weight of rats), by using the gavage technique, during the eight weeks. In order to investigate activity levels of SOD and CAT enzymes, the mice were anesthetized in Chloroform chamber. Then, liver tissues were separating and examined by kits of Zelbio Co. (made in Germany). Based on the design principles of HIIT schedules, in order to maximize the cardio performance (both oxygen uptake and oxidative capacity of skeletal muscles) the HIIT schedule was designated with intensity of almost 90 to 95 percentage of VO_2 Max, 2-4 min span, and 2-3 min active recovery time (9). The data (derived from tissue examination) were analyzed using the two-way ANOVA on independent variables, by application of SPSS (version 16), and at the significance level of 0.05. In addition, the Figures were sketched by Excel. Table 1 demonstrates the HIIT protocol of the present study (3).

Results

The results of the present study indicated a significant effect of exercise on GPX activity level, which leads to elevate its level ($M=225.13$, $SD=7.44$), ($P\leq 0.05$). In addition, the effect of curcumin on the activity level of GPX was significant ($M=195.43$, $SD=20.52$), ($P\leq 0.05$). In addition, the effect of exercise along with curcumin supplementation on the activity level of GPX was significant ($M=276.71$, $SD=4.28$), ($P>0.05$), (figure 1). The present results showed a significant effect of exercise on MDA concentration level, which leads to attenuate its amount ($M=31.97$, $SD=22.34$), ($P\leq 0.05$), (diagram 2). Also, the effect of curcumin on MDA concentration level was significant, ($M=34.38$, $SD=45.11$), ($P\leq 0.05$). In addition, the effect of exercise along with curcumin supplementation on

Table 1. HIIT protocol

Exercise* Steps Exercise Element	Warm-up	Main Part of Exercise		Cool-down
		High Intensity Interval (Four sets of 2 min)	Low Intensity Interval (1 min active rest)	
Span (min)	3	8(4 Set- 2 Minute)	2	5
Intensity (VO_2 Max) (%)	50-60	90-100	50-60	50-60

*Slope of treadmill was 0° throughout the exercise steps

MDA concentration level was significant (M=21.28, SD=17.86), ($P \geq 0.05$).

Discussion

The results of the present study indicated that taking curcumin accompany with eight weeks HIT would lead to an elevation and another attenuation in activity level of GPx and MDA concentration. It was identified the activity levels of GPx in training, supplementary, and training + supplementary groups increased more than control group, significantly. In the

other word, the elevation of power and capacity of the antioxidant system was observed. Hence, the prominent hand of nutritional supplements for proficiency enhancement and enrichment of the endogenous antioxidant system against oxidative stress is patent, during training and some diseases such as diabetes.

Some studies indicate the effects of exercise with supplementary consumption on enrichment of the antioxidant system of the body (10). Barbara et al (2006) observed a

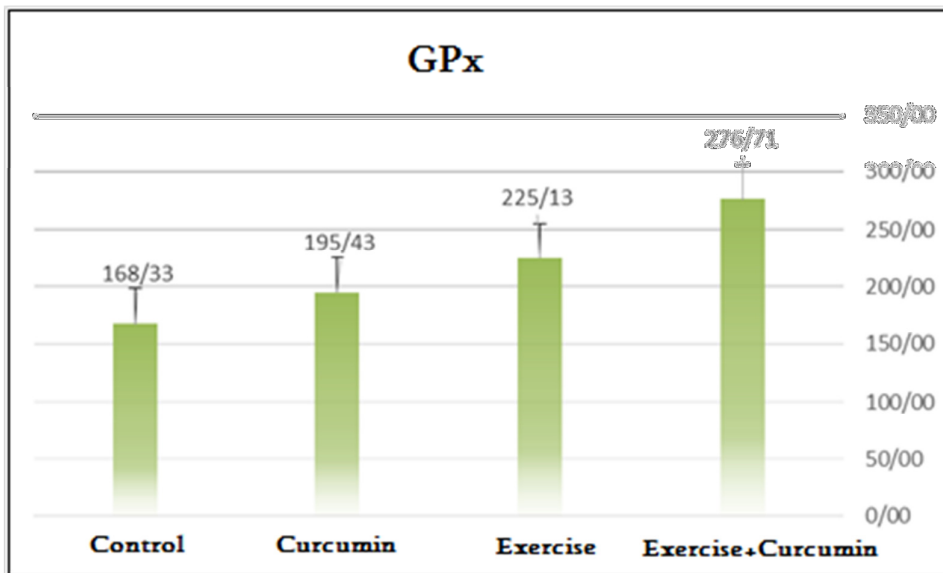


Figure 1. GPx activity levels, following eight weeks HIT

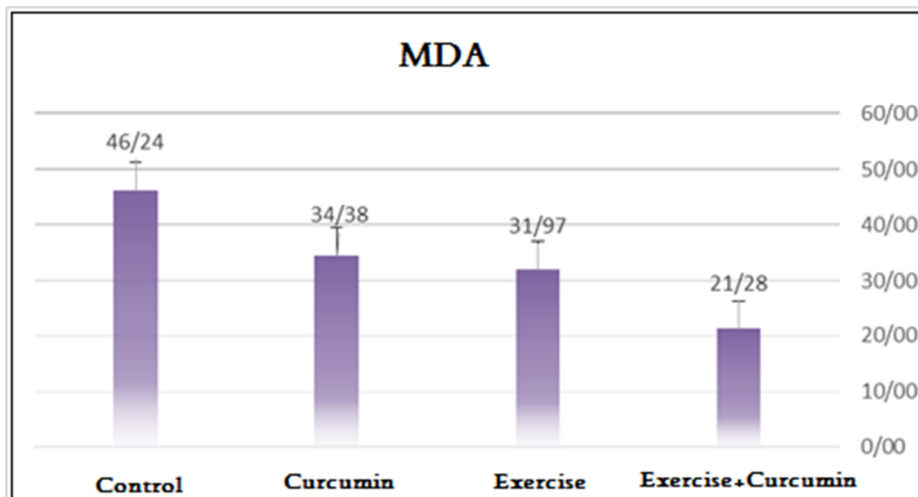


Figure 2. MDA Activity levels, following eight weeks HIT

significant elevation in the GPX activity level of trained old mice than untrained rats (either young or old), after a running schedule (five 45 min weekly sessions with simultaneous supplementary consumption, during six and eight weeks (11). Some researchers declared lipid peroxidation is one of the harmful effects of free radicals attack on cell membrane, which might occur after exhaustive exercise (12,13).

Intensity and span of exercise are paramount parameters that influence approach of exercise on oxidative stress indices and antioxidant status of the body (14). Several studies were done about effects of various exercises on oxidative stress and the body's antioxidant defense. Though, there is no congruence between their results. Those incongruities are rather evident, when various exercises with different intensities are compared to each other (15,16,17,18).

The results denote animals (three months male rats), those had regular daily exercise sessions, revealed less amounts of MDA within their active tissues, in comparison with control (19). In order to justify those incongruous results, it could be denoted oxidative stress indices did not show any significant change, in researches, in which trained subjects performed exercises with moderate intensities. Even, some of studies showed reductions in oxidative stress (20). Referred incongruous results to different laboratory methods for measuring MDA index (21). chevin et al (2003) stated that the oxidative stress response to exercise is affected to the other parameters such as health situation, age, gender, physical readiness condition, span and intensity of exercise, nutrition, duration and manner of measurement, besides oxygen delivery to tissues (22). Thus, the absence of any significant increase in lipid peroxidation

(MDA) cannot be interpreted as the absence of oxidative stress process. It can be expressed the generated reactive oxygen species have been vanished by anti-oxidative system of the body. Overall, further intensity and longer span of exercise would lead to increase in lipid peroxidation (23).

Conclusions

Due to the significant diversities in subjects, types, intensities and spans of exercises, durations of trainings periods and supplements doses of most relevant studies, surveying the effects of exercise training with curcumin on antioxidant enzymes is a controversial issue. Hence, the appearance of contradicted results about activity levels of antioxidant enzymes, between diverse studies, is not an unexpected matter. Generally, the results of the present study have indicated that either HIT or curcumin could solely effect on activity levels of antioxidants enzymes, in diabetic rats. Though, those two interventions do not have any synergetic effect, in combination. Therefore, both exercise and curcumin can be recommended as effective interventions on improvement of antioxidant performance of the liver, in diabetic condition.

Acknowledgement

This research is a part of a doctoral thesis in the field of exercise physiology, which has been approved by the faculty of Physical Education and Exercise Sciences in Islamic Azad University (Central Tehran branch). We would like to specifically thank the Animal Clinic of Yazd University of Medical Sciences (International Branch). We would also like to show our gratitude to other good people, who helped and assisted with us, for accomplishment of the present study.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 2004;27(5):1047-53.
2. Ceriello A, Testa R. Antioxidant anti-inflammatory treatment in type 2 diabetes. *Diabetes care*. 2009;32(2):232-6.

3. Gibala MJ, Little JP, MacDonald MJ, Hawley JA. Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of physiology*. 2012;590(5):1077-84.
4. Corson TW, Crews CM. Molecular understanding and modern application of traditional medicines: triumphs and trials. *Cell*. 2007;130(5):769-74.
5. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. In the molecular targets and therapeutic uses of curcumin in health and disease 2007;1-75.
6. Fujiwara H, Hosokawa M, Zhou X, Fujimoto S, Fukuda K, Toyoda K, et al. Curcumin inhibits glucose production in isolated mice hepatocytes. *Diabetes research and clinical practice*. 2008;80(2):185-91.
7. Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. *Annals of the New York Academy of Sciences*. 2005;1043(1):440-51.
8. Seo KI, Choi MS, Jung UJ, Kim HJ, Yeo J, Jeon SM, et al. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. *Molecular nutrition & food research*. 2008;52(9):995-1004.
9. Holman RR, Turner RC. Oralagents and insulin in the treatment of NIDDM. In: Pickup J, Williams G. (Eds). *Textbook of Diabetes*. Blackwell, Oxford. 1991.: 467-469.
10. Robertson JD, Maughan RJ, Duthie GG, Morrice PC. Increased blood antioxidant systems of runners in response to training load. *Clinical science*. 1991 Jun 1;80(6):611-8.
11. Barbara R, Usberti M, Gerardi GM, Micheli AM. Exercise training affects age-induced changes in SOD and heat shock protein expression in rat heart. *J. Experimental Gerontology*. 2006;41:764-70.
12. Afzalpour ME, Gharakhanlou R, Gaeini AA, Mohebbi H, Hedayati M, Khazaei M. The effects of aerobic exercises on the serum oxidized LDL and total antioxidant capacity in non-active men. *CVD prevention and control*. 2008 Apr 30;3(2):77-82.
13. Brites F, Travacio M, Gambino G, Jaita G, Verona J, Llesuy S, Wikinski R. Regular exercise improves lipid and antioxidant profile. *Atherosclerosis*. 2000;151(1):261.
14. Ashton T, Rowlands CC, Jones E, Young IS, Jackson SK, Davies B, et al. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. *European journal of applied physiology and occupational physiology*. 1998;77(6):498-502.
15. Alessio HM, Nagy S, Byrnes R, Philip B, Hagerman AE, Wiley RL. Effects of physical activity or exercise on cardiovascular parameters and oxidative stress in rats. *Medicine & Science in Sports & Exercise*. 2002;34(5):81.
16. Chicco AJ, Hayward R, Schneider CM, Turner RT, Westerlind KC. Both endurance and resistance exercise training attenuate ethanol-induced cardiac oxidative stress. *Medicine & Science in Sports & Exercise*. 2003;35(5):119.
17. Goldfarb AH, Bloomer RJ, McKenzie MJ. Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Medicine and science in sports and exercise*. 2005;37(2):234-9.
18. Jamurtas AZ, Fatouros IG, Deliconstantinos G, Viliotou V, Fotinakis P, Magiria T, et al. Chronic endurance and resistance exercise effects on oxidative stress and antioxidant status of inactive older adults. *Medicine & Science in Sports & Exercise*. 2003;35(5):96.
19. Kelle M, Diken H, Şermet A, Atmaca M, Tümer C. Effect of exercise on blood antioxidant status and erythrocyte lipid peroxidation: Role of dietary supplementation of vitamin E. *Turkish Journal of Medical Sciences*. 1999;29(2):95-100.
20. Cooper CE, Vollaard NB, Choueiri T, Wilson MT. Exercise, free radicals and oxidative stress. 2002;280-5.
21. McBride JM, Kraemer WJ. Free Radicals, Exercise, and Antioxidants. *The Journal of Strength & Conditioning Research*. 1999;13(2):175-83.
22. Chevion S, Moran DS, Heled Y, Shani Y, Regev G, Abbou B, et al. Plasma antioxidant status and cell injury after severe physical exercise. *Proceedings of the National Academy of Sciences*. 2003;100(9):5119-23.
23. Schneider CD, Barp J, Ribeiro JL, Belló-Klein A, Oliveira AR. Oxidative stress after three different intensities of running. *Canadian journal of applied physiology*. 2005;30(6):723-34.