ORIGINAL ARTICLE

Effects of High Intensity Interval Training and Curcumin Supplement on Antioxidant Enzyme in Heart Tissue of Diabetic Rats

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Abstract

Objective: Exercise and herbs can possess synergistic effects to control diabetes side-effects. The purpose of the present study was to investigate the effects of high intensity interval training (HIIT) and curcumin supplement on activity levels of superoxide dismutase (SOD) and catalase of heart tissue in diabetic rats.

Materials and Methods: Thirty-two male Wistar rats were randomly divided in to four groups of curcumin, training-placebo, training-curcumin and control. The subjects were trained to do HIIT on treadmill and in the fashion of increasing manner. Diabetes was induced via injection of 50 (ml/Kg rat weight) STZ. Fifty (ml/kg rat weight) curcumin was nourished to the animals of the curcumin groups, by using the gavage technique during eight weeks (five days a week). The rats were anesthetized via intraperitoneal injection of ketamine, 24 hrs after the intervention. Then heart tissues were separated from bodies of the rats to examine changes in SOD and catalase enzymes.

Results: Activity levels of SOD and CAT enzymes of the three groups of curcumin, training-placebo, and training-curcumin increased significantly more than control group. But no significant difference was observed between those first three groups ($P \leq 0.05$).

Discussion: The results of the present study indicated that either HIIT or taking curcumin supplement could solely contribute significant enhancement to activity levels of antioxidant enzymes within heart tissues of diabetic mice. Though, those two interventions would not strengthen the influences of each other.

Keywords: Superoxide dismutase, Catalase, Curcumin, High intensity interval training, Heart, Diabetes mellitus

Introduction

Diabetes is an important metabolic disorder which involved a large population of the world people. The prevalence of diabetes is estimated about three hundred millions people, until 2050 (1). Cardiovascular diseases have been recognized as the leading cause of deaths in 8% diabetic patients (2). Diabetes can progress cardiac hypertrophy, ischemic injuries, heart failure and stroke eventually (4). In addition,

cardiovascular disorders which are originated from metabolic diseases such as diabetes mellitus may suffer older patients (2,3).

The increase of blood glucose accompanies with increment of oxidative stress which would lead to many complications of diabetes mellitus. Increases in serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes following damage tissues like heart increases the levels of some markers, such as Malondialdehyde (MDA; as an authentic index of oxidative stress), and decreases in levels of antioxidant enzymes such as superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT) (5).

Coronary artery disease (CAD), myocardial infarction (MI) and heart failure are the leading cause of deaths among diabetic patients. Acute myocardial infarction (AMI) would cause myocardial ischemia and consequently lead to myocardial necrosis and generation of reactive oxygen species (ROS). Markers of oxidative stress of MI (such as MDA, CAT, SOD and GPx) increase in diabetic patients in comparison with non-diabetic patients. The results of a study have indicated the increment of antioxidants of diabetic patients might contribute to reduction of diabetes-induced oxidative stress (6).

Curcumin is the principal ingredient of turmeric. Turmeric is a member of the ginger family (Zingiberaceae) and its protective effects on the liver was defined (7). Curcumin ((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) is a bright yellow pigment spice. Curcumin and other natural-based ingredients can prevent insulin resistance and consequently prohibit diabetes progress and its complications in people at risk of diabetes (8).

Exercise could remarkably decrease MDA level in heart tissue and blood sample (10). In addition, exercise would enhance SOD and GOx within heart tissue (25). Diabetes would lead to significant increases in MDA, nitrite levels and insignificant reduction in activity level of SOD in heart. High dose of curcumin would significantly decrease levels of the both mentioned enzymes and insignificantly reduce activity level of SOD in heart (10-11).

Several studies investigated effects of various training schedules which were mostly about aerobic trainings. The benefits of exercise on health are clear but few people do regular exercises. Among many pleas of ignoring exercise “lack of time” is often excused (12). The recent understanding about advantages and effectiveness of low-volume high-intensity interval training (HIIT) can aid to overcome that obstacle. HIIT would significantly decrease subcutaneous fat, especially abdominal fat (14) and the body fat percentage (BFP) (15-16), increase the amount of VO_2 Max (15) and even improve insulin sensitivity (17). It was reported HIIT consumes more calories than moderate-intensity continuous trainings and would enhance post-exercise lipid oxidation. In addition, HIIT does own greater amounts of energy expenditure than continuous trainings. Effects of such trainings on oxidative factors of heart and antioxidant enzymes certainly require further studies.

The process of increases in the levels of antioxidant enzymes of heart is recognized as an inhibitive mechanism, to control diabetes-induced heart damages. Past studies showed exercise could be effective on recent mentioned matter especially the aerobic ones. In the other hand, taking herbs like curcumin was recommended due to its antioxidant influences. Previous researches have emphasized on long-term endurance trainings which might nowadays hold minor importance, according to the lack of time.

Therefore, the purpose of the present study was investigation of the effects of HIIT and curcumin supplement on oxidative properties and antioxidant enzymes in heart tissue of diabetic rats.

Materials and Methods

The present research was conducted in animal laboratory of Shahid Sadoughi University of Medical Sciences (The International Branch), in March 2016. Thirty-two adult Wistar rats between 302 to 382 gr were selected. Diabetes induced via single dose of STZ dissolved in normal saline (55 ml/kg rat weight) through intraperitoneal injection. Fasting glucose levels were assessed four days after the injection. Blood samples were taken from tails of the mice. Blood glucose levels were measured by glucometer. Blood glucose level of 300 (mmol/dl) was considered as diabetes index (19). The mice were randomly divided into four groups of curcumin, training-placebo, training-curcumin, and control. The rats were kept in separated cages and in environmental condition of 12 hrs illumination/12 hrs dark, temperature of 22±3 °C and relative humidity of 40 to 50%. Their nutrition had being carried out through pelleted food packages that were provided in a standard fashion (contain chewing seeds, consisted of calcium and phosphor) and free access water.

The training-placebo and training-curcumin groups participated in eight weeks HIIT (five weekly sessions) which involved running on a 10 lines animal treadmill with treadmill slope of 0°, intensity of 80% VO_2 Max (after three min warm up with speed of five (m/min)) and spans sessions of 10 to 20 min (Table 1). In order to make the training groups familiar with the treadmill, those mice had being forced to run on the device, with speed of 5 (m/min), slop of 0°, and span of 5 min. The treadmill orientation training sessions had gradually increased to speed of 10 (m/min), spans sessions of 15 min, at the ending of the orientation period. Though, the treadmill slope remained 0°. The rats of the two supplementary groups had being fed on daily amount of 50 (mg/ kg rat weight) curcumin supplement (based on mean weight of the rats) by using the gavage technique, during the eight week. In order to examine variation of SOD and CAT enzymes, the rats were anesthetized with ketamine/xylazine containing mixtures (80 ml ketamine to 10 ml xylazine per kg rat weight), 24 hrs after ending of the training period. Then, heart tissues were separated from the bodies and the measurements were carried out by using ZellBio (made in Germany) kits.

The statistical analysis was done via two-ways ANOVA at significance level of 0.05, utilizing SPPS version 22.0. Besides, the relative diagrams were drafted by Excel.

Results

The results of the present study have indicated HIIT has a significant influence on SOD concentration in heart tissue after eight weeks ($P \leq 0.05$). Curcumin supplement has significant influences on increase of SOD concentration both in the curcumin groups and the training-curcumin one ($P \leq 0.05$). There was no significant difference between exercise influence and that of curcumin, on SOD concentration of heart tissue ($P \leq 0.05$). Concentration of SOD in training-curcumin group has significantly increased, which denoted the influences of training and curcumin on SOD concentration (but not a synergetic effect). The evolution of mean increments of the three groups has indicated that there was no significant difference between the three intervention groups of curcumin, training-placebo, and training-curcumin ($P > 0.05$) (figure 1 and table 2).

Our findings indicated that training has a significant influence on CAT concentration in

Table 1. Protocol of HIIT[ф]

Exercise Element \ Exercise Step	Warm up	Main step (3 intervals) HIIT[φ]	Cool down
Span (min)	3 min	10 to 20 min	5 min
Intensity (% VO_2 Max)	50 to 60%	80% averagely (10% weekly increase)	50 to 60%

[ф]Slop of the treadmill remains 0°, during the entire steps/stages of the training

[φ]From four 2 min sets at the 1st weeks to eight 2 min sets at the 8th week, and with 1 min interval rest span between each two successive sets

heart tissue after eight week. HIIT effect on concentration of CAT and enhance its level, significantly ($P \leq 0.05$). Also, Curcumin does own a significant influence on CAT concentration and would effect on concentration of CAT and increase its level, significantly ($P \leq 0.05$). In addition training-curcumin do possess a significant influence on CAT concentration. Though, there was not observed any significant difference, between the three interventions ($P > 0.05$) (figure 2 and table 3).

Discussion

Our purpose was to investigate the effect of eight weeks HIIT in companion with taking curcumin on antioxidant enzymes of heart tissue (SOD & CAT). Many studies showed the protective effects of regular exercise on cardiovascular diseases prevention. Influential cellular mechanisms of those affirmative effects were not appropriately regained yet. The induced damage tissue with free radicals on development of diabetes complications were noticed (20). Moreover, increase in ROS and dysfunction of antioxidant situation was shown in clinical and experimental studies (21).

The present results indicated HIIT did own significant effects on concentration levels of SOD and CAT. The present results are in confirmation with Oláh et al (2015), Naderi et al (2015), and some others (9,10). Our findings indicated that the training-induced increases in tissue concentrations of SOD and CAT express either the glycation reduction in antioxidant enzymes or decrease in the accumulation of advanced glycation end-products (AGEs) within heart tissue were originated from exercise (20). Furthermore, chronic effects of either resistance training or running on a rotating wheel on decrease in oxidative stress index and increase in antioxidant activity were reported (21). The recent mentioned issue is another approval to the cardio-protective role of exercise for handling stressful conditions of diabetes. Various mechanisms interfere in the cardio-protective effects which might partly be mediated through oxidation-reduction (redox) variations and could consist of the induction of heat shock proteins (Hsps), development of

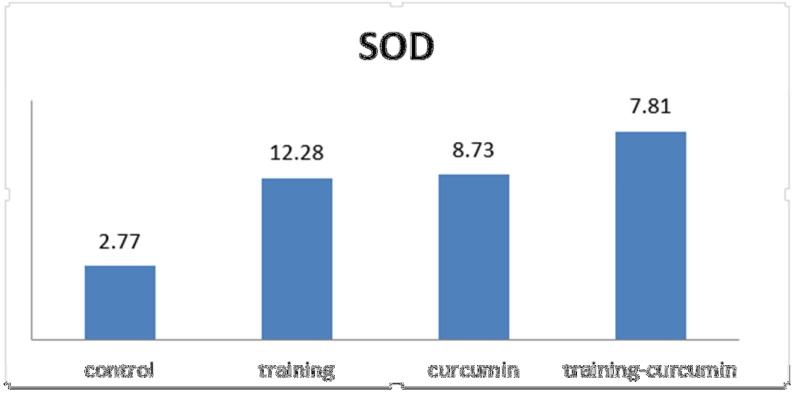

Figure 1. Mean SOD levels of different intervention groups, after the HIIT period, compared to that of the control group ($P \leq 0.05$)

Table 2. Mean SOD levels of different intervention groups, after the HIIT period, compared to that of the control group ($P \leq 0.05$)

Groups	Mean	Standard Deviation(SD)	*P*-value
TRAINING	24.48	12.28	0.001
CURCUMIN	27.60	8.73	0.000
TRAINING+CURCUMIN	36.24	7.81	0.003
CONTROL	12.35	2.77	0.908

antioxidant capacity or enhancement in other cardio-protective molecules (22). SOD reduces the toxic effects of those radicals on secondary reactions via transformation of superoxide anion to hydrogen peroxide (23). In addition, CAT enzyme is a determining factor for heart antioxidant status. CAT takes a paramount part for erasing toxic properties of hydrogen peroxide through deactivating superoxide ion (24). Therefore, the increases in CAT and SOD activity following training and/or curcumin interventions may increase the effects of HIIT and curcumin supplement on reduction (or inhibition) of oxidative stress in heart tissues of diabetic rats through enhancement of antioxidant level. However, the present research did not investigate structural changes in the heart which could be counted as one of the limitations of the present research. Nevertheless, it can be stated that the decrease in oxidative stress which has occurred because of the intervention might lead to some desirable structural changes in heart tissues of the diabetic rats, due to the hand of oxidative stress on induction of structural changes in the heart, through direct damage from apoptosis induction and changes in the extracellular matrix (ECM), DNA, cellular proteins, and hypertrophic cardiomyopathy (HCM) (25).

In the other way, the eight weeks curcumin consumption would cause significant increases in capacity levels of SOD and CAT enzymes, in heart tissues of diabetic rats. The effects of curcumin on the enzyme could be addressed to its anti-diabetic and hypoglycemic influences. In this regard, Gupta et al (2011) studied hypoglycemic effects of curcumin and showed that long-term prescription for curcumin would enforce a prominent hypoglycemic effect (which is a desirable anti-inflammatory and anti-oxidative stress influence) on brain tissues of diabetic rats (26). Natural ingredients, like curcumin, may lead to the possible regeneration of the islets of Langerhans in conditions related to diabetes associating with other treatments (27). Perhaps, the recent topic might likely occur in the present study. Moreover, it was reported curcumin reduce the tissue insulin resistance,

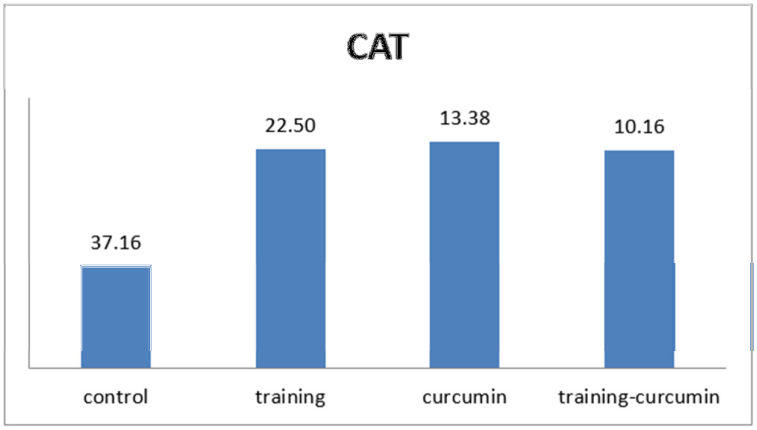

Figure 2. Mean CAT levels of different intervention groups, after the HIIT period, compared to that of the control group ($P \leq 0.05$)

Table 3. Mean SOD levels of different intervention groups, after the HIIT period, compared to that of the control group ($P \leq 0.05$)

Groups	Mean	SD	*P*-value
TRAINING	284.12	22.50	0.001
CURCUMIN	225.71	13.38	0.001
TRAINING+CURCUMIN	286.14	18.16	0.000
CONTROL	132.50	37.16	0.908

and would decrease tissue need for insulin through intensification of the activity of glucose transporters (GLUT), within muscle tissues (28). Also, such ingredient functions as a reducer of blood glucose level through modulation of hepatic enzymes which are responsible for carbohydrates metabolism including decrease in activity level of liver phosphorylases enzyme and increases in activity levels of glucokinase and glycogen synthase (29).

It was showed curcumin can increase non-enzymatic antioxidants levels and activity intensification of enzymatic antioxidant. In addition, curcumin decreases in lipid peroxidation and oxidative stress, within tissues of STZ-induced diabetic rats (5). Another beneficial aspect of curcumin can be addressed to its hypoglycemic properties, in the present study. Accordingly, curcumin decreases oxidative stress and its symptoms (such as MDA) within tissues (like heart tissues), through reducing AGEs.

The eight weeks HIIT with curcumin supplement keep significant effects on concentrations of SOD and CAT antioxidant enzymes. Though, the present results indicated that the training and the supplement do not strengthen influence of each other to increase levels of SOD and CAT enzymes. The simultaneous exploitation of training and curcumin does not own any further significant influence, than those of the separated interventions.

Conclusions

Our findings demonstrated that either HIIT or curcumin supplement can effect on activity levels of antioxidant enzymes within heart tissue of diabetic rats. Two interventions would not strengthen effects of each other. Therefore, it can be concluded that both training and curcumin are effective interventions for improvement of the antioxidant performance within heart tissue, in diabetic conditions.

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