

Ameliorative effect of sixteen weeks endurance training on biochemical and oxidative damage in high fat diet induced obese rats

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Obesity is mainly caused by consumption of high fat diet (HFD) and a lack of physical activity. Physical activity is an efficient strategy to delay development of obesity. In this study we tried to evaluate attenuating properties of 16 weeks endurance training on plasma oxidative stress and some biochemical parameters in HFD induced obese rats. Twenty-four male Wistar rats were randomly divided into four groups: standard diet group (SD), standard diet with endurance training group (ESD), HFD group and HFD with endurance training group (EHFD). After sixteen weeks, plasma was prepared and evaluated for measurement of different parameters. The results showed that HFD significantly decreased the activities of superoxide dismutase (33.58%), catalase (26.51%) and glutathione S-transferase (22.77%), while endurance training increased these enzymes activities. However, exercise ameliorated the increased malondialdehyde level and depletion of glutathione. In addition, it significantly reduced the increased levels of liver enzymes activities and lipid profiles. These findings suggest that endurance training has found to have beneficial effects against HFD-induced oxidative damage through increasing reduced antioxidants levels and inhibition of lipid peroxidation due to its antioxidant property. Thus, it can be considered an interesting strategy for the management of obesity related diseases.

Keywords: Antioxidants, Lipid peroxidation, Lipid profiles, Obesity, Physical exercise

Obesity is becoming a major challenge for public health that increases the risk for a number of diseases including diabetes, cardiovascular diseases, cancer, cognitive dysfunction and anxiety disorders. The prevalence of obesity is progressively increasing worldwide and one-third of the world population is currently classified as obese/overweight¹⁻³. It has been estimated that worldwide, 650 million adults (overall prevalence of 13.0%) were obese in 2016. The worldwide prevalence of obesity nearly tripled between 1975 and 2016⁴. In 2015, globally, a burden of about 4.0 million deaths (7.1% of all deaths) and 120 million disability-adjusted life years (4.9% of all DALYs) were attributed to overweight and obesity. Overall, about 22.7% of the Iranian's adult population (15.3% of men and 29.8% of women) was obese in 2016⁵.

Obesity is mainly caused by a sedentary lifestyle excess energy intake, the consumption of high fat diet

(HFD), lack of physical activity and exposure to stress^{6,7}. The consumption of a HFD is considered a major cause of the development of obesity-related health complications¹. Oxidative stress caused by the consumption of a HFD is evident in most experimental models and patients with clinical conditions^{1,2}. HFD-induced obesity stimulates the production of inflammatory cytokines, which is associated with synthesis of reactive oxygen species (ROS). Overproduction of ROS can cause extensive damage to macromolecules, including proteins, DNA and membrane phospholipids, which leads to lipid peroxidation and oxidative stress and finally cell death^{2,6}.

Exercise is useful way to prevent the development of obesity and its associated complications^{6,7}. It is associated with numerous health benefits. It reduces body weight and adiposity, improves physiological and functional capacity in the humans, increases bone density, decreases tissue lipid content and enhances fatty acid oxidation and insulin activity^{2,8}. Exhaustive exercise increases the production of ROS via an electron leak of the mitochondrial electron transport

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chain, which leads to lipid peroxidation and oxidative stress⁸. However, regular exercise activates an adaptation of the antioxidant system response by increasing the activities of antioxidant enzymes and its resistance to oxidative stress^{8,9}. Many cells are capable of responding to exercise-induced increases in ROS via endogenous protective mechanisms, including changes in enzymatic endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) and non-enzymatic antioxidants such as glutathione (GSH) and vitamins E and C^{8,10}. Numerous studies have shown that exercise induces changes in SOD, CAT and GST activities, an increase in malondialdehyde (MDA) level and a decrease in GSH content in tissues, which can lead to oxidative stress in animals and humans^{8,9}.

Despite evidence that exercise training may improve obesity-related disorders, there is still a lack of knowledge about the underlying cellular and molecular mechanisms⁶. Due to different ability to neutralize oxidant species in various high fat diets formulas and exercise models¹¹, further studies are required to investigate these effects in variety of cell types using an *in vivo* system. Therefore, the present study was designed to obtain the more deeper and detailed insight into the relationship between oxidative stress and long-term exercise in HFD rat model with assessing the activities of free radical scavenging enzymes such as SOD, CAT and GST, as well as MDA level as an important index of lipid peroxidation and GSH content and biochemical parameters measurement after sixteen weeks in rat plasma.

Materials and Methods

Chemicals

Nitroblue tetrazolium (NBT), GSH, 1-chloro-2, 4-dinitrobenzene (CDNB) and 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Company (Germany). All other chemicals used were of extra pure grade and obtained from Sigma and Merck.

Animals

Twenty-eight male Wistar rats weighting between 160 and 180 g (5-6 weeks old) were purchased from Pasteur Institute (Tehran, Iran) and acclimated for at least 1 week prior to experimental use. The animals were housed in normalized light-polyethylene cages in a room with 12/12 hours light/dark cycle at 22±2°C

and a relative humidity of 60±5%. Water was available *ad libitum*.

Study design

Twenty-eight male Wistar rats were randomly divided into four groups, each comprising of seven animals: the standard diet (SD) group, endurance training group with a standard diet (ESD), High-fat diet (HFD) group and endurance training group with high-fat diet (EHFD). The rats in the standard diet group were daily fed with a laboratory chow (containing 407 kcal/100 g total energy, 57% carbohydrate, 16% protein, and 25% fat based on percentage of total calories; Razi Institute, Iran)¹². The rats in the HFD group were fed with HFD (containing 457kcal/100 g total energy, 37% carbohydrate, 13% protein, and 50% fat based on percentage of total calories; Razi Institute, Iran)^{12,13}. Each trained rat was exercised between 8 and 10 am, 5 days a week for 16 weeks. The rats progressively ran on a motor-driven rodent 4-channel treadmill from 15 min/day at 15 m/min speed, 0% slope, up to 60 min/day at 25 m/min speed, 0% slope¹². Animals from sedentary groups were placed for the same period on a turned-off treadmill.

Body weight and the food intake

Body weight of the rats in each group was measured and recorded every day. Food intake was estimated daily by differential weighting for each group of seven rats and summed.

Plasma preparation

Twenty-four hour after the last training session, rats in each group were sacrificed following an overnight fasting. Blood samples were collected by cardiac puncture in heparin (0.2 mg per 1 mL of blood) as the anticoagulant and immediately centrifuged at 300×g for 15 min at 4°C. Plasma were removed and stored in 0.5 mL aliquots at -70°C freezer until biochemical analysis.

Biomarkers of oxidative damage

SOD activity assay

Plasma SOD activity was measured based on the inhibition of the reduction of NBT by superoxide⁸. For assay, 0.067 M potassium phosphate buffer with pH=7.8, 0.1 M EDTA in 0.3 mM sodium cyanide and 1.5 mM NBT were added to an appropriate volume of plasma and after mixing, it was incubated at 37°C for 5 min. Then, 0.12 mM riboflavin was added and it was placed at room temperature (22±2°C) below 40 W lamps in a dark

box. After 12 min of exposure to light, the absorbance of samples was read on a Genesys 10 UV spectrophotometer at 560 nm. The amount of enzyme required to produce 50% inhibition was taken as 1 U and results were expressed as U/mg protein.

CAT activity assay

Plasma CAT activity was measured by monitoring the initial rate of disappearance of H_2O_2 ¹⁴. Reaction mixture contained 0.93 mL potassium phosphate buffer 50 mM, pH 7.0 and 0.02 mL plasma. Reaction was initiated by addition of 0.05 mL H_2O_2 (30 mM prepared in potassium phosphate buffer, pH 7.0) and the decrease in absorbance was recorded for 3 min at 240 nm. Specific activity was expressed as U/mg protein.

GST activity assay

GST activity was assayed based on monitoring the formation of the thioether product of the reaction between GSH and CDNB⁸. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 6 mM GSH, 1 mM CDNB and 0.02 mL of plasma in a final volume of 1.0 mL. The formation of CDNB-GSH conjugate was evaluated by monitoring the increase in absorbance at 340 nm for 5 min. Results were expressed as μmol of conjugate produced per minute per mg of protein.

Measurement of GSH level

GSH level was estimated based on the method of Tietz, who reported that the DTNB is reduced by SH group to form 1 mol of 2-nitro-5-mercaptobenzoic acid per mole of SH¹⁵. Cellular protein of plasma was precipitated by addition of 5% sulfosalicylic acid and removed by centrifugation at $2000\times g$ for 10 min. 0.81 ml of 0.3 M Na_2HPO_4 and 0.09 ml of 0.4% DTNB in 1% sodium citrate were added to 0.1 mL of the protein-free supernatant of plasma, and the sample was shaken. The absorbance was read within 5 min at 412 nm. The GSH concentrations of the samples were derived from the standard curve prepared using GSH as standard and were expressed as nmol/mg protein.

Measurement of MDA level

The end product of lipid peroxidation was estimated by measuring the level of MDA⁸. 0.5 mL of plasma was added to 1.5 mL of 10% trichloroacetic acid, vortexed and incubated for 10 min at room temperature. One and 1.5 mL of supernatant and 2 mL of thiobarbituric acid (0.67%) were added and followed by 30 min incubation in boiling water bath then cooled at room temperature. About 2 mL of *n*-

butanol was added, vortexed, and centrifuged at $3000\times g$ for 15 min. The colored pink layer of supernatant was measured at 532 nm. The concentration of MDA was calculated using a standard curve from 1, 1, 3, 3-tetraethoxypropane and expressed in as nmol/mg protein.

Protein level assay

The total protein concentration in tissues was measured by Bradford's method using bovine serum albumin as standard¹⁶. Protein concentration was used to normalize enzyme activities and levels of GSH and MDA.

Plasma biochemical parameters

Plasma glucose, urea, uric acid and creatinine levels and activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ -glutamyl transferase (GGT) and creatin kinase (CK) were measured using Pars Azmoun Company kits (Tehran-Iran).

Plasma lipid profile

Total cholesterol (TC), triglyceride (TG) and high-density-lipoprotein-cholesterol (HDL-C) concentrations were measured using Pars Azmoun Company kits (Tehran-Iran). Very low-density-lipoprotein (VLDL), low-density-lipoprotein-cholesterol (LDL-C) and atherogenic indexes were calculated using the Friedwald equation as follows: $VLDL = TG/5$; $LDL-C = TC - HDL-C - (TG/5)$ ¹⁷.

Ethical statement

All Experimental procedures were carefully complied with the guidelines for use and care of laboratory animals and were approved by the ethical committee of the Baqiyatallah University of Medical (IR.BMSU.REC.1393.220).

Statistical analysis

All calculations were performed using INSTAT statistical software version 3.3. All the statistical analyses were performed using one-way analysis of variance (ANOVA) with post hoc Tukey multiple comparison tests applied across treatment groups for each group. Significance level was based on $P < 0.05$. Results are expressed as mean \pm SEM of seven different rats.

Results and Discussion

Effects of treatments on body weight and food intake of rats

Figure 1 displays the effect of HFD and 16 weeks endurance training on the weekly body weight and food intake of rats in the control and the experimental

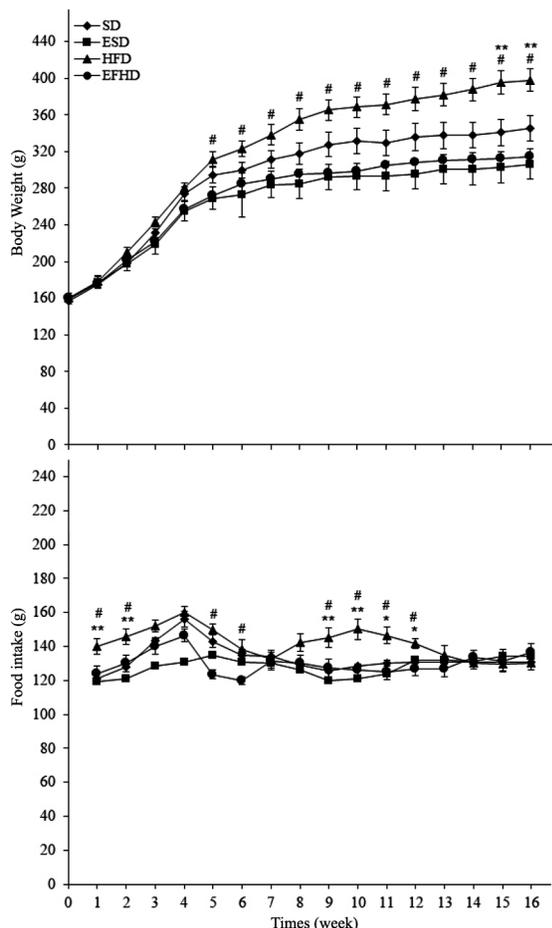


Fig. 1— Effect of 16 weeks endurance training and high-fat diet on the weekly (A) Body weight; and (B) Food intake of rats in control and experimental groups of rats. SD, standard diet group; ESD, endurance training plus standard diet; HFD, high-fat diet group; and EHFD, endurance training plus high-fat diet. [Values are expressed as mean \pm SEM (n=7). * P < 0.05 and ** P < 0.01 vs. SD group and # P < 0.05 ## P < 0.01 vs. ESD and EHFD groups]

groups. The body weight in the HFD group was significantly increased at weeks 15 and 16 compared to the SD group, while in the ESD and EHFD groups it significantly decreased between weeks 5 to 16 compared to the HFD group (P < 0.05).

Body weight gain mainly depends on the food and calorie intake in every animal species and must be recorded to obtain insights into the development of obesity¹⁸. The food intake in the HFD group significantly increased at weeks 1 and 2 and between weeks 9-12 compared to the SD group, but significantly decreased in the ESD and EHFD groups compared to the HFD group (P < 0.05).

Effects of treatments on plasma biochemical parameters

The effect of HFD and 16 weeks endurance training on biochemical parameters in different

groups are summarized in Table 1. HFD significantly increased plasma AST, ALT, LDH, ALP, GGT and CK activities and glucose level when compared to the SD group. These parameters in the ESD and EHFD groups significantly decreased compared to the HFD group (P < 0.05). The decreased glucose level and LDH and CK activities in ESD group were observed compared to the EHFD group. However, the levels of urea and creatinine were not affected in different group. In addition, TG, TC, VLDL, LDL-C, LDL/HDL and TC/HDL levels in HFD group were higher than SD group. These parameters in the ESD and EHFD group were significantly lower than HFD group (P < 0.05). The HDL-C level was decreased in HFD group compared to the SD group, while it was increased in the ESD and EHFD group comparing with the HFD group.

Effects of treatments on plasma oxidative stress biomarkers

Figure 2 displays the effect of HFD and 16 weeks endurance training on oxidative stress biomarkers in the control and the experimental groups. SOD, CAT and GST activities in plasma were decreased in the HFD group after 16 weeks, while these enzymes were increased in the ESD and EHFD groups comparing with the SD group. Antioxidant enzymes activities in the ESD and EHFD groups were significantly increased compared to the HFD group (P < 0.001). In addition, GSH level in the HFD and EHFD groups was decreased by 35.79 and 15.88%, while MDA level was increased by 45.32 and 18.09% in comparison to the SD group, respectively. The increased GSH level and decreased MDA level in the ESD and EHFD group were observed compared to the HFD group (P < 0.01). MDA level in ESD group were observed compared to the EHFD group (P < 0.01).

The rising prevalence of obesity is associated with an increase in the consumption of HFD¹⁹. In the present study, rats that consumed a HFD induced glucose intolerance, dyslipidemia and oxidative stress in the plasma after 16 weeks. Moreover, Exercise prevented the glucose intolerance and hyperlipidemia in HFD-fed rats. It also prevented oxidative stress and decreased lipid peroxidation in the plasma by restoring the cellular antioxidants.

Rat models are useful tools for inducing obesity as they will readily gain weight with HFD feeding and has many features common with human obesity^{13,20}. The current study demonstrated that regular exercise significantly decreased the increase in body weight

Table 1 — Effect of 16 weeks endurance training and high-fat diet on plasma biochemical parameters in control and experimental groups of rats

Parameters	SD	ESD	HFD	EHFD
Glucose (mg/dL)	137.13±4.98	129.54±4.75 ^{†††}	184.49±3.94 ^{***, #}	158.38±3.93 [*]
Urea (mg/dL)	27.13±1.15	26.38±3.18	33.5±1.75	29.13±1.19
Creatinine (mg/dL)	0.51±0.025	0.50±0.044	0.59±0.024	0.57±0.025
AST (U/L)	31.28±2.83	30.41±2.04	45.04±1.77 ^{***, #}	32.09±1.88
ALT (U/L)	22.06±1.90	21.88±0.61	32.16±1.57 ^{***, #}	23.81±1.99
LDH (U/L)	155.53±8.49	149±4.96 [‡]	225.54±13.63 ^{***, #}	186.87±9.16
GGT (U/L)	30.80±2.20	27.29±3.15 ^{††}	41.70±1.75 [*]	32.44±3.46
ALP (U/L)	160.13±3.41	154.49±3.44	178.85±4.75 [#]	156.5±6.32
CK (U/L)	37.97±1.79	39.55±2.22 ^{†, ‡}	53.61±3.20 ^{**}	52.13±3.86 ^{**}
TG (mg/dL)	70.5±6.58	62±4.31	94.38±3.49 ^{**}	70.25±3.03
TC (mg/dL)	82.5±3.59	77.5±2.29	120.88±3.97 ^{***, #}	85.5±2.78
VLDL (mg/dL)	14.11±1.32	12.4±0.86	18.88±0.70 ^{**}	14.05±0.61
LDL-C (mg/dL)	36.53±5.33	29.85±2.76	78.95±3.37 ^{***, #}	38.83±3.54
HDL-C (mg/dL)	31.88±2.05	35.25±2.25	23.05±1.89 [#]	32.63±1.73
L-DL-C/HDL-C (mg/dL)	1.15±0.9	0.85±0.39	3.42±0.42 ^{**}	1.19±0.22
TC/HDL-C (mg/dL)	2.59±0.38	2.20±0.39	5.24±0.64 ^{**}	2.62±0.61

[Groups: SD, standard diet; ESD, Endurance training plus standard diet; HFD, high-fat diet; and EHFD, Endurance training plus high-fat diet. Parameters: AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; GGT, γ -glutamyl transferase; ALP, alkaline phosphatase; CK, creatin kinase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density-lipoprotein-cholesterol; VLDL, very low-density-lipoprotein and LDL-C, low-density-lipoprotein-cholesterol. Values are expressed as mean±SEM (n=7). * P <0.05, ** P <0.01 and *** P <0.001 vs. SD group; # P <0.05 vs. ESD and EHFD groups; † P <0.05 and †† P <0.01 vs. HFD and ‡ P <0.05 and ‡‡ P <0.001 vs. EHFD group]

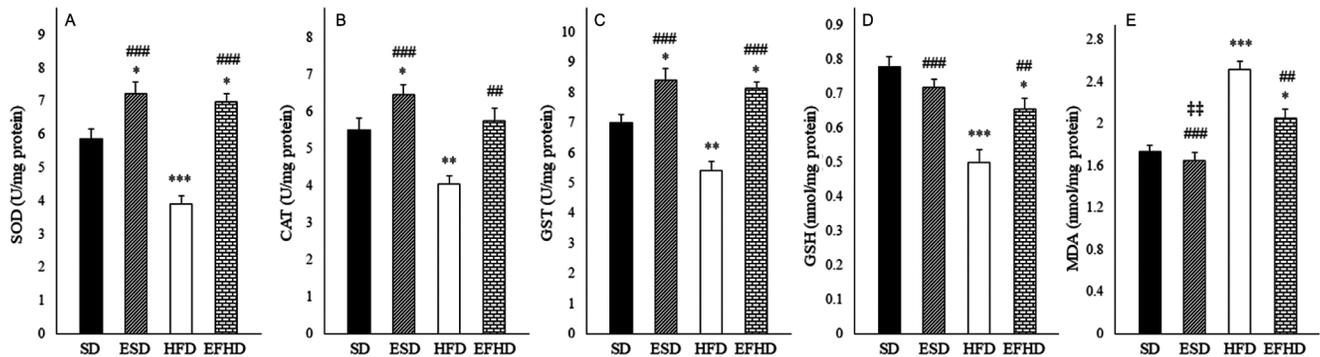


Fig. 2 — Effect of 16 weeks endurance training and high-fat diet on plasma oxidative stress biomarkers in control and experimental groups of rats. SD, standard diet group; ESD, endurance training plus standard diet; HFD, high-fat diet group; and EHFD, endurance training plus high-fat diet; SOD, superoxide dismutase; CAT, catalase; GST, glutathione S-transferase; GSH, glutathione; and MDA, malondialdehyde. [Values are expressed as mean±SEM (n=7). * P <0.05, ** P <0.01 and *** P <0.001 vs. SD group; # P <0.01 and ### P <0.001 vs. HFD group; † P <0.05 and †† P <0.01 vs. EHFD group]

gain and food intake, which was in agreement with the various previously published studies^{11,21}. This significant lower body weight in trained rats may be due to the reduction of the amount of adipose tissue resulting in decreased generation of sex hormones, glucose, leptin, the increase appetite-suppressing neuropeptide hormones levels, and negative energy and fat balance linked with increased energy expenditure and fat oxidation during the exercise^{12,19}. Although a study showed that no significant difference was observed on average body weight and/or food intake of rats fed the HFD and under exercise²². Chaolu *et al* showed that the food intake of

the mice in the exercise groups significantly increased as compared to the non-exercise groups²³.

HFD increases the generation of ROS by stimulating proinflammatory cytokines such as tumor-necrosis factor- α (TNF- α), interleukin-6 (IL-6) and interleukin-8 (IL-8), the well-known stimulators of NADPH oxidase as the main enzyme responsible for ROS generation^{19,24}. Many cells are capable of responding to HFD-induced increases in ROS via endogenous protective mechanisms, including changes in enzymatic endogenous antioxidants such as SOD and CAT. SOD scavenges superoxide radicals to less reactive H₂O₂ and O₂, while CAT

scavenges H_2O_2 to water and molecular oxygen²⁵. A significant decreased plasma SOD and CAT activities were noted in the present study in HFD group, which was the result of high oxidative stress. Inhibition of antioxidant enzyme activities may be a consequence of inactivation of enzyme proteins from increased ROS production²⁶. In addition, the depletion of SOD activity increases the endogenous superoxide anion, which would have further consequences. It reacts with NO to form peroxynitrite radical and generates hydroxyl radical in conjunction with H_2O_2 through the Haber–Weiss and Fenton reactions, all exacerbating lipid peroxidation, DNA damage and cell death¹⁴. These results are consistent with previous studies showing that tissue antioxidant defenses may be compromised in high-fat diet-fed animals^{2,27}. Cavaliere and coworkers showed that SOD activity was elevated after 1 week of HFD, while markedly decreased after 12 weeks. They suggested that the elevated SOD activity was able to balance ROS levels only for the first week and ROS levels increased progressively during the treatment reaching high values at 12 and 18 weeks when SOD activity was low²⁸. In addition, both exercise groups in the present study increased SOD and CAT activities due to the capability to develop a compensation oxidative stress in tissue by means of an adaptation of the antioxidant and repair systems²⁹. Exercise training reduces the white adipose tissue mass and the expression of NADPH oxidase and stimulate the expression of nuclear factor kappa B (NF- κ B), which leads to increase the expressions of antioxidant enzymes like SOD and CAT^{2,24}. Several studies have demonstrated that antioxidant enzymes activities were increased in blood or in tissues after aerobic exercise^{2,30}. However, several other studies have reported a decrease in antioxidant enzymes activities in the muscle, liver, kidney and blood of animals by exhaustive exercise^{8,9}, and other study have found no change in antioxidant enzymes activities after exercise³¹. The response of training on oxidative stress would appear different according to the type, duration and intensity of exercise, previous exercise exposure, subject age and species, nutritional status, type of tissue, animal species and assay technique employed⁸.

GST is a group of enzymes capable of conjugating GSH with ROS for protecting tissue from lipid peroxidation and oxidative stress and its levels can reflect the antioxidant capacity of the body^{14,32}. In present study, the GST activity was decreased in rats

fed HFD, while it was increased in both exercise groups. There were significant differences in the GST activity between HFD and exercise groups. The decreased GST activity in HFD-treated rats may be due to inactivation of enzyme by increased free radicals and depletion of GSH (Figure 2), which is essential for GST activity. These findings are in agreement with the results of the previous reports that HFD feeding significantly decreased the activity of GST in various tissues²⁰. A study has showed that GST activity was significantly increased in the muscle and no change in liver after exercise⁸. Vukovic et al. showed that liver GST activity was significantly decreased in HFD and physical activity³³.

GSH as powerful antioxidant in the cells acts as an ROS scavenger and as a substrate for several enzymes in different metabolic pathways, including GST and glutathione peroxidase. It is synthesized in the liver cells and then distributed to different organs through blood³⁴. Our data showed that a GSH depletion of 35.79% in HFD feeding rat may be due to increased utilization of GSH for participation of GSH in neutralizing free radicals produced by HFD, diminished GSH synthesis in liver and limited intracellular reduction of oxidized GSH level (GSSG). Diminished GSH indicates impair the cells defense against the toxic actions of ROS and may lead to oxidative stress and cytotoxicity. Depletion of GSH leads to the production of GSSG and finally decreased the ratio of GSH/GSSG in tissues of rats, which is indicative of oxidative stress⁸. The increased GSH content in training groups in comparison to the HFD group could be due to the antioxidant potential of the exercise³⁵. Yol and coworkers reported that regular aerobic exercise for 4–6 weeks could improve total antioxidant capacity in male soccer athletes³⁶.

One of the molecular mechanisms involved in obesity-induced toxicity is lipid peroxidation leading to the damage of the cellular membrane structure and function. MDA is most frequent marker of lipid peroxidation and oxidative stress^{8,37}. Elevation of MDA level in the present study leads to inactivation of antioxidant enzymes by cross-linking with MDA, which further cause increased accumulation of ROS, which further enhances more lipids peroxidation³⁸. A significant decrease in lipid peroxidation in ESD and EHFD groups was observed. Several studies have reported an increase in lipid peroxidation products in animals by HFD feeding and endurance training in animals^{27,33}. However, other study in rats has reported

no change in MDA level in response to physical activity³¹. The discrepancy between these results may be due to the exercise intensity, participant's age and body status³¹.

Oxidative damage also leads to hepatocytes damage in HFD-fed rats, as evidenced by the increased plasma activities of the AST, ALT, ALP, GGT and LDH enzymes²⁷. HFD-induced membrane lipid peroxidation leads to an increased permeability of liver and the leakage of these enzymes into the plasma¹⁴. The high levels of these enzymes in our study are attributed to fatty liver induced by HFD and return of their activities to its normal levels in both exercise groups, suggesting the amelioration of fatty liver⁸. This decrease may be due to the consequence of prevention of liver damage from the lipoperoxidation by the antioxidant potential of the exercise³⁵. Adiponectin secretion is inhibited by several factors including high level of TNF- α and oxidative stress. Decreased adiponectin level in the HFD was demonstrated to be associated with decreased insulin sensitivity^{2,28}. Exercise training significantly reduced the increased levels of blood glucose due to the increased adiponectin level and insulin sensitivity^{6,22}.

Hyperlipidemia, oxidative stress and obesity are the important causative factors for the development of cardiovascular diseases. The LDL to HDL and TC to HDL ratios are considered to be good prognostic indicators for atherosclerosis extent^{21,39}. In present study, the plasma levels of TC, TG, VLDL and LDL-C in the HFD group were increased, while HDL-L level was decreased, all of which eventually trigger the development of lipotoxicity and lipid accumulation in the liver²⁷. The data in our study showed that an increase in LDL-C was accompanied with a decrease in HDL-C, which may suggest a change from HDL-C into LDL-C. HFD induces the production of ROS, which react with lipoproteins to produce oxidation states, thus diminishing the cellular uptake of lipids from the blood^{27,40}. This results are similar to results other studies^{22,27}. However, the decreased these parameters in training groups in comparison to the HFD group could lead to a lowering of the atherogenicity and therefore a significant reduction in the potential incidence of coronary heart disease². This decreased of lipids may be caused by the inhibition of impaired lipid digestion and absorption, improvement in glucose and lipid metabolism, increased antioxidant defense, and down-regulation of lipogenic enzymes²².

Several studies have demonstrated that exercise decreases blood lipid and lipoprotein levels lipids in humans and rats^{6,41,42}. Huang and coworkers showed that the increases in lipid indices induced by the administration of HFD were not affected by the endurance exercise intervention²¹.

Our results suggest that the inhibition of antioxidant enzyme activities by HFD increases the endogenous free radicals and lipid peroxides, leading to intracellular Ca²⁺ influx and the release of cytochrome c from mitochondria to cytosol, which is a crucial step in apoptotic signaling through the activation of caspases^{43,44}. In addition, overproduction of ROS by HFD activate NF- κ B, which in turn increases the expression of cytokines genes such as TNF- α , leadings to inflammation and depletion of GSH and increased MDA levels^{8,14}. Depletion of GSH induces apoptosis in cells by interacting with proapoptotic and antiapoptotic signaling pathways and activating several transcription factors, such as AP-1 and NF- κ B⁴³. Exercise training has been shown to reduce cell size of adipocytes, and decrease the level of inflammation in adipose tissue in mice². Long-term exercise reduces the apoptosis in muscles by increasing the concentration of Bcl-2 as the antiapoptotic marker^{43,44}. However, further studies are required to investigate the effects of various high fat diets formulas and exercise models on induction of cell death in variety of cell types using an *in vivo* system.

Conclusion

According to our results, oxidative stress is an essential mechanism involved in HFD-induced adversity effect, as evidenced by decreased GSH level and antioxidant enzymes activities as well as enhanced lipid peroxidation. Endurance training has ameliorative effect against HFD-induced oxidative damage through increasing the level of antioxidants and attenuating lipid peroxidation due to its antioxidant property. Thus, it can be considered an interesting strategy for the management of on development of obesity and obesity-related metabolic diseases. Further *in vivo* studies are required to investigate the effects of various high fat diets formulas and exercise models on induction of cell death.

Acknowledgment

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Conflict of Interest

Authors have no competing interests.

References

- 1 Gerardi G, Cavia-Saiz M, Rivero-Perez MD, Gonzalez-SanJose ML & Muniz P, Wine pomace product modulates oxidative stress and microbiota in obesity high-fat diet-fed rats. *J Funct Foods*, 68 (2020) 103903.
- 2 Al-thepyani M, Algami S, Gashlan H, Elzubier M & Baz L, Evaluation of the Anti-Obesity Effect of Zeaxanthin and Exercise in HFD-Induced Obese Rats. *Nutrients*, 14 (2022) 4944.
- 3 Moholdt T, Parr EB, Devlin BL, Debik J, Giskeodegard G & Hawley JA, The effect of morning vs evening exercise training on glycaemic control and serum metabolites in overweight/obese men: a randomised trial. *Diabetologia*, 64 (2021) 2061.
- 4 Pourfarzi F, Sadjadi A, Poustchi H & Amani F, Prevalence of overweight and obesity in Iranian population: A population-based study in northwestern of Iran. *J Public Health Res*, 11 (2022) 2475.
- 5 Djalalinia S, Saeedi Moghaddam S, Sheidaei A, Rezaei N, Naghibi Irvani SS, Modirian M, Zokaei H, Yoosefi M, Gohari K, Kousha A, Abdi Z, Naderimagham S, Soroush AR, Larijani B & Farzadfar F, Patterns of obesity and overweight in the Iranian population: findings of steps 2016. *Front Endocrinol*, 11 (2020) 42.
- 6 Juszcak F, Vlassembrouck M, Botton O, Zwakhals T, Decarnoncle M, Tassin A, Caron N & Decleves AE, Delayed exercise training improves obesity-induced chronic kidney disease by activating AMPK pathway in high-fat diet-fed mice. *Int J Mol Sci*, 22 (2020) 350.
- 7 Wang J, Zhang Q, Xia J & Sun H, Moderate Treadmill Exercise Modulates Gut Microbiota and Improves Intestinal Barrier in High-Fat-Diet-Induced Obese Mice via the AMPK/CDX2 Signaling Pathway. *Diabetes Metab Syndr Obes*, 15 (2022) 209.
- 8 Mousavi SR, Jafari M, Rezaei S, Agha-alinejad H & Sobhani V, Evaluation of the effects of different intensities of forced running wheel exercise on oxidative stress biomarkers in muscle, liver and serum of untrained rats. *Lab Animal*, 49 (2020) 119.
- 9 Baek KW, Gim JA & Park JJ, Regular moderate aerobic exercise improves high-fat diet-induced nonalcoholic fatty liver disease via monoacylglycerol O-acyltransferase 1 pathway suppression. *J Sport Health Sci*, 9 (2020) 472.
- 10 Henriquez-Olguin C, Renani LB, Arab-Ceschia L, Raun SH, Bhatia A, Li Z, Knudsen JR, Holmdahl R & Jensen TE, Adaptations to high-intensity interval training in skeletal muscle require NADPH oxidase 2. *Redox Biol*, 24 (2019) 101188.
- 11 Maharjan BR, Martinez-Huenchullan SF, McLennan SV, Twigg SM & Williams PF, Exercise induces favorable metabolic changes in white adipose tissue preventing high-fat diet obesity. *Physiol Rep*, 9 (2021) e14929.
- 12 Haghshenas R, Jafari M, Ravasi A, Kordi M, Gilani N, Shariatzadeh M, Hedayati M & Rahimi M, The effect of eight weeks endurance training and high-fat diet on appetite-regulating hormones in rat plasma. *Iranian J Basic Med Sci*, 17 (2014) 237.
- 13 Hariri N & Thibault L, High-fat diet-induced obesity in animal models. *Nutr Res*, 23 (2010) 270.
- 14 Eshrati R, Jafari M, Gudarzi S, Nazari A, Samizadeh E & Ghafourian Hesami M, Comparison of ameliorative effects of Taraxacum syriacum and N-acetylcysteine against acetaminophen-induced oxidative stress in rat liver and kidney. *J Biochem*, 169 (2021) 337.
- 15 Tietze F, Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem*, 27 (1969) 502.
- 16 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 72 (1976) 248.
- 17 Friedewald WT, Levy RI & Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, 18 (1972) 499.
- 18 Kruk J & Duchnik E, Oxidative stress and skin diseases: possible role of physical activity. *Asian Pac J Cancer Prev*, 15 (2014) 561.
- 19 Fang J & Tang M. Exercise improves high fat diet-impaired vascular function. *Biomed Rep*, 7 (2017) 337.
- 20 Ansari JA, Bhandari U, Pillai K & Haque S. Effect of rosuvastatin on obesity-induced cardiac oxidative stress in Wistar rats—A preliminary study. *Indian J Exp Biol*, 50 (2012) 216.
- 21 Huang WC, Xu JW, Li S, Ng XE & Tung YT, Effects of exercise on high-fat diet-induced non-alcoholic fatty liver disease and lipid metabolism in ApoE knockout mice. *Nutr Metab*, 19 (2022) 1.
- 22 Kumar P, Bhandari U & Jamadagni S, Fenugreek seed extract inhibit fat accumulation and ameliorates dyslipidemia in high fat diet-induced obese rats. *Biomed Res Int*, 2014 (2014) 606021.
- 23 Chaolu H, Asakawa A, Ushikai M, Li YX, Cheng KC, Li JB, Zoshiki T, Terashi M, Tanaka C & Atsuchi K, Effect of exercise and high-fat diet on plasma adiponectin and nesfatin levels in mice. *Exp Ther Med*, 2 (2011) 369.
- 24 Celik H, Kilic T, Kaplan DS, Eren MA, Erel O, Karakilcik AZ & Bageci C, The effect of newly initiated exercise training on dynamic thiol/disulphide homeostasis in sedentary obese adults. *An Acad Bras Cienc*, 91 (2019) e20180930.
- 25 Chakraborty R & Sen S, Nephroprotective activity of *Pisonia aculeata* L. leaf extract against cisplatin induced nephrotoxicity and renal dysfunction in experimental rodents. *Indian J Exp Biol*, 58 (2020) 770.
- 26 Madhavan S, Rajkumar J & Dorai S, Evaluation of polyherbal formulation against isoniazid and rifampicin induced oxidative stress in rat kidney. *Indian J Tradit Know*, 20 (2021) 671.
- 27 Lasker S, Rahman MM, Parvez F, Zamila M, Miah P, Nahar K, Kabir F, Sharmin SB, Subhan N & Ahsan GU, High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. *Sci Rep*, 9 (2019) 1.
- 28 Cavaliere G, Viggiano E, Trinchese G, De Filippo C, Messina A, Monda V, Valenzano A, Cincione RI, Zammit C & Cimmino F, Long feeding high-fat diet induces hypothalamic oxidative stress and inflammation, and prolonged hypothalamic AMPK activation in rat animal model. *Front Physiol*, 9 (2018) 818.

- 29 Vezzoli A, Pugliese L, Marzorati M, Serpiello FR, La Torre A & Porcelli S, Time-course changes of oxidative stress response to high-intensity discontinuous training versus moderate-intensity continuous training in masters runners. *PLoS ONE*, 9 (2014) e87506.
- 30 Berzosa C, Cebrian I, Fuentes-Broto L, Gomez-Trullen E, Piedrafita E, Martinez-Ballarín E, Lopez-Pingarrón L, Reiter R & García J, Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. *J Biomed Biotechnol*, 2011 (2011) 540458.
- 31 Saritas N, Uyanik F, Hamurcu Z & Coksevrim B, Effects of acute twelve minute run test on oxidative stress and antioxidant enzyme activities. *Afr J Pharm Pharmacol*, 5 (2011) 1218.
- 32 Kazmi S, Abbas K, Shah S, Nisar U, Arif M, Ahmad R, Pavase T & Soomro M, Partial characterization of glutathione S-transferase (GST) isolated from hepatocytes of *Cyprinus carpio* from river Ravi Punjab, Pakistan. *Indian J Geo-Mar Sci*, 49 (2020) 1017.
- 33 Vukovic R, Blazetic S, Orsolcic I, Heffer M, G Vari S, Gajdos M, Krivosikova Z, Kramarova P, Kebis A & Has-Schon E, Impact of ovariectomy, high fat diet, and lifestyle modifications on oxidative/antioxidative status in the rat liver. *Croat Med J*, 55 (2014) 218.
- 34 Tahmasebi K, Jafari M, Heydari J, Asgari A, Salehi M, Khazaie S & Abedini MS, Tissues toxicity attenuation by vitamin E on oxidative damage induced by diazinon. *J Environ Anal Health Toxicol*, 37 (2022) e2022036.
- 35 Ye Y, Lin H, Wan M, Qiu P, Xia R, He J, Tao J, Chen L & Zheng G, The effects of aerobic exercise on oxidative stress in older adults: A systematic review and meta-analysis. *Front Physiol*, 2021 (2021) 701151.
- 36 Yol Y, Turgay F, Yigittürk O, Asikovalı S & Durmaz B, The effects of regular aerobic exercise training on blood nitric oxide levels and oxidized LDL and the role of eNOS intron 4a/b polymorphism. *Biochim Biophys Acta Mol Basis Dis*, 1866 (2020) 165913.
- 37 Upadhyay SD, Ahmad Y, Kohli S & Sharma R, Synergistic effect of folic acid and galantamine against experimentally induced oxidative stress in IMR 32 cells. *Indian J Exp Biol*, 60 (2022) 286.
- 38 Rana A, Kumar NR & Kaur J, Therapeutic effect of propolis on *Staphylococcus aureus* induced oxidative stress in spleen of Balb/c mice: A biochemical and histopathological study. *Indian J Nat Prod Resour*, 13 (2022) 346.
- 39 Chuffa LgdA & Seiva FRF, Combined effects of age and diet-induced obesity on biochemical parameters and cardiac energy metabolism in rats. *Indian J Biochem Biophys*, 50 (2013) 40.
- 40 Motshakeri M, Goh YM & Ebrahimi M, Metabolic effects of high sucrose and saturated oil feeding on insulin resistance in Sprague-Dawley rats. *Indian J Exp Biol*, 53 (2015) 264.
- 41 Hsu YJ, Chiu CC, Lee MC & Huang WC, Combination of treadmill aerobic exercise with bifidobacterium longum OLP-01 supplementation for treatment of high-fat diet-induced obese Murine model. *Obes Facts*, 14 (2021) 306.
- 42 Zhao S, Zhong J, Sun C & Zhang J, Effects of aerobic exercise on TC, HDL-C, LDL-C and TG in patients with hyperlipidemia: A protocol of systematic review and meta-analysis. *Medicine*, 100 (2021) e25103.
- 43 Salomao R, Neto IVdS, Ramos GV, Tibana RA, Durigan JQ, Pereira GB, Franco OL, Royer C, Neves FdAR & Carvalho ACAd, Nobrega OT, Haddad R, Prestes J & Marqueti RdC, Paternal resistance exercise modulates skeletal muscle remodeling pathways in fathers and male offspring submitted to a high-fat diet. *Front Physiol*, 12 (2021) 706128.
- 44 Ruan L, Li F, Li S, Zhang M, Wang F, Lv X & Liu Q, Effect of different exercise intensities on hepatocyte apoptosis in HFD-induced NAFLD in rats: the possible role of endoplasmic reticulum stress through the regulation of the IRE1/JNK and eIF2 α /CHOP signal pathways. *Oxid Med Cell Longev*, 2021 (2021) 6378568