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ORIGINAL ARTICLE

Effects of High Cholesterol Diet and Parallel Chronic Exercise on Erythrocyte Primary Antioxidant Enzymes and Plasma total Antioxidant Capacity In Dutch Rabbits

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Exercise is a deterrent of cardiovascular disease and atherosclerosis, but the mechanisms by which exercise reduces atherogenic risk remain unknown. The aim of the present study was to investigate the effects of chronic exercise and/or high cholesterol diet on primary antioxidant enzymes and plasma total antioxidant capacity in Dutch rabbits.

Materials and Methods: 60 male Dutch white rabbits were divided into four groups: The normal diet control (NC), normal diet with exercise (NE), high-cholesterol diet control (HC) and high cholesterol diet with exercise (HE). Animals in high cholesterol diet groups were fed 2% cholesterol rabbit chow for 8 weeks. Animals of exercise groups ran on a treadmill at 0.88 km/h for 10 –60 min/day, 5 day/week, and 8 weeks in total. At the end of experiments, blood samples were drawn from vena cavae and were used for determination of Glutathione Peroxidase (GPX), Superoxide Dismutase (SOD) and Catalase (CAT) activities in red cells, plasma Total Antioxidant Capacity (TAC), Malondialdehyde (MDA) and serum cholesterol profile. Thoracic aorta and carotid arteries were isolated for histological examination to evaluate atherosclerosis.

Results: We found that 8 weeks of chronic exercise reduced atherogenic diet-induced atherosclerotic lesions in all arteries along with positive changes in cholesterol profile especially increase of serum HDL-C level. Plasma MDA and TAC concentrations were enhanced by exercise in the both normal and hypercholesterolemic groups. Erythrocyte catalase activity was increased significantly by chronic exercise ($P<0.05$), whereas total SOD activity rose with exercise only in the control group. Surprisingly, GPX activity was significantly decreased in response to exercise in the control group and also in the high cholesterol diet group.

Conclusion: It is speculated that exercise is an appropriate method for prevention and regression of atherosclerosis that accompanies enhancement of plasma TAC and positive changes in serum cholesterol profile; the exercise effects on red cell antioxidant activities is however more limited in hypercholesterolemic animals as compared to normal ones possibly in part because of alterations in the ability to adapt to exercise–induced oxidative stress in the high cholesterol diet.

Key words: Chronic exercise, Erythrocyte antioxidant enzymes, Total antioxidant capacity, Lipid peroxidation, High fat diet, Atherosclerosis.

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Introduction

Atherosclerosis, a principal cause of death in many countries,[1] is a complex process, and is possibly caused by high-fat diets and a sedentary lifestyle.[2,3] The enrichment of the diet with cholesterol is an accepted model to induce atherosclerosis in rabbits,[4] probably by cholesterol-mediated oxidative stress and attenuation in the antioxidant defense system.[5] Contradictory results however have been reported about activities of antioxidant enzymes in hypercholesterolemic models. For example it has been reported that glutathione peroxidase and catalase activities in erythrocytes remained unchanged under hypercholesterolemic conditions in rat.[6] There are also differing results including increase, decrease or unchanged antioxidant enzymes activities in red cells, plasma and other tissues in animal models.[7-13]

On the other hand, the studies address a paradox in relation to the role of exercise in antioxidant defense systems.[14-15] Exercise (with unknown mechanism) has received widespread acclaim and is recommended as a deterrent for atherosclerosis,[15] and its antiatherogenic effects have been described in humans and in different animal models. It can also positively influence risk factors associated with cardiovascular disease (CVD).[15] Paradoxically, it has been reported as an oxidative stress factor in animals and humans. Oxidative stress is an imbalance between the free radicals production especially reactive oxygen species (ROS) and antioxidants systems and has been implicated in accelerated atherosclerosis.[14] During moderate exercise oxygen consumption increases 8-10 fold and oxygen flux through the muscle may increase 9-100 fold. Even moderate exercise may increase free radical production and overwhelm antioxidant defenses, resulting in oxidative stress,[16] and this would appear incompatible with its antiatherogenic effects.[14] On the other hand, enzymatic and non-enzymatic antioxidants form part of the body defense mechanisms to suppress the formation of free radicals and to scavenge radicals as well to reduce their damaging effects. Primary antioxidant enzymes in cells include (SOD), (GPX) and (CAT), and each detoxifies a particular reactive oxygen species.[17]

Although physical exercise may acutely induce oxidative stress, regular exercise appears to enhance antioxidant defenses, and it also has been shown to decrease lipid peroxidation in some animal and human studies.[18-19] Despite many studies about the effects of exercise on antioxidants in various tissues, the response of erythrocyte SOD, GPX and CAT activities as well as plasma lipid peroxidation to exercise are mostly controversial in literature.[16,20-23] While many studies have investigated the effects of exercise on antioxidant enzymes in various tissues, only limited studies regarding the effects of exercise on plasma total antioxidant capacity are available[24] and to our knowledge, no studies have been performed regarding exercise-induced changes in total antioxidant capacity in the hypercholesterolemic rabbit model.

Therefore the present study was designed to obtain a deeper understanding of the mentioned parameters affected by chronic exercise in the hypercholesterolemic Dutch rabbit model. We also hypothesized that a high cholesterol diet would decrease the activities of antioxidant enzymes and total antioxidant capacity and we aim to determine whether these changes could be attenuated by chronic exercise.

Materials and Methods

Animals and Diet: This study was conducted in the Physiology lab of the Drug Applied Research Center, Tabriz University of Medical Sciences. 60 male Dutch white rabbits (1.3 kg at the beginning) divided into four groups, constituted the normal diet control (NC) group, the normal diet with exercise (NE) group, the high-cholesterol diet control (HC) group and the high-cholesterol diet with exercise (HE) group. The control groups were fed normal rabbit chow, whereas the

high cholesterol diet groups were fed with a high cholesterol diet (2%). All animals were housed in an environmentally controlled room.

Exercise Protocol: The exercise protocol was the same as that used by Jen et al.[3] After 1 week of familiarization, the exercise training groups ran on a leveled treadmill (Danish Yakhteh Co, Tabriz Iran) at a speed of 0.88 km/h for 10 minutes for the first week. The running time was extended 5-10 min each week until they could run for 60 minutes per day. They were exercised for 5 days per week for a total of 8 weeks. This exercise intensity was approximately 75% of their maximal exercise capacity.[3]

In contrast, the sedentary groups were placed on the treadmill for 10 minutes each day without receiving any exercise training. Rabbits were anesthetized at the end of experiments by injecting ketamine (25mg/kg, i.v) and sodium pentobarbital (20mg/kg, i.v) via the margin ear vein. To avoid the acute effect of exercise, animals were sacrificed, 48 h after training. Blood samples were drawn from the inferior vena cava and were stored in tubes for determination of plasma cholesterol profile, MDA, TAC concentration and erythrocyte SOD, GPX and CAT activities.

Histological Studies of Blood Vessels: Thoracic aorta and carotid arteries immediately isolated and placed in formalin 10%. Briefly, after tissue processing steps, several serial sections of blood segments (6µm thick) were stained using standard hematoxylin-eosin and studied by light microscopy. Atherosclerotic lesions were assessed on a scale from 0 to 5. A segment of vessel that did not have visible lesion was given a score of 0, and a segment that was completely covered by atherosclerotic lesions was given a score of 5.[25] Then the area of thickened intima was assessed and calculated and was expressed as the percentage of luminal area of the vessel ring.

Serum cholesterol profile: Serum cholesterol profiles including low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), triglyceride (TG) and total cholesterol were determined using automatic analyzer (Abbott Alcyon 300, USA). Intra-assay and inter-assay CV% was <10% in all of the cholesterol fractions.

Determination of Antioxidant Enzymes and TAC: For determination of GPX, SOD and CAT erythrocyte lysates were used. Briefly, blood was collected in tubes containing EDTA and centrifuged (1500g) for 15 min at 4˚c. The sediment containing erythrocyte was suspended in normal saline and recentrifuged, a process that was repeated twice. Sediment red cells were added to ice-cold distilled water and mixed thoroughly. GPX activity was determined using washed red cells based on the Palia and Valentine method using the GPX kit (Randox Co, Germany), according to the instructions provided by the manufacturer. 50µL sample volume was used. The decrease in absorbance was measured in 340 nm spectrophotometrically (Pharmacia Biotec; England). The intra-assay and inter-assay coefficients of variation (CV %) were 7.4% and 6.8% respectively. The sensitivity of the GPX assay was 82.86 U/L. SOD activity in red cells was determined using the SOD kit (Randox Co, Germany) at 505 nm by spectrophotometer according to the manufacturer instructions. SOD activity that could cause 50% inhibition of superoxide produced by reaction nitroblue tetrazolium was defined as 1 unit (U). The concentration of total SOD was calculated from a semi-logarithmic standard curve of standard samples vs. absorbance. The intra-assay and inter-assay coefficients of variation (CV %) were 6.3% and 8% respectively. The sensitivity of the SOD assay was 0.06 U/ml. Plasma TAC was measured by spectrophotometer according to kit guidelines (Randox CO, Germany). 20 µL sample volume was used. Absorbance was measured 600 nm at 37˚c. The intra-assay and inter-assay coefficient

variation (CV%) were 5.8% and 6.4% respectively. The sensitivity of the TAC assay was 0.21 mmol/l.

Red cell catalase activity was determined by monitoring the decrease in absorbance at 240 nm in presence of 10 mM hydrogen peroxide at 25˚C. One unit of catalase activity was defined as the decomposition of 1 M hydrogen peroxide min^{-1} at 25˚C.[26] Intra-assay CV% was 7%.

The amount of Malondialdehyde (MDA) was determined by the TBA (Tthiobarbitoric acid) assay. All reagents that were used in this assay were obtained from Merck (Darmstadt Germany). Briefly, 0.50ml of plasma was added to 3ml of 1% phosphoric acid, 1 ml of 0.60% TBA, and 0.15ml of 0.20% butylated hydroxytoluene (BHT) in 95% methanol. The samples were heated in a boiling water bath for 45 minutes, cooled and 4 ml of 1- butanol was added. The butanol phase was separated by centrifugation at 3000 rpm for 10 minutes and absorbance was measured at 532 nm. The concentration of MDA was expressed as μM.[27-28]

Statistical Analysis: Data was expressed as means±SD; statistical computations were calculated using SPSS 10 for windows software (SPSS INC, Chicago, IL, USA). Sample size (animal numbers) was indicated by n (n=15 rabbits for each group). Results of four groups was analyzed by ANOVA and further by Tukey HSD as post hoc test. Student- t-test was used for comparison of two groups or two methods. Differences were considered significant at $p<0.05$.

Table 1. Comparison of the serum lipid profile changes among four groups of Dutch rabbits by chronic exercise and /or high cholesterol

Variable	NC	NE	HC	HE
Total cholesterol (mg/dL)	74.6±3.9	69.2±3.5	1970±84*	2001±104
LDL-C (mg/dL)	28.23±3.8	19354±2.41	1630±67.57*	1592±61.89
HDL-C (mg/dL)	32.3±2.8	39.3±2.03	312.6±45.4*	439.6±42‡§
VLDL-C (mg/dL)	1736±4.5	16±2.8	51.6±15*	33±9.2‡§
TG (mg/dL)	88±22.5	8±14.4	266±69.6*	169±13.3‡§
HDL/LDL	1.15±0.20	1.92±0.41†	.188±.02*	.269±.05‡

Data are expressed as mean ± SD (n=15) for each group; Differences of $p<0.05$ were Considered significant.
* HC vs. NC and NE; † NE vs. NC; ‡ HE vs. NC and NE; § HE vs. HC
Abbreviations: NC, normal diet control; NE, normal diet with exercise; HC, high cholesterol diet control; HE, high cholesterol diet with exercise; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; TG, triglyceride

Results

Body Weight: Body weight was significantly increased by normal and high cholesterol diet feeding, but it showed no dramatic change in animals under chronic exercise (Data not shown).

Histological Examination: 8 weeks of 2% high cholesterol diet feeding induced atherosclerotic lesions and thickening of the intima in all thoracic aorta and to a lesser extent in the carotid arteries in the HC group (n=15). Chronic exercise reduced atherosclerotic lesions (20-35%) in HC group significantly ($p<0.05$). There was no lesion in the normal diet groups (Data not shown).

Serum Lipid Profile: Our results clearly demonstrated that eight weeks of 2% of high cholesterol diet feeding significantly increased serum total cholesterol, LDL-C, HDL-C, VLDL-C, and TG. These observations indicated that the atherogenic diet indeed induced hypercholesterolemia in our experimental Dutch rabbit model. 8 weeks of concomitant chronic exercise considerably reduced diet-increased serum levels of VLDL-C and TG in the HE group without significant changes in total cholesterol and LDL-C. Although chronic exercise tended to reduce the mentioned parameters in the control group, the effects were not statistically significant. The observed increases in plasma HDL-C or HDL-C to LDL-C ratio are considered to be the main effect of chronic exercise on serum cholesterol profiles (Table 1).

Antioxidant Enzymes TAC and MDA: All erythrocyte antioxidant enzymes were decreased by high cholesterol diet consumption. Red cell SOD activity rose significantly with chronic exercise only in the control group (Fig 1B). Erythrocyte catalase activity was significantly enhanced by chronic exercise in the NE and HE groups (Fig 2A). In addition, erythrocyte GPX activity was reduced significantly in response to chronic exercise and/ or high cholesterol diet feeding (Fig. 1A). Based on these findings, erythrocyte primary antioxidant enzymes indicated different and sometimes paradoxical changes by exercise and / or high cholesterol diet feeding. Plasma MDA and TAC levels were significantly increased in response to chronic exercise and /or high cholesterol diet feeding (Figs 2B and 3 respectively). These observations are possibly indicative of lipid peroxidation and induction of non-enzymatic antioxidants by both high cholesterol diet feeding and /or chronic exercise.

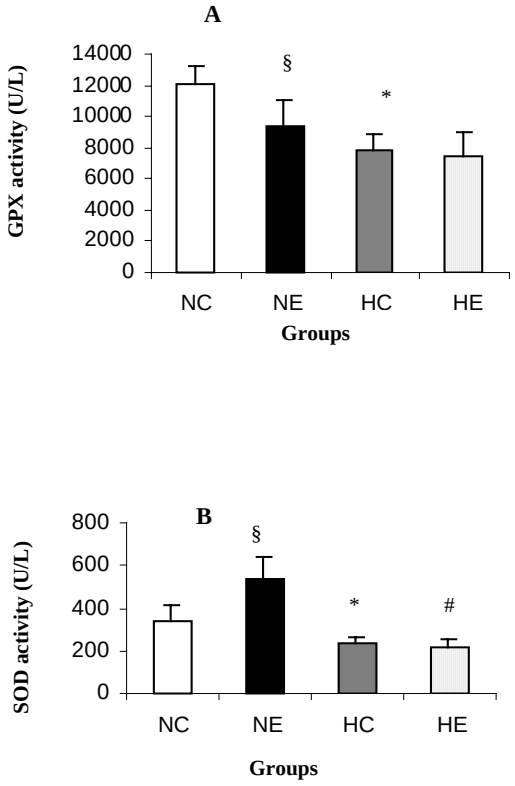

Fig.1. Comparison of the chronic exercise effect and /or high cholesterol diet on red cells GPX (A) and SOD (B) activities among four groups of rabbits. Data are expressed as mean±SD (n=15 for each group). Differences of $p<0.05$ were considered significant. $ NE vs. NC * HC vs. NC and NE # HE vs. NC and NE † HE vs. HC Abbreviations: NC, normal diet control; NE, normal diet with exercise; HC, high cholesterol diet control; HE, high cholesterol diet with exercise. SOD, superoxide dismutase; CAT, catalase

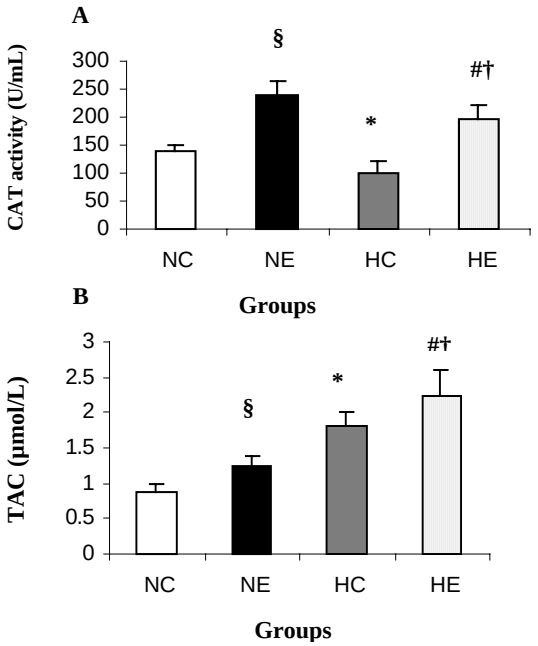

Fig.2. Comparison of the chronic exercise effect and /or high cholesterol diet on plasma CAT (A) activity and TAC concentration among four groups of rabbits. Data are expressed as mean±SD (n=15 for each group). Differences of p<0.05 were considered significant. $ NE vs. NC * HC vs. NC and NE # HE vs. NC and NE † HE vs. HC. Abbreviations: NC, normal diet control; NE, normal diet with exercise; HC, high cholesterol diet control; HE, high cholesterol diet with exercise. TAC, total antioxidant capacity.

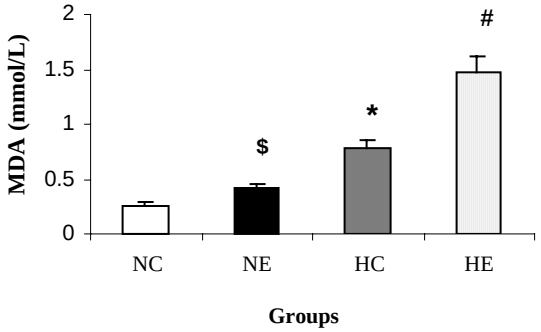

Fig.3. Comparison of the chronic exercise effect and /or high cholesterol diet on plasma MDA concentration among four groups of rabbits. Data are expressed as mean±SD (n=15 for each group). Differences of p<0.05 were considered significant $ NE vs. NC * HC vs. NC and NE # HE vs. NC and NE † HE vs. HC Abbreviations: NC, normal diet control; NE, normal diet with exercise; HC, high cholesterol diet control; HE, high cholesterol diet with exercise. TAC, total antioxidant capacity.

Discussion

Our results clearly demonstrated that 8 weeks of high cholesterol diet feeding induced hypercholesterolemia along with atherosclerotic plaques in aorta and carotid arteries. After 8 weeks of concomitant exercise intervention, atherosclerotic plaques were reduced in both the arteries associated with positive change in cholesterol profile. None of the arteries in the NC and NE groups showed any signs of fatty streaks.

Although it is still unclear exactly how exercise improves atherosclerosis, several possible mechanisms of exercise–induced atheroprotective effect have been proposed including antithrombotic, anti-inflammatory and antioxidant properties of HDL-C, decrease in plasma LDL-C[29] and positive changes due to exercise-induced oxidative stress such as induction of antioxidant systems as a defensive mechanism of the cells under oxidative stress.[14] In contrast to studies in rabbit[30-31] and mice,[14] in this study chronic exercise increased HDL-C or proportion of HDL-C to LDL-C. According to our results, it is thought that increases in HDL-C level are the main effect of chronic exercise on serum cholesterol profile. Based on recent studies,[32] it seems HDL-C is protected from exercise-induced oxidative stress by the paraoxonase antioxidant enzyme. Probably exercise results finally in decrease of LDL-C, VLDL-C and TG in plasma and then atherosclerosis.[33] through increase in efficacy of HDL-mediated reverse cholesterol transport system and lipoprotein lipase activity.

Some studies have reported that exercise decreases LDL-C and total cholesterol in humans and rats.[14,15,34,35] In this work, LDL-

C, total cholesterol, LDL-C, VLDL-C and TG showed a tendency to decrease by exercise in the control group but data were not significant statistically. On the other hand, VLDL-C and TG were reduced remarkably by exercise in the hypercholesterolemic group. The importance of LDL-C, HDL-C and total cholesterol is well documented in the pathogenesis of atherosclerosis,[36] but VLDL-C and TG should not be ignored. Lipoproteins are not static and it has been reported that enhancing the concentrations of these lipoprotein fractions increase the production of LDL-C. VLDL-C, like as LDL-C, is taken up by the endothelium and delivered to the various cell types of the arterial wall. TG-rich fractions are essential in the exchange of cholesterol particles and increase of HDL-C in the exercise animals may be a consequence of enhanced catabolism of TG-rich lipoproteins.[29-33]

In this study, chronic exercise reduced atherosclerotic lesions in the thoracic aorta more than in the carotid artery. These changes are similar to those reported as occurring in thoracic aorta and carotid with exercise in the New Zealand white rabbit.[3,38] However, there is little information about the susceptibility of the different arteries to atherosclerosis and their improvement by exercise in literature.[3,30,38]. According to vasorelaxation studies in rabbit arteries, different responses of thoracic aorta and carotid to atherosclerosis and exercise may result from vascular function and differences in exercise-induced flow-mediated nitric oxide production.[31-38] It is well known that the blood flow in aorta increases severalfold during exercise, whereas, the flow in carotid arteries remains relatively constant due to efficient cerebral autoregulation. In addition, the blood chemical composition is presumably identical within the major arterial systems. Therefore, the differences in exercise-induced changes between carotid and aorta are likely due to local increases in blood flow or sheer stress instead of the systemic changes in the plasma hormone level.[31-38]

Although there is a close relationship between hypercholesterolemia and atherosclerosis, it has been suggested that atherosclerotic lesions might depend on enhanced oxidative stress. Hypercholesterolemia increases the levels of ROS and elevated ROS can stimulate atherosclerosis pathogenesis.[40] Exercise is also known to impose oxidative stress on the body due to the generation of ROS and probably depletion of antioxidants that may result in atherosclerosis.[15]

In this study we found that erythrocyte activities of total SOD, GPX and CAT were significantly decreased by the high cholesterol diet. Red cell CAT activity was increased by chronic exercise but total SOD activity rose with exercise only in the control group whereas GPX activity was reduced by exercise and /or high cholesterol diet consumption. It has been proposed that high cholesterol diet induces free radical production and may result in oxidative stress.[8,14,20] On the other hand, regular exercise has been shown to strengthen antioxidant defense in normal humans and animals and may decrease the effects of deleterious oxidative stress.[16-21] There have been conflicting results about effects of exercise on changes mentioned in the antioxidant enzymes in various tissues of human and animal models.[8,14,16,19-21] While upregulation of GPX in response to acute exercise has been reported in skeletal muscle in animal experiments[18,41] and erythrocytes of some normal subjects,[18,42] no changes have been found in erythrocyte GPX activity in some human studies[19] or in rabbit heart after chronic exercise;[8,19] In the systemic circulation, this is less clear.[43] Majority of studies have shown an increase in GPX activity following exercise[18,20,21] and the response of erythrocyte GPX activity in our study agrees with some previous studies also showing a decrease after exercise.[42-43] The decrease of GPX activity in our study seems to be a result of lipid peroxidation,[44] sensitivity to exercise-induced peroxide and proxy radical formation according to cell location,[18] exercise intensity,[45] and finally the type of

animal. It has been reported that GPX activity in the blood of intact mammals predisposed to atherosclerosis (rabbits, mini-pig) is considerably less in comparison to the resistance species (rats). Hypercholesterolemia also produces an abrupt decrease in GPX activity in whole blood and plasma in the susceptible animals and exercise may impose an additional stress decreasing its activity.[7] It has been reported that intense exercise did not change red cell CAT activity in human subjects,[19,43] rat heart muscle[43] and small mammals.[46] CAT activity of erythrocytes has been reported to increase in professional cyclists compared with amateur cyclists and sedentary controls.[47] Exercise-induced CAT expression in C57BL/6 mouse arterial wall has been reported after chronic and acute exercise.[14] It is thought that decrease of CAT activity in the HC group as well as increased CAT in exercised groups depends on oxidative stress intensity. In the other words, oxidative stress has dual effects on inducing antioxidant enzymes. SOD has been studied to a greater extent than other antioxidant enzymes, but there is no consensus in literature about response of erythrocyte SOD activity to exercise.[16,19,21,22,43] In our study, total SOD activity in erythrocyte rose with exercise only in the control group, similar to results of some studies in rabbits.[19] With a few exceptions, most studies indicate that acute exercise increases SOD, this activation of SOD resulting from increased superoxide production during exercise.[21] On the other hand, many studies have reported no increase in SOD activity following short-term and prolonged exercise in tissues including muscle, heart, lung, liver, brain, plasma and red cells.[41-43] In our study decreased SOD activity under the concomitant effects of chronic exercise and high cholesterol diet may result from high oxidative stress through increase of superoxide production. Superoxide may react with other ROS such as NO to form highly toxic species such as peroxynitrite in addition to direct toxic effects. Alternatively, superoxide can be dismuted to much more reactive hydrogen peroxide which can then lead to highly toxic radical formation.[18] In addition, decreased CAT activity can also contribute to the oxidative stress found in hypercholesterolemic animals due to the dual effects of oxidative stress. Understanding of the relationship among exercise, oxidative stress and free radicals, or in the other words, the mechanisms involved in the changes of antioxidant enzymes activity during exercise remains a challenge.[21,43] Thus, although some of the antioxidant enzymes are activated during chronic exercise, the protective margin could be quite limited depending on individual enzymes and the tissues involved.[21,22,43] Antioxidant enzymes may be activated selectively during exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. Also different characteristics of the different antioxidant enzymes adaptation to exercise may be multifaced and depend on the specific pattern of gene expression for each enzyme, the threshold required for induction and their interaction.[21,22]

In this study we found that plasma MDA and TAC levels increased significantly following chronic exercise and /or high cholesterol diet feeding. Although MDA as a marker of oxidative damage has been studied extensively, generally very differing and conflicting results have also been reported in various tissues and plasma of animal models and in humans;[4,8,15,22,23,43] our results are similar to some of these.[21,43] This inconsistency of results may be a reflection of differences in exercise intensity and duration, type of animal or method used for assessment and also maximal oxygen uptake.[43,44] Increased MDA in our results may be attributable to high sensitivity of rabbits to free radical production by high cholesterol and exercise. Elevated MDA is not thought to be a negative exercise effect because oxidants and oxidized-lipids are not necessarily deleterious.[48] According to recent studies it has been suggested that some of the oxidized lipids could also elicit "antioxidant, antiatherogenic" responses

from cells.[49] Exercise-induced oxidative stress and oxidation of lipids especially oxidized-LDL and possibly VLDL-C and TG might suggest an ongoing oxidative clearance of LDL-C, VLDL-C and TG from the plasma. If so, oxidation of LDL-C in the plasma itself accounts for some of the lipid-lowering effects of exercise and may actually be beneficial against atherosclerosis. In addition, although in our study activities of erythrocyte antioxidants were reduced in animals on atherogenic diet, their induction in exercised groups, based on the threshold required, may be attributable to plasma oxidized lipids specially oxidized-LDL-C and fatty acid interaction.[14,15,50]

Plasma is mostly accounted for by a number of low molecular weight antioxidant molecules either water or lipid-soluble. Evaluation of the TAC gives more biological relevant information than that of the individual levels of specific antioxidants of a given body fluid such as plasma. The overall TAC considers the cumulative effect of all antioxidants (known and unknown, measurable and not measurable) present in plasma and it is used for evaluating the effect of several physiological conditions on plasma in humans and animals.[51] In contrast to our results, it has been reported that exercise with 65% VO2max decreases plasma TAC in rat.[24] It has also been shown that people with high aerobic capacity due to extreme endurance exercise, have plasma with decreased TAC and higher susceptibility to oxidation.[52] In addition, to our knowledge no study has been published about interaction of chronic exercise and TAC in animal models. Many studies have investigated the effects of exercise on components of TAC specially GSH metabolism as a measure of TAC and have reported differing and controversial results.[21-41] Increased TAC in our study may be related to GSH metabolism. In addition, elevation of vitamin E as part of TAC due to increased mobilization of tocopherol with free fatty acid from adipose tissue or increased flux of fatty acids through the liver stimulating the secretion of LDL-C, which are rich in tocopherol and also redistribution of tocopherol toward tissues by increased ROS production. Finally increased absorption from the gastrointestinal tract may be considered as another possible mechanism for increased TAC plasma concentration affected by exercise and/ or high cholesterol diet.[43] On the other hand, alteration in thiol content as a functional sulfhydryl has been suggested as a main determinant of TAC changes, but there are limited studies regarding with the interaction of chronic exercise effect and thiol changes in literature.[53] More studies are required for evaluation of the relationship between the atherogenic diet, exercise effect, TAC and its components, especially thiols in human and animal models.

In conclusion, our findings suggest that chronic exercise is an appropriate method for prevention and regression of atherogenic diet-induced atherosclerosis along with positive changes in the serum cholesterol profile and enhancement of TAC. In contrast to TAC, the activity of red cell primary antioxidant enzymes were reduced by the atherogenic diet but the pattern of changes in these enzymes were affected differently by exercise and /or high cholesterol diet feeding possibly because of alterations in the ability to adapt to exercise-induced oxidative stress intensity. We found that exercise and /or high cholesterol diet feeding increased lipid peroxidation but this finding is not necessarily deleterious and can also be interpreted as an “antioxidant;-antiatherogenic” response.

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References

1. Meraji S, Abuja PM, Hayn M, Kostner GM, Morris R, Oraii S, et al. Relationship between classic risk factors, plasma antioxidants and indicators of oxidant stress in angina pectoris (AP) in Tehran. Atherosclerosis 2000; 150:403-12.
2. Gabriel HH, Heine G, Kroger K, Ratz M, Lichtenstern C, Schmitz A, et al. Exercise and atherogenesis: where is the missing link? Exerc Immunol Rev 1999; 5: 96-102.
3. Jen CJ, Chan HP, Chen HI. Chronic exercise improves endothelial calcium signaling and vasodilatation in hypercholesterolemic rabbit femoral artery. Arterioscler Thromb Vasc Biol 2002; 22: 1219-24.
4. Aguilera CM, Mesa MD, Ramirez-Tortosa MC, Quiles JL, Gil A. Virgin olive and fish oils enhance the hepatic antioxidant defence system in atherosclerotic rabbits. Clin Nutr 2003; 22: 379-84.
5. Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, et al. Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. Am J Physiol Endocrinol Metab 2002; 282: E1055-61
6. Kempaiah RK, Srinivasan K. Antioxidant status of red blood cells and liver in hypercholesterolemic rats fed hypolipidemic spices. Int J Vitam Nutr Res 2004; 74: 199-208.
7. Lankin VZ, Tikhaze AK. Glutathione peroxidase II activity in the blood of hypercholesteremic mammals. Biull Eksp Biol Med 1980; 89: 554-6.
8. Mantha SV, Prasad M, Kalra J, Prasad K. Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits. Atherosclerosis 1993; 101:135-44.
9. Lapenna D, Del Boccio G, Porreca E, Pennalli A, Mezzetti A, De Gioia S, et al. Effects of high fat -, cholesterol-enriched diet on the antioxidant defense mechanisms in the rabbit Heart. Free Radic Res Commun.1992; 31:87-96.
10. 10. Del Boccio G, Lapenna D, Porreca E, Pennelli A, Savini F, Feliciani P, et al. Aortic antioxidant defence mechanisms. time-related changes in cholesterol-fed rabbits. Atherosclerosis 1990; 81: 127-35.
11. Ibrahim W, Lee US, Yeh CC, Szabo J, Bruckner G, Chow CK. Oxidative stress and antioxidant status in mouse liver: effects of dietary lipid, vitamin E and iron. J Nutr 1997; 127: 1401-6.
12. Lu YF, Chiang CF. Effect of dietary cholesterol and fat levels on lipid peroxidation and the activities of antioxidant enzymes in rats. Int J Vitam Nutr Res 2001; 71: 339-46.
13. Hsu HC, Lee YT, Chen MF. Effects of fish oil and vitamin E on the antioxidant defense system in diet-induced hypercholesterolemic rabbits. Prostaglandins Other Lipid Mediat 2001; 66: 99-108.
14. Meilhac O, Ramachandran S, Chiang K, Santanam N, Parthasarathy S. Role of arterial wall antioxidant defense in beneficial effects of exercise on atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2001; 21: 1681-8.
15. Shern-Brewer R, Santanam N, Wetzstein C, White-Welkley J, Parthasarathy S. Exercise and cardiovascular disease. A new perspective. Arterioscler Thromb Vasc Biol 1998; 18: 1181-7.
16. Atalay M, Laaksonen DE. Diabets, oxidative stress and physical exercise. J Sports Sci and Med 2002; 1: 1-14.
17. Marlin DJ, Fenn K, Smith N, Deaton CD, Roberts CA, Harris PA, et al. Changes in circulatory antioxidant status in horses during prolonged exercise. J Nutr 2002; 132: 1622S-7S.
18. Atalay M, Laaksonen DE, Niskanen L, Uusitupa M, Hanninen O, Sen CK. Altered antioxidant enzyme defences in insulin-dependent diabetic men with increased resting and exercise-induced oxidative stress. Acta Physiol Scand 1997; 161:195-201.
19. Duthie GG, Robertson JD, Maughan RJ, Morrice PC. Blood antioxidant status and erythrocyte lipid peroxidation following distance running. Arch Biochem Biophys 1990; 282:78-83.
20. Sen CK. Oxidants and antioxidants in exercise. J Appl Physiol 1995;79: 675-86.
21. Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999; 222 : 283-92.
22. 22. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003; 189: 41-54.
23. Mantha SV, Prasad M, Kalra J, Prasad K. Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits. Atherosclerosis. 1993; 101: 135-44.
24. Ficicilar H, Zergeroglu AM, Tekin D, Ersoz G. The effects of acute exercise on plasma antioxidant status and platelet response. Thromb Res 2003; 111: 267-71.
25. Simonet S, Porro de Bailliencourt J, Descombes JJ, Mennecier P, Laubie M, Verbeuren TJ. Hypoxia causes an abnormal contractile response in the atherosclerotic rabbit aorta. Implication of reduced nitric oxide and cGMP production. Circ Res. 1993; 72: 616-30.
26. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121-6.
27. Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. Free Radic Biol Med 2000; 28: 1745-50.
28. Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM.

thentic plasma hydroperoxides in NIDDM. Diabetes 1995; 44: 1054-8.
29. Leaf DA. The effect of physical exercise on reverse cholesterol transport. Metabolism 2003;52: 950-7.
30. Yang AL, Chen HI. Chronic exercise reduces adhesion molecules/iNOS expression and partially reverses vascular responsiveness in hypercholesterolemic rabbit aortae. Atherosclerosis 2003; 169: 11-7.
31. Yang AL, Jen CJ, Chen HI. Effects of high-cholesterol diet and parallel exercise training on the vascular function of rabbit aortas: a time course study. J Appl Physiol 2003; 95:1194-200.
32. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101: 1581-90.
33. Ensign WY, McNamara DJ, Fernandez ML. Exercise improves plasma lipid profiles and modifies lipoprotein composition in guinea pigs. J Nutr Biochem 2002; 13:747-53
34. Holloszy JO, Skinner JS, Toro G, Cureton TK. Effefcts of a six-month program of endurance exercise on the serum lipids of middle-aged man. Am J Cardiol. 1964; 14: 753-60.
35. Goldberg L, Elliot DL. The effect of physical activity on lipid and lipoprotein levels. Med Clin North Am. 1985; 69: 41-55.
36. Pereira B, Costa Rosa LF, Safi DA, Medeiros MH, Curi R, Bechara EJ. Superoxide dismutase, catalase, and glutathione peroxidase activities in muscle and lymphoid organs of sedentary and exercise-trained rats. Physiol Behav 1994; 56:1095-9.
37. Graham TE. Exercise, postprandial triacylglyceridemia, and cardiovascular disease risk. Can J Appl Physiol 2004; 29: 781-99.
38. Chen HI, Li HT. Physical conditioning can modulate endothelium-dependent vasorelaxation in rabbits. Arterioscler Thromb 1993; 13: 852-6.
39. Sherman DL. Exercise and endothelial function. Coron Artery Dis 2000; 11:117-22.
40. Ling WH, Cheng QX, Ma J, Wang T. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. J Nutr 2001; 131: 1421-6.
41. Clarkson PM, Thompson HS. Antioxidants: what role do they play in physical activity and health? Am J Clin Nutr 2000; 72: Suppl 2: 637S-46S.
42. Balakrishnan SD, Anuradha CV. Exercise, depletion of antioxidants and antioxidant manipulation. Cell Biochem Funct 1998; 16: 269-75.
43. Deaton Ch.m, Marlin DJ. Exercise-associated oxidative stress. Clin Tech in Equin Practice. 2003; 2: 278-291.
44. Thirunavukkarasu V, Balakrishnan SD, Ravichandran MK, Anuradha CV. Influence of 6-week exercise training on erythrocyte and liver antioxidant defense in hyperinsulinemic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003; 135: 31-7.
45. Powers SK, Lennon SL. Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. Proc Nutr Soc 1999; 58: 1025-33.
46. Selman C, McLaren JS, Collins AR, Speakman JR. Voluntary exercise has only limited effects on activity of antioxidant enzymes and does not cause oxidative damage in a small mammal. J Nutr 2002; 132: Suppl 6:1784S-6S.
47. Aguilo A, Tauler P, Pilar Guix M, Villa G, Cordova A, Tur JA, et al. Effect of exercise intensity and training on antioxidants and cholesterol profile in cyclists. J Nutr Biochem 2003; 14: 319-25.
48. Sen CK. Update on thiol status and supplements in physical exercise. Can J Appl Physiol 2001; 26: Suppl: S4-12.
49. Parthasarathy S, Santanam N, Ramachandran S, Meilhac O. Oxidants and antioxidants in atherogenesis. An appraisal. J Lipid Res 1999; 40: 2143-57.
50. Leaf DA, Kleinman MT, Hamilton M, Barstow TJ. The effect of exercise intensity on lipid peroxidation. Med Sci Sports Exerc 1997; 29: 1036-9.
51. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med 2000; 29: 1106-14.
52. Sharman JE, Geraghty DP, Shing CM, Fraser DI, Coombes JS. Endurance exercise, plasma oxidation and cardiovascular risk. Acta Cardiol 2004; 59: 636-42.
53. Inayama T, Oka J, Kashiba M, Saito M, Higuchi M, Umegaki K, et al. Moderate physical exercise induces the oxidation of human blood protein thiols. Life Sci 2002; 70: 2039-46.