

Research article

INFLUENCE OF CHRONIC EXERCISE ON RED CELL ANTIOXIDANT DEFENSE, PLASMA MALONDIALDEHYDE AND TOTAL ANTIOXIDANT CAPACITY IN HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT

Despite the knowledge on the antiatherogenic effects of exercise, the mechanism by which exercise reduces atherogenic risk remains unknown. In this study, we investigated the hypothesis that chronic exercise-induced oxidative stress may increase plasma total antioxidant capacity and antioxidant defense in the red cells. For 8 weeks, 60 male Dutch rabbits were fed rabbit chow with or without the addition of 2% cholesterol. The animals were further divided into rest and exercise groups (n = 15 for each group). Animals in exercise groups ran on a rodent treadmill at 15 m/min for 10 to 60 minutes gradually for 5 days per week for a total of 8 weeks. At the end of experiments, blood samples were collected and glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) activities were determined in red blood cells. Total antioxidant capacity (TAC), malondialdehyde (MDA) and total thiol (T-SH) levels were measured in plasma. Thoracic aorta and carotid arteries were isolated for histological examination to evaluate atherosclerosis. Eight weeks of chronic exercise reduced atherogenic diet-induced atherosclerotic lesions in all the arteries studied, along with positive changes in cholesterol profile, especially increase of serum HDL-C level. Plasma MDA, TAC and T-SH concentrations were enhanced by exercise in both control and hypercholesterolemic diet groups. Erythrocyte catalase activity was significantly increased by chronic exercise ($p < 0.05$), whereas total SOD activity rose with exercise only in the control group. Surprisingly, GPX activity was significantly reduced ($P < 0.05$) in response to exercise in the control group and also in the high cholesterol diet group. Exercise is a useful tool for the prevention and regression of atherosclerosis which is evident by our findings of the enhancement of plasma TAC and positive change in serum cholesterol profile. However, the effect of exercise on red cell antioxidant activities is limited in the hypercholesterolemic animals compared to control animals, possibly in part because of alterations in the ability to adapt to exercise-induced oxidative stress in high cholesterol diet.

KEY WORDS: Chronic exercise, antioxidant, malondialdehyde, thiol, atherosclerosis..

INTRODUCTION

Atherosclerosis is the leading cause of mortality and morbidity in the developed world and most of

developing countries (Meraji et al., 2000). Atherosclerosis is a complex process, and it is possibly caused by high-fat diet and sedentary

lifestyle (Jen et al., 2002). It has been proposed that oxidative stress plays an important role in atherosclerosis process (Stocker and Keaney, 2004). On the other hand, exercise has been suggested as a deterrent of cardiovascular disease (CVD) and atherosclerosis, and its antiatherogenic effects have been described in human and in different animal models. It can also positively influence risk factors that are associated with atherosclerosis, but all the mechanisms by which exercise might protect against CVD are not known (Aguilo et al., 2003; Meilhac et al., 2001). Paradoxically, exercise can also induce oxidative stress in animals and humans. Oxidative stress is an imbalance between the free radical production and antioxidant defense systems of the body and has been implicated in the accelerated atherosclerosis (Atalay and Laaksonen, 2002; Shern et al., 1998; Urso et al., 2003). Since the oxidation hypothesis of atherosclerosis was suggested, a plethora of experiments involving cell culture, animal and human studies have shown that oxidized lipids could exhibit in vitro proatherogenic effects.

Although physical exercise may acutely induce oxidative stress, regular exercise appears to enhance antioxidant defenses, and decrease lipid peroxidation in animal and human studies (Atalay et al., 1997; Duthie et al., 1990). However, the responses of the primary antioxidant enzymes in red cells including superoxide dismutase (SOD), glutathion peroxidase (GPX) and catalase (CAT) activities and also plasma MDA level to exercise have been inconsistent in the literature (Atalay and Sen, 1999; Atalay and Laaksonen, 2002; Clarkson and Thompson, 2000; Gul et al., 2002; Mantha et al., 1993; Pereira et al., 1994; Sen, 1995).

Despite a large number of studies on the effects of exercise on antioxidant enzymes in various tissues, only limited studies concerned with the effect of exercise on plasma total antioxidant capacity and also total thiol have been reported (Ficicilar et al., 2003, Kinnunen et al. 2005). To our knowledge, evaluation of effects of chronic exercise and/or high cholesterol diet on total antioxidant capacity and total thiol has not been done in rabbit model before. Therefore, the present study was scheduled for attaining a closer view of the mentioned parameters affected by chronic exercise in hypercholesterolemic Dutch rabbits.

METHODS

Animals and diet

Sixty male Dutch white rabbits (1.3 kg at the beginning) were divided into four groups: The normal diet control (NC) group, normal diet with

exercise (NE) group, high-cholesterol diet (HC) group and high-cholesterol diet with exercise (HE) group. The control groups were fed normal rabbit chow, whereas the high cholesterol diet groups were fed with high cholesterol diet (2%). All animals were housed in an environmentally controlled room.

Exercise protocol

The exercise protocol was described elsewhere (Jen et al., 2002). After 1 week of familiarization, the exercise training groups ran on a leveled treadmill (Danish Yakhteh Co, Tabriz, Iran) at a speed of 15 m/min for 10 minutes for the first week. The running time was extended 5-10 min each week until the rabbits could run for 60 minutes per day. They were exercised for 5 days per week for a total of 8 weeks. This exercise intensity is approximately 70% of their maximal exercise capacity (Jen et al., 2002; Urso and Clarkson, 2003). In contrast, the sedentary groups were placed on the treadmill for 10 minutes each day without receiving any exercise training. The rabbits were anesthetized at the end of experiments by injecting ketamine (25mg/kg, i.v.) and sodium pentobarbital (20 mg/kg, i.v.) via the margin ear vein. To avoid the acute effect of exercise, the animals were sacrificed 48 h after exercise. Blood samples were drawn from the inferior vena cava and were stored in tubes for determination of serum lipid profile, erythrocyte SOD, GPX and CAT activities, and plasma MDA, TAC and T-SH concentrations.

Histological studies of blood vessels

Thoracic aorta and carotid arteries were immediately isolated and were placed in formalin 10%. Briefly, after tissue processing steps, several serial sections of blood vessel segments (6 μ m thick) were stained by 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 core of 0, and a segment that was completely covered by atherosclerotic lesion was given a core of 5 (Simonet et al., 1993). Then the area of thickened intima was assessed and calculated statistically among animals and was expressed as the percentage of luminal area of the vessel ring.

Serum lipid profile

Serum lipid profile including total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglyceride (TG) were determined by enzymatic methods using automatic analyzer (Abbott, Alcyon 300, USA).

Table 1. Comparison of the serum lipid profile changes (mg/dl) among four groups of Dutch rabbits under chronic exercise and /or high cholesterol diet. Data (n = 12) are means (\pm SD).

Variable	NC (1)	NE (2)	HC (3)	HE (4)
Total cholesterol	74.6 (3.9) ^{3,4}	69.2 (3.5) ⁴	1970 (84) ^{1,2}	2001 (104) ^{1,2}
LDL-C	28.2 (3.8) ^{3,4}	19.5 (2.4) ⁴	1630 (68) ^{1,2}	1592 (62) ^{1,2}
HDL-C	32.3 (2.8) ^{3,4}	39.3 (2.0) ⁴	313 (45) ^{1,2,4}	440 (42) ^{1,2,3}
VLDL-C	17.6 (4.5) ^{3,4}	16.0 (2.8) ⁴	52 (15) ^{1,2,4}	33 (9) ^{1,2,3}
TG	88.0 (22.5) ^{3,4}	80.0 (14.4) ⁴	266 (70) ^{1,2,4}	169 (13) ^{1,2,3}
HDL/LDL	1.15 (.20) ^{2,3,4}	1.92 (.41) ¹	.19 (.02) ^{1,2}	.27 (.05) ^{1,2}

Superscripts denote significantly ($p < 0.05$) differences between the groups.

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-cholesterol, HDL-C = high density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, TG = triglyceride.

Determination of antioxidant enzymes

Erythrocyte lysates were used for determination of GPX, SOD and CAT. Briefly, blood was collected in tubes containing EDTA and centrifuged (1500 g) for 15 min at 4°C. The sediment containing erythrocyte was suspended in normal saline and recentrifuged. This washing process was repeated twice. Sediment red blood cells were added to ice-cold distilled water and mixed thoroughly for hemolysis. GPX activity was determined using red blood cell lysates based on Palia and Valentine method using a commercial kit (Randox Laboratories GmbH, Krefeld, Germany), according to the instructions provided by the manufacturer. A sample volume of 50 μ l was used. The decrease in absorbance was measured in 340 nm spectrophotometrically (Pharmacia Biotec, Cambridge, England). Also SOD activity in red cell was determined by SOD kit (Randox Laboratories GmbH, Krefeld, Germany) in 505 nm by spectrophotometer. The activity of SOD 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.

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 (Aebi, 1984).

Determination of serum malondialdehyde (MDA)

The amount of MDA was determined by the TBA (thiobarbituric acid) assay. All reagents used in this assay were obtained from Merck (Darmstadt, Germany). Briefly, 0.50 ml of plasma was added to 3 ml of 1% phosphoric acid, 1 ml of 0.60% TBA, and 0.15ml of 0.20% butylated hydroxytoluene 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 calculated and was expressed as μ M (Dandekar et al., 2002; Nourooz-Zadeh et al., 1995).

Determination of plasma total thiol (T-SH)

A spectrophotometric assay based on 2, 2-dithiobisnitrobenzoic acid (DTNB) was used for the thiol assay. In brief, an aliquot of plasma (50 μ l) was mixed with 1 ml of the Tris-EDTA buffer, and the absorbance was measured at 412 nm (A_1). To this was added 20 μ l of 10 mM DTNB. After 15 minutes at ambient temperature the absorption was measured again (A_2), together with a DTNB blank (B). Then total SH groups were calculated as (Aebi, 1984):

$$(A_2 - A_1 - B) \times (1.07/0.05)/13.6 = (A_2 - A_1 - B) \times 1.57 \text{mM}$$

Statistical analysis

Data was expressed as means \pm SD; statistical computations were calculated using SPSS 10 for windows software (SPSS INC, Chicago, IL, USA). Sample size numbers of animal was indicated by n (n = 15 rabbits for each group). Result among four groups was analyzed by ANOVA and student's t-test and further by least significant difference (LSD). Significant differences were considered at $p < 0.05$.

RESULTS

Body weight

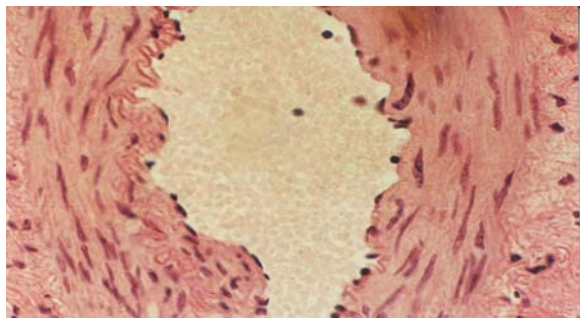
Body weight was significantly increased using normal and high cholesterol diet but it did not show any significant change in animals under chronic exercise (Data not shown).

Histological findings

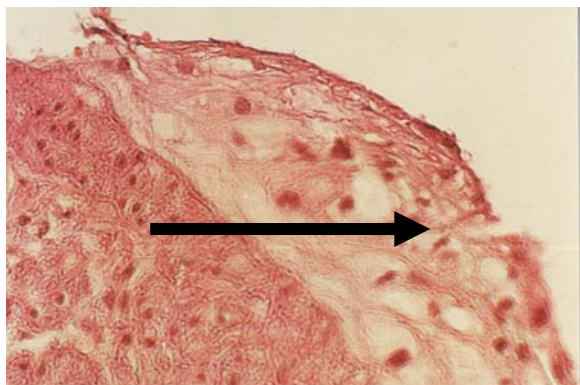
Eight weeks of 2% high cholesterol diet induced atherosclerotic lesions and thickening of the intima in all thoracic aorta and with less extent in carotid arteries in HC group. Chronic exercise reduced

significantly atherosclerotic lesions (20-35%) in HE group. There was no lesion in normal diet groups or the normal diet with exercise group (Figure 1A, B, C and 2A, B).

A



B



C

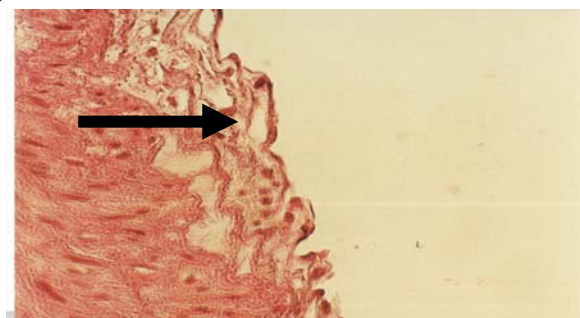


Figure 1. Example of standard H&E staining of aorta for evaluating atherosclerotic lesions among groups. No lesions were observed in NC or NE groups (A). 2% high cholesterol diet induced atherosclerotic lesions in 86% of thoracic aorta in HC group (B) while chronic exercise reduced atherosclerotic lesions significantly (mean=35%) in HE group (C).

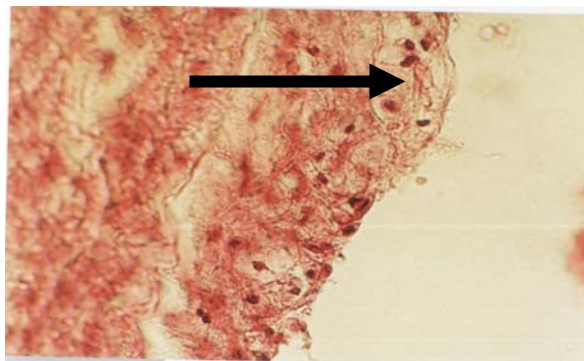
Abbreviations: NC = normal diet control, NE = normal diet with exercise, HC = high cholesterol diet control, HE = high cholesterol diet with exercise.

Serum lipid profile

Our results clearly demonstrate that eight weeks of 2% of high cholesterol diets significantly increased serum total cholesterol, LDL-C, HDL-C, VLDL-C, and TG. These observations indicate that atherogenic diet indeed induced hypercholesterolemia in our experimental Dutch

rabbit model. Eight weeks of concomitant chronic exercise considerably reduced diet-increased serum level of VLDL-C and TG in HE group without significant changes in total cholesterol and LDL-C. Although chronic exercise tended to reduce the mentioned parameters in the control group, these 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 effects of chronic exercise on serum cholesterol profile (Table 1).

A



B

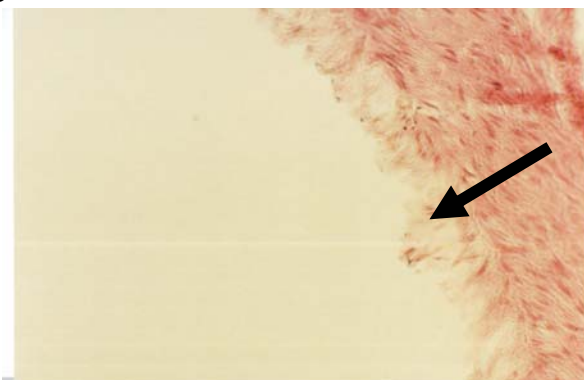


Figure 2. Examples of standard H&E staining of carotid for evaluating of atherosclerotic lesions with comparison of HC (A) with figure 1A (control) and HE (B) with HC (A). It was shown that approximately 68% of intimal surface has been covered with the lesions (A) and reduced by chronic exercise significantly (mean=20%) (B).

Abbreviations: NC = normal diet control, NE = normal diet with exercise, HC = high cholesterol diet control, HE = high cholesterol diet with exercise.

Antioxidant enzymes activities

All of the erythrocyte antioxidant enzymes were decreased by high cholesterol diet. Red blood cell SOD activity rose significantly with chronic exercise only in the control group (Figure 3A). Erythrocyte catalase activity was significantly enhanced by chronic exercise in NE and HE groups (Fig. 3B). In addition, erythrocyte GPX activity was reduced in response to chronic exercise and/ or high cholesterol diet significantly (Figure 4A).

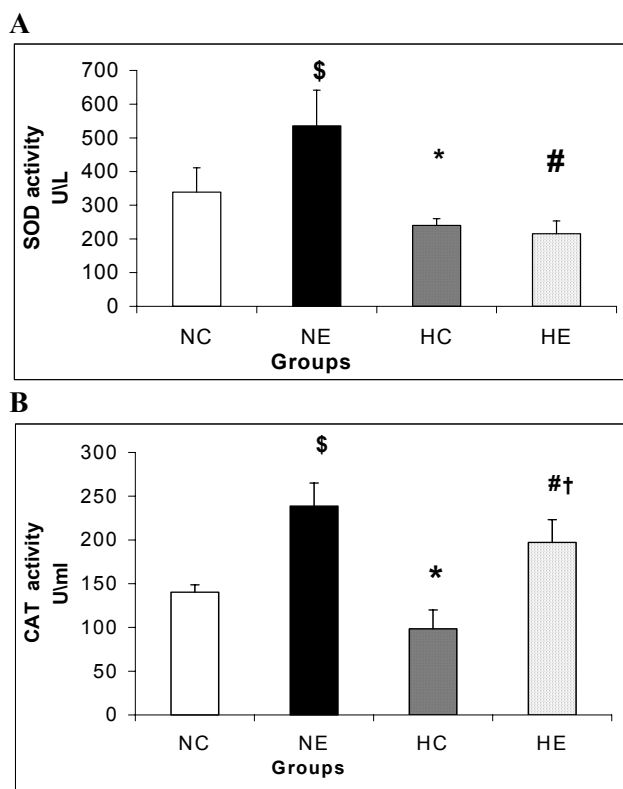


Figure 3. Comparison of the chronic exercise effect and /or high cholesterol diet on red cell SOD (A) and CAT (B) activities among four groups in rabbits. Data are expressed as mean \pm SD (n=15 for each group). Differences of $p < 0.05$ were considered significant differences.

\$ 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.

Plasma MDA, T-SH and TAC levels

Plasma MDA, TAC and T-SH levels were significantly increased in response to chronic exercise and /or high cholesterol diet (Figures 4B, 5A and 5B respectively). These observations are possibly indicator of lipid peroxidation and induction of non-enzymatic antioxidants by both high cholesterol diet and /or chronic exercise.

DISCUSSION

Our results indicated that 8 weeks of 2% high cholesterol diet increased all serum cholesterol profile fractions and induced formation of atherosclerotic lesions in thoracic aorta and to less extent in carotid artery. After 8 weeks of concomitant exercise intervention, atherosclerotic plaques were significantly reduced in both arteries. None of the arteries in NC and NE groups showed any sign of fatty streaks.

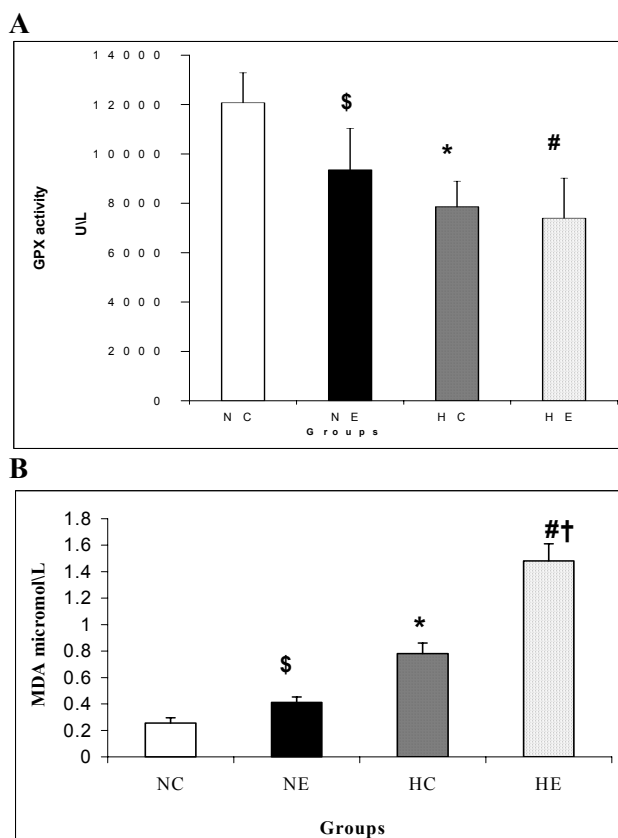


Figure 4. Comparison of the chronic exercise effect and /or high cholesterol diet on red cell GPX activity (A) and plasma TAC concentration (B) among four groups in rabbits. Data are expressed as mean \pm SD (n=15 for each group). Differences of $p < 0.05$ were considered significant.

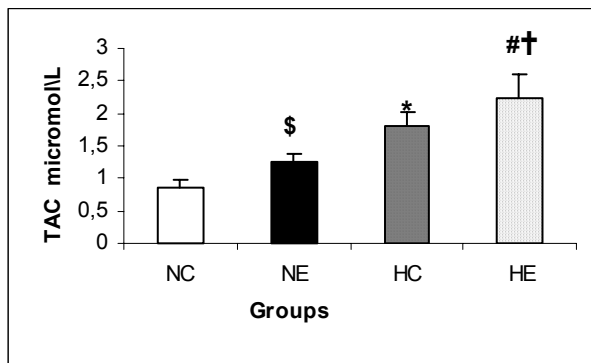
\$ NE vs. NC * HC vs. NC and NE
HE vs. NC and NE † HE vs. HC

Abbreviations: NC = normal diet control, NE = normal diet with exercise, HC= high cholesterol diet control, HE = high cholesterol diet with exercise, GPX = glutathione peroxidase, MDA = malondialdehyde.

Although exactly how exercise improves atherosclerosis is still unclear, several possible mechanisms of the anti-atherogen effects of exercise have been proposed: including antithrombotic, anti-inflammatory and antioxidant properties of HDL-C, decrease in plasma LDL-C (Leaf et al., 2003) and positive changes resulting from exercise-induced oxidative stress such as induction of antioxidant systems as a defensive mechanism of the cell under oxidative stress (Meilhac et al., 2001). In contrast to studies in rabbit (Yang et al., 2003) and mice (Meilhac et al., 2001), in this study, chronic exercise increased HDL-C or proportion of HDL to LDL. According to our results and others (Leaf et al., 2003; Price et al., 2002), increase in HDL-C level can be the main effect of chronic exercise on serum cholesterol profile. Based on the recent studies, it seems that HDL-C is protected from exercise-induced oxidative stress by paraoxonase antioxidant

enzyme (Aviram et al., 1998). Probably exercise through increase in efficacy of HDL-mediated reverse cholesterol transport system and lipoprotein lipase activity results in finally to decrease of LDL-C, VLDL-C and TG in plasma and then atherosclerosis (Ensign et al., 2002).

A



B

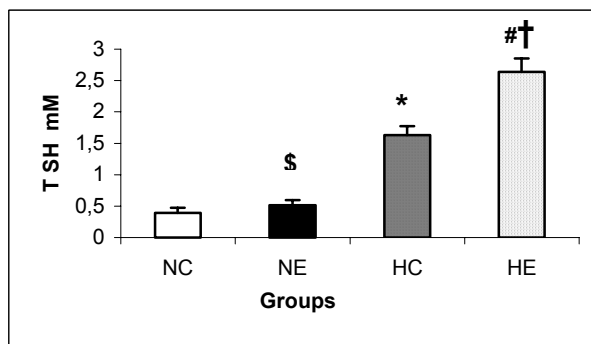


Figure 5. Comparison of the chronic exercise effect and/or high cholesterol diet on plasma TAC and T-SH concentrations among four groups in rabbit. Data are expressed as mean \pm SD (n=15 for each group). Differences of $p < 0.05$ were considered significant.

\$ 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, T-SH = total sulfhydryl (total thiol).

In this study, chronic exercise reduced atherosclerotic lesions in thoracic aorta more than in carotid artery. These changes are similar to those reported in thoracic aorta and carotid artery in New Zealand white rabbit by exercise (Chen et al., 1993; Delp et al., 1993; Jen et al., 2002). There is little information about the susceptibility of the different arteries to atherosclerosis and their improvement by exercise in the literature (Chen and Li, 1993; Laborgne et al., 2003; Yang et al., 2003). According to the 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 (Chen and Li 1993; Jen et al., 2002). It is well known that the blood flow in aorta increases several folds during exercise, whereas, the flow in carotid arteries remains relatively constant due to efficient cerebral autoregulation. Therefore, the 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 (Chen and Li, 1993; Yang et al., 2003). Shear stress undergone during acute and chronic exercise may result in an improvement in vasomotor function and may deter atherogenesis. Blood vessels with higher areas of shear stress are usually found to be free of atherosclerotic changes while areas of lower shear stress such as arterial branch points are more prone to atherogenesis (Ross, 1993). Our data also show that amelioration of chronic exercise-induced antioxidant defense systems may be considered as one of the important defensive mechanisms for prevention and regression of atherosclerosis.

Although there is a close relationship between the hypercholesterolemia and atherosclerosis, it has been suggested that atherosclerotic lesions might depend on increased oxidative stress (Leborgne et al., 2003). Hypercholesterolemia increases the levels of ROS and elevated ROS can stimulate the progression of atherosclerosis pathogenesis. Exercise is also known to induce oxidative stress on the body due to the increased generation of ROS and probably depletion of antioxidants that may result in atherosclerosis (Shern-Brewer et al., 1998). In this study we found that erythrocyte activities of total SOD, GPX and CAT were significantly decreased by high cholesterol diet. Red blood cell CAT activity was increased by chronic exercise but total SOD activity rose with exercise only in the normal diet group whereas GPX activity was reduced by exercise and/or high cholesterol diet.

It has been proposed that high cholesterol diet induces free radical production and may result in oxidative stress (Mantha et al., 1993; Meilhac et al., 2001; Sen, 1995). Most of the studies but not all have shown that regular exercise strengthens antioxidant defense in healthy humans and animals (Beckman, 2002; Clarkson, 2000; Ji, 1999). On the other hand these results are highly conflicting with each other. Upregulation of GPX in response to acute exercise has been reported in skeletal muscle in animal experiments (Ji 1993) and in erythrocytes of some normal humans (Atalay et al., 1997; Balakrishnan et al., 1998). On the other hand, no changes have been found in erythrocyte GPX activity in human (Duthie et al., 1990) and animal studies after chronic exercise (Bejma et al., 2000; Ji,

1999). However, the majority of studies have shown an increase in GPX activity with exercise and the response of erythrocyte GPX activity in our study agrees with some previous studies also showing a decrease after exercise (Balakrishnan et al., 1998; Deaton and Marlin, 2003). It seems that decrease of GPX activity in our study may result from: lipid peroxidation (Thirunavukkarasu et al., 2002); sensitivity to exercise-induced peroxide and proxy radical formation according to its location in the cell. In addition, exercise intensity can also influence lipid peroxidation because high intensity exercise has been shown to be generally superior to low intensity exercise in the up-regulation of GPX and SOD activities (Powers et al., 1999). Furthermore, type of animal can be a determinant, it has been reported that GPX activity in the blood of intact mammals predisposed to atherosclerosis (rabbits, mini-pigs, men) is considerably lower in comparison to the resistance species (rats). Hypercholesterolemia also produces an abrupt decrease in GPX activity in the whole blood and plasma in the susceptible animals and exercise may impose an additional stress for decreasing its activity (Lankin and Tikhaze, 1980).

It has been reported that exercise has not changed the red blood cell CAT activity in human subjects following exhaustive exercise (Duthie et al., 1999), and in small mammals (Selman et al., 2002). Conversely, another study has shown an increase around 20% with exercise (Deaton and Marlin, 2003). CAT activity of erythrocyte has been reported to be increased in professional cyclists compared with amateur cyclists and sedentary controls (Aguilo et al., 2003). Exercise-induced CAT expression in C57BL/6 mouse arterial wall has been reported after chronic and acute exercise (Meilhac et al., 2001). It is thought that decrease of CAT activity in HC group as well as increased CAT in exercised groups depends on oxidative stress intensity.

SOD has been studied to a greater extent than other antioxidant enzymes, but there is not a consensus about response of erythrocyte SOD activity to exercise in the literature (Atalay and Laaksonen 2002; Clarkson and Thomson, 2000; Ji 1999). In our study, total SOD activity in erythrocyte rose with exercise only in the control group and this observation is in agreement with some studies in rabbit (Fukai et al., 2000) and human (Duthie et al., 1990). Most of the studies with a few exceptions indicate that acute exercise increases SOD and this activation of SOD results from increased superoxide production during exercise (Ji, 1999). On the other hand, many studies have reported no increase or unchanged SOD

activity following short-term and prolonged exercise in tissues including muscle, heart, lung, liver, brain, plasma and red cells (Clarkson and Thompson, 2000; Deaton and Marlin, 2003). In our study decreased SOD activity under concomitant effect 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 converted to much more reactive hydrogen peroxide which can then lead to highly toxic radical formation (Hunt and Wolff, 1991). In addition, decreased CAT activity can also contribute to the oxidative stress found in hypercholesterolemic animals.

Understanding of the relationship between exercise, oxidative stress and the changes of antioxidant enzyme activity during exercise remains a challenge (Deaton and Marlin, 2003; Ji, 1999). 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 (Ji, 1999; Powers and Lennon, 1999). 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.

In this study we found that plasma MDA, TAC and T-SH levels were significantly increased by chronic exercise and /or high cholesterol diet. Although MDA as a marker of oxidative damage has been studied extensively, generally very variable and conflicting results have also been reported in various tissues and plasma of animal model and human (Atalay and Laaksonen, 2002; Clarkson and Thompson, 2000; Deaton and Marlin, 2003; Ji, 1999; Mantha et al., 1993; Meilhac et al., 2001; Urso and Clarkson, 2003). This inconsistency of results may be a reflection of differences in exercise intensity and duration, type of animal or training or assay method used (Deaton and Marlin, 2003; Ji, 1999). Increased MDA in our results may be attributed to high sensitivity of rabbit to free radical production by high cholesterol and exercise. Since recently it has been reported that oxidized lipids can also induce some of the antioxidant enzymes such as CAT in aorta and Mn-SOD in mitochondria (Meilhac et al., 2001), increased lipid peroxidation by exercise may also be interpreted as an antioxidant or antiatherogenic effect.

The overall TAC considers the cumulative effect of all antioxidants present in plasma and it is used for evaluating the effect of several physiological conditions on plasma in human and animals (Ghiselli et al., 2000). In contrast to our

results, it has been reported that exercise with 65% VO₂max decreased plasma TAC in rat (Ficiclar et al., 2003). It has also been shown that high intensity endurance exercise, decreased plasma TAC and increased susceptibility to oxidation in human (Sharman, 2004). While the plasma TAC is mainly accounted for uric acid and vitamin C (Balcerczyk and Bartosz, 2003), based on our results, alteration in thiol content can also be considered as determinant of TAC changes and indirect index of protein oxidation. It has been reported that moderate exercise induces the oxidation of human blood protein thiols (Inayama et al., 2002). Most of the exercise studies related to thiols have investigated GSH metabolism and have reported different and controversial results (Clarkson and Thompson, 2000; Sen and Packer, 2000), but GSH account for only half of all cell thiols. Studies on exercise-induced protein oxidation have mainly used the formation of carbonyls as a marker and information about the effect of exercise on protein sulfhydryl is scanty (Sen and Packer, 2000). The direct scavenging of hydroxyl radicals by thiols have been suggested as their main protective function (Sagrasta et al., 2002). One of the properties of most thiols is their ability to act as reducing agents. While exerting its antioxidant function, thiols such as glutathione are transformed from a reduced sulfhydryl (-SH) state to an oxidized disulfide (-s-s-) state. However, in biological systems, disulfides are recycled to thiols by specific reductase enzymes using cellular-reducing equivalents such as NADH or NADPH (Sen and Packer, 2000). Amelioration of the mentioned process by chronic exercise may lead to the accumulation of the reduced state of thiols. More studies are required for evaluation of the relationship between the atherogenic diet, exercise effect, TAC and its components specially thiols in human and animal models.

In conclusion, our findings suggest that chronic exercise is a proper method for prevention and regression of atherogenic diet-induced atherosclerosis along with positive change in serum cholesterol profile and enhancement of TAC. The late case, based on our data, is probably due to the elevation of total thiol concentration. In contrast to TAC, the activity of red blood cell primary antioxidant enzymes were reduced by atherogenic diet but the pattern of changes in these enzymes were differently affected by exercise and /or high cholesterol diet possibly because of alterations in the ability to adapt to exercise-induced oxidative stress intensity. We found that exercise and /or high cholesterol diet increased pro-oxidants evident by lipid peroxidation but this finding may not be

necessarily deleterious and can also be interpreted as an "antioxidant;-antiatherogenic" response.

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KEY POINTS

- Plasma MDA, TAC and T-SH concentrations were enhanced by exercise in both control and high cholesterol diet groups.
- GPX activity was significantly reduced in response to exercise in the control group and also in the high cholesterol diet group.
- Eight weeks of chronic exercise reduced atherogenic diet-induced atherosclerotic lesions in all the arteries studied.

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