Role of Arterial Wall Antioxidant Defense in Beneficial Effects of Exercise on Atherosclerosis in Mice

Olivier Meilhac, Sumathi Ramachandran, Kenneth Chiang, Nalini Santanam, Sampath Parthasarathy

Abstract—The mechanism(s) by which exercise reduces atherogenic risk remains unknown. This study tested the hypothesis that sustained exercise-induced oxidative stress may increase antioxidant defense in the arterial wall. Acute exercise induced an increase in antibodies to oxidatively modified proteins and catalase in the aortic walls of normal mice compared with sedentary control mice. In male atherogenic diet-fed low density lipoprotein (LDL) receptor-deficient mice, exercise lowered plasma cholesterol (15%) and decreased atherosclerotic lesions by 40% compared with values in sedentary control mice, with a concomitant increase in arterial catalase and endothelial NO synthase. Because these mice lack the LDL receptor, the results indicate that the LDL receptor might not be responsible for the exercise-induced lowering of plasma cholesterol. Vitamin E supplementation to exercising LDL receptor-deficient mice did not reduce atherosclerotic lesion formation significantly as opposed to lesion formation in untreated exercised mice. Moreover, vitamin E counteracted the beneficial effects of exercise by preventing the induction of aortic catalase activity and endothelial NO synthase expression. These results might indicate that although vitamin E might have prevented the exercise-induced oxidative stress, its availability in the artery was insufficient to prevent the atherosclerotic process. These results indicate that exercise-induced plasma oxidative stress could be responsible for the prevention of atherosclerosis by stimulating arterial antioxidant response. Furthermore, vitamin E supplementation could be deleterious in exercisers by inhibiting antioxidant enzyme buildup in the arterial wall. (Arterioscler Thromb Vasc Biol. 2001;21:1681-1688.)

Key Words: catalase □ NO synthase □ vitamin E □ oxidative stress □ LDL receptors

Exercise is a deterrent of cardiovascular disease, and its antiatherogenic effects have been described in different animal models.\(^1,2\) Exercise can also positively influence risk factors that are associated with cardiovascular disease: hypertension, diabetes mellitus, obesity, increased plasma lipids, and endothelial dysfunction.\(^3\) However, the mechanism(s) by which exercise might benefit cardiovascular disease is not known.

Ever since the “oxidation hypothesis” of atherosclerosis was suggested about 15 years ago,\(^4,5\) a plethora of experiments involving cell culture, animal, and human studies has shown that oxidized lipids could exhibit numerous in vitro proatherogenic effects.\(^6-9\) Paradoxically, exercise also induces an oxidative stress\(^10,11\) in animals and humans, and this would appear incompatible with its antiatherogenic effects. As a resolution to this paradox, on the basis of our human studies, we recently proposed that either the overall beneficial effects of exercise would overwhelm the deleterious effects of oxidative stress, or the exercise-induced oxidative stress might itself be beneficial by inducing arterial antioxidant enzymes.\(^9,10,12\)

Our hypothesis is supported by evidence from studies involving the induction of antioxidant enzymes in vitro by oxidants. Enzymes associated with antioxidant defense, such as manganese superoxide dismutase, endothelial NO synthase (eNOS), heme oxygenase, and catalase,\(^13-16\) as well as the synthesis of glutathione,\(^17\) can be induced by oxidants in cell culture studies. Moreover, in vivo studies have shown that exercise as well as other oxidant stimulation could induce antioxidant enzymes in different tissues: heart, liver, blood, or muscle.\(^18-20\) Taken together, these observations alerted us to the possibility that extracellular oxidative stress could be potentially beneficial for atherosclerosis through the induction of arterial antioxidant response in the artery. This induction of antioxidant enzymes would not only minimize oxidative damage but also reduce in situ the generation of oxidants.

If this hypothesis of compartmentalization between plasma and arterial wall oxidative stress is true, then antioxidant supplementation during exercise-induced oxidative stress should be counterprotective. In other words, it would prevent the induction of antioxidant enzymes and thwart the exercise-induced beneficial changes in atherosclerosis.

In the present study, which used C57BL/6 and LDL receptor-deficient (LDLr\(^{-/-}\)) male mice, we have provided evidence for the induction of catalase and eNOS in the arterial...
wall and the lowering of atherosclerotic lesions by exercise. More important, vitamin E supplementation to exercising LDLr−/− mice inhibited the plasma oxidative stress induced by exercise and prevented its antiatherogenic properties as well as the induction of arterial antioxidant defense.

Methods

Mice, Exercise Protocol, and Vitamin E Supplementation

Six-week-old male C57BL/6 (n = 32) or LDLr−/− mice (n = 64) (Jackson Laboratories, Bar Harbor, Me) were fed a regular or atherogenic mouse chow (Harlan Teklad). The exercised groups were trained on a treadmill (Columbo Instruments) for 30 minutes per day (at 15 min/d) during the late afternoon. The 1-week exercise group was trained for 8 consecutive days before euthanasia. The 6-week and 12-week (LDLr−/−) exercise groups were trained 5 days per week. Vitamin E-supplemented animals were fed daily (5 days per week, in the morning) with 0.5 IU of (±)-α-tocopherol (synthetic, from Sigma Chemical Co), which is equivalent to ~1000 IU for a 70-kg man in relation to a 35-g mouse. Animals were treated in compliance with Emory University animal committee (Institutional Animal Care and Use Committee) regulations.

Preparation of Arterial Samples

Mice were euthanized by CO2 asphyxia, and then the blood was drawn into heparinized tubes from the inferior vena cava. Red blood cells and plasma were separated by centrifugation (3000 rpm, 10 minutes at 4°C) and then frozen. The aortic tissue was washed by perfusion with PBS containing 10 μg/mL aprotinin and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF) through the left ventricle. The dissection of the aorta was performed under a stereomicroscope from the iliac bifurcation up to the heart, including the beginning of the carotid and subclavian arteries. The adventitia was carefully removed.

For the C57BL/6 mice, a 2-mm cross section was saved for immunohistochemical analysis (in Bouin’s fixative solution, Sigma), whereas the remaining aorta was homogenized and sonicated in 250 μL of protein extraction buffer (50 mmol/L Tris [pH 8], 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 5 mmol/L EDTA, containing 1 mmol/L dithiothreitol, 10 mmol/L β-glycerophosphate, 10 μg/mL aprotinin, 10 μg/mL trypsin inhibitor, 2 μg/mL leupeptin, and 0.1 mmol/L PMSF). After 15 minutes on ice, protein extract was centrifuged (13 000g for 15 minutes at 4°C), and an aliquot of the supernatant (10 μL) was assayed for protein estimation before Western blot analysis.

For LDLr−/− mice, the aorta was opened longitudinally and pinned on black wax for en face observation.21 Briefly, the aorta was continuously soaked with PBS containing 10 μg/mL aprotinin and 0.1 mmol/L PMSF from the time of the dissection until the computerized determination of the lesion area was completed. To save the aorta for catalase activity and Western blot analysis, neither fixative nor staining was used for the identification of the lesions. Pictures of different segments of the aorta observed under the microscope were captured. Areas of the lesions were printed and circled under the microscope. The aorta was then cut longitudinally; one half was placed in 250 μL of protein extraction buffer (as described above), and the other half was saved in 1 mL of KH2PO4 buffer (containing 10 μg/mL aprotinin and 0.1 mmol/L PMSF) for further determination of eNOS protein expression (Western blot) and catalase activity, respectively.

Western Blot Analysis

Approximately 10 to 15 μg of protein extract was separated by a 10% SDS-PAGE. The gel was transblotted onto a nitrocellulose membrane, blocked with 10% milk powder in Tris-buffered saline (pH 7.4) with 0.1% Tween 20 (TBS-T) overnight, incubated with rabbit polyclonal anti-human catalase or anti-eNOS antibody (1:1000 dilution for 1.5 hours at room temperature), washed with TBS-T, and incubated with secondary antibody (anti-rabbit IgG conjugated to horseradish peroxidase, 1:1500) for 1 hour at room temperature. After 5 washes with TBS-T, the signal was detected by using a chemiluminescence kit (Amersham ECL kit). The membrane was then stripped in 62.5 mmol/L Tris (pH 6.7), 2% SDS, and 0.75% β-mercaptoethanol for 30 minutes at 50°C. After 3 washes in TBS-T, the membrane was reprobed as described above with anti-β-actin primary and anti-mouse secondary antibodies (1:2000 and 1:1000 dilution, respectively) to adjust the amount of protein loaded at the moment of quantification.

Immunohistochemistry

Aortic cross sections (2-mm) were fixed in Bouin’s solution (Acustain, Sigma) for 12 hours, dehydrated, and then embedded in paraffin. Tissue sections (5 μm) were blocked for endogenous peroxidase activity by 15 minutes of incubation with 10% H2O2 in methanol. Slides were incubated with 3% BSA for 30 minutes, and then the anti-catalase antibody was applied to the sections for 1 hour at room temperature in a moisture chamber (1:50 dilution [0.05 mg/mL] in 3% BSA-PBS, Calbiochem). The primary antibody was detected with horseradish peroxidase-conjugated goat antibody to rabbit IgG (1:100 dilution in 3% BSA-PBS). After a wash with PBS, the slides were incubated with diaminobenzidine, which was used as a substrate for peroxidase (Sigma Fast). When the slides were incubated with rabbit IgG (used as a negative control) instead of the primary antibody, no nonspecific staining was observed.

Quantification of Atherosclerotic Lesion Area

The lesions were quantified by using Adobe Photoshop software. To use the aortas for Western blot analysis and enzyme assays, no staining was used to visualize the lesions. After capturing of different areas of the aorta, the lesions were circled on a printout under direct microscopic observation. Accordingly, the lesions were further delimited and quantified by using Adobe Photoshop. Pixels were converted to square millimeters by using a microscopic standard scale treated under the same condition as the aortas.

Aortic Catalase Activity

Aortas were homogenized and sonicated in 50 mmol/L KH2PO4 containing 10 μg/mL aprotinin and 0.1 mmol/L PMSF. After centrifugation (13 000 rpm for 10 minutes at 4°C), an aliquot was saved for protein estimation, and the assay for catalase activity was performed on the remaining extract. The initial rate of disappearance of H2O2 was recorded at a wavelength of 240 nm during 1 minute (ΔA240 nm/min) according to the method of Aebl.22

Detection of LOOH-Protein Abs in Plasma by ELISA

One hundred microliters of a 1:50 dilution of plasma from each mouse was tested for autoantibodies against lipid peroxide–modified proteins (LOOH-protein Abs). Every step of the following method was separated by 3 washes with PBS. A 96-well plate was coated overnight at 37°C with a solution of LOOH-modified rabbit serum albumin (50 μg/mL), blocked 3 hours with 3% BSA in PBS, and incubated with the diluted plasma (1 hour at 37°C). The wells were initially incubated for 1 hour at 37°C with an anti-mouse IgG-conjugated alkaline phosphatase (1:10 000 dilution), incubated with the substrate (p-nitrophenyl phosphate) for 30 minutes, and read at 405 nm by use of an ELISA plate reader. Results are expressed as the optical density (OD) equivalent of p-nitrophenol formed at 405 nm.

Statistical Analysis

Results are expressed as mean±SEM or mean±SD as stated in the legend. The difference in each group was evaluated by a 1- or 2-factor ANOVA when applicable (Statview software). Statistical significance was accepted at P<0.05. Linear regression analysis was performed by the least squares method.

Results

Exercise-Induced Catalase Expression in C57BL/6 Mouse Aorter Wall

The effect of acute (1-week) or chronic (6-week) exercise was studied on the expression of catalase protein in the aortic wall of C57BL/6 mice. After removal of the adventitia, aortic
protein extracts were immunoblotted with anti-catalase and anti-β-actin (used as an internal control) antibodies. Films were quantified by densitometry (Bio-Rad densitometer), and catalase was normalized with β-actin (n=9 for 1-week groups, n=6 and n=5 for sedentary and exercised 6-week groups, respectively). Two-factor ANOVA yielded the following: ***P=0.0007 for exercise (+) vs sedentary (-); P=0.64 for period of training; and P=0.6 for interaction. Two-factor ANOVA was performed. B, Five micrograms of each aortic protein extract from the same group was pooled, and a Western blot detection for catalase and β-actin was performed. C through F, Immunohistochemistry for catalase in C57BL/6 mice aortas is shown. Representative immunostaining shows that catalase induction appears to be predominantly located in the intima and proximal media.

Figure 2. Detection of plasma autoantibodies against LOOH-protein Abs. After 1 week of exercise, plasma samples from each group were pooled and assessed in triplicate for detection of LOOH-protein Abs (n=9). Results are expressed as mean±SD of values obtained in triplicate. **P<0.01 for exercise (+) vs sedentary (−) by 1-factor ANOVA.
Lesions were mainly localized in the aortic arch and at the beginning of the carotid and subclavian arteries (Figure 3A). No appreciable lesions were observed in normal diet-fed animals (data not shown).

### Effect of Exercise on Catalase and eNOS Aortic Levels in LDLr$^{-/-}$ Mice

Antioxidant defense levels were estimated in the same aortas by Western blotting for eNOS and by determining the catalase activity. eNOS levels (Western blot densitometric quantification) were much higher in aortas of animals fed an atherogenic diet than in aortas of animals fed a normal diet (Figure 4A). Exercise caused an increase of eNOS protein expression only when animals were on high fat diet. Aortic catalase activity was increased by exercise in the normal diet- and atherogenic diet-fed groups (Figure 4B).

### Effect of Vitamin E Supplementation on Protection From Atherosclerosis by Exercise

The induction of aortic antioxidant response and the prevention of atherosclerosis by exercise, regardless of the individual physiological relevance of each, could be correlative in nature. To make a link between these 2 events and to test the hypothesis that exercise-induced oxidative stress might be involved in the induction of arterial antioxidant defenses as well as the prevention of atherosclerosis, we supplemented exercising LDLr$^{-/-}$ mice with vitamin E.

The presence of LOOH-protein Abs was assessed in plasma by using an ELISA. As expected, the atherogenic diet induced an increase of LOOH-protein Ab (OD$_{405}$ nm $=1.02 \pm 0.64$ versus $0.31 \pm 0.042$ in normal chow-fed animals; data not shown). The high plasma or arterial lipid concentration enhanced the formation of lipid peroxides and the subsequent generation of LOOH-protein Ab. In high fat diet-fed animals, exercise did not significantly affect the body weight and plasma cholesterol levels.

### Body Weight and Plasma Cholesterol Levels in LDLr$^{-/-}$ Groups

<table>
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<th>Diet</th>
<th>Exercise</th>
<th>Body Weight, g</th>
<th>Cholesterol, mg/dL</th>
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<tr>
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<td>35.06±1.04‡</td>
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<td>19</td>
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<tr>
<td>Atherogenic+vitamin E</td>
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<td>8</td>
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<tr>
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<td>1440.83±178.27</td>
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</tbody>
</table>

ND indicates not determined. Values are mean±SEM. P values were calculated by 1-factor ANOVA test, comparing sedentary group with corresponding exercised group.

*P<0.01; †P<0.001.

**Figure 3.** Effect of exercise on atherosclerotic lesion progression in LDLr$^{-/-}$ mice. Computerized quantification of lesion area was performed on aortas of atherogenic-fed animals (no lesions were observed in normal diet-fed mice). A, Pictures of 3 aortas (arch and beginning of carotid and subclavian arteries), representative of each group, illustrate the smaller size of the lesions observed in the exercised (Ex., n=19) vs sedentary (Sed., n=17) group. B, Exercise induced a 40% decrease of the lesion area in high fat diet-fed animals. Lesion area results are expressed as mean±SD (mm$^2$). ***P=0.0002 for exercise (+) vs sedentary (−) by 1-factor ANOVA.
LOOH-protein Ab level (OD\textsubscript{405 nm}=1.19±0.59 versus 1.02±0.64). However, vitamin E supplementation afforded a partial protection against oxidative stress induced by the atherogenic diet and exercise (OD\textsubscript{405 nm}=0.80±0.07 and 0.66±0.08, respectively; P=0.0276; Figure 5A).

In our experimental conditions, vitamin E supplementation (0.5 IU per mouse per day, 5 days per week) did not prevent atherosclerosis on its own, but it did thwart the protective effect of exercise on lesion progression (Figure 5B). In addition, vitamin E inhibited the induction of aortic catalase activity and eNOS protein by exercise (Figure 5C and 5D). Finally, the cholesterol lowering observed in exercised atherogenic diet-fed animals is impaired by vitamin E supplementation (Table).

**Discussion**

In the present study, we tested the hypothesis that exercise-induced plasma oxidative stress may induce aortic antioxidant response, which in turn could eventually be beneficial against atherosclerosis. We chose catalase as a representative antioxidant enzyme because of our previous findings that oxidants such as H\textsubscript{2}O\textsubscript{2} and lipid peroxides could induce catalase gene expression in cultured cells\textsuperscript{16} and because H\textsubscript{2}O\textsubscript{2} might be a common mediator among all the proposed mechanisms of the oxidation of LDL\textsuperscript{26–28}.

We evaluated the effect of exercise on the expression of aortic catalase in male C57BL/6 mice. Western blot and immunohistological analysis showed that catalase protein is increased after exercise in the arterial wall (Figure 1) and particularly in the proximal media (Figure 1C through 1F). Catalase promotes the dismutation of H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O and O\textsubscript{2}. Because H\textsubscript{2}O\textsubscript{2} has been shown to participate in the oxidation of LDL\textsuperscript{26,27,29} and cell toxicity,\textsuperscript{30} an increased catalase in this precise area could be important in preventing proatherogenic oxidative damage in the intima.

We showed an increase in LOOH-protein Abs in C57BL/6 mice by acute exercise (Figure 2). The presence of oxidative stress markers after a bout of exercise has been extensively reported in humans and animals.\textsuperscript{10,11,23} In the present study, we provide evidence of an acute response to oxidative stress induced by exercise.

Using LDL\textsuperscript{−/−} male mice on a high fat diet, we have shown that exercise could reduce atherosclerotic lesions by 40% (Figure 3). The demonstration that exercise can prevent the progression of atherosclerosis in mice validates this model for future studies on exercise with the use of C57BL/6 mice or the derived LDL\textsuperscript{−/−} mice. Moreover, catalase and eNOS were increased in the aorta of these animals (Figure 4). Many studies report the induction of antioxidant enzymes by exercise in different species, including humans.\textsuperscript{31} In the present study, we show for the first time the induction of catalase in the aorta by exercise in a mouse model of atherosclerosis. The induction of eNOS by exercise has been previously reported in the coronary vessels of dogs.\textsuperscript{32} We observed an exercise-induced increase of aortic eNOS protein only in atherogenic diet-fed animals. The high fat diet itself seemed to stimulate eNOS expression, which could be attributed to the presence of plasma lipid peroxides, as it is described in in vitro models.\textsuperscript{14} Exercise could increase the level of plasma oxidized fatty acids in high fat diet-fed mice and further upregulate eNOS levels. It is interesting to point out that most of the studies on exercise are performed immediately after a bout of exercise and show subsequent changes in various tissues or in blood. In the present study, we provide evidence that chronic exercise induces a sustained activation of aortic antioxidant defenses from 1 week up to 12 weeks of training (because the animals were euthanized after resting overnight). The benefits of exercise on atherosclerosis cannot exclusively be attributed to an induction of aortic antioxidant defenses alone because several risk factors for coronary heart disease are favorably modified by physical activity.\textsuperscript{33} However, aortic catalase activity is found to be negatively correlated to the lesion area (r=0.41), whereas total plasma cholesterol and eNOS expression were only poorly or not correlated to atherosclerotic lesion size (r=0.29 and 0.06, respectively).

We showed that exercise decreased total plasma cholesterol in atherogenic diet-fed mice as well as the body weight in both normal diet- and high fat diet-fed animals (Table). This decrease in cholesterol is unlikely to be due to an activation of the LDL receptor pathway because the animals used in the present study lack the receptor. A number of studies,\textsuperscript{33,34} including ours,\textsuperscript{12} have suggested an increase in plasma myeloperoxidase during exercise. Because myeloper-

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Figure 4. Determination of eNOS protein expression and catalase activity in the aortic wall of LDL\textsuperscript{−/−} mice. A, eNOS protein expression was estimated by quantification of the Western blot of the aortic extracts with the use of eNOS and β-actin antibodies. The ratio eNOS OD/β-actin OD is given for each group as mean±SD of all the samples. Two-factor ANOVA yielded the following: P=0.0011 for normal vs atherogenic diet; P=0.21 for interaction. *P=0.21 for atherogenic diet vs sedentary group (−) vs exercised (+) group by 1-factor ANOVA. B, Catalase activity in the aorta was determined individually for each animal according to the method of Aebi\textsuperscript{22} (n=8 for atherogenic diet/sedentary group and n=11 for atherogenic diet/exercised group). Two-factor ANOVA yielded the following: *P=0.02 for sedentary (−) vs exercised (+) group; P=0.09 for atherogenic vs normal diet; and P=0.057 for interaction.
oxidase has been shown to oxidize LDL in in vitro experiments, we recently postulated that exercise-induced oxidative stress could promote plasma oxidation of circulating LDL and its clearance by the liver. Indeed, because a liver pathway for the catabolism of modified LDL has been proposed, many studies have implicated hepatic scavenger receptors in the clearance of oxidized LDL and oxidized cholesterol from plasma. The lack of the LDL receptor in mice used for the present study makes liver scavenger receptors good candidates for an explanation of the decrease in plasma cholesterol observed in exercised high fat-fed animals. In the present study, we provide for the first time a clear demonstration that cholesterol lowering by exercise could be independent of the classical LDL receptor pathway.

We hypothesize a compartmentalization between oxidative stress occurring in plasma and in the arterial wall. The oxidative stress induced by exercise could be the mediator of its beneficial effects toward atherosclerosis by inducing vascular parietal antioxidant defenses. To put to test the hypothesis, we supplemented LDL−/− mice with vitamin E during the exercise period. As expected, vitamin E could decrease the oxidative stress in plasma induced by an atherogenic diet and exercise, as determined by the lower titer of autoantibody to LOOH-modified proteins (Figure 5A). High fat diet-fed animals have a high concentration of plasma lipids, which could be taken up and oxidized by surrounding tissues. Oxidized lipids in tissues and blood vessel walls can modify proteins and generate new epitopes, leading to the formation of autoantibodies. The decrease of antibodies to LOOH-modified proteins in vitamin E-supplemented groups (sedentary and exercised) could be attributed to the well-documented antioxidant effect of vitamin E as a lipid peroxidation chain-breaking agent. Studies attempting to determine the effects of vitamin E on atherosclerosis are still

Figure 5. Effect of vitamin E supplementation and exercise on oxidative stress, lesion area, and aortic antioxidant enzymes in LDL−/− mice on atherogenic diet (n=17 for sedentary group, n=19 for exercised group, n=15 for vitamin E-supplemented group, and n=13 for exercised/vitamin E-supplemented group, except for panel D). A, Detection of LOOH-protein Abs in plasma (ELISA). Plasma from each mouse was assessed in triplicate for detection of LOOH-protein Abs, after a 1:50 dilution. Results are expressed as mean±SD of values obtained for animals in the same group. Two-factor ANOVA yielded the following: P=0.0276 for effect of vitamin E supplementation; P=0.93 for exercise (+) vs sedentary (−); and P=0.34 for interaction. B, After en face preparation, computer-quantified lesion areas are expressed in square millimeters for each animal (open circles); solid diamonds represent the mean. Two-factor ANOVA did not show statistically significant interaction between vitamin E supplementation and exercise status (P=0.16). However, 1-factor ANOVA showed statistical differences between groups: without vitamin E supplementation, ***P<0.01 for sedentary vs exercise; with vitamin E supplementation, **P=0.01 for exercise vs sedentary; and *P=0.039 for exercise without vitamin E vs exercise with vitamin E. C, Aortic catalase activity was assessed according the method of Aebi for each animal and expressed in units per milligram protein. Two-factor ANOVA test shows an interaction between vitamin E supplementation and exercise status (P=0.015). One-factor ANOVA show the following: without vitamin E supplementation, *P<0.05 for sedentary vs exercise; ***P<0.01 for exercise without vitamin E vs exercise with vitamin E. D, eNOS expression was determined by Western blot analysis of aortic protein extracts. Samples from the same group were pooled before electrophoresis and immunodetection (n=8 for sedentary, exercised, and vitamin E-supplemented groups; n=7 for exercised/vitamin E-supplemented group). Films were quantified by densitometry (Bio-Rad densitometer), and catalase was normalized with β-actin used as an internal control.
in conflict, especially in mouse models. Under experimental conditions used in the present study, vitamin E did not afford protection against the progression of atherosclerotic lesions. More interestingly, vitamin E supplementation in the exercised group seems to thwart the beneficial effects of exercise. Accordingly, levels of catalase and eNOS in this group were significantly lower relative to the levels in the exercised group without vitamin E supplementation. Atalay et al have recently shown that oxidative stress induced by fish oil supplementation could stimulate antioxidant enzymes (catalase, glutathione peroxidase, and glutathione-S-transferase) in the liver, heart, and skeletal muscle. They have reported that vitamin E supplementation markedly decreased fish oil–induced antioxidant enzyme activities in all tissues. The present results are consistent with the results of that study and suggest that vitamin E supplementation could be deleterious in exercisers by inhibiting exercise-mediated oxidative stress induction of aortic antioxidant enzymes.

Exercise provides antioxidant protection in the arterial wall in LDL<sup>−/−</sup> mice and normal C57BL/6 mice. Whereas acute exercise induces an increase in plasma antibodies to LOOH-modified proteins, this oxidative stress marker was not significantly increased in the group that underwent chronic (12-week) exercise compared with the sedentary group (Figure 5A). We suggest that the induction of aortic antioxidant defenses initiated after 1 week of exercise could be effective by inhibiting further oxidative modification of proteins and subsequent generation of autoantibodies. Catalase and NO synthase are more likely to be important in the context of atherosclerosis and endothelial function. According to the present study, antioxidant supplementation in beginning male exercisers could abolish the arterial antioxidant response to oxidative stress and thereby counteract the effects of exercise. Conversely, antioxidants that reach the artery might be needed for those who have genetic deficiencies in these enzymes. More studies combining antioxidant supplementation and exercise are necessary to verify this hypothesis.

Acknowledgments

This work was supported by National Institutes of Health grant HL-52628-01A4 (Molecular Mechanisms of Oxidation of LDL). S.P. acknowledges the support of American Heart Association SRC Grant-in-Aid 94120115. S.N. acknowledges the support of American Heart Association SRC Beginning Grant-in-Aid 97103029. The authors thank Dr Wulf Palinski for training O.M. in characterizing atherosclerotic lesions.

References


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doi: 10.1161/hq1001.097106
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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