運動對動脈硬化的影響(3/3)

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Effects of Chronic Exercise on Endothelial Calcium Signaling and Vascular Responses in Hypercholesterolemic Rabbit Femoral Artery at Different Time Period

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Abstract

The time course of chronic exercise effects on vasodilatation and endothelial intracellular calcium (EC \([\text{Ca}^{2+}]_i\)) signaling was examined in atherosclerotic animals. Male New Zealand White rabbits were fed for 2, 4 or 6 weeks with rabbit chow with or without the addition of 2% cholesterol. They were further divided into control and exercise groups. Animals in exercise groups ran on a leveled treadmill at 0.88 km/h for 10-60 minutes gradually, 5 days per week for 2-6 weeks. At the end of experiments, femoral arteries were dissected, loaded with fura 2-AM, and mounted in a tissue flow chamber. Phenylephrine-precontracted vessel specimens were exposed to acetylcholine (ACh). The EC \([\text{Ca}^{2+}]_i\) elevation and vasorelaxation were determined simultaneously under an epifluorescence microscope equipped with ratio imaging capability. Our results showed that 1) high cholesterol diet feeding for \(\geq 4\) weeks caused lipid deposition on vascular surface, reduced the ACh-evoked EC \([\text{Ca}^{2+}]_i\) elevation, and impaired both endothelium-dependent and endothelium-independent vascular responses in a time-dependent manner; 2) vasorelaxation at given levels of EC \([\text{Ca}^{2+}]_i\) elevation decreased in hypercholesterolemia; 3) concomitant exercise program had reverse effects. In conclusion, high cholesterol diet intervention for at least 4 weeks induces vascular structural changes, impairs EC \([\text{Ca}^{2+}]_i\) signaling and vasodilatation, whereas chronic exercise eliminates these adverse effects.

**Key Words**: calcium ■ exercise ■ hypercholesterolemia ■ nitric oxide ■ vasodilation
Atherosclerosis is a leading cause of mortality in the developed world, and it is possibly caused by a high-fat diet and a sedentary lifestyle. \(^1\) Risk factors of atherosclerosis, ie, elevated LDL cholesterol levels, diabetes mellitus, hypertension, homocystinemia, and smoking, are associated with vascular dysfunction, such as monocyte adhesion and invasion, smooth muscle proliferation and migration, platelet activation, and extracellular matrix formation. \(^2\) Moreover, the atherogenesis-related endothelial impairment occurs well before any detectable structural changes of the vessel wall. \(^3\) Normally, the vascular endothelial cells release NO. \(^4\) NO relaxes vascular smooth muscle, inhibits platelet adhesion/aggregation, inhibits monocyte adhesion and migration, reduces the production of superoxide, inhibits oxidation of LDL, and inhibits smooth muscle proliferation and migration. Therefore, it is considered to be an endogenous antiatherosclerotic factor. \(^5\) It is likely that the impairment of endothelium-derived NO activity is a cause, not a consequence, of atherosclerosis.

Current consensus indicates that regular exercise reduces incidence of cardiovascular diseases and death or causes regression of symptoms. \(^1,6-8\) Several possible mechanisms of these exercise effects have been proposed; such as an increase in HDL cholesterol level, \(^9,10\) a decrease in the oxidation of LDL cholesterol or total cholesterol levels, \(^10,11\) a decrease in the production of atherogenic cytokines (eg, interleukin-1\(\alpha\), tumor necrosis factor-\(\alpha\), and interferon), and an increase in the production of atheroprotective cytokines (eg, interleukin-4 and transforming growth factor-\(\beta1\)). \(^12\) We and others have reported that exercise training increases agonist-stimulated NO release and enhances endothelium-dependent vasodilatation in vessels obtained from normal or hypertensive animals. \(^13-15\) Whether the improvement of endothelial function by chronic exercise occurs in atherosclerosis has not been
proved yet until recently. In our recent studies, 8 weeks of 2% cholesterol diet feeding caused severe vessel damage in rabbit aortas, but moderate structural changes in the femoral arteries. In these vessels, endothelium-dependent vascular function was impaired. These impairments could be completely reversed by concomitant exercise training in femoral arteries, but only partially recovered in the aortas. Therefore, the severity of vascular structural changes seems to be associated with the extent of exercise-improved endothelial function. In order to find out how long the high cholesterol diet feeding could induce vascular dysfunction, and how early the exercise effects occurred, we conducted this study to investigate the time course of diet and/or exercise effects in rabbit femoral arteries.

Many receptor-mediated agonists, such as acetylcholine (ACh), affect cellular function via generating intracellular calcium signals. Besides, the endothelial NO synthase (eNOS) is known to be a calcium-dependent enzyme. It is likely that agonist-evoked endothelial intracellular calcium (EC \([\text{Ca}^{2+}]\)) signaling is involved in the exercise-induced vascular adaptation. Our previous studies have demonstrated that high cholesterol diet feeding for 8 weeks reduces, whereas chronic exercise increases, ACh-evoked EC \([\text{Ca}^{2+}]\), elevation response in normal rats and hypercholesterolemic rabbits. Therefore, the second purpose of the present study was to evaluate the time course of ACh-evoked EC \([\text{Ca}^{2+}]\), signaling changes affected by diet or exercise intervention.
Methods

Animals and Diet
The present study was conducted in conformity with the policies and procedures detailed in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health, and the procedures followed were in accordance with institutional guidelines. Male New Zealand White rabbits (weighing ~1 kg at the beginning) were divided into 4 groups; the normal diet control (N) group, the normal diet with exercise training (NE) group, the high cholesterol diet control (H) group, and the high cholesterol diet with exercise training (HE) group. The control groups were fed normal rabbit chow, whereas the high cholesterol diet groups were fed a 2% high cholesterol diet (PMI Feeds Inc) for 2, 4, or 6 weeks. They were housed in an environmentally controlled room.

Exercise Protocol
A training protocol similar to that discussed in our previous studies\(^{16,17}\) was used. After 1 week of familiarization, the exercise training groups ran on a leveled treadmill (model Q55, Quinton Instrument Co) at the speed of 0.88 km/h for 10-40 minutes per day, 5 days per week for 2, 4, or 6 weeks. In contrast, the sedentary groups were placed on the treadmill for 10 minutes each day without receiving any exercise training.

At the end of experiments, rabbits were anesthetized by injecting ketamine (25 mg/Kg) and pentobarbital (20 mg/Kg) into their ear veins. Blood samples were drawn from the inferior vena cava for lipid profile determination. Femoral arteries were then isolated for various experiments described below.

An increase in citrate synthase activity is a common biochemical method to
confirm the exercise training effect.\textsuperscript{16,17} Therefore, we examined citrate synthase activity of the soleus muscle for all animal groups. Mitochondrial citrate synthase activity was determined spectrophotometrically at 412 nm.

**Vessel Preparation and Fura 2 Loading**

The femoral arteries were excised and cut into rings (5 mm long), which were stored in an organ chamber containing Krebs-Ringer solution bubbled with 95% O\textsubscript{2}/5% CO\textsubscript{2} (22 °C, pH 7.4). This solution had the following composition (in mmol/L): NaCl 118.0, KCl 4.8, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 24, Na\textsubscript{2}-EDTA 0.03, and glucose 11.0. Vessel rings were fluorescently labeled by incubating with 10 \textmu mol/L of fura 2-AM and 0.025% Pluronic F-127 in Krebs-Ringer solution for 1 hour.\textsuperscript{23} Extracellular fura 2-AM was washed away afterwards. As in rat aortas, our preliminary results indicated that the endothelial fluorescence signals in rabbit femoral arteries were basically free of contaminated signals from the smooth muscle layer underneath.

**Simultaneous Measurements of ACh-Induced EC [Ca\textsuperscript{2+}]\textsubscript{i} Responses and Vasorelaxation in Phenylephrine-Precontracted Vascular Segments**

To allow simultaneous measurements of vascular EC [Ca\textsuperscript{2+}]\textsubscript{i} and smooth muscle contraction, the vessel segment was mounted as described previously.\textsuperscript{24,25} In brief, fura 2-loaded vessel rings were opened longitudinally and pinned onto the base plate of a tissue flow chamber. One side of the longitudinally opened vessel segment was fixed in the direction of blood flow with insect pins. The corners on the opposite side were passively stretched and pinned onto the base plate. This arrangement allowed free movement of the central portion of the specimen when vasoactive chemicals were added. After vessel mounting, the tissue flow chamber was mounted on an inverted microscope with epifluorescence attachments (Diaphot 300, Nikon), and perfused
with Krebs-Ringer buffer at 30 °C under a constant flow rate of 0.7 ml/min for an equilibration period of 1 hour.

For the measurement of EC $[\text{Ca}^{2+}]_i$, the 510-nm fluorescence images excited at 340 or 380 nm were recorded by a high-sensitivity SIT camera (model C2400-08, Hamamatsu). Axon image workbench software (Axon Instruments) was used to acquire, digitize, and store the experimental results for offline processing. The average value of EC $[\text{Ca}^{2+}]_i$ was calculated by monitoring a large area in the mainstream region of an opened femoral segment, covering ~ 0.15 mm$^2$ of tissue surface, or >200 cells. At the end of each experiment, the calcium concentration was calibrated by the established methods. In brief, the calcium concentration was calibrated by applying ionomycin (5 µmol/L) in the presence of 5 mmol/L EGTA, followed by 10 mmol/L CaCl$_2$. Finally, the background autofluorescence was determined by exposing the tissue to 5 mmol/L manganese to quench cytosolic fura 2 fluorescence. EC $[\text{Ca}^{2+}]_i$ was calculated after subtracting the background autofluorescence by using the following equation: $[\text{Ca}^{2+}]_i = K_d[(R-R_{\text{min}})/(R_{\text{max}}-R)]B$; where $K_d$ is the dissociation constant (~224 nmol/L), R is the ratio of 340 to 380 nm during measurements, $R_{\text{max}}$ is the 340/380 ratio in the presence of saturating calcium levels, $R_{\text{min}}$ is the 340/380 ratio in calcium-free solution, and B is the ratio of the fluorescence at 380 nm in calcium free solution to the fluorescence at 380 nm in saturated CaCl$_2$ solution.

The dose responses of ACh-induced EC $[\text{Ca}^{2+}]_i$ elevation and vascular displacement were determined in the phenylephrine (PE, 5x10$^{-7}$ mol/L)- precontracted vessel segment by subsequent exposure to cumulative ACh (10$^{-8}$ to 10$^{-5}$ mol/L). The relative movement of endothelial cells was used as an index of vascular tone, whereas fluorescence ratio images from fura 2-labeled endothelial cells provided quantitative
To calculate the vascular displacement, MetaMorph software (Universal Imaging Corp) was used to trace and analyze a series of images. The fluorescence image was focused sharply, and a particular endothelial cell in the initial image was selected as a location marker. In the presence of PE, this image was shifted inward during experiments. After the marker had reached its final equilibrium location, the total displacement represented the extent of vascular contraction induced by PE. When the preparation was subsequently exposed to ACh, the marker moved back toward its initial location, indicating vasorelaxation. The ACh-induced vasorelaxation was expressed as percentage of PE-induced contractile displacement for normalization.

To evaluate the contribution of NO to the effects of exercise, some specimens were pretreated with $10^{-4}$ mol/L of $N^\omega$-nitro-L-arginine (L-NNA) for 15 minutes to block NO synthesis.\(^\text{13}\)

**Vascular Responses to A23187 or SNP**

In normal rabbit aortas, chronic exercise does not alter the vascular responses either to A23187, a calcium ionophore that induces endothelium-dependent vasodilatation without receptor activation, or to sodium nitroprusside (SNP), an endothelium-independent vasodilator.\(^\text{14}\) In contrast, these responses are impaired in rabbit femoral arteries by 8 weeks of high cholesterol diet feeding, and can be reversed by concomitant exercise.\(^\text{16}\) Whether the diet- or exercise-altered these vascular responses happens in the early stage needs to be verified. Therefore, the vascular responses to A23187 ($2\times10^{-8}$ mol/L) or SNP ($10^{-6}$ mol/L) were evaluated in some PE ($5\times10^{-7}$ mol/L)-precontracted femoral arteries. The level of vasorelaxation was expressed by the percentage of the PE precontraction value.

**Determination of Serum Cholesterol Levels**
Blood samples were collected from the inferior vena cava when the animals were under general anesthesia. The serum cholesterol levels, including total cholesterol, HDL and LDL, were determined by using an automatic analyzer (model 747, Hitachi Ltd).

**En Face Oil Red O Staining of Blood Vessels**

En face oil read O staining was used to evaluate lipid deposition on the inner surface of longitudinally opened vessel segments (5 mm long). The image analysis of lipid deposition was performed by using Image-Pro Plus (Media Cybernetics), and the results of stained areas were normalized against the total measured surface area.

**Statistical Analysis**

Data are expressed as means ± SEM. Sample sizes are indicated by the letter n. Dose responses of ACh-induced EC [Ca^{2+}]i elevation or vasorelaxation were analyzed by ANOVA with a repeated-measures design. Others were analyzed by ANOVA and further evaluated by Scheffe F test. If only 2 groups were compared, the unpaired Student t test was applied. Differences were considered at P<0.05.
Results

Chronic exercise over 2 weeks in this study significantly increased citrate synthase activities of soleus muscles in both normal diet groups and high cholesterol diet groups (data not shown), indicating that our training protocol was effective.

Serum Cholesterol Levels
High cholesterol diet feeding for ≥ 2 weeks elevated serum levels of total cholesterol, HDL and LDL in a time-dependent manner (Table 1). These observations indicated that high cholesterol diet feeding indeed induced hypercholesterolemia in our animal models. Six weeks of chronic exercise significantly lowered total cholesterol, HDL and LDL levels (Table 1). In addition, 4 weeks, but not 2 weeks, of concomitant exercise also tended to lower serum cholesterol levels.

Oil Red O Staining
Table 2 shows that 2 weeks of high-cholesterol diet feeding induced little fat deposits in rabbit femoral arteries, whereas the diet intervention over 4 weeks caused scattered lipid deposition in a time-dependent manner. Furthermore, the concomitant exercise program reduced the area of lipid deposits, especially in 6 week-intervention groups. In contrast, there was no lipid deposition in either N or NE groups.

Comparison of ACh-Induced EC [Ca^{2+}]i Responses and Vasorelaxation in PE-Precontracted Vascular Segments
Two weeks of diet intervention did not affect ACh-evoked EC [Ca^{2+}]i elevation and vasorelaxation in PE-precontracted rabbit femoral arteries (data not shown). However, high cholesterol diet feeding for 4 or 6 weeks significantly reduced ACh-evoked responses (Fig. 1). On the contrary, chronic exercise in parallel with the diet intervention enhanced these responses nearly to the normal levels (Figs. 2 and 3). In
normal diet groups, the exercise effects occurred only after 6 weeks of intervention. When NO synthesis was blocked by L-NNA pretreatment, the responses of ACh-induced vasorelaxation were largely inhibited, and the calcium responses were also diminished. In this condition, diet/exercise effects were abolished (data not shown). These results imply that high cholesterol diet feeding for more than 4 weeks impairs NO release, and that chronic exercise can recover it back to normal.

When the ACh-evoked vasorelaxation was plotted against the corresponding EC [Ca^{2+}]_i elevation by using data from N and H groups after 6 weeks of diet intervention, the slope of logarithmically fitted curve in the H group was lower than that of the N group (Fig. 4). Similar results, but to a less extent, were observed when the diet intervention lasted for 4 weeks (data not shown). In normal diet groups, 6 weeks of chronic exercise extended the curve to the higher-level range without alter the curve shape (Fig. 5A; \( r^2 = 0.855 \) and 0.918 for N and NE groups, respectively; EC_{50}~52 nmol/L for both groups). In high cholesterol diet groups, however, 4 or 6 weeks of concomitant exercise training not only extended the curve to nearly normal ranges but also elevated the slope of the relation curve (Fig. 5B for 6 weeks of intervention; \( r^2: 0.845 \) and 0.900, EC_{50}: 64 and 55 nmol/L, for H and HE groups, respectively). These results imply that high cholesterol diet feeding for 4 or 6 weeks reduced the sensitivity of ACh-evoked vasodilation to a given level of EC [Ca^{2+}]_i elevation in femoral arteries in a time–dependent manner, and that concomitant exercise training could ameliorate the relationship between vasorelaxation and EC [Ca^{2+}]_i elevation.

**Vascular Responses to SNP or A23187**

In accordance with our previous report,^{14} exercise in the normal diet group (ie, the NE group) did not significantly alter these responses (Table 3). However, high cholesterol diet feeding for 4 or 6 weeks, but not for 2 weeks, impaired the SNP- or
A23187-evoked vasorelaxation, and this impairment was almost reversed by chronic exercise (Table 3).
Discussion

Our results indicated that (1) 2% high cholesterol diet feeding for more than 2 weeks increased serum levels of total cholesterol, HDL, and LDL in rabbits, and induced scattered lipid deposition in a time-dependent manner; (2) 6 weeks of chronic exercise significantly lowered cholesterol levels, and reduced lipid deposition in femoral arteries; (3) high cholesterol diet feeding for \( \geq 4 \) weeks reduced, whereas concomitant exercise reversed, the responses of ACh-evoked EC \([Ca^{2+}]_i\) elevation and agonist-induced vasorelaxation; (4) the effects of diet/exercise on ACh-induced EC \([Ca^{2+}]_i\) elevation and vasorelaxation were abolished by L-NNA pretreatment, indicating that NO was the major contributing factor for these effects; (5) ACh-induced vasorelaxation was well associated with EC \([Ca^{2+}]_i\) elevation in all groups; and (6) in normal diet groups, chronic exercise did not change the relationship between vasorelaxation and EC \([Ca^{2+}]_i\) elevation but extended the curve to a higher level range. However, high cholesterol diet feeding for \( \geq 4 \) weeks reduced the extent of vasorelaxation at a certain level of EC \([Ca^{2+}]_i\) elevation.

It has been reported that chronic exercise increases endothelium-dependent vasodilatation in the aortas of normal rabbits or rats.\(^{14,15}\) Our previous results suggested that 8-week chronic exercise improved endothelium-dependent vasodilating responses in the femoral arteries and thoracic aortas of hypercholesterolemic rabbits as well.\(^{16,Yang}\) In addition, the reduced lipid deposition and intimal thickening that occurred after chronic exercise indicated that our exercise protocol could ameliorate the progression of atherosclerosis. Endothelial dysfunction occurs in the early stage of atherosclerosis.\(^2\) In the present study, atherosclerotic lesion and endothelial dysfunction in femoral arteries were observed after 4 weeks of high cholesterol diet feeding.
feeding in rabbits. On the contrary, atherosclerotic changes occurred as early as 2 weeks of diet intervention in rabbit aortas (data not shown). It seems that the time course of atherosclerotic vessel changes induced by high cholesterol diet feeding varies with different vascular regions.

To clarify whether the receptor-independent or endothelium-independent vasodilating responses were also affected by high cholesterol diet feeding or exercise, we examined vascular responses to A23187 or SNP. Our results indicated that vasodilating responses to these agonists were also impaired in femoral arteries after 4 or 6 weeks of diet intervention. These data coincide with our observation that cholesterol diet feeding alters the relationship between EC [Ca^{2+}]i elevation and vasorelaxation (Figure 4). These results were consistent with our previous study in which diet intervention lasted for 8 weeks. Therefore, not only endothelium but also smooth muscle cells of femoral arteries are affected by high cholesterol diet intervention. Nevertheless, a previous report demonstrated that cholesterol diet feeding did not affect SNP-elicited vasorelaxation in rabbit aortas. In our pilot study, we also found that cholesterol diet feeding did not change vascular responses to SNP or A23187 in rabbit aortas (data not shown). This discrepancy may be due to regional differences.

According to our previous study and the present one, chronic exercise for more than 6 weeks increases the ACh-evoked EC [Ca^{2+}]i elevation and vasorelaxation without altering the relationship between these 2 parameters in normal diet groups. However, concomitant exercise training for 4 or 6 weeks in hypercholesterolemia not only enhances the ACh responses, but also improves the relationship between vasorelaxation and EC [Ca^{2+}]i elevation in rabbit femoral arteries. We also noticed that 4 or 6 weeks of chronic exercise improved SNP- or A23187-induced
vasorelaxation, and reduced lipid deposition in hypercholesterolemic rabbit femoral arteries, indicating that the structural/functional alterations of these vessels were improved by chronic exercise as well. In comparison, exercise in rabbits fed a normal diet enhances only ACh-induced, but not SNP- or A23187-induced, vasorelaxation. Thus, the exercise effects in normal animals are most likely focused on the endothelium, and they are receptor-mediated. In fact, previous studies using normal rats have provided evidence that endothelial receptors are upregulated by exercise. Similar mechanisms may also partially explain the endothelium-dependent part of the exercise effects seen in hypercholesterolemia. Taken together, it appears that the exercise effects in normal animals were mediated by improving endothelial function. In high cholesterol-fed animals, however, the exercise effects were due to improvements of endothelial function and vascular structure.

EC [Ca^{2+}]_{i} signaling has been shown to serve as an integrating signal for endothelium-dependent vasorelaxation in rat aortas. In the present study, we found that ACh-evoked EC [Ca^{2+}]_{i} elevation and vasorelaxation were impaired in hypercholesterolemic rabbit femoral arteries, whereas chronic exercise enhanced ACh-evoked EC [Ca^{2+}]_{i} response and improved the endothelium-dependent vasorelaxation. Because eNOS is a calcium-dependent enzyme, this increased EC [Ca^{2+}]_{i} signaling could be a key factor responsible for the enhanced NO-dependent vasodilation after chronic exercise. Because EC [Ca^{2+}]_{i} signaling has also been reported to be improved in normal rats given a single bout of exercise, there may be a common pathway or stimulus for these exercise effects, such as elevated blood flow or shear stress. Previously, we have shown that either flow pretreatment or exercise enhances ACh-evoked EC [Ca^{2+}]_{i} elevation by facilitating the calcium influx. Moreover, gadolinium, the mechanosensitive cationic channel blocker, reduces the
calcium response to ACh in the exercise groups but has little effect in the control groups. This suggests that mechanosensitive cationic channels upregulated by flow during exercise may partially account for the enhanced EC [Ca\textsuperscript{2+}]\textsubscript{i} signaling after chronic exercise.

It is interesting to learn that the pretreatment of L-NNA not only inhibit ACh-induced vasorelaxation but also reduce the evoked calcium signaling, indicating that these 2 intracellular messengers may interact with each other. Our previous study\textsuperscript{25} suggests that a high concentration of ACh-stimulated NO after exercise is capable of evoking EC [Ca\textsuperscript{2+}]\textsubscript{i} elevation. Therefore, large amounts of ACh-stimulated endothelial NO release in the exercise-trained animals may promote the EC [Ca\textsuperscript{2+}]\textsubscript{i} response, which further activates eNOS and enhances endothelium-dependent vasorelaxation in a positive-feedback manner. In addition, eNOS gene upregulation by chronic exercise may be partially responsible for the elevated vascular responses as well. Recent studies suggest that exercise training upregulates NO synthase gene expression in normal animals.\textsuperscript{33-35} In contrast, eNOS expression is impaired in atherosclerosis.\textsuperscript{36} It is plausible to assume that exercise-induced NO synthase gene expression may compensate for the defect of eNOS gene expression in hypercholesterolemic rabbits. However, this viewpoint needs to be further clarified.

In conclusion, high cholesterol diet feeding for at least 4 weeks induces significant vascular structural alterations and impairs EC [Ca\textsuperscript{2+}]\textsubscript{i} signaling and vasodilatation in a time-dependent manner, whereas a concomitant exercise program can reverse these adverse effects.
Acknowledgments

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References


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29. Chen HI, Cheng SY, Jen CJ. Chronic exercise enhances vascular responses to


Figure Legends

Figure 1. Comparison of ACh-evoked EC [Ca^{2+}]_i elevation (A) and vasorelaxation (B) at different time points of high cholesterol diet feeding (ie, 2, 4 or 6 weeks) with the normal control (ie, 0 week). W- week. * P<0.05 (4W vs. 0W), # P<0.05 (6W vs. 0W). N=5-7.

Figure 2. Comparison of dose-response relations of ACh-induced EC [Ca^{2+}]_i elevation (A) and vasorelaxation (B) after four weeks of diet/exercise intervention. It was noticed that 4 weeks of diet intervention reduced ACh responses (* P<0.05), whereas chronic exercise in parallel enhanced these responses in hypercholesterolemic groups (# P<0.05, N=5-6), but not in normal diet groups.

Figure 3. Comparison of dose-response relations of ACh-induced EC [Ca^{2+}]_i elevation (A) and vasorelaxation (B) after six weeks of diet/exercise intervention. It was noticed that 6 weeks of diet intervention drastically reduced ACh responses (* P<0.05), whereas chronic exercise in parallel enhanced these responses in both hypercholesterolemic and normal groups (# P<0.05, N=6-7).

Figure 4. Effects of cholesterol diet feeding on the correlations between ACh-evoked EC [Ca^{2+}]_i elevation and vasorelaxation in rabbit femoral arteries. Data from N and H groups after 6weeks of diet intervention were plotted with logarithmic curve fitting. It is noticed that high cholesterol diet feeding reduced the sensitivity of ACh-evoked vasodilation to EC [Ca^{2+}]_i elevation (EC_{50}: 64 and 50 nmol/L, for H and N groups, respectively).

Figure 5. Comparison of relationship between ACh-evoked EC [Ca^{2+}]_i elevation and
vasorelaxation between control and exercise groups after 6 weeks of intervention. Panel A shows data for normal diet groups, and panel B is for high cholesterol diet groups. It was noticed that in cholesterol diet groups, chronic exercise not only had higher maximal values of EC [Ca\(^{2+}\)]\(_{i}\) and vasorelaxation, but also elevated the curve to nearly normal ranges.
<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
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<tbody>
<tr>
<td>2 W:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>67±8</td>
<td>31±4</td>
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</tr>
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<td>NE</td>
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</tr>
<tr>
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<td>1094±209*</td>
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<td>1087±228*</td>
</tr>
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<td>1213±117*</td>
<td>54±8@</td>
<td>1164±115*</td>
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<td>1444±146*</td>
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<td>2205±257*</td>
<td>123±25*</td>
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<td>HE</td>
<td>1622±183*#</td>
<td>80±13*#</td>
<td>1524±179*#</td>
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*P<0.05 (H vs. N, HE vs. NE); # P<0.05 (HE vs. H); @ P<0.05 (HE vs. NE). N=6-9.

Abbreviations: W- week; N- normal diet control; NE- normal diet with exercise; H high cholesterol diet control; HE- high cholesterol diet control.
Table 2. Comparison of the Areas of Lipid Deposit in the Femoral Arteries between HE and H groups after High-Cholesterol Diet Feeding for 2, 4, or 6 Weeks

<table>
<thead>
<tr>
<th>Lipid Deposit (% surface area)</th>
<th>Group</th>
<th>2 W</th>
<th>4 W</th>
<th>6 W</th>
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<td></td>
<td>H</td>
<td>0.4±0.3</td>
<td>3.6±0.9</td>
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<tr>
<td></td>
<td>HE</td>
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<td>1.7±0.8</td>
<td>4±2*</td>
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* P<0.05 (HE vs. H). N=6-7.

Abbreviations: the same as in Table 1.
Table 3. Comparison of SNP- or A23187-Evoked Vasorelaxation among Four Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>SNP:</th>
<th>A23187:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
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<td>39±2</td>
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<td>47±6</td>
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<tr>
<td>H</td>
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<td>30±2*</td>
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<tr>
<td>HE</td>
<td>42±8</td>
<td>37±2#</td>
</tr>
</tbody>
</table>

Abbreviations are the same as in Table 1.

Vasorelaxation (% precontraction)

- P<0.05 (H vs. N), # P<0.05 (HE vs. H). N=4-6
Fig. 1
Fig. 2
Fig. 3

(A) Calcium elevation (nmol/L) vs. [ACh] (log mol/L)

(B) Relaxation (% precontraction) vs. [ACh] (log mol/L)
Fig. 4
Fig. 5