Exercise Training Enhances Endothelial Function in Young Men

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OBJECTIVES

The present study was designed to assess whether exercise training can enhance endothelium-dependent dilatation in healthy young men.

BACKGROUND

Exercise has been shown to reduce cardiovascular morbidity and mortality, but the mechanisms for this benefit are unclear. Endothelial dysfunction is an early event in atherogenesis, and animal studies have shown that exercise training can enhance endothelial function.

METHODS

We have examined the effect of a standardized, 10-week, aerobic and anaerobic exercise training program on arterial physiology in 25 healthy male military recruits, aged 17 to 24 (mean 20) years, of average fitness levels. Each subject was studied before starting, and after completing the exercise program. Baseline vascular reactivity was compared with that of 20 matched civilian controls. At each visit, the diameter of the right brachial artery was measured at rest, during reactive hyperemia (increased flow causing endothelium-dependent dilation) and after sublingual glyceryltrinitrate (GTN; an endothelium-independent dilator), using high-resolution external vascular ultrasound.

RESULTS

At baseline, flow-mediated dilatation (FMD) and GTN-mediated dilatation were similar in the exercise and control groups (FMD 2.2±2.4% and 2.4±2.8%, respectively, p=0.33; GTN 13.4±6.2 vs. 16.7±5.9, respectively, p=0.53). In the military recruits, FMD improved from 2.2±2.4% to 3.9±2.5% (p=0.01), with no change in the GTN-mediated dilation (13.4±6.2% vs. 13.9±5.8%, p=0.31) following the exercise program.

CONCLUSION

Exercise training enhances endothelium-dependent dilation in young men of average fitness. This may contribute to the benefit of regular exercise in preventing cardiovascular disease. (J Am Coll Cardiol 1999;33:1379–85) © 1999 by the American College of Cardiology

Epidemiologic studies have indicated that high levels of physical activity and cardiorespiratory fitness are associated with lower morbidity and mortality from coronary heart disease (1–3). However, the mechanisms that mediate these beneficial effects remain unknown.

Endothelial dysfunction is both a key early event in atherogenesis and has been linked to the clinical manifestations of established coronary artery disease (CAD) (4–6). Several studies have shown that endothelial function is abnormal in the presence of classical cardiovascular risk factors such as smoking, hypercholesterolemia and insulin-dependent diabetes in young subjects without evidence of established atherosclerotic disease, and these risk factors may interact at an early stage of the disease process (7–9). In such subjects endothelium-dependent responses can be improved by a number of interventions, including low-density lipoprotein (LDL) cholesterol reduction, antioxidant therapy and L-arginine supplementation, and these may represent novel antiatherogenic strategies (10,11).

Reduced bioavailability of nitric oxide (NO) is an important consequence of endothelial damage and is thought to contribute to the development of atherosclerotic vascular disease (4). Physical exercise augments blood flow and shear stress, resulting in increased NO production and upregulation of endothelial NO synthase activity (12). Short periods of regular, localized exercise training has been shown to restore flow-dependent dilatation of the systemic arteries of patients with chronic heart failure (13). However, the ability to alter endothelium-dependent vascular function using a generalized exercise regime, appropriate for the general population, in subjects without clinical disease and with low risk factor profiles remains unknown.
We have therefore investigated the effects of a 10-week physical training program on systemic artery vascular function in young men of average fitness levels and have shown an improvement in endothelium-dependent dilatation, which may represent an important mechanism by which physical exercise provides cardiovascular benefit.

METHODS

Study subjects. Between September 1994 and February 1995, 35 male military recruits of average fitness levels (aged 16–25 years, mean 18 years), who had undergone a medical examination to exclude hypertension, diabetes mellitus, and clinical evidence of atherosclerotic disease, were selected from recruits to the Army Training Regiment, Bassingbourn, United Kingdom. We studied military subjects because they had homogeneous risk factor profiles (apart from cigarette smoking) and fitness levels (comparable with those of the general population), and could be supervised closely through a standardized exercise program in a manner not possible in the civilian population. None of the recruits had participated in regular exercise above that expected in the general population. Subjects were assessed at the beginning of their 10-week basic military training, with a detailed medical, smoking and drug history (15 were cigarette smokers and 20 life-long nonsmokers). Height, weight and supine blood pressure (the mean of three readings 1 min apart, after 5 min rest) were measured and a salivary cotinine levels were estimated (enzyme-linked immunosorbent assay technique; Gamex Laboratories, London, United Kingdom). Venous blood was taken for total cholesterol (cholesterol C-system high-performance CHOD-PAP [cholesterol oxidase-para-amino-phenazone] method) and high-density lipoprotein (HDL) cholesterol, after precipitation of apoprotein B–containing lipoproteins (GPO-PAP [glycerol phosphate oxidase-para-amino-phenazone] high-performance enzymatic colorimetric test; Boehringer-Mannheim GmbH, Mannheim, Germany). Lipoprotein(a) was measured using an immunoradiometric assay (Pharmacia, Milton Keynes, UK) and fibrinogen levels by HPLC (Phar-}

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Abbreviations and Acronyms

- CAD = coronary artery disease
- FMD = flow-mediated dilatation
- GTN = glyceryl trinitrate
- HDL = high-density lipoprotein
- LDL = low-density lipoprotein
- NO = nitric oxide
- NOS = nitric oxide synthase
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The subjects undertook 10 weeks of supervised, standardized, aerobic and anaerobic training, consisting of daily 3-mile runs and upper-body strength and endurance exercises. Due to injury or discharge, only 25 recruits (15 lifelong nonsmokers and 10 current smokers) completed the training regime and underwent re-assessment. Supine blood pressure measurement was repeated and blood was taken for cholesterol subfraction, lipoprotein(a) and fibrinogen analysis. The Basic Fitness Test was repeated to assess the degree of improved fitness after the exercise training program, and on the following day brachial artery vascular reactivity was measured.

It was not possible to included a concurrently studied control group of nonexercising troops as all those available for study were undertaking the basic military training. We therefore also studied 20 civilian men, matched for age and fitness levels, recruited from hospital staff and their relatives. All were life-long nonsmokers, normotensive, nondiabetic and had average cholesterol levels. Venous blood samples were taken for total cholesterol and HDL cholesterol, measured as before. Because of the difficulty in matching accurately for smoking exposure, only nonsmoking controls were studied.

All military recruits and civilian subjects who participated in the study provided written informed consent. The study was approved by the Army Medical Services Research Executive (ethics committee) and was overseen by the Regimental Medical Officer.

Vascular reactivity study. Arterial endothelial and smooth muscle function were studied noninvasively by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli as we have previously described (14). The reproducibility and repeatability of this technique over the time course of this study has been established (15–17). Arterial diameter was measured using a high-resolution ultrasonic vessel wall-tracking device (AMA, Netherlands) at rest, after reactive hyperemia (with increased flow causing an endothelium-dependent vasodilation) again at rest and after sublingual glyceryl trinitrate (GTN; an endothelium-independent vasodilator) (15). A 7-MHz linear array transducer and standard (128XP/10; Acuson, Mountain View, California) were used to image the brachial artery in longitudinal section 5–7 cm above the antecubital fossa. A stereotaxic clamp was employed to maintain the transducer position. When a suitable B-mode image was found, with a clear anatomical marker, the image was recorded using a video graphic printer so that a similar transducer position could be obtained for follow-up measurements. A suitable point where clear images of both anterior and posterior artery walls was selected, and an M-mode image obtained. The radio frequency signals from the M-mode output were digitized, relayed to the wall-tracking system and then displayed on the computer screen.
The operator marked the anterior and posterior vessel wall with sample volume markers, after which the exact position of each wall was determined by an interpolation technique. Positioning of the ultrasound probe and sample volume markers was always performed by the same operator, after which calculation of the brachial artery diameter was performed automatically. At each stage of the experiment, continuous wall motion was recorded for 5 s, allowing the mean diameter of between four and eight cardiac cycles to be measured at end-diastole automatically by the computer with a resolution of approximately 3 μm.

Subjects were rested in a supine position for 15 min before the first baseline measurement. After this, a pneumatic tourniquet was inflated above the wrist for 4.5 min to a pressure of 300 mm Hg. The cuff was then rapidly deflated (leading to reactive hyperemia in the hand and increased brachial artery blood flow), and brachial artery diameter and wall motion was recorded 55 s after cuff deflation. After a 10-min rest period, a further baseline measurement of the brachial artery was recorded and then a 400-μg metered dose of GTN was then administered by sublingual spray. A final brachial artery recording was then made after 3 min.

Blood flow was measured using pulse-wave Doppler with sampling gate set in the center of the artery. Measurements were taken at baseline and again at the time of maximum flow increase after forearm cuff deflation (approximately 15 s after cuff deflation). The vessel diameter after reactive hyperemia and GTN administration was expressed as a percentage relative to the average diameter of the artery in the two resting (baseline) scans (100%). Blood flow was estimated using the pulse-wave Doppler measurements and calculated arterial cross-sectional area. By taking the ratio of the maximum flow after cuff deflation and resting flow, an accurate estimate of relative increase in brachial artery blood flow can be made.

Statistics. Descriptive data were expressed as mean ± standard deviation unless otherwise stated. Baseline characteristics of the exercise subjects and controls were compared using independent student $t$ tests. The parameters measured in the exercise subjects, before and after exercise training, were compared using a paired student $t$ test (only data from the 25 subjects completing the training program were included). To test whether the changes in flow-mediated dilatation might be related to changes in other measured parameters, such as baseline vessel diameter, blood flow, hyperemia response, cholesterol, lipoprotein(a) and fibrinogen, a linear regression model was created, combining pre- and postexercise results, with exercise as a dummy variable. Possible determinants of flow-mediated dilatation, pre- and postexercise training and the degree of change in flow mediated dilatation were explored using univariate and multivariate linear regression analysis. To compare vascular responses in the smokers and nonsmokers, independent student $t$ tests were employed. Appropriate corrections were made for multiple $t$ tests. Statistical significance was inferred at $p < 0.05$.

RESULTS

The physical characteristics, baseline biochemistry and vascular reactivity of the 10 military recruits who dropped out were not significantly different from the 25 who completed the program. Subsequent results are given for the latter group of subjects only.

Baseline studies. PHYSICAL CHARACTERISTICS. The exercise subjects were of average physique (body mass index $22 ± 2$ kg/m$^2$) and fitness at commencement of training. Resting heart rate ($69 ± 8$ beats/min) and blood pressure (systolic $119 ± 12$ mm Hg, diastolic $72 ± 12$ mm Hg) were within normal limits and cotinine levels were appropriate for reported smoking history (nonsmokers $1.1 ± 0.4$ ng/mL, smokers $233.8 ± 44.3$ ng/mL). The control group was of similar physique (body mass index $22 ± 3$ kg/m$^2$) and had similar resting heart rate and blood pressure (heart rate $70 ± 9$ beats/min, systolic and diastolic, $120 ± 14$ mm Hg and $73 ± 13$ mm Hg, respectively).

BIOCHEMICAL STUDIES. The exercise subjects had normal total cholesterol, HDL cholesterol levels, fibrinogen and lipoprotein(a) levels for their age ($4.0 ± 0.7$ mmol/liter, $1.4 ± 0.2$ mmol/liter, $278 ± 48$ mg/dL and $0.17 ± 0.17$ g/liter, respectively). In the control group, total cholesterol and HDL cholesterol levels were not significantly different (total cholesterol $4.4 ± 1.0$ mmol/liter, $P = 0.29$ and HDL cholesterol $1.3 ± 0.2$ mmol/liter, $P = 0.58$). Lipoprotein (a) and fibrinogen were not measured.

VASOcular STUDIES. Baseline vessel diameter, resting blood flow and hyperemia response for the exercise group are shown in Table 1. Brachial artery diameters (exercise group $4.3 ± 0.5$ mm, controls $4.3 ± 0.4$ mm, $p = 0.64$) and flow responses were not significantly different in the recruits and the civilians. Baseline flow mediated dilatation was $2.2 ± 2.4\%$ in the exercise group and $2.4 ± 2.8\%$ in the controls ($p = 0.33$). In the exercise group, there was a trend towards lower flow-mediated dilatation in the smokers (10 subjects), but this did not reach statistical significance (smokers $1.9 ± 3.1\%$, nonsmokers $2.4 ± 1.8\%$, $p = 0.31$). Dilatation in response to sublingual GTN was similar in exercise subjects and controls ($13.4 ± 6.2$ vs. $16.7 ± 5.9$, respectively, $p = 0.53$).

Effects of exercise training. PHYSICAL CHARACTERISTICS. After exercise training, fitness levels, as measured by the Basic Fitness Test 1.5-mile run time, improved markedly (preexercise training $598 ± 30$ s, postexercise training $564 ± 28$ s, $p < 0.001$), reflecting increased aerobic fitness. Anaerobic fitness also improved in the upper limb as measured by increased ability to perform heaves to a bar (preexercise training $2 ± 1$ heaves, postexercise training $4 ± 2$ heaves, $p < 0.005$). Resting heart rate (preexercise
training 69 ± 8 beats/min, postexercise training 71 ± 11 beats/min, supine systolic (pre 119 ± 12 mm Hg, post 120 ± 10 mm Hg) and diastolic blood pressure (pre 72 ± 12 mm Hg, post 73 ± 10 mm Hg) were unchanged after exercise training (Table 1). Smoking habits during the 10-week period were not changed and there was no difference either in the peak fitness achieved (1.5-mile run time: nonsmokers 570 ± 27 s, smokers 551 ± 28 s, p = 0.24) or in the change of fitness levels between the nonsmoking subjects and smoking subjects (27 ± 19 s vs. 52 ± 36 s, p = 0.21).

BIOCHEMICAL STUDIES. After exercise training total cholesterol increased (pre 4.0 ± 0.7 mmol/liter, post 4.2 ± 0.7 mmol/liter, p = 0.02) and HDL cholesterol levels fell (pre 1.4 ± 0.2 mmol/liter, post 1.3 ± 0.3 mmol/liter, p = 0.01), while lipoprotein(a) levels (pre 0.17 ± 0.17 g/l, post 0.17 ± 0.17 g/liter, p = 0.64) and fibrinogen levels (pre 278 ± 48 mg/dL, post 301 ± 62 mg/dL, p = 0.07) were unchanged.

VASCULAR STUDIES. Baseline vessel diameter, resting blood flow and hyperemia response were similar before and after exercise training (Table 1). Flow-mediated dilatation increased significantly after the training program from 2.2 ± 2.4% to 3.9 ± 2.5%, p = 0.01. In contrast, dilation to sublingual GTN was unchanged (13.4 ± 6.2 vs. 13.9 ± 5.8, p = 0.31) (Fig. 1). There was no difference in the degree of improvement in flow-mediated dilatation after the exercise training program in smokers or nonsmokers (difference in smokers 1.8 ± 3.5%, difference in nonsmokers 1.6 ± 3.1%, p = 0.74).

DETERMINANTS OF CHANGE IN ENDOTHELIUM-DEPENDENT RESPONSES. On multivariate regression analysis, the change in flow-mediated dilatation was only related to exercise training, and was independent of changes in baseline vessel size, blood flow, hyperemia response, total cholesterol, HDL cholesterol, lipoprotein(a) or fibrinogen. Similarly, the magnitude of the change in flow-mediated dilatation was found to be independent of smoking history, lipid levels, fibrinogen levels, resting blood pressure, family history of coronary artery disease, baseline or posttraining, 1.5-mile run time or heaves to a bar.

**DISCUSSION**

This study has shown that endothelium-dependent responses in the brachial artery of healthy young men can be improved after just 10 weeks of regular physical exercise of an intensity that might be reasonably undertaken by the general population. The beneficial effects were not mediated by the known influences of exercise on total cholesterol, HDL cholesterol, lipoprotein(a), fibrinogen levels or resting blood pressure.

**Table 1. Characteristics of the 25 Subjects Completing the Exercise Training Program**

<table>
<thead>
<tr>
<th></th>
<th>Preexercise Training</th>
<th>Postexercise Training</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>1.5-mile run time (s)</td>
<td>598 ± 30</td>
<td>564 ± 24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69 ± 8</td>
<td>71 ± 11</td>
<td>0.78</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>119 ± 12/72 ± 12</td>
<td>120 ± 10/73 ± 10</td>
<td>0.73/0.68</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>278 ± 48</td>
<td>301 ± 62</td>
<td>0.06</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td>4.0 ± 0.7</td>
<td>4.2 ± 0.7</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/liter)</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Lipoprotein(a) (g/l)</td>
<td>0.17 ± 0.17</td>
<td>0.17 ± 0.17</td>
<td>0.61</td>
</tr>
<tr>
<td>Brachial artery diameter (mm)</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Baseline blood flow (mL/min)</td>
<td>43 ± 16</td>
<td>49 ± 19</td>
<td>0.15</td>
</tr>
<tr>
<td>Reactive hyperemia (%)</td>
<td>308 ± 111</td>
<td>269 ± 74</td>
<td>0.09</td>
</tr>
<tr>
<td>Flow-mediated dilation (%)</td>
<td>2.2 ± 2.4</td>
<td>3.9 ± 2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>GTN-mediated dilation (%)</td>
<td>13.4 ± 6.2</td>
<td>13.9 ± 5.8</td>
<td>0.31</td>
</tr>
</tbody>
</table>

GTN = glyceryltrinitrate, HDL = high-density lipoprotein.

**Figure 1.** Flow-mediated dilation (a) and GTN-mediated dilation (b) before and after the 10-week exercise training program (expressed as mean ± SD). Flow-mediated dilation was significantly increased after the exercise program, whereas GTN-mediated dilation remained unchanged. GTN, glyceryltrinitrate.
Previous studies of exercise training. Convincing evidence of the benefits of regular exercise on cardiovascular morbidity and mortality has been provided by epidemiologic studies (1–3). As a result, regular physical aerobic activity is frequently incorporated into primary and secondary prevention programs (18,19). The beneficial effects of exercise may be mediated in a number of ways, including changes in lipid profile, fibrinogen, carbohydrate metabolism, neurohormonal release as well as effects on blood pressure (20–24). Recently, however, an improvement in brachial artery endothelium-dependent relaxation has been reported after forearm exercise in patients with congestive cardiac failure, although this was confined to the vascular bed of the exercised limb (13). Our study extends these observations on the effects of exercise on the endothelium, by demonstrating that endothelial function can be enhanced by generalized exercise from a young age in the absence of clinical disease. Animal experiments have also shown the effects of exercise training on endothelium-dependent responses, both in the resistance and conduit vessels of the coronary and peripheral circulation (25–28). While studies employing pharmacological agonists have yielded conflicting results (12,25–28), flow-mediated dilation has consistently been improved by exercise training in a variety of models (29).

Possible mechanisms. Our protocol examines flow-mediated dilatation in the human brachial artery, which is known to depend on the ability of the vascular endothelium to release NO (30). Shear stress is a potent physiologic stimulus for NO release (31) (not only mediating vasodilation but also endothelial cell adhesion molecule expression, monocyte binding and superoxide production [32,33]), and repeated episodes of increased blood flow with exercise may be the basis for the improved endothelial function seen in our subjects and the long-term benefits of regular exercise in reducing the complications of atherosclerotic vascular disease (34). The mechanism is likely to involve chronic increases in NO production mediated by an increase in the expression of nitric oxide synthase (NOS). NOS mRNA is upregulated in cultured endothelial cells exposed to laminar shear stress, and similar observations have been reported from animal studies with both short- and long-term exercise (12,29,35,36). A transcription factor binding site in the NO synthase promoter gene has recently been described linking shear stress to changes in vascular endothelial NO production (37).

Study limitations. We selected young male military recruits as they represented a homogenous population of men within a narrow age range with comparable exercise ability. We excluded subjects with a number of conditions known to have early effects on endothelial function, including glucose intolerance, hypercholesterolemia and hypertension. We did, however, include cigarette smokers and, interestingly, the beneficial effects of exercise were similar in the presence of this major cardiovascular risk factor. It was not possible to study a control group of nonexercising troops as all recruits were undertaking basic military training. We have therefore compared the baseline responses in the exercise subjects with those of 20 civilian men matched for age, vessel size and fitness studied concurrently. Flow-mediated dilation was similar in the exercise and control groups, but values were lower than those that we have previously reported. This, in part, is related to the large resting diameter of the brachial arteries in these healthy young men (flow-mediated dilation is inversely proportional to the vessel diameter [13,14]), but also due to changes in the methodology used. These included the use of the wall-tracking technique for arterial diameter measurement and a change in the position of the pneumatic cuff (to minimize artifactual brachial artery movement), which resulted in a decreased flow stimulus. Despite the lower baseline levels of flow-mediated dilation, we were able to show a significant improvement in endothelium-dependent vascular reactivity after just 10 weeks of regular exercise. This did not result from a change in reactive hyperemia, consistent with the observations of Hornig et al. (13), who found no changes in hyperemic response after localized exercise training. In our young clinically well population, the improvement of flow-mediated dilation observed was smaller than that found in the only other clinical study of brachial artery responses in congestive cardiac failure patients (13). The latter, however, examined the impact of intense localized exercise in a sedentary population with markedly impaired baseline vascular function. Nevertheless, the improvements found after exercise are likely to be based on a similar enhancement of endothelium-derived NO bioavailability. It remains to be determined whether further improvements in flow-mediated dilatation could be achieved in our study with a longer or more intense period of exercise training. Smoking did not appear to diminish the improvement in flow-mediated dilation in the exercise group, but further studies are required to establish whether exercise can improve vascular function in association with other cardiovascular risk factors, such as diabetes and hypercholesterolemia. Our civilian control group did not undergo restudy, and we cannot be certain that unmeasured confounding factors did not account for the observed changes in vascular function seen in the military recruits. However, we and others have previously demonstrated the reproducibility and repeatability of flow-mediated dilation measurements over periods of up to 24 weeks (15–17).

Clinical implications. Our study is the first to demonstrate an improvement in endothelial function that is not confined to the exercising limb. As brachial artery vasoreactivity has been shown to parallel agonist-induced endothelial responses in the coronary circulation, the benefits of exercise are likely to be present in the clinically relevant vascular beds not merely in the forearm (38). Endothelium-derived NO appears to be a key antiatherogenic molecule. In addition to its effects on vasomotor tone, evaluated in this study, it inhibits platelet adhesion and aggregation, monocyte adhe-
sion, vascular permeability and smooth muscle proliferation (39). We have previously reported impairment in flow-mediated dilation, in association with classical cardiovascular risk factors known to predispose to atherosclerosis and its complications in later life, many years before the clinical manifestations of vascular disease (7–9). Thus, increased NO bioavailability may represent an important mechanism by which exercise affects the progression of atherosclerotic vascular disease and cardiovascular morbidity and mortality (36).

Conclusions. Our study demonstrates an improvement of brachial artery endothelium-dependent responses in young healthy subjects, after 10 weeks of generalized exercise training at levels that might be achieved by the general population. This provides support for the incorporation of physical exercise in cardiovascular disease prevention strategies.

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