Enhanced endothelium-dependent vasodilation in older endurance-trained men

MORTON R. RINDER, ROBERT J. SPINA, AND ALI A. EHSANI
Division of Gerontology and Geriatrics and Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri 63110

Rinder, Morton R., Robert J. Spina, and Ali A. Ehsani. Enhanced endothelium-dependent vasodilation in older endurance-trained men. J. Appl. Physiol. 88: 761–766, 2000.—We hypothesized that abnormal endothelium-dependent vasodilation (EDD) found in older otherwise healthy subjects can be attenuated with long-term endurance training. Ten endurance-trained men, 68.5 ± 2.3 yr old, and 10 healthy sedentary men, 64.7 ± 1.4 yr old, were studied. Aerobic exercise capacity (VO$_{2\text{max}}$), fasting plasma cholesterol, insulin, and homocysteine concentrations were measured. Master athletes had higher VO$_{2\text{max}}$ (42 ± 2.3 vs. 27 ± 1.4 ml·kg$^{-1}$·min$^{-1}$, P < 0.001), slightly higher total cholesterol (226 ± 8 vs. 199 ± 8 mg/dl, P = 0.05), similar insulin, and higher homocysteine (10.7 ± 1.3 vs. 9.2 ± 1.4 µmol/ml, p = 0.02) concentrations. Brachial arterial diameter, determined with vascular ultrasound, during the hyperemic response was greater in the master athletes than in controls (P = 0.005). Peak vasodilatory response was 109.1 ± 2 vs. 103.6 ± 2% (P < 0.05) in the athletes and controls, respectively. Endothelium-independent vasodilation in response to nitroglycerin was similar between the two groups. The increased arterial diameter during the hyperemic response correlated significantly with the VO$_{2\text{max}}$ in the entire population (r = 0.66, P < 0.002). Our results suggest that long-term endurance exercise training in older men is associated with systemic enhanced EDD, which is even detectable in the conduit arteries of untrained muscle.

training; elderly

INCREASED SHEAR STRESS in arterial segments induces release of endothelial-derived mediators of vasodilation, such as nitric oxide (NO) (3–5, 12, 17). Endothelium-dependent arterial dilation (EDD) plays a major role in the regulation of vasomotor tone and regional blood flow. Impaired EDD has been observed in patients with coronary artery disease and those with risk factors for coronary artery disease such as diabetes mellitus, hypertension, hypercholesterolemia, smoking, and hyperhomocysteinemia (3, 15, 19, 22, 23, 26, 28). Advancing age also appears to be associated with impaired EDD (7, 27).

Reversibility of impaired EDD and restoration of a normal vasodilatory response have been demonstrated after pharmacological reduction of serum cholesterol (1, 24). Further evidence that reversibility is possible comes from longitudinal data, which demonstrated that EDD is enhanced after estrogen supplementation in middle-aged men and that estrogen appears to improve impaired EDD found in postmenopausal women (9, 14, 16, 18, 20). The effects of short- and long-term exercise on EDD is controversial. EDD in young athletes and in healthy volunteers after short-term training has been studied previously (8, 11, 13, 25). Several investigators found that short-term training does not seem to improve EDD-mediated blood flow in subjects with normal baseline responses to vasodilators, such as ACh, whereas other investigators have found that single bouts of exercise and long-term endurance training can enhance EDD in subjects with abnormal or normal baseline EDD (8, 11, 13, 25). Because aging is associated with a deterioration of EDD and EDD may improve with endurance exercise training, we hypothesized that the abnormal EDD associated with aging may be attenuated with long-term endurance training.

The purpose of the present study was to determine whether long-term endurance training is associated with enhanced EDD in older, healthy men. We measured the brachial arterial vasodilatory response during hyperemia after the release of a 5-min arterial occlusion as a measure of EDD and vasodilation after sublingual nitroglycerin (GTN) administration in the same vessel as a marker of endothelium-independent vasodilation (EID).

METHODS

Subjects. Study subjects consisted of 1) 10 older endurance-trained healthy men (68.5 ± 1.4 yr old) and 2) 10 sedentary healthy men (64.5 ± 2.3 yr old). The master athletes were training regularly (at least 3 times/wk for at least 1 h/day) and were participating in local and national athletic competitions. There were seven runners, two triathletes (running, swimming, and cycling), and one cyclist in this group. Most subjects had been training for years, and all had been training in the past 4 mo. Some of these master athletes have been studied previously in our laboratory to assess the effects of endurance exercise on the age-related decline in maximal aerobic power (21). Subjects were excluded if there was a history of hypertension, coronary artery disease, diabetes, or peripheral vascular disease. Subjects in the sedentary group were normotensive men over 60 yr old who were not performing any endurance or resistance exercise on a regular basis. They were nonsmokers and free of cardiovascular disease, risk factors for coronary artery disease, or any of the above illnesses. All subjects had normal cardiovascular examinations and electrocardiograms and were not taking any antihy-
ENHANCED ARTERIAL VASODILATION IN OLDER TRAINED MEN

Maximal oxygen consumption. Maximal exercise testing was performed on a motor-driven treadmill or cycle ergometer, depending on the mode of training of the master athletes, for measurement of the $V\dot{O}_{2\text{max}}$ as previously described (21). After a 5-min warm-up, the subjects began to exercise on a treadmill at 0% grade with the speed adjusted to increase their heart rate to ~70–75% of the age-predicted maximal heart rate. The grade was then increased by 2% every 2 min until exhaustion. Cycle ergometer maximal exercise testing was also performed with the use of an incremental protocol in which the work rate was increased every 2 min. Oxygen consumption ($V\dot{O}_2$) was measured with the MAX1 metabolic cart (FITCO, Farmingdale, NY). Subjects breathed through a Daniel’s valve, and expired gases were sampled from a mixing chamber. Inspiratory volume was measured with a pneumotac. $V\dot{O}_{2\text{max}}$ was defined as the mean of the two highest consecutive 30-s $V\dot{O}_2$ measurements that met the following criteria: 1) attainment of a plateau $V\dot{O}_2$ with increasing exercise intensity and 2) a respiratory exchange ratio (RER) exceeding 1.10.

Vascular ultrasound. Vascular imaging and induction of brachial arterial hyperemia were performed using a protocol described by others (4, 16, 18). The subjects were brought to the laboratory during the morning on a day when no previous exercise had been performed and rested for at least 10 min in a supine position. The right upper arm was used for vascular ultrasound. A blood pressure cuff was placed on the upper right arm just below the axilla, and the resting blood pressure was measured. Subsequently, a 7.5-mHz vascular ultrasound probe (Hewlett-Packard Sonos 2000) was placed over the brachial artery to visualize the conduit artery. The probe was placed longitudinally ~5 cm above the antecubital crease and held in place by a technologist. The transducer position was adjusted by mediolateral movement to obtain the largest diameter at each time point. However, proximal-distal movement of the probe was not allowed once the data collection started to ascertain measurement from the same segment of the brachial artery.

Endothelium-dependent vasodilator response. For the hyperemic experiments, images were obtained first at baseline and recorded on videotape. The blood pressure cuff was then inflated to 50 mmHg above systolic pressure for 5 min. Color flow Doppler confirmed the absence of flow through the conduit artery. After 5 min, the cuff was rapidly deflated and arterial images were obtained at 20 s and then each minute for the remaining 10 min. The baseline flow and the flow increase induced by cuff occlusion were calculated from pulsed Doppler recordings of the resting and the immediate postocclusive brachial artery flow velocities.

To determine whether cuff occlusion of the upper arm elicits a more pronounced hyperemic response compared with cuff occlusion of the forearm, we measured the brachial artery diameter after both occlusions (upper arm and forearm) in seven healthy subjects randomly selected from the staff in our laboratory. These subjects were not in the master athlete or sedentary control groups. Cuff occlusion of the upper arm with measurements just distal to the cuff were performed as described above. In addition, a blood pressure cuff was placed around the forearm in these subjects and inflated to 300 mmHg with the transducer placed proximal to the cuff occlusion as described by previous investigators (26). After release of the cuff, measurements were obtained similarly to the previous protocol.

RESULTS

Clinical and physiological characteristics. Values for two groups are shown in Table 1. The master athletes had significantly higher peak $V\dot{O}_2$ than sedentary controls ($P < 0.001$). The mean RER was sufficiently high in both groups such that most subjects achieved $V\dot{O}_{2\text{max}}$. Serum total cholesterol levels were different among the two groups; the older trained men had marginally higher total serum cholesterol. LDL cholesterol concentration was also higher in the older trained men, but the difference was not statistically significant. However, HDL cholesterol fractions were significantly higher in the master athletes than in the sedentary men, resulting in relatively similar atherogenic indexes (i.e., total cholesterol/HDL cholesterol) in the two groups. Serum homocysteine levels were higher in the master athletes than in the sedentary men (Table 1).
Table 1. Clinical and physiological characteristics of master athletes and sedentary controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Master Athletes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.7 ± 1.4</td>
<td>68.5 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline diameter, cm</td>
<td>0.52 ± 0.02</td>
<td>0.48 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>131 ± 15</td>
<td>130 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>70 ± 2.7</td>
<td>82 ± 2.1</td>
<td>0.003</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>90 ± 2.8</td>
<td>98 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>11.5 ± 1.8</td>
<td>15.7 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Homocysteine level, µmol/l</td>
<td>9.2 ± 1.4</td>
<td>10.7 ± 1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol ratio</td>
<td>4.5 ± 1.3</td>
<td>3.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>46 ± 3.3</td>
<td>61 ± 3.1</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>127 ± 7.5</td>
<td>142 ± 6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>199 ± 7.6</td>
<td>226 ± 7.7</td>
<td>0.05</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·min⁻¹</td>
<td>27 ± 1.4</td>
<td>42.4 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RER</td>
<td>1.16</td>
<td>1.17</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; V̇O₂, O₂ consumption; RER, respiratory exchange ratio. NS, not significant.

although this difference is unlikely to be of any clinical significance. There were no significant differences in baseline plasma insulin concentration between the trained and untrained men (Table 1).

Systolic blood pressure was similar in the two groups, but diastolic blood pressure was higher in master athletes (Table 1). Mean arterial pressures were not significantly different between the two groups (Table 1).

EDD. Arterial diameter was similar at baseline in the two groups (Table 1) and increased significantly in both groups (P = 0.02), consistent with the hyperemic response (Fig. 1). Furthermore, the overall brachial artery diameter was strikingly larger in the endurance-trained older men with significant interaction between group and time (P = 0.005). The highest vasodilation during hyperemia was also significantly greater in the master athletes than in the older sedentary men (109.1 ± 1.7% vs. 103.6 ± 1.5%, P < 0.05). There was a similar degree of reactive hyperemia in the untrained and trained groups (737 ± 132 vs. 780 ± 150% of baseline, P = 0.84).

EID. Because an abnormal EDD may represent either abnormal production of vasodilators in response to shear stress or an abnormal response of medial smooth muscle cells to substances released from the endothelium, we measured the arterial diameter after sublingual administration of GTN, an endothelium-independent vasodilator. Unlike the EDD responses, the EID response to GTN did not differ between the two groups. The EDD-to-EID ratio was significantly greater in the older endurance-trained men (0.97 ± 0.03 vs. 0.88 ± 0.02, P = 0.01) (Fig. 2).

Relation between physical activity and EDD. We also sought to determine whether the magnitude of changes in EDD correlates with maximal aerobic exercise capacity. EDD at 1 min during the hyperemia response correlated significantly (r = 0.66 and P < 0.002) with V̇O₂max in the subjects (Fig. 3). HDL, LDL, total cholesterol, and plasma insulin concentrations, however, did not correlate significantly with EDD (data not shown).

Comparison of forearm vs. upper arm occlusion. The maximal arterial diameter in the first 2 min after release of cuff occlusion was not different between proximal (upper arm) and distal (forearm) cuff occlusions (104.3 ± 1.3 vs. 104.9 ± 2.3%, P = not significant). There was a similar degree of reactive hyperemia for upper arm and forearm occlusions (693 ± 84 vs. 579 ± 74% of baseline, P = 0.34).

DISCUSSION

Our results suggest that long-term endurance exercise in older men is associated with systemic enhancement of EDD as it is detectable even in the conduit arteries of untrained muscles. Because only two of the athletes performed any upper extremity training (swimming), this adaptive response does not appear to be mediated exclusively by local adaptations. The significant and reasonably good correlation between V̇O₂max and EDD provides evidence suggesting that, within the
Several investigators have found an inverse relationship between serum total or LDL cholesterol concentrations and EDD (15, 22, 27). In addition, several other risk factors for coronary artery disease are associated with abnormal EDD, such as hyperinsulinemia and hyperhomocysteinemia, which we measured in our subjects. In our subjects, the adaptive increase in EDD was independent of cholesterol levels and the average serum concentration of total cholesterol was higher in the trained older men. The endurance-trained older men also had higher average diastolic blood pressure, serum cholesterol, and homocysteine concentrations, all of which have been associated with impaired EDD (3, 8, 23, 26). Although the difference in serum homocysteine levels between the two groups is clinically small, the serum concentrations in the athletes are comparable to previously reported levels in elderly sedentary subjects who have documented impaired EDD (23). Although the serum HDL cholesterol concentration was higher in the master athletes, we found no correlation between HDL and EDD by linear regression analysis. Our observations can be explained in several ways: 1) it is possible that endurance exercise training in elderly men increases serum HDL cholesterol concentrations, which protects the endothelium from the detrimental effects of LDL cholesterol, although, similar to our findings, previous larger studies have not demonstrated an association between HDL cholesterol and EDD (27); 2) endurance exercise training induces significant endothelial adaptations independent of cholesterol concentrations that can actually overcome or at least attenuate any negative effects of high-cholesterol concentrations; or 3) some other genetic or other variables may be responsible for the difference observed between the two groups. We believe, however, that the observed enhancement of EDD in the conduit artery is likely to be a result of training adaptations, since other risk factors known for their adverse effects on EDD were in fact either elevated or similar to controls in our master athletes. Therefore, it seems likely that endurance exercise training can attenuate the abnormal EDD response associated with these risk factors. Although the EDD measured in the endurance-trained older men appears to approach maximal responses found in young healthy men from previous investigations using similar techniques, cross-study comparisons cannot be validated (4).

We compared upper and lower arm occlusions to determine whether there is a significant difference between the two techniques. Because the occlusion causes 5 min of ischemia to local tissues, endothelium-independent vasodilators, such as adenosine, probably contribute to postocclusion vasodilation. Other investigators have clearly demonstrated that the hyperemic response is at least partially mediated by NO, as evidenced by a reduced vasodilatory response when NO synthase inhibitors are locally infused (5, 17). In addition, investigators have recently used a forearm blood pressure cuff occlusion in an attempt to eliminate the contribution of adenosine from the vasodilatory response seen after release of the occlusion in the upper arm (12, 16, 18). We utilized an upper arm occlusion in our investigation, as has been reported in previous investigations (4, 14, 17). The one comparison performed previously comparing upper arm and forearm occlusion revealed no significant differences in the maximal vasodilatory effect in the first 2 min following release of the occlusion in normal volunteers (4). Our present comparison study measuring arterial diameter after forearm and upper arm occlusion, in seven healthy subjects, confirms these findings. These findings suggest that tissue ischemia and release of endothelium-independent vasodilators are not significantly different among the two techniques.

Our sedentary subjects had smaller increases in the brachial artery diameter at 20 s and 1 min compared with trained men, and, furthermore, the diameter changes peaked at 2 min posthyperemia. This phenomenon probably occurred as a result of a prolonged increase in flow velocity and thus shear stress, well into the first minute after occlusion release. The first measurement, which was taken at 20 s, clearly demonstrated high flow velocity (data not shown) and did not seem to decrease until the end of the first minute. We believe that this prolonged increase in flow velocity and shear stress resulted in a continuous stimulus for NO synthesis and release and is most likely responsible for the observed increase in arterial diameter despite a brief NO half-life. Furthermore, this phenomenon is not new. Coretti et al. (4) found a very similar time course for diameter and flow changes after occlusion release in normal volunteers that was independent of occlusion placement.

One limitation of the present study is that we have made discontinuous measurements of the arterial diameter at selected intervals. Therefore, we may have missed the maximal vasodilation in some of our subjects. However, with the measurements made as frequently as possible in this study, i.e., at 20 s and 1 min, we believe that we were able to measure at least near
the maximal NO-induced vasodilator responses. The use of unblinded investigators, a small sample size, and similar gender characteristics (i.e., all men) limits the ability to generalize our data. In addition, the cross-sectional nature of our study does not allow evaluation of significant genetic differences between the two groups or metabolic changes that may occur with time. The rationale for the use of a cross-sectional study was because our objectives were to assess the effects of long-term endurance training in older men. Longitudinal studies will obviously be necessary to further evaluate our observation and determine whether factors other than exercise are responsible for improved EDD.

In summary, our findings suggest that endurance-trained older men have a better EDD in comparison to age-matched sedentary controls. Although this adaptive increase in EDD seems to be attributable to endurance exercise, the influence of genetic factors or even metabolic adaptations cannot be excluded. Because previous longitudinal studies have demonstrated improvement in EDD after endurance exercise training in young subjects, the findings of this study suggest that age-related impairment in EDD is potentially reversible or preventable by increased habitual physical activity. Longitudinal studies will be necessary to evaluate this possibility.

This study was supported by the Claude D. Pepper Older American Independence Center (National Institute on Aging Grant AG-13629) and by National Institutes of Health Grants R01-AG-12822 and MO1-RR-00036. M. R. Rinder was supported by National Institutes of Health Institutional Training Grant 5-T32-HL-07081-22. Address for reprint requests and other correspondence: M. R. Rinder, Washington Univer. School of Medicine, 660 S. Euclid Ave, Campus Box 8086, St. Louis, MO 63110. Received 21 August 1998; accepted in final form 6 October 1999.

REFERENCES


