Enhanced endothelium-dependent vasodilation in older endurance-trained men

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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 (VO2max), fasting plasma cholesterol, insulin, and homocysteine concentrations were measured. Master athletes had higher VO2max (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 VO2max 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.

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 antihypertensive medication.

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pertensives or lipid-lowering medications. Informed consent was obtained from all participants. The study was approved by the Human Studies Committee at the Washington University School of Medicine.

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 V̇O₂max, 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 (V̇O₂) 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 pneumotach. V̇O₂max was defined as the mean of the two highest consecutive 30-s O₂ measurements that met the following criteria: 1) attainment of a plateau V̇O₂ 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 inflated to 300 mmHg above systolic pressure for 5 min. Color Doppler-derived blood flow velocity was also recorded at each time point.

Endothelium-independent vasodilator response. After a 10-min rest interval, a second set of baseline images were obtained followed by sublingual administration of 0.4 mg GTN spray. Blood pressures were recorded every 2 min in the left arm after administration of GTN. Images of the brachial artery were recorded every minute for 10 min on videotape. Doppler-derived blood flow velocity was also recorded at each time point.

Measurements. All measurements were made off-line using the same Hewlett-Packard Sonos 2000 calibrated to the enlarged image of the artery. Five arterial diameters were measured in evenly spaced segments, approximately every 0.25 cm, perpendicular to the long axis of the arterial segment, using B-mode images. The diameter was defined as the distance between the junction of the endothelial layer and the media of the anterior wall and that of the posterior wall of the brachial artery.

Metabolic assessment. Serum concentrations of insulin, cholesterol, and homocysteine were measured in all subjects after a 10-h fast. None of the subjects had exercised before blood samples were obtained. Concentrations of total cholesterol and triglycerides were determined by commercial enzymatic kits from Boehringer Mannheim (Indianapolis, IN) on the Hitachi 917 analyzer. High-density lipoprotein (HDL) cholesterol was determined in serum supernates after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate (molecular weight of 50,000; Genzyme, Cambridge, MA) (6). Low-density lipoprotein (LDL) cholesterol was estimated by the method of Friedewald et al. (2). Homocysteine was measured by a modification of the method of Araki and Sako (2). Briefly, serum was reduced with tributylphosphine, extracted in dimethylformamide, and deproteinized with TCA. The free thios was derivitized with 7-fluoro- benzofurazane-4-sulfonic acid, ammonium salt (Fluka) and separated by HPLC. Plasma insulin concentrations were assayed by RIA.

Statistics. Data are expressed as means ± SE. Unpaired t-tests were used to compare characteristics between groups for statistics with normal distributions. Mann-Whitney rank sum tests were utilized to compare variables that were not normally distributed. Repeated measures two-way ANOVA (SigmaStat Software, Inc) and Scientific, San Rafael, CA) was utilized to compare multiple means for the brachial arterial diameters before and during hyperemia. Linear regression analysis was used to assess the relationship between V̇O₂max and the EDD response (Prism 2.01, Graphpad Software, San Diego, CA).

RESULTS

Clinical and physiological characteristics. Values for two groups are shown in Table 1. The master athletes had significantly higher peak V̇O₂ than sedentary controls (P < 0.001). The mean RER was sufficiently high in both groups such that most subjects achieved V̇O₂max. 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),
There was a similar degree of reactive hyperemia for proximal (upper arm) and distal (forearm) cuff occlusions (84 vs. 69% and 20% vs. 15%, respectively, for sedentary 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-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 V\textsubscript{O}2\textsubscript{max} 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 V\textsubscript{O}2\textsubscript{max} and EDD provides evidence suggesting that, within the age range...
age group of our subjects, a higher exercise capacity is associated with a greater EDD, perhaps resulting in a greater blood flow in the exercising muscle.

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 postocclusive 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.

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