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Endothelial Flow-Mediated Dilatation and Exercise Capacity in Highly Trained Endurance Athletes

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Kasikcioglu, E., Oflaz, H., Kasikcioglu, H.A., Kayserilioglu, A., Umman, S. and Meric. M. *Endothelial Flow-Mediated Dilatation and Exercise Capacity in Highly Trained Endurance Athletes*. Tohoku J. Exp. Med., 2005, **205** (1), 45-51 —— Brachial artery ultrasound during reactive hyperemia is a noninvasive method of assessing peripheral endothelium-dependent vasodilatation. Aerobic exercise has the potential to improve local endothelial function. We sought to analyze the effects of regular aerobic training on brachial artery endothelial function in endurance athletes. We studied diameter and blood flow of the brachial artery in 32 endurance male athletes and 30 healthy male subjects. In the same subjects flow-mediated dilatation of the brachial artery was recorded by inducing an ischemia through a forearm arterial occluding cuff. Maximal oxygen consumption was significantly higher in the athletes group than in the controls (61.24 ± 5.43 vs 44.49 ± 2.68 ml/kg/min, $p < 0.001$). Flow-mediated dilatation of the brachial artery induced by forearm arterial occlusion in athletes was also higher than that of the control subjects (17.1 ± 2.3 vs 11.2 ± 1.7, $p = 0.002$). Furthermore, there was an association between flow-mediated dilatation and $\dot{V}O_{2max}$ ($r = 0.69$, $p < 0.001$). Baseline measurements of the diameter and the blood flow volume of the brachial artery were similar in both groups. During reactive hyperemia period, the percent of the changes of endothelial diameters and flow were significantly higher in athletes than in controls. Higher flow-mediated dilatation levels in athletes reflect better vascular adaptation to habitual aerobic exercise. ———— endothelium; athlete; exercise; nitric oxide

Endothelium is an active barrier between the vascular wall and the blood. The main functions of the endothelium are control of coagulation, fibrinolysis, vascular tone and immune response (Glasser et al. 1996). Flow-mediated dilatation (FMD), a non-invasive method of assessing endothelial function, is the measurement of in response to forearm ischemia (Celermajer et al. 1992). The evaluation of FMD induced by reactive hyperemia following the release of a forearm-occluding cuff is an established method for assessing endothelial function (Celermajer et

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al. 1992). The main cause of FMD has been shown as an endothelial release of nitric oxide (NO) (Nakano et al. 2000) to regulate vascular diameter, and reflects the severity of atherosclerosis (Celermajer 1997). FMD is used as a tool for quantifying endothelium-dependent vasodilatation, and impaired peripheral endothelial function may also be a marker of increased cardiovascular risk (Celermajer 1997).

The initial rapid component of hyperemia is considered to be mediated by direct cell-to-cell conduction in a functional syncytium of the microcirculation that is embedded in the interstitial fluid and subjected to changes in the level of the metabolic by-products (Koller and Kaley 1990). FMD represents a plausible mechanism for vasodilatation induced by exercise (Koller and Kaley 1990; Tschakovsky et al. 1996). Although these studies have shown that aerobic exercise has the potential to improve local endothelial function, there is the lack of knowledge between endothelium-dependent vasodilatation and maximal oxygen consumption in athletes. Thus, the scope of the present study was to assess the relationship between flow-mediated dilatation and aerobic capacity in athletes compared with sedentary subjects.

Materials and Methods

Study population

We screened 32 male Caucasian runners and 30 Caucasian sedentary males for this study. We regarded the subjects as runners when they had trained at least 10 hours and a per week (averaging 65 km/week) for at least 8 years. Subjects were regarded as sedentary controls, when they exercised (walking) for less than 2 hours per week. Both the athletes and control subjects have no habitual training on forearm exercise with muscle strength. Subjects having acute or chronic illnesses by either history or physical examination were excluded. No subject had a history of hypertension, diabetes mellitus, smoking and alcohol abuse, none had electrolyte disturbances. No subjects were taking any form of medication. All subjects were free of cardiovascular disease as determined with detailed history and physical examination.

The investigation conforms to principles outlined in the Declaration of Helsinki and was approved by the Local Research Ethics Committee. Written informed consent was obtained from all subjects before participation.

Assessment of vascular function

Endothelial functions of the brachial artery were evaluated by using high-resolution vascular ultrasound (10 MHz transducer, attached to a standard Vingmed System Five, Norway). Athletes and controls did not receive vasoactive drugs and coffee or other caffeine rich beverages for 12 hours preceding testing. After resting in supine position for 10 min, internal diameter of the brachial artery was assessed at the end of diastole (timed by the QRS complex) and arterial flow volume was measured by using the pulsed Doppler sample volume. The diameter was measured in the ultrasound M-mode and flow was calculated from Doppler velocity and vessel diameter by the following formulae: mean velocity × arterial area ($\pi \times \text{radius}^2$). In practice, after deflating the pneumatic forearm cuff that had already been inflated up to 250 mmHg for 5 min, the arterial blood flow volume (BFV) was measured first 15 seconds and the diameter was measured at 45 to 60 seconds. This method of inducing increased blood flow by an ischemia-producing forearm occlusion is valid for assessing endothelium-dependent (FMD) and the reproducibility of the method have previously been described (Corretti et al. 2002; Mercanoglu et al. 2004).

Diameter changes were derived as percent change relative to the first (baseline) scan. Thus, flow-mediated dilation equalled:

([diameter after cuff deflation-resting diameter]/ resting diameter) × 100%

Flow change (reactive hyperemia) was calculated as

([flow after cuff deflation-resting flow]/ resting flow]) × 100%

Two experts blinded to the clinical data performed arterial diameter and BFV measurement.

The inter-observer and intra-observer variability for measurements of brachial artery diameter were < 3%.

Cardiopulmonary exercise testing

The exercise was performed in a quiet air-conditioned room with an average temperature of 21 ± 2°C and full resuscitation facilities. All participants underwent a standard Bruce multistage maximal treadmill protocol with metabolic measurements. A standard 12-lead electrocardiogram was monitored continuously, and was recorded at rest, during the last 10 seconds of each exercise stage, at the end of the graded

exercise test, and at the end of each minute of the subsequent 3-min recovery. Oxygen uptake was measured every 10 seconds using a metabolic cart (2900C B × B, Sensormedics, CL, USA). Respiratory gas was analysed using a zirconium oxygen analyser and a non-dispersive infrared sensor for carbon dioxide. Before each test, the gas analysers were calibrated with two mixtures of gases of known oxygen and carbon dioxide concentration. Basic gas and flow measurements were also corrected for ambient temperature, barometric pressure, and water vapour. Subjects breathed ambient air through a Rudolph two-way valve during the exercise. To ensure that maximal oxygen consumption ($\dot{V}O_{2max;}$ ml/kg/min) was reached, three criteria had to be met: 1) a levelling off of $\dot{V}O_2$ despite an increase in exercise power over the final stages of the test, 2) attainment of age-predicted maximal HR (210 – 0.65 × age) ± 10 beat.min-1, 3) R (respiratory exchange ratio) ≧ 1.10 (Wasserman et al. 1973; Kasikcioglu et al. 2004).

Statistical analysis

Statistical results are presented as the means ± S.D. Independent *t*-test was used for statistical analysis. The difference was considered statistically significant when $p < 0.05$. Correlations were assessed by Pearson's coefficients.

Results

There were not any statistically significant differences between athletes and controls, regarding to demographic characteristics, which have been summarized in Table 1. Detailed laboratory and hemodynamic parameters are provided in Table 2. $\dot{V}O_{2max}$ values were significantly higher in the athletes than in controls (63.4 ± 5.7 vs 43.4 ± 3.5 ml/kg/min, $p < 0.001$).

The measurements of brachial artery endothelium-dependent (flow-mediated) and their statistical analyses are presented in Table 3. Baseline measurements of the diameter and the blood flow volume of the brachial artery were similar in both groups. During reactive hyperemia period, the percent of the changes of endothelial diameters and flow were characterized by significantly higher in athletes than in controls.

The change in endothelium-dependent vasodilatation was significantly correlated with $\dot{V}O_{2max}$

Table 1. *Demographic characteristics of the groups*

	Athletes (n = 32)	Controls (n = 30)	p value
Age (year)	23.2 ± 3.7	21.1 ± 4.3	0.21
Height (cm)	174.3 ± 6.1	173.1 ± 5.4	0.19
Weight (kg)	67.3 ± 5.4	65.8 ± 6.3	0.37
BMI (kg/m^2)	22.1 ± 3.2	21.7 ± 2.7	0.41

BMI, body mass index.

Table 2. *The laboratory and hemodynamic parameters in two groups*

	Athletes (n = 32)	Controls (n = 30)	p value
Heart rate (/min)	58.1 ± 7.6	77.1 ± 11.4	< 0.001
$\dot{V}O_{2max}$ (ml/kg/min)	63.4 ± 5.7	43.4 ± 3.5	< 0.001
SBP (mmHg)	104 ± 13	111 ± 14	0.06
DBP (mmHg)	71 ± 4	72 ± 5	0.91
Total cholesterol (mg/100 ml)	168 ± 7	179 ± 11	< 0.001
HDL-cholesterol (mg/100 ml)	47 ± 3	42 ± 3	0.03
LDL-cholesterol (mg/100 ml)	89 ± 6	98 ± 9	0.003
Triglyceride (mg/100 ml)	170 ± 11	174 ± 14	0.57

$\dot{V}O_{2max}$, maximal oxygen consumption; SBP, systolic blood pressure;
DBP, diastolic blood pressure.

TABLE 3. *The diameter and the flow volume of brachial artery at baseline and after the ischemic*

	Athletes (n = 32)	Controls (n = 30)	p value
B-DIA (mm)	5.32 ± 0.37	4.79 ± 0.41	0.07
H-DIA (mm)	6.22 ± 0.71	5.41 ± 0.59	0.72
FMD (%)	17.1 ± 2.3	11.2 ± 1.7	0.002
B-BFV (ml/min)	185.2 ± 64.7	183.3 ± 55.6	0.91
% incBFV-RH	114.2 ± 12.2	79.95 ± 16.8	0.04

B-DIA, baseline artery diameter; B-BFV, baseline brachial artery flow; FMD, flow-mediated dilatation; RH, reactive hyperemia; inc, increase.

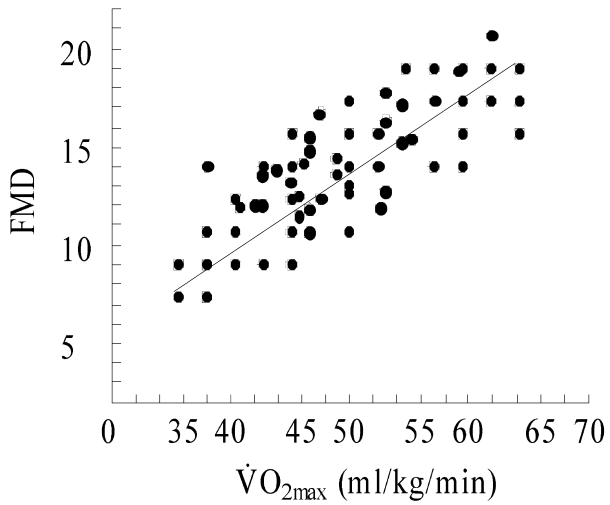

Fig 1. The plots show the relation of flow-mediated dilatation (FMD) and maximal oxygen consumption ($\dot{V}O_{2max}$) (r = 0.69, $p < 0.001$).

(r = 0.69, $p < 0.001$) (Fig. 1). Furthermore, there was a positive correlation between the percent of blood flow changes during hyperemia period and $\dot{V}O_{2max}$ (r = 0.41, $p = 0.03$).

DISCUSSION

The vascular endothelium plays an important role in the regulation of vascular tone and the maintenance of cardiovascular homeostasis (Glasser et al. 1996). Endothelial dysfunction, especially impaired endothelium-dependent vasodilatation, has been linked to the pathogenesis of atherosclerotic vascular disease and acute cardiovascular events. Furthermore, reduced endothelial vasodilator function occurs early in atherogenesis before histological and angiographic evidence of arteriosclerosis (Mano et al. 1996).

No prior study has determined whether endothelial function is associated with $\dot{V}O_{2max}$ in elite endurance athletes. The present study may show the effects of chronic aerobic training on vascular endothelial function that the increase in the FMD is positively correlated with $\dot{V}O_{2max}$ level in endurance athletes.

Exercise and endothelial function

Our results showed that the degree of change in diameters after the ischemic period was significantly higher than that of the controls although brachial artery diameters at baseline and hyperemia period were not different between two groups. We speculated that chronic regular exercise may improve the endothelium-dependent function, leading to enhancements in vascular adaptation. Available evidence in humans indicates that physical training is associated with increased endothelium-dependent vasodilatation. Clarkson et al. (1999) demonstrated that 10 weeks of lower-limb endurance training in healthy subjects with normal endothelial function have systemic enhanced on endothelium-dependent vasodilatation. Shoemaker et al. (1997) also reported that high-intensity handgrip exercise resulted in a diameter increase of the brachial artery. Furthermore, Kingwell et al. (1996) demonstrated that enhanced vascular reactivity to acetylcholine in young endurance-trained athletes compared with age-matched sedentary control

subjects. 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 (Wang et al. 1993; Muller et al. 1994; Oltman et al. 1995). FMD, has consistently been improved by exercise training in a variety of models (Koller et al. 1995).

In the present study, total cholesterol and LDL cholesterol values were lower in athletes than in controls; in contrast, HDL cholesterol value was higher. It is known that these changes have beneficial effects on the endothelium. Kingwell et al. (1996) suggested that the discrepancy of endothelial function is likely due to the differences in plasma cholesterol concentrations among subjects in each study. Other several studies also showed that elevations in plasma cholesterol levels, at any age, are associated with impaired endothelium-dependent vasodilatation (Steinberg et al. 1997). In contrast, Clarkson et al. (1999) found that these beneficial effects were not mediated by the known influences of exercise on total cholesterol, HDL cholesterol, lipoprotein (a), fibrinogen levels or resting blood pressure.

Endothelial function and $\dot{V}O_{2max}$ in athletes

The present data indicate that changes in brachial artery diameter may be related to $\dot{V}O_{2max}$. In contrast, although DeSouza et al. (2000) found that three months of regular aerobic exercise evoked a 30% increase in endothelium-dependent vasodilatation in previously sedentary middle aged and older men, this improvement occurred without concomitant changes in $\dot{V}O_{2max}$. In the present study we have observed that, improvements in endothelium-dependent vasodilator capacity were related to aerobic capacity. The correlation between improvement of endothelial vasodilator function and functional work capacity implies that the endothelial dysfunction is improving.

Hypothetical mechanisms

These results suggest that chronic aerobic physical activity may change the endothelial function to better level, because the restoration of nitric oxide availability may prevent production of oxidative stress. Release of nitric oxide represents the central factor responsible for FMD (Gillian et al. 1994). The exercise-induced increase in blood flow causes endothelial shear stress that is sensed and transduced by the endothelium, resulting in vasodilatation being proportional to the increase of shear stress. 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 (Sessa et al. 1994). 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. It is plausible that mechanical alteration/deformation of the endothelium during exercise as a result of increased systemic arterial pressure and pulsatile flow contributes to NOS up regulation.

Clinical applications

It is known that regular aerobic exercise is associated with a reduced risk of atherosclerotic vascular disease and acute cardiovascular events. In addition to favourably modifying traditional risk factors such as blood pressure, a mechanism by which regular exercise may confer this protection is through improved vascular endothelial function (Niebauer and Cooke 1996; Tanaka et al. 2000). Indeed, recent evidence suggests that regular aerobic exercise is an effective intervention strategy for improving endothelium-dependent vasodilatation in disease states such as chronic heart failure and hypertension (Hambrecht et al. 1998; Higashi et al. 1999). In this respect, it has recently been demonstrated that dynamic exercise reduces cardiovascular risk in the elderly (Hakim et al. 1999). It is tempting to speculate that the beneficial effect of this physical training program could be related in part to an improvement in endothelial function and depends on changes of

aerobic capacity level or changes of peak oxygen consumption value.

Study limitations

We did not administrate of nitroglycerin (an endothelium-independent vasodilator) due to ethical restriction. For this reason, we do not know change of endothelium-independent function in endurance athletes. Furthermore, this study was performed in inactive males and endurance-trained athletes. It may be of interest to examine the effects of different exercise type and gender.

Conclusions

The present study has shown a positive relation between $\dot{V}O_{2max}$ levels, a measure of aerobic capacity, and the endothelial vasodilatation response to exercise stress in endurance athletes. Based on these results, higher FMD levels in athletes may reflect better vascular adaptation to habitual aerobic exercise. FMD may also provide information regarding exercise capacity and the effect of cardiac risk factors on arterial function in athletes.

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