Endothelial function in highly endurance-trained and sedentary, healthy young women

Ingvild T Moe, Heidi Hovena, Eva V Hetland, Øivind Rognmo, and Stig A Slørdahl

Abstract: Endothelial function is reduced by age, chronic heart failure, coronary artery disease, hypertension or type 2 diabetes, and it is shown that aerobic exercise may reverse this trend. The effect of a high aerobic training status on endothelial function in young, healthy subjects is however less clear. The present study was designed to determine whether endothelial function is improved in highly endurance-trained young women compared to sedentary, healthy controls. Brachial artery diameter was measured in 16 endurance-trained (age: 23.7 ± 2.5 years, maximal oxygen uptake (VO2max): 60.6 ± 4.5 ml/kg per min) and 14 sedentary females (age: 23.7 ± 2.1 years, VO2max: 40.5 ± 5.6 ml/kg per min) at rest, during flow-mediated dilation (FMD) and after sublingual glycerol trinitrate administration, using high-resolution ultrasound. FMD did not differ between the endurance-trained and the sedentary females (14.8% vs 16.4%, p = NS), despite a substantial difference in VO2max of 50% (p < 0.001). The endurance-trained group possessed however, a 9% larger resting brachial artery diameter when adjusted for body surface area. The results of the present study suggest that endothelial function is well preserved in young, healthy women, and that a high aerobic training status due to long term aerobic training does not improve the dilating capacity any further.

Key words: aerobic exercise; flow mediated dilation; oxygen uptake

Introduction

Regular aerobic exercise and higher levels of aerobic capacity reduce cardiovascular morbidity and mortality. Physical exercise improves health by modifying classical risk factors associated with cardiovascular disease, such as reducing blood pressure, serum triglycerides, LDL-cholesterol, and glucose intolerance. In addition, insulin sensitivity and HDL-cholesterol are elevated in physically active subjects compared to sedentary individuals. Dysfunction of the endothelium is found to occur in the presence of elevated cardiovascular risk factors and play an initial part in the progress of cardiovascular disease. Thus, endothelial dysfunction represents one of the earliest events in the pathogenesis of atherosclerotic disease and it is possible to demonstrate impaired endothelial function at an early stage of disease, before histological and angiographical evidence of atherosclerosis.

Aerobic exercise improves endothelial flow-mediated dilation (FMD) in chronic heart failure, coronary artery disease, hypertension, type 2 diabetes and prevents age-related declines in endothelial function. The influence of long-term aerobic training in young, healthy individuals is however less clear because it has been previously reported that training may enhance, decrease and have no effect on endothelial function. To our knowledge, no studies have compared endothelial function between well-trained and sedentary, healthy young women. The present study was therefore designed to determine whether endothelial function in highly endurance-trained females is improved compared to sedentary, healthy females.

Methods

Subjects

Sixteen endurance-trained females and 14 sedentary females, with no habit of exercise, were recruited among university students. Subject characteristics are presented in Table 1. The athletes were cross-country...
skiers, track and field or orienteering runners, and were recruited if they exercised regularly for a minimum of 3 times per week. None of the subjects were smokers, frequently passive smokers, pregnant or taking cardiovascular or antioxidant medication. Subjects who had a history of diabetes, dyslipidemia, hypertension, thyroid illness, or a family history of premature cardiovascular disease were excluded. Six of the endurance-trained and 7 of the sedentary women were taking oral contraception. All subjects who participated in the study provided written, informed consent, and the regional committee for medical research ethics approved the study protocol.

Testing
Height and body weight were measured and body mass index (BMI) was calculated as BMI = weight (kg)/height2 (m2). The subject’s body surface area (BSA) was determined according to the formula developed by Haycock et al.14

\[
\text{BSA (m²)} = 0.024265 \cdot \text{weight (kg)}^{0.5378} \cdot \text{height (cm)}^{0.3964}
\]

Blood samples were taken from venous blood after a minimum 12-h overnight fast.

Brachial artery vasoreactivity
Endothelium-dependent and -independent dilation were studied using the method originally described by Celermajer et al.15 The guidelines for determination of the method described by Corretti et al16 were strictly followed. The reproducibility and repeatability of the method have been established previously,17 and the coefficient of repeatability18 of baseline brachial diameter measurements in our laboratory is 4%. Since variation in FMD with estrogen level and menstrual cycle has been described,19,20 vasoreactivity was measured during the first 6 days of the menstrual cycle, when the oestrogen level is lowest. Those using oral contraception (n = 13) were measured when taking placebo or not taking pills. All subjects were studied during the afternoon, after at least 4 h of fasting. Everyone was instructed to avoid exercise for 48 h before the test. After a 10-min rest in supine position, blood pressure (BP) and heart rate (HR) were measured in the right arm, using an automatic sphygmomanometer (Criticare Systems Inc. Waukesha, WI, USA). The BP and HR were reported as means of 3 measurements.

Thereafter, the brachial artery diameter at baseline was obtained in the left arm approximately 4.5 cm above the antecubital fossa, using a 12 MHz probe (Vivid 7 system, GE Vingmed Ultrasound AS, Horten, Norway). The artery was scanned when a longitudinal clear image of the lumen-intima line was found. A pneumatic tourniquet, placed on the upper arm, was inflated to 250 mmHg for 5 min, and thereafter rapidly deflated, producing reactive hyperaemia. Diameter measurements were recorded every minute for 5 min after deflation, and values at 1 min were used in assessment of FMD. The subjects then rested until the baseline diameter was restored (maximum 10 min) before endothelium-independent response was studied. After sublingual administration of 500 µg glycerol trinitrate (GTN) the diameter of the artery was measured for 4 min. All ultrasound images were analyzed in random order, using EchoPAC™ (GE Vingmed Ultrasound AS, Horten, Norway).

The analyzer of the images was blinded, unaware of the training status of the subjects. All measurements were obtained at the peak of the R-wave in the ECG using calipers with a 0.1 mm resolution. Diameters were measured from intima to intima, using the trailing to leading convention. The mean of 5 diameter measurements was used in the calculations of FMD and GTN responses.

Maximal oxygen uptake (VO2max)
Measurements of maximal oxygen uptake (VO2max) were conducted using an individualized treadmill ramp protocol. After a 10-min warm-up period at a

Table 1 Subject characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Trained group</th>
<th>Sedentary group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23.7 ± 2.5</td>
<td>23.7 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.8 ± 5.0</td>
<td>167.4 ± 7.8</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.4 ± 5.2</td>
<td>67.1 ± 14.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4 ± 1.5</td>
<td>23.7 ± 3.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.72 ± 0.91</td>
<td>1.77 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>54 ± 11</td>
<td>68 ± 11</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124 ± 11</td>
<td>124 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67 ± 6</td>
<td>69 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.2 ± 0.8</td>
<td>13.2 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>87 ± 8</td>
<td>79 ± 9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.43 ± 0.70</td>
<td>4.05 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.85 ± 0.32</td>
<td>1.45 ± 0.27</td>
<td>&lt;0.001</td>
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<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.24 ± 0.53</td>
<td>2.17 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.7 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>VO2max (ml/kg per min)</td>
<td>60.6 ± 4.5</td>
<td>40.5 ± 5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory exchange ratio (RER)</td>
<td>1.14 ± 0.06</td>
<td>1.17 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean values ± standard deviation. NS, not significant.
speed (7–10 km/h) and an inclination (0–5%) adjusted to the subject’s fitness level, the inclination was increased to 10%. The speed was thereafter increased by 1 km/h every minute until exhaustion. VO\(_{2}\text{max}\) was defined as the mean of the 3 highest 10 s measurements during a plateau of the VO\(_2\)-curve, or a respiratory exchange ratio (RER) above 1.10 if a plateau did not appear.

**Statistical analysis**

Values are expressed as means ± standard deviations (SD). An independent sample *t*-test was used to make comparisons between groups. Analysis of covariance (ANCOVA) was used to compare FMD- and GTN-data to control for possible baseline diameter imbalance. To test whether FMD might be related to arterial diameter and VO\(_{2}\text{peak}\), Pearson’s correlation coefficient was used. Statistical significance was defined as a two-tailed *p* < 0.05.

**Results**

Despite 50% (*p* < 0.001) difference in VO\(_{2}\text{max}\), there was no difference in brachial artery FMD between the sedentary and the trained group. The endurance-trained group showed a FMD of 14.8% compared to 16.4% in the sedentary group (Figure 1). The GTN responses were 22.2% for the trained group and 25.8% for the sedentary group, respectively. There was a trend towards larger absolute baseline diameters in the trained group (*p* = 0.08) (Figure 2), and when adjusting for BSA, the trained females showed a 9% larger baseline diameter (2.01 ± 0.19 mm/m\(^2\)) than the sedentary females (1.84 ± 0.21 mm/m\(^2\), *p* < 0.05).

There was no correlation between FMD and VO\(_{2}\text{max}\) (*r* = 0.014, *p* = NS), but a significant inverse correlation was found between FMD and baseline arterial diameter (*r* = −0.36, *p* < 0.05). Also, GTN mediated dilation varied inversely with baseline arterial diameter (*r* = −0.57, *p* < 0.001). There was no difference in endothelium-dependent or endothelium-independent dilation between those using and those not using oral contraception. HDL cholesterol and creatinine were higher in the endurance-trained women, and BMI and resting heart rates were higher in the sedentary women (Table 1).

**Discussion**

The present study suggests that long-term endurance training does not improve FMD of the brachial artery above normal levels in young, healthy women. Despite the 50% difference in aerobic capacity between the two groups, there was no difference in FMD between highly trained and sedentary individuals. To our knowledge this study is the first to determine the relationship between aerobic capacity and endothelial function in young, healthy females.

Similar FMD in highly trained and sedentary subjects, suggest that endothelial function is being well
preserved in healthy, young individuals. Our results correspond with other cross-sectional comparisons of endothelial function between active and sedentary young subjects. DeSouza et al.\textsuperscript{11} reported no differences in forearm vascular responses to acetylcholine between trained (VO\textsubscript{2max}: 58.4 ml/kg per min) and sedentary (VO\textsubscript{2max}: 41.8 ml/kg per min) men aged ~27. Similarly, Taddei et al.\textsuperscript{21} reported equal forearm blood flow modifications induced by intrabrachial acetylcholine in male athletes (VO\textsubscript{2max}: 68.2 ml/kg per min) and sedentary subjects (VO\textsubscript{2max}: 40.2 ml/kg per min), aged ~27, and concluded that endothelial function is well preserved and cannot be affected by potentially beneficial intervention such as aerobic exercise. These findings contrast those of Kingwell et al.\textsuperscript{22} who found greater vasodilatory response to acetylcholine in ~35 year old male endurance athletes compared with age-matched sedentary men. A covariance analysis suggested that a lower total cholesterol level in the endurance-trained group was a major contributor to the enhanced vasodilation. Elevation in total- and LDL-cholesterol levels is associated with impaired endothelium-dependent dilatation, also within the high normal range.\textsuperscript{23} In our study, the endurance-trained females had similar total- and LDL-cholesterol and higher HDL-cholesterol, compared to the sedentary subjects. However, the total cholesterol in the sedentary group was considerably lower compared to Kingwell et al.’s study (4.05 vs 5.24 mmol/l).\textsuperscript{22} It is worth mentioning that DeSouza et al.\textsuperscript{11} found similar endothelial function and similar blood lipid profiles between the trained and sedentary young men, while Taddei et al.\textsuperscript{21} measured the same endothelial dilation despite higher LDL- and lower HDL-cholesterol in the sedentary group.

Aerobic exercise interventions are known to improve endothelial function when it is reduced by age, chronic heart failure, coronary artery disease, hypertension or type 2 diabetes.\textsuperscript{7–11} However, longitudinal studies also indicate that exercise training has relatively little or no impact when endothelial function is well preserved at baseline.\textsuperscript{24} Kingwell et al.\textsuperscript{25} found no change in endothelial dilation in the forearm after 4 weeks of cycling in 13 sedentary ~24 year old males. Also, a study by Maiorana et al.\textsuperscript{26} found that 8 weeks of exercise-training did not alter forearm acetylcholine response in middle-aged healthy, sedentary subjects. In contrast, Clarkson et al.\textsuperscript{12} found that a 10-week aerobic and anaerobic exercise programme improved FMD from 2.2% to 3.9% in male military recruits. This study included smokers, who most likely influenced the results, as smoking is associated with impaired FMD.\textsuperscript{27} The military recruits had low FMD before training (2.2%), and significantly lower than our sedentary subjects (16.7%). The lower values may have been caused by the greater baseline arterial diameter (4.3 mm) of the recruits, because FMD is inversely proportional to the vessel size.\textsuperscript{15} Another distinction was the use of distal placement of the pneumatic cuff, which according to Uehata et al.\textsuperscript{28} causes a smaller hyperemic stimulus to produce FMD, compared to our study. The sum of the discussed studies, together with our results, suggest that aerobic exercise improves endothelial function only when it is impaired at baseline, and that the dilatory response is unaltered in healthy, young individuals. The phenomenon of improved FMD caused by long-term aerobic training thus seems to be valid only when investigations include subjects who possess cardiovascular risk factors or established cardiovascular disease.

To our knowledge, the only previous study of exercise and endothelial function among females is a study of postmenopausal women by McKechnie et al.\textsuperscript{29} They found an association between self-reported physical activity and brachial artery change to cold pressor testing. The response to reactive hyperaemia did not reach statistical significance, but there was a tendency for greater FMD among the more physically active women. These results are not inconsistent with our findings since impairment of endothelial function increases with age.\textsuperscript{11,21} Also, estrogens, which are at a much higher level in premenopausal than in postmenopausal women, have a beneficial effect on endothelium-dependent vasodilation.\textsuperscript{30} Postmenopausal women thus have reduced endothelial function compared to the premenopausal women of the present study, and are therefore most likely to improve it by exercise.

The highly-trained women in our study possessed a very high VO\textsubscript{2max} (60.6 ml/kg per min). Bergholm et al.\textsuperscript{13} suggested that hard exercise, i.e. to reach such a high VO\textsubscript{2max}-level, may induce oxidative stress that may adversely affect endothelial function. They showed that 12 weeks of aerobic training at an intensity of 70–80% of VO\textsubscript{2max} resulted in decreased levels of circulating antioxidants, and that the endothelial function in the forearm vessels was impaired after exercise training. The authors suggested that high intensity training impairs endothelial function through a decrease in levels of antioxidants and an increase in reactive oxygen species, resulting in a reduction in NO bioavailability. It may be argued if exercise at 70–80% of VO\textsubscript{2max} is a high intensity, but a highly trained individual will always exercise at a higher absolute intensity compared to a sedentary subject exercising at the same relative exercise intensity (i.e. % of maximal HR or VO\textsubscript{2max}), causing possible enhanced oxidative stress. It is also important to be aware that some of the measured effects of training on endothelial function may be acute, and to a lesser extent, long-term effects. Some studies do not report when the vascular measurements have been performed in relation to the last training session. The subjects of our study were not allowed to exercise in the last 48 h before the vascular measurements, and in the study of Bergholm et al.\textsuperscript{13} the nonexercise period was 36 h. It is then inviting to speculate if a possible
adverse effect due to intensive exercise for a highly trained subject may be turned into a favorable state after some period of time.

The highly-trained females showed a 9% larger resting brachial arterial diameter when adjusting for BSA. This corresponds with former studies where specifically trained athletes possess larger conductive arteries compared to untrained counterparts.\(^{31,32}\) The inverse correlation between both FMD and GTN-mediated dilation among all subjects manifest earlier findings that larger arteries will dilate less compared to smaller arteries.\(^{15,16}\) One may then speculate if a larger brachial arterial diameter is a stronger indicator of improved endothelial function rather than FMD, when comparing highly-trained and sedentary young subjects.

**Study limitations**

The comparative design of our study does not allow for determination of a possible relationship between long-term training and endothelial function as opposed to the short-term impact assessed in clinical trials. The study population is also relatively small. The data, however, are based on highly selected well-trained females, which limit the enrolment of larger numbers. However, since no indication towards improved FMD was found among the highly-trained women, this strongly suggests that long-term aerobic exercise will not improve FMD beyond normal among young, healthy individuals.

The design of the study attempted to diminish the known variations in endothelial function with menstrual cycle by measuring during the first 6 days of the cycle. Since timing of the measurements was based on self-reports, instead of oestrogen-measurements, abnormal variations in hormonal fluctuations may have confounded the results. Inclusion of individuals using oral contraceptives could also have influenced the results, but there were approximately equal numbers using oral contraceptives in the two groups, and there was no difference in FMD or GTN-mediated dilation between those using and those not using oral contraception.

**Conclusion**

Although the highly-trained females showed a larger baseline brachial artery diameter which should be considered, the results of the present study indicate FMD to be similar in highly-trained and sedentary females. This suggests that endothelial function is being well preserved in untrained, though healthy young women.

**References**


