Enhanced oxygen extraction and reduced flow heterogeneity in exercising muscle in endurance-trained men

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1.2 performed immediately after an intravenous injection of CTI, Knoxville, TN) was used for image acquisition. For the described (28, 32). An ECAT 931/08 tomograph (Siemens/Turku, Finland) and the PET scanner, as previously described (28). The delay between the input curve and the tissue curve was solved by fitting, and muscle blood flow was calculated by the autordiagnostic method (35) pixel by pixel into flow images with a 250-s tissue integration time, as previously described (28). Blood flow values in the exercising muscles were corrected to similar workloads by dividing measured blood flows with exercise intensity (in newtons).

For the femoral muscle blood volume studies, the subjects inhaled 2.7 ± 0.3 GBq (73 ± 8 mCi) $[^{15}O]CO$ (0.14% CO mixed with room air) for 2 min. Two minutes after the inhalation, a static scan for 4 min was started, during which three blood samples were taken and their radioactivity concentration was measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland), as previously described (32). Muscle blood volume was calculated under steady-state conditions by dividing the concentration of tissue $[^{15}O]CO$ radioactivity by the concentration of $[^{15}O]CO$ in the blood. The regional tissue-to-large vessel hematocrit ratio (0.91) was taken into account, as previously described (32).

Skeletal muscle oxygen consumption was measured by the bolus inhalation technique, as previously described (27). Measurement of muscle oxygen consumption began by pumping $[^{15}O]O_2$ into a rubber bladder and mixing it with room air. Thereafter the subjects inhaled the gas as a single bolus.

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Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Trained</th>
<th>Untrained</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26 ± 3</td>
<td>24 ± 3</td>
<td>0.23</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76 ± 10</td>
<td>73 ± 10</td>
<td>0.49</td>
</tr>
<tr>
<td>Height, cm</td>
<td>183 ± 7</td>
<td>180 ± 3</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.9 ± 2.6</td>
<td>22.6 ± 2.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Fasting serum glucose, mmol/l</td>
<td>5.2 ± 0.3</td>
<td>5.3 ± 0.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Fasting serum cholesterol, mmol/l</td>
<td>4.3 ± 0.9</td>
<td>4.4 ± 0.7</td>
<td>0.80</td>
</tr>
<tr>
<td>Fasting serum triglycerides, mmol/l</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Fasting serum HDL cholesterol, mmol/l</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Fasting serum HDL cholesterol/total cholesterol ratio</td>
<td>38 ± 9</td>
<td>33 ± 10</td>
<td>0.38</td>
</tr>
<tr>
<td>Fasting serum LDL cholesterol, mmol/l</td>
<td>2.4 ± 0.9</td>
<td>2.6 ± 0.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Blood hemoglobin</td>
<td>140 ± 5</td>
<td>145 ± 6</td>
<td>0.16</td>
</tr>
<tr>
<td>Blood hematocrit</td>
<td>40.1 ± 0.02</td>
<td>40.4 ± 0.02</td>
<td>0.16</td>
</tr>
</tbody>
</table>

BMI, body mass index; HDL and LDL, high- and low-density lipoprotein, respectively.

regular basis ≥5 times and >7 h weekly. Untrained subjects exercised occasionally and less than twice a week (0–2 h/wk). Written informed consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The Joint Commission on Ethics of the Turku University and Turku University Central Hospital approved the study protocol.

Study design. Subjects reported after an overnight fast (>10 h) and were instructed to avoid exercise and caffeinated beverages 24 h before the studies. Before PET studies, maximal isometric force of the right knee extensors was measured with a dynamometer (KinCom; Chattex, Chattanooga, TN) as will be described. Thereafter, two catheters were inserted, one in an antecubital vein for injection of tracers, and the other in the opposite radial artery for blood sampling. The subjects were positioned supine in the PET scanner with the femoral regions of both legs in the gantry. The right leg was fastened to a dynamometer (I-KON, Chattanooga Group, Oxfordshire, UK) at a knee angle of 50°, while the other leg rested in an extended position, as previously described (20, 27) (Fig. 1). Care was taken to fasten subjects carefully to the imaging table to avoid any movements in the femoral region during the study. Each study started with a 30-min resting period, during which a transmission scan for the correction of photon attenuation was performed (Fig. 1). After that, a 60-min intermittent isometric exercise period was started. The exercise consisted of intermittent 2-s isometric contractions (10% of maximal isometric power) followed by 2 s of rest for a duration of 60 min. The subjects performed the contraction after a sound signal, and exercise intensity was controlled by red, green, and yellow lights. Muscle blood flow, muscle oxygen uptake, and muscle blood volume were measured in the femoral region during the exercise, as will soon be described in detail.

Measurements of blood flow, oxygen uptake, and blood volume in skeletal muscle. Positron-emitting tracers $[^{15}O]H_2O$, $[^{15}O]O_2$, and $[^{15}O]CO$ were produced as previously described (28, 32). An ECAT 931/08 tomograph (Siemens/CTI, Knoxville, TN) was used for image acquisition. For the femoral muscle blood flow studies, a 6-min dynamic scan was performed immediately after an intravenous injection of 1.2 ± 0.2 GBq (34 ± 6 mCi) $[^{15}O]H_2O$. Input function was obtained from arterial blood, which was continuously with-
was calculated from $^{15}$O$\text{O}_2$ studies and blood flow from SD of blood flow values was calculated. Relative dispersion pixel flow data from four planes were pooled, and the mean for the CO images (1).

coefficient 0.3 for the flow and oxygen uptake images and 0.9 recently developed iterative median root prior (MRP) reconstruction method was used (20).

**Calculation of blood transit time.** The blood transit time in muscle was calculated by dividing muscle blood volume (ml/kg) by muscle blood flow (ml·kg$^{-1}$·min$^{-1}$) in the ROIs (33).

**Other measurements.** Maximal isometric force of the right knee extensors was measured with a dynamometer. Three repetitions consisting of 5 s of continuous maximal isometric tension were performed after two warm-ups. Highest tension (in newtons) during the three repetitions was taken as the maximal isometric force value. Arterial blood samples for the clinical laboratory tests were taken before the PET measurements. Plasma glucose was determined by the glucose oxidase method with an Analox GM7 (Analox Instruments, London, UK) glucose analyzer. Serum total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were measured using standard enzymatic methods (Boehringer Mannheim, Mannheim, Germany) with a fully automated analyzer (Hitachi 704, Hitachi, Tokyo, Japan). The low-density lipoprotein cholesterol concentration was calculated by Friedewald’s formula (13). Heart rate and blood pressure were measured with an automatic oscillometric blood pressure analyzer (Omron, Tokyo, Japan) before and during the PET measurements. Maximal oxygen uptake ($\dot{V}_\text{O}_2 \text{max}$) was determined by cycle ergometry or by treadmill running or pole-walking with direct respiratory measurements. The criteria used to establish the $\dot{V}_\text{O}_2 \text{max}$ were a plateau in oxygen uptake despite an increase in intensity and a respiratory quotient $>1.1$.

**Statistical methods.** Statistical analyses were done using SAS/STAT statistical analysis software release 6.12 (SAS Institute, Cary, NC). ANOVA for repeated measurements with contrasts was used for the analysis of statistical differences between groups and between exercising and resting muscles. $P$ values $<0.05$ were considered statistically significant. All data are shown as means ± SD.

**RESULTS**

Maximal oxygen uptake, isometric force, blood pressure, and heart rate. The $\dot{V}_\text{O}_2 \text{max}$ and resting heart rates differed in trained and untrained subjects (Table 2). Endurance-trained subjects tended to have lower blood pressure values than untrained subjects, and the maximal isometric force of one leg was 10% higher in trained subjects, but neither difference was statistically significant because of large group variability (Table 2).

**Table 2. Maximal oxygen uptake, heart rate, blood pressures, and isometric forces in study subjects**

<table>
<thead>
<tr>
<th></th>
<th>Trained</th>
<th>Untrained</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}_2 \text{max}$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>$67 \pm 3$</td>
<td>$46 \pm 6$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Resting heart rate, beats/min</td>
<td>$44 \pm 8$</td>
<td>$61 \pm 9$</td>
<td>0.003</td>
</tr>
<tr>
<td>Resting systolic blood pressure, mmHg</td>
<td>$111 \pm 4$</td>
<td>$118 \pm 7$</td>
<td>0.06</td>
</tr>
<tr>
<td>Resting diastolic blood pressure, mmHg</td>
<td>$67 \pm 5$</td>
<td>$75 \pm 9$</td>
<td>0.07</td>
</tr>
<tr>
<td>Maximal isometric force, N</td>
<td>$680 \pm 132$</td>
<td>$622 \pm 149$</td>
<td>0.33</td>
</tr>
<tr>
<td>Force during exercise, N</td>
<td>$68 \pm 13$</td>
<td>$61 \pm 15$</td>
<td>0.22</td>
</tr>
</tbody>
</table>

$\dot{V}_2 \text{max}$, maximal oxygen uptake.

containing 1.2 ± 0.2 GBq (32 ± 6 mCi) of $^{15}$O$\text{O}_2$. Dynamic PET imaging of the femoral region was started simultaneously and performed for 7 min with time frames of 6 × 5 s, 6 × 15 s, 6 × 30 s, and 2 × 60 s. The input function was measured as described above in the blood flow measurements. Muscle oxygen uptake was calculated from the equation $r\text{MRO}_2 = [\text{O}_2]_a \cdot r\text{OEF} \cdot r\text{MBF}$, where $r\text{MRO}_2$ is regional tissue oxygen uptake, $[\text{O}_2]_a$ is arterial oxygen concentration, $r\text{OEF}$ is regional oxygen extraction fraction, and $r\text{MBF}$ is regional blood flow. The parameters $r\text{OEF}$, $r\text{MBF}$, and $r\text{MRO}_2$ were quantified with nonlinear fitting from the $^{15}$O$\text{O}_2$ data by use of a model that includes separate compartments for oxygen bound to myoglobin and hemoglobin (27). Oxygen uptake values in the exercising muscles were corrected to similar workloads by dividing measured oxygen uptake values with exercise intensity (in newtons). Oxygen extraction fraction was calculated by the equation $\text{OEF} = \text{O}_2$ uptake/blood flow·$\text{O}_2$ content in blood, where $\text{O}_2$ uptake was calculated from $^{15}$O$\text{O}_2$ studies and blood flow from $^{15}$O$\text{H}_2\text{O}$ studies. The total effective radiation dose for the subjects was 3.7 ± 0.3 mSv.

All data were corrected for dead time, decay, and measured photon attenuation. PET images were processed using a recently developed iterative median root prior (MRP) reconstruction algorithm with 150 iterations and the Bayesian coefficient 0.3 for the flow and oxygen uptake images and 0.9 for the CO images (1).

**Regions of interest.** Regions of interest (ROIs) surrounding the quadriceps femoris (QF) muscle group and the individual muscle regions of the QF group were drawn into four subsequent cross-sectional planes (each 6.75 mm thick) in both thighs, avoiding the area of great vessels (Fig. 2), as described previously (20). The muscle areas were defined as rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius. Localization of the different muscle compartments of the QF group was done on the basis of individual transmission scans performed in all subjects.

**Calculation of muscle blood flow heterogeneity.** Pixel-by-pixel flow data from four planes were pooled, and the mean ± SD of blood flow values was calculated. Relative dispersion (RD) of blood flow heterogeneity was calculated as RD = (SD/mean)·100% (38). Heterogeneity due to methodological factors, estimated from phantoms, averaged 10% at the activity levels of the resting muscles and 6% at the activity levels of the exercising muscles for the flow images when the MRP reconstruction method was used (20).

Fig. 2. Examples of blood flow (top), oxygen uptake (middle), and blood volume (bottom) PET images in one trained (left) and one untrained subject (right). In each image, resting leg is on left and exercising leg is on right. Red and yellow colors define areas of highest blood flow, oxygen uptake, and blood volume.
Skeletal muscle oxygen uptake. Examples of blood flow, oxygen uptake, and blood volume images are shown in Fig. 2. Resting muscle oxygen uptake was not different between trained and untrained subjects. Oxygen uptake was four- to sixfold higher in the exercising muscle than in the resting contralateral muscle in both groups, and higher in the trained than in the untrained subjects (Fig. 3A). When oxygen uptake values were individually corrected to similar workload (i.e., oxygen uptake per one newton), they were not significantly different between groups (Fig. 4A).

Skeletal muscle blood flow, blood volume, and transit time. Blood flow in the resting muscle was similar in the trained and the untrained subjects. In the exercising contralateral muscle, blood flow was significantly higher in both trained and untrained subjects without significant differences between the groups (Fig. 3B). When muscle blood flow rates were individually corrected to similar workloads (i.e., oxygen uptake per one newton), a tendency ($P = 0.10$) toward lower values in athletes was found (Fig. 4B).

Muscle blood volume was similar in resting ($41 \pm 10$ and $43 \pm 5$ ml/kg) and in exercising muscle ($45 \pm 6$ and $43 \pm 3$ ml/kg) for trained and untrained subjects, respectively. Resting muscle blood transit time was also similar between groups, but during exercise, it was significantly longer in trained than in untrained subjects (Fig. 5A).

Skeletal muscle oxygen extraction fraction and blood flow heterogeneity. Oxygen extraction fraction was similar between groups in the resting muscle, but in the exercising muscle it was significantly higher in trained than in untrained subjects (Fig. 3C). Resting muscle perfusion was similarly heterogeneous in both groups but more heterogeneous in the exercising muscle in untrained than in trained subjects (Fig. 5B). In further analysis, flow was found to be
was more homogeneous in the exercising muscle in the trained subjects. Taken together, these changes may be associated with improved exercise efficiency in endurance-trained subjects.

Use of PET for quantification of blood flow and oxygen uptake in skeletal muscle offers advantages over previous techniques. It allows direct assessment of these parameters regionally in muscle without interference from other leg tissues (e.g., bone, skin, and other muscles outside the region). In addition, invasive catheterizations of the femoral region are not needed. The blood flow method applied in the present study has previously been compared with plethysmography (28) and the steady-state PET method (35). The method applied for the measurement of oxygen uptake was recently validated against Fick’s principle in our laboratory (27).

Mean resting blood flow values in the present study were in concordance with previous values in humans (25) and in animals (7). Exercising flow values in the present study were comparable with the values achieved during dynamic exercise with cycle ergometry at an exercise intensity of 24% of VO₂ max (14). However, blood flow and oxygen uptake values in the exercising muscles in the present study were less than one-tenth of the estimated values during maximal dynamic one-legged knee-extension exercise (6). Therefore, the results of the present study reflect the conditions of mild submaximal exercise.

Oxygen uptake relative to workload in the exercising muscle was similar between groups; however, blood flow tended to be lower in the trained subjects, thus indicating an increased oxygen extraction efficiency in the exercising muscle of trained subjects. Higher peripheral arteriovenous oxygen difference has previously been found in trained compared with untrained legs (21, 31). However, in those studies, arteriovenous oxygen difference has represented whole limb oxygen extraction, including various tissue components and both resting and exercising muscles.

One potential explanation for the observed higher oxygen extraction fraction that was found in the trained subjects is a longer blood transit time in exercising muscle. Muscle capillary density is related to degree of training, and it has been higher in trained subjects (2, 9, 18). Therefore, in conditions of similar or lower blood flow, the time blood stays in capillaries

### Table 3. Relative dispersion (index of heterogeneity) of blood flow in different parts of quadriceps femoris muscle group

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>Rest Untrained</th>
<th>Trained</th>
<th>Exercise Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus intermedius</td>
<td>30 ± 9</td>
<td>29 ± 5</td>
<td>34 ± 8</td>
<td>25 ± 6*</td>
</tr>
<tr>
<td>Rectus femoris</td>
<td>41 ± 11</td>
<td>39 ± 5</td>
<td>58 ± 10‡</td>
<td>42 ± 13*</td>
</tr>
<tr>
<td>Vastus lateralis</td>
<td>40 ± 5</td>
<td>35 ± 5</td>
<td>57 ± 16†</td>
<td>46 ± 8‡</td>
</tr>
<tr>
<td>Vastus medialis</td>
<td>34 ± 7</td>
<td>32 ± 5</td>
<td>35 ± 6</td>
<td>31 ± 13</td>
</tr>
<tr>
<td>Whole quadriceps femoris</td>
<td>43 ± 3</td>
<td>40 ± 7</td>
<td>65 ± 13</td>
<td>50 ± 9*</td>
</tr>
</tbody>
</table>

Values are in percentages ± SD. *P < 0.05 between groups; †P < 0.05 rest vs. exercise within group, ‡P < 0.01 rest vs. exercise within group.
(capillary transit time) is longer in the trained muscle, thereby potentially allowing more efficient oxygen extraction. Another potential explanation for the observed higher oxygen extraction fraction is differences in perfusion heterogeneity. In the present study, flow was less heterogeneous in the trained compared with the untrained subjects during exercise. Furthermore, analysis of different muscle compartments suggested that observed lower heterogeneity in exercising QF was due not to differences in absolute flow levels in different muscle compartments but rather to real vascular adaptation. No previous studies regarding the effects of training on perfusion heterogeneity in skeletal muscle are available. Sexton and Poole (36) investigated the effects of an acute exercise bout and long-term endurance training on flow heterogeneity in the rat diaphragm, and they found no changes in flow heterogeneity from rest to exercise or between trained and untrained rats. However, several studies have shown an increased capillary-to-fiber ratio in skeletal muscle after endurance training (2, 9, 18), which could potentially affect flow heterogeneity.

It has been demonstrated previously that muscle $\dot{V}O_2_{\text{max}}$ is limited by O$_2$ supply rather than by biochemical limitations (24, 34). In addition, O$_2$ transfer efficiency is reduced with heterogeneous blood flow and O$_2$-diffusing capacity (29). Therefore, the more homogeneous perfusion observed in the trained subjects compared with the untrained might be an adaptive mechanism to better supply oxygen to muscles. Recently, Walley (39) studied the effects of heterogeneity of oxygen delivery on oxygen extraction in tissues by using a theoretical model. According to his model, mismatch between oxygen demand and supply leads to impaired oxygen extraction by the tissues (39). Therefore, heterogeneous perfusion may impair oxygen extraction by the muscles. A previous model of Piiper and Haab (30) also supports this theory of impaired oxygen uptake with heterogeneous blood flow.

The use of PET provides a unique method to measure flow heterogeneity in humans in vivo. Methodological variation of flow values of pixels with PET and the MRP reconstruction method (20) has been estimated to be in the same order as that with the microsphere method used in animals (5, 22). However, the PET method we used has its own limitations related to resolution and pixel size. Because dimensions of pixels were $3.1 \times 3.1 \times 6.75$ mm$^3$ in the present study, heterogeneity was measured on a larger scale than the size of microvascular units, capillaries, or smaller arterioles.

The isometric exercise mode used in the present study may not be optimal when differences between trained and untrained subjects are measured. This exercise mode was chosen because of methodological limitations, because when blood flow and oxygen uptake are measured with PET, it is important that motion artifacts be avoided. However, the intermittent nature of isometric exercise allowed rhythmic contractions and a more natural exercise mode. The cross-sectional nature of this study does not exclude potential genetic differences between groups, which may have contributed to the results. Also, the low exercise intensity used in the present study may not necessarily reflect differences at higher workloads. Further studies during higher intensity and potentially during dynamic exercise are needed.

In conclusion, endurance-trained subjects have a higher oxygen extraction fraction in the exercising muscles, which could potentially be associated with the observed longer blood transit time and more homogeneous perfusion. These changes could contribute to the enhanced oxygen supply to muscle cells and improved exercise efficiency in the endurance-trained subjects.

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