Peak muscle perfusion and oxygen uptake in humans: importance of precise estimates of muscle mass

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Rådegran, G., E. Blomstrand, and B. Saltin. Peak muscle perfusion and oxygen uptake in humans: importance of precise estimates of muscle mass. J. Appl. Physiol. 87(6): 2375–2380, 1999.—The knee extensor exercise model was specifically developed to enable in vivo estimates of peak muscle blood flow and O₂ uptake in humans. The original finding, using thermodilution measurements to measure blood flow in relation to muscle mass [P. Andersen and B. Saltin. J. Physiol. (Lond.) 366: 233–249, 1985], was questioned, however, as the measurements were two- to threefold higher than previous anthropometric-based estimates. The initial criticism was mainly based on traditional findings measuring blood flow with ¹³³Xe clearance and plethysmography. ¹³³Xe clearance has, however, been shown to be nonlinear and to underestimate blood flow (3, 5, 6, 23). In addition, plethysmography is subject to inherent limitations such as motion and occlusion artifacts confined to application at rest (4, 7, 28). Furthermore, the plethysmography measurements performed after or in between contractions only reflect an immediate postexercise value and not exercise per se. Moreover, as the magnitude of the thermodilution measurements has now been verified by other independent methods such as ultrasound Doppler and indocyanine green dye infusion (2, 10, 16, 26), and as these three techniques enable equivalent measurements during both the contraction and relaxation phases, they seem today to offer the best regional measurements of blood flow during exercise in humans (17).

In the previous estimates of muscle perfusion, the size of the knee extensor muscle engaged has often been determined from anthropometric estimates (2). The mathematical formulas (2, 8) applied for the anthropometric estimates are based on the original model derived by Jones and Pearson (9). The quadriceps muscle mass is determined from a size relationship between the quadriceps and hamstring muscle in relation to the total limb volume, on the basis of a small number of autopsy studies (2). There are comparisons between such anthropometric estimates and imaging techniques, but the number of image scans have generally been too few (8). Recently, Ray and Dudley (15) also...
used MRI to determine the muscle volume engaged in contractions, but instead of measuring blood flow, they used data in the literature to estimate muscle perfusion. Thus, to obtain accurate values of muscle perfusion, not only blood flow but also the muscle volume engaged in the exercise needs to be accurately determined in the same subjects. As the thermodilution measurements today have been verified as accurate by other methods, we aimed to address the impact of the muscle mass estimates. In the present study, muscle blood flow was determined with thermodilution in the femoral vein. The size of the knee extensor muscle was determined by multiple computed tomography (CT) image cross sections along the full length of the muscle. Moreover, to evaluate their validity, three different estimates of the quadriceps muscle length were applied to anthropometrically estimated knee extensor muscle volume. The present study, therefore, provides a guideline for the accuracy of muscle mass estimates and an improved knowledge of peak perfusion and oxygen uptake in human skeletal muscle.

**METHODS**

Subjects. Nine healthy human subjects, eight men and one woman, with a mean ± SE age of 26 ± 1 (range 22–35) yr, height of 178 ± 3 (range 158–186) cm, and weight of 78 ± 4 (range 49–98) kg, volunteered to participate in this study. The subjects’ engagement in exercise varied from daily activities to regular endurance training. Before the experiments, the subjects were informed about the experimental procedures and potential risks and discomfort and were advised that they could withdraw at any time without consequences. They participated after first giving signed informed consent. The experiments were carried out with the approval of the Ethical Committees of Copenhagen and Frederiksberg (KF-01-403/95).

Experimental procedures. Before the experiments all subjects were familiarized with the one-legged, dynamic, knee extensor exercise model (1) by training at 60 rpm until they were comfortable and could fully relax the hamstring muscles, so that the work was done solely by the knee extensors. The isolation of the quadriceps muscle was supported by the kicking-force tracings collected during exercise. The peak power output that the subjects could sustain at 60 rpm was determined before the experiments by an incremental test starting at 10 W and increasing by 5–10 W every third minute until they could no longer sustain the rhythm.

The subjects reported to the laboratory on the morning of the experimental day. The femoral artery and vein of one leg were cannulated under local anesthesia (Lidokain, 20 mg/ml, Sygehus Apotekerne, Copenhagen, Denmark). The arterial (Ohmeda, Wiltshire, UK) and the venous catheter (Cook) were inserted proximally ~2–5 cm below the inguinal ligament (IL). A thermistor (model 94–030–2.5F, TD probe, Edwards Edslab, Baxter, Irvine, CA) was inserted in the venous catheter and connected to a cardiac output computer (model 9520A, Harvard Apparatus, American Edwards Laboratories, Irvine, CA) for thermodilution blood flow measurements (2). A Harvard mechanical syringe pump (model 44, Harvard Apparatus) was used for constant infusion of cold saline (2). Arterial and venous blood samples were taken and analyzed for hemoglobin, oxygen saturation, and PO2 (AVL 912 CO-oxylite, AVL Medical Instruments, Schaffhausen, Switzerland), as well as hematocrit. Limb oxygen uptake was calculated by multiplying the blood flow measurements in the femoral vein (outflow) with the arterial and venous difference in oxygen content (Fick principle). Heart rate (electrocardiogram) and intra-arterial blood pressure were recorded via a Kontron patient data monitor (model 565A, Medicoline, Valley, Denmark). The knee extensor force was measured with a strain gauge attached to the ergometer lever arm. All variables were recorded on a Gould recorder (model TA200, Gould) or in a personal computer (IBM compatible, Pentium based). The pulmonary oxygen uptake was determined online by a CPX Medical Graphics instrument (Spiropharma, Klampenborg, Denmark).

In the experiments, the subjects exercised for ~10 min at an absolute workload of 30 W and then for 5–7 min at a relative workload corresponding to 75–80% of their individual peak power output. The relative workload was included as a comparison to the peak power output to ensure that all subjects reached their peak intensity. The subjects were then rested for ~30–40 min before performing an experimental ramp protocol starting at 20–30 W for 3 min and increasing by 5 W every 30 s up to their peak power output. The peak power output was defined as the highest workload at which the subjects could maintain the pace for at least 1 min. Measurements of blood flow and pulmonary oxygen uptake as well as sampling of the blood were performed at rest and during steady-state exercise and during the last minute at peak intensity. To minimize blood flow contributions from the lower leg, a cuff was placed just below the knee of the working leg and temporarily inflated to a suprasystolic blood pressure (~240 mmHg) during the measurements.

**Table 1. Average values of peak work rate, \( Q_\text{peak} \), and \( V_\text{O}_2 \text{peak} \)**

<table>
<thead>
<tr>
<th>Study, Year (Ref. No.)</th>
<th>Peak Work Rate</th>
<th>( Q_\text{peak} )</th>
<th>( V_\text{O}_2\text{peak} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute, W</td>
<td>Per kg muscle, W/kg</td>
<td>Absolute, l/min</td>
</tr>
<tr>
<td>Andersen and Saltin, 1985 (2)</td>
<td>−54</td>
<td>−23.5*</td>
<td>−5.7</td>
</tr>
<tr>
<td>Rowell et al., 1986 (24)</td>
<td>−52</td>
<td>−23.6*</td>
<td>−5.8</td>
</tr>
<tr>
<td>Richardson et al., 1993 (21)</td>
<td>−99</td>
<td>−41.9*</td>
<td>−9.1</td>
</tr>
<tr>
<td>Present study, 1999</td>
<td>−67</td>
<td>−27.0†</td>
<td>−6.0</td>
</tr>
</tbody>
</table>

\( Q_\text{peak} \): peak blood flow; \( V_\text{O}_2\text{peak} \): peak \( O_2 \) consumption. Absolute and knee extensor muscle mass-related peak average values for work rate, leg blood flow (\( Q_\text{peak} \)) and leg oxygen uptake (\( V_\text{O}_2\text{peak} \)) during knee extensor exercise obtained previously (2, 21, 24) and in the present study are shown. * and †: Muscle mass estimates performed with anthropometry and computer tomography, respectively.
35–45 cm were used, giving a spatial resolution in the range of 0.59–0.87 mm. In total, 21–25 serial segments, with a 10-mm thickness, were obtained every 20 mm along the thigh, starting distally from the patella and going in a proximal direction toward the spina iliaca anterior superior (SIAS). All segments taken of the first subject were analyzed by two experienced persons who were independent of the study. After comparison, verification, and agreement as to the definition of the different tissue structures, one of the analysts continued to analyze data from the remaining subjects.

The cross-sectional tissue areas, obtained from computer measurements on consecutive CT images along the thigh, are shown in Fig. 1. The figure emphasizes the fact that, because the muscle configuration markedly varies along its length, a series of frequent cross-sectional images has to be obtained to make true volume estimates. The tissue volume was calculated from the mean area of two neighboring sections multiplied by the section distance, summed over the full length of the tissue. Moreover, the muscle mass of the CT measurements were calculated by assuming a muscle tissue density of 1.049 kg/l (14).

The mean quadriceps muscle volume measured by CT was specifically compared with traditional anthropometric measurements (2, 8, 9). The anthropometric thigh volume (V) was calculated from the formula

\[ V = L \cdot \frac{(12\pi)^{-1} \cdot (O_1^2 + O_2^2 + O_3^2)}{(S - 0.4) \cdot 2^{-1} \cdot L \cdot (O_1 + O_2 + O_3) \cdot 3^{-1}} \]

where L is the estimated muscle length (see below); O_1, O_2, and O_3 are the circumferences 10 cm above the middle, at the middle, and 10 cm below the middle of the segment of the muscle length, respectively; and S is the correction for subcutaneous fat measured by skinfold callipers at each circumference (2, 8). The anthropometric quadriceps femoris muscle mass (M_d) was calculated as previously described, where \( M_d = 0.307 \cdot V \) (l) + 0.353 kg (2). Because the muscle length, for the volume and mass calculation, has been estimated previously with muscle insertion points from patella to either the pubic bone (PB), inguinal ligament (IL), or spina iliaca anterior superior (SIAS), all three lengths were measured and separate calculations were performed.

Statistics and data analysis. Parametric statistics were used for data analysis. Analysis of variance for repeated measures was used when more than two data groups were compared, and Tukey's honestly significant difference post hoc tests were used to distinguish the differences. Linear regression and Pearson's correlation were employed. P < 0.05 was considered as statistically significant. A nonstatistically significant comparison is indicated by P = NS. The values given in the text are means ± SE.

RESULTS

Estimates of muscle volume and mass. The CT quadriceps femoris muscle volume averaged 2.36 ± 0.17 (range 1.31–3.27) liters, which corresponded to a mass (M_d,CT) of 2.48 ± 0.18 (range 1.37–3.43) kg. The anthropometric muscle mass estimates overestimated (P < 0.02) the CT measurement by ~21% (M_d,PB, 3.00 ± 0.18 kg, range 2.60–3.99), 29% (M_d,IL, 3.21 ± 0.19 kg, range 2.15–4.20), and 46% (M_d,SIAS, 3.61 ± 0.22 kg, range 2.26–4.64), respectively (Fig. 2A). However, they all correlated with and were linearly related to each other (0.93 ≤ r ≤ 0.98, P < 0.001)

\[ M_d = M_d,CT \approx 0.924 \cdot M_d,PB - 0.292 \text{ kg} \]

\[ M_d = M_d,CT \approx 0.882 \cdot M_d,IL - 0.352 \text{ kg} \]

\[ M_d = M_d,CT \approx 0.792 \cdot M_d,SIAS - 0.382 \text{ kg} \]

Knee extensor exercise. Leg blood flow (Q_{peak,qf}) and leg oxygen uptake (V\dot{O}_{2,leg}) increased (P < 0.05) during submaximal exercise at 30 W from their resting baseline values of 0.24 ± 0.02 (range 0.17–0.35) l/min and 18.7 ± 2.6 (range 9.4–34.2) ml/min, respectively, to 3.57 ± 0.25 (range 2.69–5.01) l/min and 434 ± 24 (range 361–528) ml/min, respectively. A further increase (P < 0.05) was observed at peak effort (67 ± 7, range 55–100 W) to 5.99 ± 0.66 (range 4.15–9.52) l/min and 856 ± 109 (range 590–1,521) ml/min for Q_{peak,qf} and V\dot{O}_{2,leg}, respectively.

As a consequence of the different muscle mass estimates using the anthropometric procedure, peak muscle perfusion (Q_{peak,qf}) was underestimated compared with the CT scan measurement (Q_{peak,qf,CT}, 246.2 ± 24.2 ml·min⁻¹·100 g⁻¹, range 149.2–373.0) by ~19% (Q_{peak,qf,PB}, 199.5 ± 16.6 ml·min⁻¹·100 g⁻¹, range 136.6–2377).
Previous anthropometrically based estimates of peak muscle perfusion and oxygen uptake in humans, using various types of blood flow measurements, are underestimated by up to ~30% (2, 21, 24). In light of the fact that the initial thermodilution blood flow measurements were criticized for being too high, as peak muscle perfusion and oxygen uptake were studied in a single active muscle group (2), these findings are of major importance because they emphasize that the peak values are even higher than previously found. As the magnitude of the thermodilution measurements also have been verified by ultrasound Doppler and indocyanine green dye infusion (2, 10, 16, 26), the low peak values found by 133Xe clearance and plethysmography seem to be even less believable.

In this study multiple CT image scans were taken along the full length of the quadriceps muscle (Fig. 1) of subjects with a large variation in the length (L) of the quadriceps muscle (LCT 42.4 ± 0.73 cm, range 38–46) and its points of insertion. Furthermore, the large variation in muscle length with the three different anthropometric approaches (LPB 38.5 ± 0.62 cm, range 36–41; LIL 41.0 ± 0.54 cm, range 39–43.5; LSIAS 45.3 ± 0.65 cm, range 40.5–47) induces a great source of error in the muscle volume and mass calculations. Moreover, the configuration of the muscle varies markedly along its length (Fig. 1). Thus only three circumference measurements or CT image scans along the thigh may not give an accurate estimate of muscle volume (2, 8, 27). In addition, the mathematical formulas used to estimate the muscle volume on the basis of anthropometry do not account for the variation in muscle configuration, because they are based on the fact that the muscle is constructed from two symmetrical “cones” that narrow toward the ends of the muscle. More importantly, the anthropometric formulas do not account for either the relative area that the quadriceps muscle group occupies in the thigh region or the fact that the quadriceps muscle volume may vary. In the present study, this variation in quadriceps muscle volume ranged from ~1.31 to 3.27 liters. The present study thus provides a set of formulas that relate the anthropometric estimates to the CT measurements, which potentially can be used to correct for the anthropometric overestimations. However, one should be careful with such a generalization because the formulas are based on results from nine subjects only and the relationship is likely to be affected by different types and levels of activity, inactivity, gender, and age. If anthropometry is used to estimate muscle mass, muscle length estimated from the patella to the PB induces the smallest error in the muscle volume and mass estimate.

Besides providing a relationship between anthropometric and CT estimates of muscle volume, knowing the number of CT or magnetic resonance image (MRI) sections that need to be performed is also of interest to allow a reasonable measure of accuracy. In the present data, the maximum width of the quadriceps muscle was located approximately one-half the distance between the greater trochanter and the top of patella. Thus, if...
muscle volume is estimated by using an area from the section of maximum width and from two other sections, imaged at one-half the distance between the maximum width and the greater trochanter, respectively, the three sections would give a muscle volume of ~2.20 liters, i.e., an underestimation of ~7%. If an estimate of the missing muscle volume proximally and distally, respectively, to the two outer sections is added, assuming a decrease in muscle size toward its end points, as in the case of two narrowing cones, then the muscle volume would be ~2.64 liters, i.e., an overestimation of ~12%. If the estimate is based on five sections, the first section halfway between the greater trochanter and patella and then four additional sections measured every 8 cm, i.e., two on each side of the first section, then the muscle volume would be ~2.37 liters, i.e., an overestimation of ~0.5%. By adding the missing outer cone-shaped muscle volumes, the volume would be ~2.46 liters, i.e., an overestimation of ~4.2%. Similarly, 13 sections taken every 4 cm, starting from the same midpoint between the greater trochanter and patella, would render an overestimation of only ~0.4%. Thus, to carefully describe muscle volume, as many imaged sections as are in the range of every 2–4 cm are needed, because the shape of the muscle varies individually along its full length. However, five sections may give a reasonable estimate, but one should be aware that our sample of subjects is fairly small.

There is ample evidence for the whole quadriceps muscle to be engaged in very intense knee extensor exercise (11, 15, 18). However, at submaximal efforts the recruitment pattern has been less clear. Electromyography tracings from the various portions of the quadriceps muscle suggest a gradual and equal involvement. Ray and Dudley’s (15) recent findings using T2-weighted MRI scans support this notion but more importantly add a value for the muscle mass being recruited at different intensities. On the basis of their findings, with a muscle volume “engagement” of ~16% at rest, ~54% at 20 W, and ~94% at 40 W, and assuming ~100% at peak effort, a linear relationship can be expressed as the recruited muscle volume (% of total muscle volume) = 25.187 + 1.285·load (W). Interpolation gives the notion that in the present study ~64% of the muscle volume could have been engaged at 30 W, rendering values for muscle perfusion and oxygen uptake of ~235 ± 24 (range 154–383) and 28 ± 2.3 (range 21–41) ml·min⁻¹·100 g⁻¹, respectively, on the basis of blood flow and oxygen uptake in the present study (Fig. 3). This can be compared with ~151 ± 15 (range 99–245) and 18 ± 1.4 (range 14–26) ml·min⁻¹·100 g⁻¹, respectively, values that are found if, as in the past (2), blood flow and oxygen uptake in the present study are related to the whole quadriceps muscle mass. Thus, as pointed out by Ray and Dudley, peak muscle perfusion may be achieved in certain regions at already submaximal intensities. However, muscle oxygen uptake appears to be further elevated at peak effort, possibly due to a larger oxygen extraction (Fig. 3). A tentative explanation for higher oxygen uptake per unit of muscle at increasing work intensities despite recruitment of more muscle to perform the work could be that first the frequency of activation is increased and then energy turnover.

In conclusion, anthropometric overestimates of muscle volume in the past have markedly underestimated peak muscle perfusion and oxygen uptake. Thus peak perfusion and oxygen uptake in human skeletal muscle may be up to ~30% higher than previous estimates on the basis of blood flow measurements with techniques equivalent to thermodilution (2, 16, 19–22, 26), making values obtained by ¹³³Xe clearance and plethysmography even less believable. Moreover, to obtain an estimate of muscle mass recruited when muscle perfusion and muscle oxygen uptake are estimated at intensities lower than at peak effort, T2-weighted MRI is a necessity, in addition to either CT or MRI muscle volume determinations.

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