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In the lungs, the concept of matching is well established, not as a mechanism for equitably supplying all regions of the lung with O2, but rather as necessary for efficient transfer of gases between the blood and the environment. Here it is how ventilation (VA) is distributed in relation to blood flow (Q) that is the important concept: when VA and Q are distributed so that their ratio (VA/Q) is everywhere similar, gas exchange is maximized (29). Correspondingly, in the muscle, it is how Q is distributed in relation to regional metabolic rate [O2 consumption (VO2)] that is important to overall muscle function (20). If the regional ratios of metabolic rate to muscle Q (VO2/Q) are similar, all regions will be optimally supplied with O2. However, if some regions are substantially underperfused in relation to their metabolic need, muscle function may suffer, whereas, if other regions are overperfused in relation to metabolic need, Q is “wasted.” While in the lungs, many methods exist for quantifying how VA and Q are distributed (24, 51, 61, 67); this is not the case in the (working) muscles (41, 66).

Positron emission tomography (PET) is an noninvasive imaging method that can be applied to measure Q and its distribution in muscle as well as muscle VO2 and O2 extraction, enabling the specific determination of local muscle O2 uptake (35, 38–40). PET has been used to evaluate heterogeneity of skeletal muscle perfusion and metabolism, either during small muscle mass exercise sustained at relatively low exercise intensity, such as one-legged knee extensor exercise (38–40), or several minutes following one-legged (50) or two-legged high-intensity cycling exercise (34). These studies (34, 38–40, 43, 50) suggested that, while at rest as well as during recovery from exercise there was some systematic regional variation in VO2, perfusion remained closely matched to metabolic demand, thereby indicating very little functional heterogeneity of muscle perfusion and metabolism. Furthermore, Heseler et al. (31) and Richardson et al. (56) developed a method using magnetic resonance spectroscopy to study metabolism/perfusion heterogeneity in exercising human calf muscle. However, while feasible during contractions, regional VO2 was inferred from 31P signals reflecting ATP breakdown and was not able to be directly measured. In addition, magnetic resonance spectroscopy does not allow simultaneous investigation of local muscle Q and metabolism.

Near-infrared spectroscopy (NIRS) offers a rapidly responsive and noninvasive method for measuring O2-related indexes in skeletal muscle and can be used during exercise (3). NIRS signals reflect the summed contributions of O2 levels in the
arterial, capillary, and venous blood of the muscle, as well as the myocyte interior, because there is a contribution from myoglobin and possibly also cytochromes (2, 5, 16).

Since all regions of a muscle receive arterial blood with the same oxygenation, regional differences in NIRS-based O2 signals must then reflect differences in venous (hemoglobin-related) and intracellular (myoglobin/cytochrome-related) O2 levels and might, therefore, be usable to empirically estimate regional venous O2 levels. Indeed, there have been studies investigating whether NIRS measurements of tissue O2 saturation (StO2) reliably reflect O2 saturation measured in forearm vein (46) or femoral vein during exercise (14, 45). Because a region of muscle with high VO2/Q must extract a higher percentage of O2 from the blood than a region with low VO2/Q, local venous O2 levels would be lower when VO2/Q is high. In this way, local NIRS O2-related signals might be used to estimate local VO2/Q.

Indocyanine green (ICG) dye has, for many years, been used as an indicator in the measurement of cardiac output and plasma volume (6, 49). Fortunately, ICG is detectable by NIRS (52) and has been used in this way to measure local Q in respiratory and locomotor muscles (27, 28). If NIRS is used to gather ICG-based Q signals and at the same time also O2 signals reflecting VO2/Q, those can be multiplied to yield an estimate of regional VO2, because Q cancels in this multiplication. In this way, NIRS becomes a tool to measure regional VO2, regional Q (using ICG), and their ratio, VO2/Q.

The aim of the present study was to first develop this approach and then utilize it to investigate heterogeneity of VO2 to Q in normal human skeletal muscle. We applied NIRS using six optodes taped to the skin over the upper, middle, and lower vastus lateralis in each of six trained cyclists. While sampling the muscle at just six locations is recognized as likely not capturing the full extent of regional function, even this required three separate near-infrared (NIR) two-channel spectrophotometers. We thus view the current outcome as addressing proof of concept and anticipate studies with many more optodes going forward.

Measurements were made at rest and during constant load exercise at various intensities in both normoxia and acute hypoxia. Varying both exercise intensity and inspired O2 fraction (FIO2) was done primarily to provide a wide range of femoral venous O2 saturation (SfvO2) and regional NIRS O2 saturation to determine the relationship between SFvO2 and the NIRS O2 saturation signals summed over the regions sampled. This relationship is the key to converting regional NIRS O2 saturation into the corresponding regional muscle venous O2 saturation (SvO2), as explained below in detail.

**METHODS**

**Theoretical Basis of the Estimation of the Regional VO2/Q, Q, and VO2**

**Estimation of the regional ratio of VO2 to Q.** For the estimation of the regional VO2/Q, the Fick principle of mass conservation is applied as follows:

\[
\frac{\text{VO}_{2}}{\text{Q}} = \frac{\text{CaO}_{2} - \text{CvO}_{2}}{\text{StO}_{2} - \text{SvO}_{2}}
\]  

Here, VO2 is the local regional VO2, Q is the local Q rate, CaO2 is the inflowing arterial O2 concentration, and CvO2 is the outflowing regional venous O2 concentration. Dividing by Q, the regional VO2/Q is calculated:

\[
\frac{\text{VO}_{2}}{\text{Q}} = \frac{\text{CaO}_{2} - \text{CvO}_{2}}{\text{Q}} = 0.000139 \times [\text{Hb}] \times (\text{SaO}_{2} - \text{SvO}_{2})
\]

where [Hb] is hemoglobin concentration in g/dl; SaO2 and SvO2 are arterial and regional muscle venous blood O2 saturations, respectively, expressed as percent. VO2 and Q are expressed in identical units (such as ml/min, l/min or ml/min-1·100 g-1). Both [Hb] and SaO2 are easily and directly measured, while regional SvO2 is estimated from local NIRS O2 saturation as follows.

To infer regional SvO2 from the regional NIRS O2 signal, we used the relationship between directly measured SFvO2 and the Q-weighted average NIRs O2 saturation over the six optodes. We established this relationship in each subject by simultaneously measuring SFvO2 and NIRS O2 saturations at rest and during exercise in normoxia and hypoxia, which provided a wide range of saturation values. As will be shown, that relationship was found to be linear in all subjects, and, from the regression line for that relationship in any subject, regional SvO2 was computed from regional NIRS O2 saturation signals of that subject and used in Eq. 2 to determine the regional VO2/Q underlying each of the six optodes.

**Estimation of regional flow (Q).** Q under each optode was determined from the rate of tissue ICG accumulation measured by NIRS, as calculated according to the Sapirstein principle (58), as previously described by Boushel et al. (4). Accordingly, after a rapid intravenous bolus injection of ICG, the rate of appearance of ICG in a region of muscle depends on Q. ICG can be detected by the same NIR spectrophotometer as used for regional muscle O2 saturation measurements, as mentioned above. We measured by NIRS the appearance rate of ICG under each of the six optodes after a bolus ICG injection to estimate regional Q:

\[
Q(\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) = \frac{k \cdot \text{[ICG]}}{\text{t}}
\]

\[
= \int_{0}^{t} \frac{\text{[ICG]}_{m} \cdot \text{dr}}{\text{f}}
\]

where \(k\) includes the molecular weight of ICG for the conversion of ICG in moles to grams per liter and the estimated muscle density for conversion of tissue volume to tissue weight; \([\text{ICG}]_{m}\) is the accumulation of ICG in tissue over time \(t\) expressed in micromoles per liter; and \(f\) is the time integral of the arterial ICG concentration expressed in milligrams per liter. To measure the quantity described by the denominator, we also recorded the arterial ICG concentration during constant withdrawal from the radial artery after injection. Experimental details are provided later in this paper.

**Estimation of regional VO2.** Regional VO2 was calculated as the product of regional VO2/Q (Eq. 2) and the corresponding value of regional Q from NIRS-based ICG appearance rate (Eq. 3) as follows:

\[
\text{VO}_{2} = \frac{\text{VO}_{2}}{\text{Q}} \times \dot{Q}
\]

**Experimental Methods**

**Subjects.** Six healthy trained male subjects (Table 1) participated in the study, which was approved by Athens University Evangelismos Hospital Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. Before participation in the study, all subjects were informed of any risks and discomfort associated with the experiments and gave written, signed, informed consent.

**Overview.** Experiments were conducted in two visits. In visit 1, subjects underwent a preliminary incremental exercise test to their highest tolerable workload [maximal work rate (WRmax)] (Table 1). In visit 2, after resting measurements, subjects completed, in a balanced-order sequence, two graded exercise tests (separated by 120 min of rest) breathing room air in one test and O2 with low FIO2 of 0.12 in the other; exercise at each FIO2 was sustained for 5 min at each of four
VTmax, l/min 3.4

V\dot{\text{O}}_2\text{max}, \text{ml/kg } 40\%, \text{ and } 60\% \text{ of normoxic WRmax when breathing room 12\% O}_2. With the resting measurements at each FIO2, this protocol, therefore, 

Max; Marquette Hellige) and a pulse oximeter (Nonin 8600; Nonin Products, Milwaukee, WI). An 8-MHz linear array was used while assessed by Ultrasound imaging (LOGIQ Book XP; GE Healthcare 

fat thickness of the upper, middle, and lower vastus lateralis was 

catheters were kept patent by periodic flushing with heparinized (1 

U/ml) saline. 

catheters were used to collect arterial and femoral venous blood samples for blood-gas analysis and also for ICG dye injection (femoral vein) and continuous ICG measurement in blood after each ICG injection (radial artery). The 

rest period, followed by 3 min of pedaling at 20 –30 W to warm up. Tests were preceded by a 3-min 

work rates, corresponding to unloaded pedaling, and then 20\%, 50\%, and 80\% of normoxic WRmax (when breathing room air) and 15\%, 40\%, and 60\% of normoxic WRmax when breathing room 12\% O}_2. With the resting measurements at each FIO2, this protocol, therefore, produced 10 measurement conditions in each of the 6 subjects. 

 Preliminary testing (visit 1). The incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergo- line 800; Sensor Medic, Anaheim, CA) starting at 30 W and increasing by 30 W every minute, with the subjects maintaining a pedaling frequency of 70–90 rpm. BMI, body mass index; WRmax, maximal work rate; V\dot{\text{O}}_{2\text{max}}, maximal oxygen uptake; HRmax, maximal 

heart rate; Vi\text{t max}, maximal minute ventilation; V\text{t max}, maximal tidal volume; \text{f max}, maximal breathing frequency. 

work rates, corresponding to unloaded pedaling, and then 20\%, 50\%, and 80\% of normoxic WRmax (when breathing room air) and 15\%, 40\%, and 60\% of normoxic WRmax when breathing room 12\% O}_2. With the resting measurements at each FIO2, this protocol, therefore, produced 10 measurement conditions in each of the 6 subjects. 

 Preliminary testing (visit 1). The incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergoline 800; Sensor Medic, Anaheim, CA) starting at 30 W and increasing by 30 W every minute to exhaustion, with the subjects maintaining a pedaling frequency of 70–90 rpm. Tests were preceded by a 3-min rest period, followed by 3 min of pedaling at 20–30 W to warm up. Heart rate and percentage O\text{2} saturation were determined using the R-R interval from a 12-lead online electrocardiogram (Marquette Max; Marquette Hellige) and a pulse oximeter (Nonin 8600; Nonin Medical, North Plymouth, MN), respectively. 

 Subject preparation (visit 2). Subjects were prepared with arterial and femoral venous catheters. Using local anesthesia (2\% lidocaine) and sterile technique, catheters were introduced percutaneously into the left femoral vein (central venous catheter model AK-04301, Arrow International, Durham, NC) and the right radial artery (Angiocath 20 gauge, 1.16 in., model 381134, Becton Dickinson), both oriented in the proximal direction. The catheters were used to collect arterial and femoral venous blood samples for blood-gas analysis and also for ICG dye injection (femoral vein) and continuous ICG measurement in blood after each ICG injection (radial artery). The 

catheters were kept patent by periodic flushing with heparinized (1 

U/ml) saline. 

Within 1 h after termination of the exercise protocol, subcutaneous fat thickness of the upper, middle, and lower vastus lateralis was assessed by Ultrasound imaging (LOGIQ Book XP; GE Healthcare Products, Milwaukee, WI). An 8-MHz linear array was used while capturing the image in B-mode. Ultrasound gel was applied to the center of the template before the transducer was placed on the skin. The skin was marked after removal of the optodes to verify the correct placement of the transducer. After a clear image was identified, the image was saved. This procedure was repeated three times for each optode location to calculate a mean value. The images were labeled with the participant’s number and optode location of measurement. 

 Regional NIRS O\text{2} saturation. Three pairs of NIRS optodes were placed on the skin over the upper, middle, and lower vastus lateralis and secured by double-sided adhesive tape (Fig. 1) to measure muscle Q and oxygenated Hb/Mb signals using a spectrophotometer (NIR 200 spectrophotometer, Hamamatsu Photonics, Hamamatsu, Japan). The light emission and collection points in each optode were 4 cm apart, corresponding to a penetration depth of 2 cm. Each pair of optodes was connected to a different NIR 200 spectrophotometer, because these devices have only two channels each, thus necessitating three separate spectrophotometers. Preliminary measurements were, therefore, made in two subjects to determine whether light emitted from the optodes of one spectrophotometer interfered with the signals recorded by the adjacent optodes of the other spectrophotometers and whether the different optodes and different spectrophotometers gave similar results from the same physical region over the muscle. This was done by 1) disconnecting (one at a time) input cables from each optode pair to its spectrophotometer and observing whether signals on the other two spectrophotometers changed; 2) switching the optode output/input between the two channels within each spectrophotometer as well as among the three spectrophotometers; and, separately, by 3) interchanging optodes over each position on the leg. To compare changes due to these manipulations with spontaneous time-dependent alterations, recordings were also performed for 20 min without any manipulations of optodes or optode output/input. 

Because muscle oxygenation was assessed by the same spectrophotometer as used for the measurement of muscle Q by ICG (see below) and because high ICG tissue concentrations during the passage of the dye bolus through the muscle may interfere with Hb signals, oxygenation data were averaged over 10 s immediately before ICG injection. The variables assessed by NIRS were the concentration changes of oxygenated, deoxygenated, and total Hb. A commonly derived parameter from NIRS studies in humans is the ratio of oxygenated Hb to total Hb, an absolute 

index of O\text{2} saturation (StiO2) (12, 18). Studies in animals, as well as among the three spectrophotometers; and, separately, by 3) interchanging optodes over each position on the leg. To compare changes due to these manipulations with spontaneous time-dependent alterations, recordings were also performed for 20 min without any manipulations of optodes or optode output/input. 

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index of O\text{2} saturation (StiO2) (12, 18). 

Regional muscle Q. To measure local Q, 0.9–1.0 ml of ICG (5 mg/ml, Pulsion ICG, ViCare Medical) was injected into the femoral vein catheter, followed by a rapid 10-ml flush of isotonic saline. As previously described (4), the ICG bolus circulates to the right heart and the lungs and enters the arterial circulation to reach the skeletal muscles. 

Radial arterial blood, withdrawn at a constant rate of ∼20 ml/min using an automated pump (Harvard Apparatus), was passed through sterile, in-line spectrophotometric cuvettes [Electronics for Medicine (E for M) model: DCCO-04 SNs: 7553-1343] connected to a Q computer (Waters CO-10, Rochester, MN) to record arterial ICG levels continuously using a data acquisition system at 20 Hz (DI-720, Dataq). This information is necessary for computation of the denominator in Eq. 4. The blood was reinfused into the femoral vein immediately on completion of the measurements. ICG calibration and

### Table 1. Anthropometrics, pulmonary function, and maximal exercise data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 8</td>
<td>21–32</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 5</td>
<td>174–183</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78 ± 4</td>
<td>76–82</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.6 ± 1.6</td>
<td>23.2–25.7</td>
</tr>
<tr>
<td>Body fat mass, %</td>
<td>18.7 ± 4.2</td>
<td>15.2–25.0</td>
</tr>
<tr>
<td>Lean body mass, %</td>
<td>81.3 ± 4.4</td>
<td>78–85</td>
</tr>
<tr>
<td>WR\text{max}, W</td>
<td>258 ± 59</td>
<td>203–303</td>
</tr>
<tr>
<td>\text{V\dot{O}}_{2\text{max}}, ml·kg\text{−1·min}\text{−1}</td>
<td>44.7 ± 3.8</td>
<td>42–47</td>
</tr>
<tr>
<td>HR\text{max}, beats/min</td>
<td>195 ± 12</td>
<td>184–206</td>
</tr>
<tr>
<td>\text{Vi}t\text{max}, l/min</td>
<td>125 ± 19</td>
<td>111–138</td>
</tr>
<tr>
<td>\text{Vt}\text{max}, l/min</td>
<td>3.4 ± 0.3</td>
<td>3.1–3.4</td>
</tr>
<tr>
<td>\text{f}\text{max}, breaths/min</td>
<td>38 ± 6</td>
<td>34–45</td>
</tr>
</tbody>
</table>

\( n = 6 \) Subjects. Exercise data depict the results of incremental exercise test starting at 30 W and increasing by 30 W every minute, with the subjects maintaining a pedaling frequency of 70–90 rpm. BMI, body mass index; WR\text{max}, maximal work rate; \text{V\dot{O}}_{2\text{max}}, maximal oxygen uptake; HR\text{max}, maximal heart rate; \text{Vi}t\text{max}, maximal minute ventilation; \text{Vt}\text{max}, maximal tidal volume; \text{f}\text{max}, maximal breathing frequency.

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Fig. 1. Positioning of six optodes over the right vastus lateralis.

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linearity were established in each subject at the end of the study by measuring the voltage deflection from four 20-ml blood samples containing various concentrations of ICG, as previously described (4).

Tissue microcirculatory ICG following the same injection was detected transcutaneously by the NIRO 200, measuring light attenuation at 775, 813, 850, and 913 nm, and was analyzed using an algorithm incorporating the modified Beer-Lambert law (4, 21, 59), as previously described (27). Since the measured light attenuation in the tissue is influenced by ICG as well as oxy- and deoxyhemoglobin and myoglobin concentration, the contribution of ICG to the light absorption signal was determined using dedicated NIRS software (N200ICG MFC Application). Figure 2 shows the simultaneous ICG appearance curves for two separate optode locations. It is apparent that the rate and degree of increase in ICG is considerably greater for the upper channel, indicating greater Q under the corresponding optode.

Blood-gas analysis and calculations. Arterial and venous tensions of O$_2$ and CO$_2$, Hb concentration, and %SaO$_2$ and %SvO$_2$ were measured from arterial and femoral venous blood samples by electrodes and CO-oximetry (ABL 625, Radiometer, Copenhagen, Denmark). CaO$_2$ and CaO$_2$ were computed as follows: $C_{O_2} = (1.39 \times Hb \times S_{O_2}) + (0.003 \times P_{O_2})$. Here the symbol $x$ denotes either arterial (a) or femoral venous (v) blood. The blood-gas analyzer was auto-calibrated every 4 h throughout the day, and calibrating gases of known concentrations were run before each set of measurements. Blood-gas measurements were corrected for subject’s tympanic temperature taken during withdrawal of each arterial blood-gas sample.

Statistical analysis. Data are reported as means ± SD, unless otherwise stated. Linear regression was performed to determine the relationship between SfvO$_2$ and StiO$_2$ in each of the six subjects. As an index of regional VO$_2$/Q heterogeneity, we calculated the coefficient of variation [also termed relative dispersion (RD)] of the three components, VO$_2$, Q, and VO$_2$/Q, individually over the six optodes for each condition in each subject. RD of StiO$_2$ was also computed. Two-way ANOVA with repeated measures was applied to detect differences 1) across the various workloads between normoxia and hypoxia for all four of these dispersion indexes; and 2) across the upper, middle, and lower vastus lateralis between the medial and lateral probe location for the assessment of subcutaneous adipose thickness. The level of significance was set at $P < 0.05$.

RESULTS

Between-Optode and Between-Spectrophotometer Variation in NIRS-Based O$_2$ Saturation

There was no interference among optodes connected to different spectrophotometers: disconnecting the emitter cables was associated with mean (interquartile range) change in StiO$_2$ by 10.220.32.246 on July 11, 2017 http://jap.physiology.org/ Downloaded from

![Fig. 2. Screen shot of the output of one two-channel near-infrared (NIR) spectrophotometer, showing the indocyanine green (ICG) dye signal (yellow) following intravenous ICG injection marked by the purple arrow. Note the different scales for ICG between the two channels, and also the upslope differences, indicating greater perfusion under the optode of channel 1. Green trace [tissue oxygenation index (TOI)] is oxygenation signal. Bottom portion overlays the flow signals from the two channels to highlight their differences. Q, blood flow; O$_2$Hb, oxyhemoglobin; cHb, total hemoglobin; HHb, deoxyhemoglobin.](http://jap.physiology.org/doi/10.1152/japplphysiol.00458.2014)
of only 0.1% (−0.5 to 0). Likewise, switching optode output/input between channels of each spectrophotometer produced a mean change in \( \text{StiO}_2 \) of 0.8% (1.6 to 0.1). Switching optode output/input among spectrophotometers resulted in a mean change in \( \text{StiO}_2 \) of 0% (1.3 to 1.2). Interchanging optodes over each position on the leg produced a mean change in \( \text{StiO}_2 \) of 0.1% (3.2 to 3.8). This compares favorably to the spontaneous temporal fluctuations of \( \text{StiO}_2 \) within 20 min of 4.3% (2.3 to 6.8). There were not any significant differences in subcutaneous adipose tissue thickness between the medial and lateral probe locations across the upper, lower, and middle vastus lateralis (Table 2).

### Relationship Between Femoral Vein \( O_2 \) Saturation and NIRS-Based Muscle \( O_2 \) Saturation

Varying both exercise intensity and \( F_{\text{IO}_2} \) provided \( \text{SfvO}_2 \) values ranging from ~10% to ~70%, and a correspondingly wide range in \( \text{StiO}_2 \). Data for all subjects are shown in Fig. 3, where, on the abscissa, the \( \text{StiO}_2 \) value is the \( Q_\dot{\text{O}} \)-weighted average of \( \text{StiO}_2 \) values from the six optodes. Similarly linear relationships were found between \( \text{SfvO}_2 \) and mean \( Q_\dot{\text{O}} \)-weighted \( \text{StiO}_2 \) in each subject (Fig. 3). The range of slopes of the regression lines across the six subjects was between 1.02 and 2.21. When all data were combined (Fig. 3, right), the similarity remains apparent. We used the empirical relationship individually for each subject to compute local muscle \( \text{SvO}_2 \) from \( \text{StiO}_2 \) recorded at each optode, from which local \( \frac{\dot{\text{V}}_\text{O}_2}{\dot{\text{Q}}_\text{O}_2} \) ratios were calculated by the Fick principle, as outlined in METHODS. Multiplying regional \( \frac{\dot{\text{V}}_\text{O}_2}{\dot{\text{Q}}_\text{O}_2} \) by \( \dot{\text{Q}}_\text{O}_2 \) yielded the corresponding regional \( \dot{\text{V}}_\text{O}_2 \).

### Distribution of \( \dot{\text{V}}_\text{O}_2, \dot{\text{Q}}, \) and \( \frac{\dot{\text{V}}_\text{O}_2}{\dot{\text{Q}}_\text{O}_2} \)

Figure 4 shows the RD values for \( \dot{\text{Q}}, \dot{\text{V}}_\text{O}_2, \) and \( \frac{\dot{\text{V}}_\text{O}_2}{\dot{\text{Q}}_\text{O}_2} \) under one condition for one subject: at rest, breathing air. The two panels show the values of \( \dot{\text{V}}_\text{O}_2 \) and \( \dot{\text{Q}} \) under each optode for these conditions plotted at their corresponding \( \frac{\dot{\text{V}}_\text{O}_2}{\dot{\text{Q}}_\text{O}_2} \). Two scales

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**Table 2. Subcutaneous adipose tissue thickness**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Upper Vastus Lateralis</th>
<th>Middle Vastus Lateralis</th>
<th>Lower Vastus Lateralis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>B1</td>
</tr>
<tr>
<td>1</td>
<td>4.30</td>
<td>4.80</td>
<td>3.70</td>
</tr>
<tr>
<td>2</td>
<td>5.20</td>
<td>5.10</td>
<td>5.20</td>
</tr>
<tr>
<td>3</td>
<td>5.70</td>
<td>4.70</td>
<td>4.70</td>
</tr>
<tr>
<td>4</td>
<td>6.70</td>
<td>6.00</td>
<td>7.50</td>
</tr>
<tr>
<td>5</td>
<td>5.00</td>
<td>5.20</td>
<td>5.10</td>
</tr>
<tr>
<td>6</td>
<td>5.00</td>
<td>4.90</td>
<td>5.80</td>
</tr>
<tr>
<td>Mean</td>
<td>5.32</td>
<td>5.12</td>
<td>5.33</td>
</tr>
<tr>
<td>SD</td>
<td>0.81</td>
<td>0.47</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Values are means ± SD in mm; \( n = 6 \) subjects. Values were recorded from the upper, middle, and lower vastus lateralis on lateral (A1, B1, C1) and medial (A2, B2, C2) sites.

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**Fig. 3.** Linear relationships between measured femoral venous \( O_2 \) saturation (\( \text{SfvO}_2 \)) and \( Q_\dot{\text{O}} \)-weighted average NIRS spectroscopy \( O_2 \) saturation over the six optodes for all subjects individually and then superimposed (panel on the right). \( \text{StiO}_2 \), tissue \( O_2 \) saturation.

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**Innovative Methodology**

Muscle \( \dot{\text{V}}_\text{O}_2/\dot{\text{Q}}_\text{O}_2 \) Heterogeneity in Exercising Healthy Humans • Vogiatzis I et al. • *J Appl Physiol* • doi:10.1152/japplphysiol.00458.2014 • www.jappl.org
are employed for the same data. On the left, the scale is expanded to show the very narrow range of $V\dot{O}_2/Q\dot{O}$ from 0.08 to 0.12, whereas the right panel replots the data on a scale corresponding to that used in normal depiction of the $V\dot{A}/Q\dot{A}$ distribution in the lung, ranging from 0.3 to 3.0 (67).

Absolute mean data for regional muscle $V\dot{O}_2$, $Q\dot{O}$, $V\dot{O}_2/Q\dot{O}$, fractional $O_2$ extraction (calculated by dividing regional $V\dot{O}_2/Q\dot{O}$ by $CaO_2$ and multiplying the result by 100), and $StiO_2$ for each subject across all exercise intensities in normoxia and hypoxia are displayed for all six probe positions in Table 3. $V\dot{O}_2$, $Q\dot{O}$, and fractional $O_2$ extraction increased with increasing exercise intensity, whereas $StiO_2$ progressively decreased. $Q\dot{O}$ and fractional $O_2$ extraction were greater in hypoxia, whereas $V\dot{O}_2$ was not different compared with normoxia. $StiO_2$ was lower in hypoxia. Furthermore, it is apparent that $Q\dot{O}$ and $V\dot{O}_2$ were lower in the upper compared with the lower part of vastus lateralis, whereas deoxygenation and fractional $O_2$ extraction were greater in the lower part of vastus lateralis.

Table 3. Effect of exercise intensity, hypoxia, and site of measurement on vastus lateralis

<table>
<thead>
<tr>
<th>Probes</th>
<th>Rest</th>
<th>Unload</th>
<th>20%</th>
<th>50%</th>
<th>80%</th>
<th>Rest</th>
<th>Unload</th>
<th>20%</th>
<th>50%</th>
<th>80%</th>
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<td></td>
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</tr>
<tr>
<td>A1</td>
<td>0.35</td>
<td>0.62</td>
<td>0.97</td>
<td>2.24</td>
<td>2.40</td>
<td>0.24</td>
<td>0.49</td>
<td>0.95</td>
<td>2.13</td>
<td>2.81</td>
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<tr>
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<td>0.63</td>
<td>0.85</td>
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<td>1.66</td>
<td>0.23</td>
<td>0.45</td>
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<td></td>
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</tr>
<tr>
<td>B1</td>
<td>0.26</td>
<td>0.66</td>
<td>1.92</td>
<td>4.19</td>
<td>4.11</td>
<td>0.21</td>
<td>0.47</td>
<td>1.66</td>
<td>5.44</td>
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<tr>
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<td>0.47</td>
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<tr>
<td>Lower</td>
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<td></td>
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<tr>
<td>C1</td>
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<td>0.37</td>
<td>1.13</td>
<td>3.32</td>
<td>3.40</td>
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</table>

Innovative Methodology

Fig. 4. $O_2$ consumption ($V\dot{O}_2$), $Q\dot{O}$, and $V\dot{O}_2$-to-$Q\dot{O}$ ratio ($V\dot{O}_2/Q\dot{O}$) under each of the six optodes for subject 1 at rest breathing room air. Left: despite threefold variation in $Q\dot{O}$, all $V\dot{O}_2/Q\dot{O}$ values lie within a narrow range. Right: same data as left but on the broader scale used for $V\dot{A}/Q\dot{A}$ inequality in the lung to underscore presence of minimal heterogeneity. The abscissa range in normal lung is typically from 0.3 to 3.0; that in muscle is here only 0.08 to 0.12.
Innovative Methodology

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DISCUSSION

Brief Overall Summary of Main Findings

This study proposes that NIRS can be used to assess the distribution of metabolism (\( \dot{V}O_2 \)) with respect to perfusion (\( Q \)) regionally within muscle. It does so with a proof-of-concept approach that examines these variables and their ratio, \( \dot{V}O_2/Q \), in just six regions of the knee extensors, fully realizing that many more positions should be sampled for a more complete picture of \( \dot{V}O_2/Q \) heterogeneity. All interpretations to follow carry this caveat.

The principal findings are as follows: 1) that regional \( StiO_2 \), the NIRS-derived oxygenation index from the six optodes placed over the muscle, when averaged, relates linearly to \( S_fV_O_2 \) across a wide range and thus may be used empirically to estimate the corresponding regional muscle \( S_vO_2 \) under each optode; 2) that using the Fick principle, muscle \( S_vO_2 \) can in turn be used to compute the local ratio of \( \dot{V}O_2/Q \) via Eqs. 1 and 2; 3) using ICG dye, which is also seen by NIRS, muscle \( Q \) under each optode can be measured, and, by multiplying \( \dot{V}O_2/Q \) by \( Q \), \( \dot{V}O_2 \) also becomes available. Armed with the values of these three variables across all sampled sites, their RD can be calculated. In the athletes studied, RD for \( \dot{V}O_2 \) and for \( Q \) were similar and \( \sim 0.4 \), while that for their ratio, \( \dot{V}O_2/Q \), was one-fourth of that, i.e., \( \sim 0.1 \). This represents a very small amount of heterogeneity. The RD values for \( \dot{V}O_2/Q \) were similar at rest and all levels of exercise studied up to near maximal and were unaffected by breathing 12% \( O_2 \).

Comparisons with Other Approaches to Muscle Heterogeneity

The study of matching local muscle \( Q \) to \( O_2 \) uptake in humans during exercise has been challenging (41). Attempts to simultaneously measure local muscle \( Q \) and metabolism have used PET during small-muscle mass exercise, such as one-legged static knee extension or one-legged or two-legged isometric or cycling exercise (34, 36, 38 – 40, 43, 60). The findings of the present study, indicating an average local muscle \( \dot{V}O_2/Q \) of \( \sim 0.10 \), are in agreement with the \( \dot{V}O_2/Q \) ratios obtained from the studies by Kalliokoski and colleagues (38 – 40), reporting a range of 0.05 to 0.08 during one-legged intermittent or continuous isometric exercise. Heinonen et al. (35) used PET imaging based on radiolabeled water and \( O_2 \) to measure muscle \( Q \) and \( \dot{V}O_2 \) during single-leg kick exercise. They examined heterogeneity of \( Q \), but not that of \( \dot{V}O_2 \) or their ratio, \( \dot{V}O_2/Q \). Heterogeneity in \( Q \) was observed, with a RD of between 0.4 and 0.5, just as found in the present study (Fig. 5). However, without simultaneous evaluation of \( \dot{V}O_2 \) heterogeneity, it was not possible for Heinonen et al. (35) to answer the question of how well \( \dot{V}O_2/Q \) was matched to metabolic need, which is the focus of the present study. Furthermore, in the study by Mizuno et al. (50) employing PET measurement 10-min after one-legged high-intensity cycling exercise to the limit of tolerance, the average quadriceps muscle \( \dot{V}O_2/Q \) ranged between 0.04 and 0.06. Collectively, the present findings and those from previous studies (38 – 40, 50) suggest that \( Q \) remains closely matched to \( \dot{V}O_2 \), and thus there is little apparent functional heterogeneity defined as the matching of \( Q \) to metabolism.
Physiological Implications

The most important finding, within the limits of optode number and placement, is that of very little heterogeneity in the $V_{O2}/Q$ and no change from rest to exercise or when $F_{O2}$ is reduced to 0.12. This finding suggests that, within the muscles, Q rate is tightly matched to metabolic need, even as metabolic and environmental conditions change profoundly. We have no data to throw light on the physiological mechanism of how such tight flow regulation occurs, because this was well beyond the objectives of this project. However, this is a well-studied topic, and regulation likely involves a variety of pathways that could include local hypoxia, nitric oxide release, influences of the metabolites of oxidative phosphorylation, and autonomic activity affecting vascular tone (19, 44, 54). One important question is whether the degree of heterogeneity observed in normal muscle materially interferes with $O_2$ transport or utilization.

Another physiological question of interest relates to the reduction in overall $O_2$ transport is barely measurable, however, in health, the degree of inequality is not great (68), whereas arterial $PO_2$ is generally well preserved high on the flat portion of the oxyhemoglobin dissociation curve. Now with finding that the degree of heterogeneity in muscle is some fourfold less than that in even the normal lung, the expectation is that regions that are significantly underperfused with respect to metabolic demand will likely not produce the required contractile responses, whereas those that are overperfused will not use all of the $O_2$ they are delivered. In the lung, the corresponding form of heterogeneity, $VA/Q$ or $VA/Q$ inequality, is well known to interfere with $O_2$ exchange (69, 70).

However, in health, the degree of inequality is not great (68), and the reduction in overall $O_2$ transport is barely measureable, whereas arterial $Po_2$ is generally well preserved high on the flat part of the oxyhemoglobin dissociation curve. Now with finding that the degree of heterogeneity in muscle is some fourfold less than that in even the normal lung, the expectation is that this will produce little disturbance to muscle $O_2$ transport/utilization.

Another physiological question of interest relates to the numerical upper limit of the $V_{O2}/Q$. Examination of Eq. 1 shows that $V_{O2}/Q = CaO_2 - CvO_2$ (that is, $V_{O2}/Q$ equals the $CaO_2-CvO_2$ difference in ml $O_2$/ml blood). The smallest possible value of $V_{O2}/Q$ is zero, seen only if a region takes up no $O_2$ at all. The highest possible value will occur if all $O_2$ is extracted such that $CvO_2$ (and saturation, $SvO_2$) is zero, and then $V_{O2}/Q$ will equal $CaO_2$, which, in the stated units, is $-0.2$ for normal [Hb] and $O_2$ saturation (see Eq. 2). By comparison, in the lung, while the lowest value for $VA/Q$ is also zero, the highest value has no limit. The constraint on the $V_{O2}/Q$ range imposed by inability to exceed the value of $CaO_2$ will in theory limit the extent of $V_{O2}/Q$ heterogeneity that can occur. That said, while breathing air, even at 80% of maximal power, no estimate of local $SvO_2$ was zero in any subject, and in only two subjects was minimal $SvO_2$ under any optode $<10$%. Such single-digit venous saturation estimates were found under only one optode each in those two subjects. Thus when breathing air, the upper limit of $V_{O2}/Q$ appears not to be commonly reached. On the other hand, while exercising at 80% of maximal effort and breathing 12% $O_2$, two subjects showed zero estimated $SvO_2$ under three and one optodes, respectively. Two additional subjects showed low single-digit $SvO_2$ values (1 and 3%) under one or more optodes, so that, with restricted $O_2$ availability, some regions of muscle appear to approach their upper $V_{O2}/Q$ limit.

Limb muscle dysfunction is an important systemic consequence of chronic diseases, such as chronic obstructive pulmonary disease (9, 22, 25, 62–64), chronic heart failure (30, 53), diabetes (1, 11), and peripheral vascular disease (15), because of its effect on daily physical activity (47), exercise intolerance (65), quality of life (26, 55), and even survival (23, 37). A common consequence of these abnormalities across the four disease entities is the limitation in $O_2$ availability caused by reduced arterial oxygenation and/or reduced muscle $Q$ (10). Reduction in either of the two may adversely impact the degree of local matching of metabolic demand to energy supply within muscle (10). It is uncertain, however, whether extreme seden-
tariness explains this, or whether intrinsic additional dysfunction exists. In the latter case, one possibility is mismatching between metabolic demand (\(V_O^2\)) and O2 supply by Q, resulting in \(V_O^2/Q\) heterogeneity in different regions within the limb muscles (7, 8, 50).

**Methodological Considerations**

Because this particular study required the simultaneous use of three spectrophotometers and six different optodes (Fig. 1), it was felt to be important to know if the same physical region in muscle gave results that varied for technical reasons due to differences in optode or photometer function. The preliminary studies provided assurance that regional differences were physiological in origin and not spurious technical variation.

An important consideration of the approach is the need to convert local NIRS-based StiO2 into corresponding values of local SvO2. In the present study, we catherized the femoral vein to measure the relationship between StiO2 and SvO2, and used this to determine local venous saturation. To apply the NIRS technique more broadly, it would be advantageous to not have to measure the relationship between femoral venous saturation and that from NIRS in every subject, thus avoiding femoral venous catheterization and also a lot of possibly unnecessary effort. The correlation between the RDs of StiO2 and \(V_O^2/Q\) across all subjects and conditions was linear, positive, and highly significant, but, with an \(R^2\) value of 0.45, it would be difficult to confidently substitute RD of StiO2 for that of \(V_O^2/Q\) to characterize dispersion of the latter. When femoral vein catheterization is unavailable, it should still be possible to use RD of StiO2 as an index of heterogeneity for group studies and comparisons among different groups or conditions. That said, Figs. 5 and 6 show that RD for StiO2 is less than that for \(V_O^2/Q\). This likely reflects the fact that StiO2 is a weighted average of, among other contributors, SaO2 and SvO2. A simple calculation assuming two muscle compartments with SvO2 of 20 and 30% and SaO2 of 100% shows that (SaO2 + SvO2)/2, while \(V_O^2/Q\) is derived from the difference between arterial and venous saturation, i.e., from SaO2 − SvO2. A simple calculation assuming two muscle compartments with SvO2 of 20 and 30% and SaO2 of 100% shows that (SaO2 + SvO2)/2 for the two compartments would be 60 and 65%, whereas SaO2 − SvO2 for the same compartments are 70 and 80%, respectively. In this example, RD for (SaO2 + SvO2)/2 will reflect a 5% saturation difference related to a mean of 62.5%, whereas for SaO2 − SvO2 will reflect a 10% saturation difference related to a mean of 75%. On this basis, RD for StiO2 should be generally less than that for \(V_O^2/Q\). Similarly, the increase in RD of StiO2, with exercise compared with the lack of increase in RD \(V_O^2/Q\) with exercise can be explained by the same arithmetically based reasoning. Figure 6 also shows that RD for Q and RD for \(V_O^2\) increase with exercise; however, RD for \(V_O^2/Q\) does not change with exercise. This may reflect increasing heterogeneity of the O2 demand (\(V_O^2\)) and O2 supply (Q) secondary to regional heterogeneity of muscle recruitment, coupled with tight regulation of Q with respect to metabolism.

In the present study, we were limited to the use of six optodes and thus six muscle regions. Thus the full extent of heterogeneity may not have been visualized as the StiO2 recorded over the upper, middle, and lower vastus lateralis reflect the O2 levels of the superficial muscle regions (Table 3). Potential contribution to heterogeneity of the deeper currently inaccessible region muscles, such as the vastus intermedius, needs to be considered in the future. In addition, the linear regression analysis relating average StiO2 to SFV2O may be inaccurate if the six areas sampled were not representative of the whole muscle. The study by Chin et al. (13) showed that the profile of muscle deoxygenation in different muscle groups of the quadriceps (vastus lateralis, rectus femoris, and vastus medialis) during cycle ramp exercise exhibits profound differences that are characterized by a rightward shift of deoxyhemoglobin in rectus femoris compared with vastus lateralis and vastus medialis. Repeating exercise after placing the same optodes over different quadriceps muscle groups may be an approach to better define the extent of heterogeneity and the relationship with SFV2O until a multiopode spectrophotometer becomes available. Thus the present results need to be interpreted cautiously in a quantitative sense. In summary, using the linear regression analysis relating average StiO2 to SFV2O to estimate local SvO2 from local StiO2 is an approximation, since the six areas sampled may not be representative of the whole muscle. The main conclusion that both Q and \(V_O^2\) are regionally quite heterogeneous, whereas both StiO2 and \(V_O^2/Q\) are much less heterogeneous, appears robust based on the consistency of the results obtained (Table 3 and Figs. 5 and 6).

Mancini et al. (46) show good linear correlation between StiO2 and SvO2 in the arm during incremental arm exercise, which is similar in principle to what we found in the present study. However, Costes et al. (14) and MacDonald et al. (45) have shown somewhat weaker correlations, but their studies were not done with an incremental exercise protocol, but rather under a constant-load design where StiO2 was followed over time. Thus both of these studies are not well matched in design to our protocol, and comparisons should be made with caution.

Another issue that merits consideration is that microvascular circulation may affect NIRS muscle monitoring (17, 34, 57). However, a recent study by Messere and Roatta (48) indicated that the NIRS method used in the present study, spatially resolved spectroscopy, effectively rejects interference by alterations in muscle blood volume and skin Q. Furthermore, it is known that subcutaneous adipose tissue Q is increased during exercise in an intensity-dependent manner; the increase is less during hypoxic exercise, in part due to the redistribution of Q to exercising muscle facing reduced O2 availability (33). However, a recent study by Messere and Roatta (48) showed that, with the use of spatially resolved spectroscopy in the present study, skin and subcutaneous tissues do not perturb the NIRS signals. Thus it is reasoned that differences in adipose tissue perfusion between normoxia and hypoxia will not lead to misinterpretation of our data. Moreover, there was only 5 mm of adipose tissue subcutaneously in our (athletic) subjects, and the PET-based data of the study by Heinenon et al. (32) show that, during even light exercise, subcutaneous adipose tissue Q is only one-tenth of muscle Q per 100 ml tissue.

**Conclusions**

In conclusion, using NIRS to measure at the same time both Q (by ICG dye appearance) and oxygenation in different regions of exercising muscle offers the potential for directly assessing muscle O2 supply/demand heterogeneity in humans. The present study offers proof of concept of this approach,
albeit accessing only six muscle regions, due to absence of a multirode NIR spectrometer. The major conclusion is that both Q and VO2 vary considerably across normal exercising muscle, but their ratio, VO2/Q, is much less variable, and its RD appears physiologically insignificant. This suggests tight matching between Q˙ and metabolism, not just at rest, but also during exercise and in hypoxia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

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