Performance of near-infrared spectroscopy in measuring local O₂ consumption and blood flow in skeletal muscle

MIREILLE C. P. VAN BEEKVELT,1,2 WILLY N. J. M. COLIER,1 RON A. WEVERS,2 AND BAZIEL G. M. VAN ENGELEN2
1Department of Physiology, Faculty of Medical Sciences, University of Nijmegen, and 2Neuromuscular Centre Nijmegen, Institute of Neurology, University Medical Centre St. Radboud, 6500 HB Nijmegen, The Netherlands

Received 3 April 2000; accepted in final form 1 September 2000

Van Beekvelt, Mireille C. P., Willy N. J. M. Colier, Ron A. Wevers, and Baziel G. M. van Engelen. Performance of near-infrared spectroscopy in measuring local O₂ consumption and blood flow in skeletal muscle. J Appl Physiol 90: 511–519, 2001.—The aim of this study was to investigate local muscle O₂ consumption (muscV˙O₂) and forearm blood flow (FBF) in resting and exercising muscle by use of near-infrared spectroscopy (NIRS) and to compare the results with the global muscV˙O₂ and FBF derived from the well-established Fick method and plethysmography. muscV˙O₂ was derived from 1) NIRS using venous occlusion, 2) NIRS using arterial occlusion, and 3) the Fick method (muscV˙O₂/Fick). FBF was derived from 1) NIRS and 2) strain-gauge plethysmography. Twenty-six healthy subjects were tested at rest and during sustained isometric handgrip exercise. Local variations were investigated with two independent and simultaneously operating NIRS systems at two different muscles and two measurement depths. muscV˙O₂ increased more than fivefold in the active flexor digitorum superficialis muscle, and it increased 1.6 times in the brachioradialis muscle. The average increase in muscV˙O₂/Fick was twofold. FBF increased 1.4 times independent of the muscle or the method. It is concluded that NIRS is an appropriate tool to provide information about local muscV˙O₂ and local FBF because both place and depth of the NIRS measurements reveal local differences that are not detectable by the more established method, but also more global, Fick method.

NEAR-INFRARED SPECTROscopy (NIRS) is a noninvasive, continuous, and direct method to determine oxygenation and hemodynamics in tissue. It enables the study of local differences in muscle O₂ consumption (muscV˙O₂) and delivery. NIRS has also shown to be a sensitive tool in the discrimination between normal and pathological states. Abnormal oxygenation due to insufficient delivery has been found with NIRS in patients with heart failure (4, 29, 30, 44) and peripheral vascular disease (6, 23, 24, 32). NIRS was also used to characterize patients with metabolic myopathies, in which abnormalities in oxygenation pattern are related to O₂ extraction instead of O₂ delivery (1, 2, 17). Our recent study showed that NIRS makes it possible to quantify differences in O₂ consumption and forearm blood flow (FBF) at rest as well as during exercise and discriminates between patients with mitochondrial myopathies and healthy persons (39).

Quantification of muscV˙O₂ and blood flow using NIRS has become possible by incorporating a differential path-length factor (DPF) in the Lambert-Beer law (8) and applying an occlusion to control circulation in the limb. muscV˙O₂ has been measured with NIRS during arterial occlusion (6, 7, 9, 10, 38) as well as during venous occlusion (9, 11, 19, 38). Muscle blood flow has been measured with NIRS during venous occlusion (11, 38) and by use of an intravascular tracer (14).

Comparison of the NIRS method to quantify blood flow with more established methods has been reported in only a few studies. NIRS blood flow measurement during rest, obtained by an intravascular tracer, was compared with venous occlusion plethysmography by Edwards et al. (14), and De Blasi et al. (11) compared NIRS flow measurement with plethysmographic flow measurement, both simultaneously measured during venous occlusion. Quantitative NIRS muscV˙O₂ measurement, however, has never been compared with a more established method. Although Homma et al. (19) showed that there was a relationship between the deoxygenation pattern estimated by NIRS during venous occlusion and the O₂ consumption obtained by a more established method, they did not calculate quantitative values for muscV˙O₂.

The present study was undertaken to determine whether quantitative measurements of NIRS muscV˙O₂ and FBF of human skeletal muscle at rest as well as during exercise correlates with the more established methods of combining blood gas analysis, pulse oximetry, and plethysmography and whether the depth and the place of the NIRS measurements reveal local differences that are not detectable by the more established, though global, Fick method.

http://www.jap.org 8750-7587/01 $5.00 Copyright © 2001 the American Physiological Society 511

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
NIRS PERFORMANCE IN HUMAN SKELETAL MUSCLE

MATERIALS AND METHODS

Subjects

Twenty-six healthy volunteers (16 men, 10 women) participated in this study. The study was approved by the Faculty Ethics Committee, and all subjects gave their written, informed consent. The subject characteristics were 28.8 ± 7.7 yr in age, 178.9 ± 10.9 cm in height, and 70.0 ± 11.1 kg in weight (means ± SD). One subject used medication (Pulmicort puffs) that, to our knowledge, does not affect muscle peripheral circulation. Skinfold thickness was measured between the NIRS optodes by use of a skinfold caliper (Holtain, Crymmych, UK) and divided by two to determine the adipose tissue thickness (fat + skin layer; ATT) covering the muscle. All but two of the subjects were right-handed.

NIRS

NIRS is based on the relative tissue transparency for light in the near-infrared region and on the O$_2$-dependent absorption changes of hemoglobin and myoglobin. By using a continuous-wave near-infrared spectrophotometer (OXYMON, University of Nijmegen, Nijmegen, The Netherlands) that generates light at 905, 850, and 770 nm (40), it is possible to differentiate between oxy- and deoxyhemoglobin/myoglobin (O$_2$Hb/O$_2$Mb and HHb/HMb, respectively). Because of identical spectral characteristics, it is not possible to distinguish between Hb and Mb. The absorption changes at the discrete wavelengths are converted into concentration changes of O$_2$Hb and HHb by using the algorithm described by Livera et al. (27). To correct for scattering of photons in the tissue, a DPF of 4.0 was used for the calculation of absolute concentration changes (12, 16). Data were sampled at 10 Hz, displayed in real time, and stored on disk for off-line analysis.

The sum of O$_2$Hb and HHb concentrations ([O$_2$Hb] and [HHb], respectively) reflects the total amount of hemoglobin ([tHb]), and changes in [tHb] can be interpreted as changes in blood volume in the tissue. The difference between [O$_2$Hb] and [HHb] (= [Hb$_{diff}$]) is used for the calculation of O$_2$ consumption during arterial occlusion.

Simultaneous NIRS measurements were done on top of the flexor digitorum superficialis (FDS) muscle and on top of the brachioradialis (BR) muscle with two independent-operating NIRS systems. This was done to obtain unique information about local differences in muscV˙O$_2$ and blood flow between the agonistic flexor muscles initiating handgrip exercise and the synergistic BR muscle. In addition, two interoptode distances (IO) of 35 and 50 mm were used to measure simultaneously at different depths, further referred to as IO$_{35}$ and IO$_{50}$, respectively.

Strain-Gauge Plethysmography

FBF was also measured by the more established method of strain-gauge plethysmography (Loosco, Amsterdam, NL) by using mercury-filled silicon gauges (45). A pneumatic arm cuff around the upper arm just above the elbow was inflated to 50 mmHg to apply venous occlusion. A wrist cuff inflated to 260 mmHg was used to exclude blood flow from the hand. The strain gauge was stretched halfway around the forearm on top of the FDS muscle and between the NIRS optodes to measure plethysmographic flow in the same region as the NIRS measurement. The strain gauge was electronically calibrated. Strain-gauge plethysmography and NIRS data were recorded simultaneously.

Blood Sampling

A Venflon catheter (BOC Ohmeda AB, Helsingborg, Sweden) was inserted into the antecubital vein. To sample blood from deep within the active muscle, thus avoiding mixture with skin circulation, the catheter was inserted in retrograde direction (18, 34). A three-way stopcock was attached to allow for drawing blood into heparinized 1-ml syringes for measurement of blood gases and Hb content (Synthesis 25, Instrumentation Laboratory). Blood gas analysis took place directly after withdrawal of the blood. The catheter was washed out with sodium chloride (0.9%) to prevent it from clotting. Arterial saturation was measured using a pulse oximeter (FOX; N200, Nellcor Puritan-Bennet) with the probe placed on the left index finger.

Protocol

The subject lay in a comfortable supine position, 15–20 min before the test. The right hand rested on a handgrip dynamometer with the upper arm at heart level and the forearm in an upward angle of 30° to avoid venous pooling of the blood. The arm was supported at the wrist and above the elbow so that there was no contact between forearm and dynamometer, and circulation in the forearm was completely unrestricted. The subject’s maximum voluntary contraction (MVC) force was determined before the test. Pneumatic cuffs were placed around the upper arm and the wrist.

The experiment started with a 5-min rest period after placement of the instruments and insertion of the catheter (Fig. 1). At 4 min of rest, the wrist cuff (260 mmHg) was inflated. One minute later, three consecutive venous occlusions (50 mmHg) were applied, followed by an arterial occlusion (260 mmHg). All venous occlusions lasted 20 s, and the arterial occlusion was maintained for 30–45 s. One minute of recovery separated the interventions. A blood sample was taken just before the first venous occlusion and again before arterial occlusion (Fig. 1).

After 5 min of recovery, the subject was asked to perform sustained isometric handgrip exercise at 10% MVC. The 10% level was marked on a display visible for the subject. The wrist cuff was inflated at the start of exercise. After 50 s of exercise, when NIRS signals had reached steady state, a blood sample was taken, immediately followed by rapid inflation of the arm cuff to apply venous occlusion while exercise was maintained (Fig. 1). Cuff inflation was kept at 50 mmHg for 20 s and then released. At the same time, the exercise was ended and the wrist cuff was released.

When NIRS and plethysmographic signals had returned to preexercise levels after 5 min, a second exercise session was performed under identical conditions to determine muscVo$_2$ during arterial occlusion. A blood sample was taken at 50 s after the start of exercise, this time immediately followed by an arterial occlusion, which was released after 30–45 s while the exercise was ended and the wrist cuff released.

Forearm Measurements

FBF. FBF was calculated from NIRS data (FBF$_{NIRS}$) by evaluating the linear increase in [tHb] within the first seconds of the venous occlusion (9, 38). Concentration changes of [tHb] were expressed in micromolars per second and were converted to milliliters blood per 100 milliliters tissue per minute by using the individual Hb concentration that was obtained from the blood samples. The molecular weight of Hb (64.458 g/mol) and the molecular ratio between Hb and O$_2$ (1:4) were taken into account.
To compare $\text{FBF}_{\text{NIRS}}$ with a more established method, $\text{FBF}$ was also measured by venous occlusion plethysmography ($\text{FBF}_{\text{pleth}}$) (45) (Fig. 1). The linear increase within the first seconds of the 20-s occlusion was considered for $\text{FBF}_{\text{pleth}}$ calculation. Volume changes were expressed in percentages and converted to milliliters per 100 milliliters per minute for the comparison with $\text{FBF}_{\text{NIRS}}$. $\text{FBF}_{\text{NIRS}}$ and $\text{FBF}_{\text{pleth}}$ were both calculated from the same time period during venous occlusion and reflect, therefore, the local ($\text{FBF}_{\text{NIRS}}$) and the total ($\text{FBF}_{\text{pleth}}$) flow in the forearm for that time period.

**O$_2$ consumption.**

$\text{muscVO}_2$ was measured by three methods (Fig. 1). First, $\text{muscVO}_2$ was derived from NIRS using venous occlusion [$\text{muscVO}_2(\text{NIRS}_{\text{VO}})$] as the rate of increase in [HHb] (9). Second, $\text{muscVO}_2$ was derived from NIRS using arterial occlusion [$\text{muscVO}_2(\text{NIRS}_{\text{AO}})$] by evaluating the rate of decrease in [Hb$_{\text{diff}}$] ([Hb$_{\text{diff}}$] = [O$_2$Hb] - [HHb]) with the assumption that [tHb] is constant (9). Concentration changes of Hb and Hb$_{\text{diff}}$ were expressed in micromolars per second and converted to milliliters O$_2$ per minute per 100 grams. A value of 1.04 kg/l was used for muscle density (42). Third, $\text{muscVO}_2$ was derived from the combination of blood samples, POX, and plethysmography by using Fick's law for the equation of $\text{muscVO}_2$ [$\text{muscVO}_2(\text{Fick})$] assuming STPD conditions (Eq. 1)

\[
\text{muscVO}_2(\text{Fick}) = \text{FBF}_{\text{pleth}} \times (\text{a-v})\text{O}_2\text{diff}
\]

where (a-v)O$_2$diff is arteriovenous O$_2$ difference.

Arterial saturation was derived from POX, whereas venous saturation was derived from the blood samples. The O$_2$ binding capacity of human Hb (Hufnér's factor = 1.39 ml O$_2$/g) was taken into account. The dissolved O$_2$ in the blood was assumed to be 0.3 ml O$_2$/100 ml.

**Statistics**

In some cases, parts of the measurements were missing because of a very low signal-to-noise ratio. A Shapiro-Wilk test was used to test all variables for normality ($P < 0.01$). Log transformation was applied on variables that failed the normality test. To test the reproducibility of the three consecutive venous occlusions for the measurement of $\text{muscVO}_2$ and $\text{FBF}$, a two-way ANOVA with a mixed model was used and followed by Scheffé’s method if significant differences were found. Statistical differences between measurement depth, measurement place, both methods for $\text{muscVO}_2$, and both methods for $\text{FBF}$ and between rest and exercise were tested by means of Student’s paired t-test. To protect against a type I error, an $\alpha$ of 0.01 was chosen. A Spearman correlation test was used to test the correlation between NIRS $\text{muscVO}_2$ and ATT. All data are reported as means ± SD. The level of statistical significance was set at $P < 0.05$.

**RESULTS**

ATT was 2.2 ± 0.8 mm on top of the FDS muscle and 2.6 ± 0.5 mm on top of the BR muscle. MVC force was 566 ± 125 N. No correlation was found between $\text{muscVO}_2$ measurements and ATT (Table 1).
Reproducibility

The reproducibility of the NIRS measurements for muscVO$_2$ and FBF as well as the plethysmographic flow measurement was investigated by means of the repetition of three venous occlusions. All values for muscVO$_2$ and FBF during the repeated measurements as well as the coefficient of variation are shown in Table 2.

O$_2$ consumption from NIRS. O$_2$ consumption, measured by NIRS during venous occlusion [muscVO$_2$(NIRSAO)] was not reproducible for FDS at IO$_{35}$ because this value decreased slightly but significantly when venous occlusion was repeated. Because no differences were expected, on the basis of the physiological background and the sufficient time for recovery, we decided that this value for muscVO$_2$(NIRSAO) was not reliable and, therefore, excluded it from further analysis. On the contrary, FDS at IO$_{50}$ and BR at IO$_{35}$ were reproducible (P > 0.05) and, therefore, were calculated as the average muscVO$_2$ from the three occlusions.

FBF from NIRS. No significant differences between the three consecutive venous occlusions during rest were found in the calculated flow measured by NIRS (FBF$_{\text{NIRS}}$). Therefore, FBF$_{\text{NIRS}}$ was calculated as the average flow of the three occlusions.

FBF from plethysmography. No significant differences between the three consecutive venous occlusions during rest were found in the plethysmographic flow measurement (FBF$_{\text{pleth}}$) either. Therefore, FBF$_{\text{pleth}}$ was calculated as the average flow value obtained from the three occlusions.

NIRS muscVO$_2$ Measurements

No significant differences between muscVO$_2$(NIRSAO) and muscVO$_2$(NIRSAO) were found in the BR muscle or for FDS at IO$_{50}$ (Table 3). During exercise, muscVO$_2$ increased significantly for all measurements (P ≤ 0.01) compared with at rest. Although muscVO$_2$(NIRSAO) showed marked differences between the different muscles, the increase in muscVO$_2$(NIRSAO) was roughly the same, independent of the measured muscle or the depth of the measurement (Table 3). This resulted in a significantly lower muscVO$_2$(NIRSAO) in FDS at IO$_{35}$ and FDS at IO$_{50}$ compared with muscVO$_2$(NIRSAO), whereas no difference was found in the BR.

We decided to focus on muscVO$_2$(NIRSAO) in the rest of this paper on the basis of 1) the nonreproducibility of FDS at IO$_{35}$, 2) the absence of expected local differences between active and inactive muscles during exercise, and 3) a substantially lower coefficient of variation for muscVO$_2$(NIRSAO) (16.2%) compared with muscVO$_2$(NIRSAO) (32.6%) that was found in another study (unpublished data) that we performed.

Influence of Depth

The muscVO$_2$(NIRSAO) at rest was significantly lower (P ≤ 0.01) in the deeper region of the FDS (IO$_{50}$) compared with the superficial region (IO$_{35}$) (Table 4). From rest to low-intensity exercise at 10% MVC, muscVO$_2$(NIRSAO) increased more than five times independent of the measurement depth (Fig. 2). This increase during exercise was highly significant for both depths (P ≤ 0.01). No significant difference (P = 0.15) was found between IO$_{35}$ and IO$_{50}$ during exercise.

The higher muscVO$_2$(NIRSAO) at IO$_{35}$ compared with IO$_{50}$ was accompanied by a significantly higher FBF$_{\text{NIRS}}$ at IO$_{35}$ compared with IO$_{50}$ (Table 4). From rest to exercise, FBF$_{\text{NIRS}}$ increased significantly (P ≤ 0.01) at both depths (1.4 times) but did not match

Table 1. Correlation between ATT and muscVO$_2$(NIRSAO) in the FDS and BR

<table>
<thead>
<tr>
<th></th>
<th>FDS IO$_{35}$</th>
<th>FDS IO$_{50}$</th>
<th>BR IO$_{35}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT, mm</td>
<td>2.2 ± 0.8</td>
<td>2.2 ± 0.8</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Spearman r</td>
<td>−0.13</td>
<td>0.11</td>
<td>−0.20</td>
</tr>
<tr>
<td>P</td>
<td>0.52</td>
<td>0.61</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Values are means ± SD (range) for adipose tissue thickness (ATT). Spearman correlation coefficient (r) and P values are given for the three near-infrared spectroscopy (NIRS) local muscle O$_2$ consumption (muscVO$_2$) measurements. FDS, flexor digitorum superficialis muscle; BR, brachioradialis muscle; IO$_{35}$ and IO$_{50}$, interoptode distances of 35 and 50 mm, respectively.

Table 2. Reproducibility of muscVO$_2$ and FBF during three consecutive venous occlusions

<table>
<thead>
<tr>
<th></th>
<th>VO-1</th>
<th>VO-2</th>
<th>VO-3</th>
<th>Comparison</th>
<th>P Value</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>muscVO$_2$(NIRSAO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDS IO$_{35}$ (n = 24)</td>
<td>0.09 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>Overall</td>
<td>*P = 0.01</td>
<td>30.6</td>
</tr>
<tr>
<td>FDS IO$_{50}$ (n = 20)</td>
<td>0.08 ± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.07 ± 0.04</td>
<td>VO-3 vs. VO-1</td>
<td>*P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>BR IO$_{35}$ (n = 26)</td>
<td>0.14 ± 0.09</td>
<td>0.14 ± 0.11</td>
<td>0.13 ± 0.10</td>
<td>VO-3 vs. VO-2</td>
<td>NS</td>
<td>25.4</td>
</tr>
<tr>
<td>FBF$_{\text{NIRS}}$</td>
<td></td>
<td></td>
<td></td>
<td>VO-2 vs. VO-1</td>
<td>†P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>FDS IO$_{35}$ (n = 25)</td>
<td>0.76 ± 0.33</td>
<td>0.70 ± 0.39</td>
<td>0.71 ± 0.35</td>
<td>Overall</td>
<td>NS</td>
<td>28.6</td>
</tr>
<tr>
<td>FDS IO$_{50}$ (n = 21)</td>
<td>0.61 ± 0.28</td>
<td>0.59 ± 0.34</td>
<td>0.54 ± 0.28</td>
<td>Overall</td>
<td>NS</td>
<td>30.3</td>
</tr>
<tr>
<td>BR IO$_{35}$ (n = 26)</td>
<td>1.41 ± 1.01</td>
<td>1.40 ± 1.09</td>
<td>1.44 ± 1.11</td>
<td>Overall</td>
<td>NS</td>
<td>20.4</td>
</tr>
<tr>
<td>FBF$_{\text{pleth}}$</td>
<td>2.07 ± 0.86</td>
<td>2.01 ± 0.73</td>
<td>2.07 ± 0.71</td>
<td>Overall</td>
<td>NS</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Values are means ± SD for muscVO$_2$ (mlO$_2$·min$^{-1}$·100 g$^{-1}$) and forearm blood flow (FBF; ml·min$^{-1}$·100 ml$^{-1}$). First, second, and third venous occlusions are represented by VO-1, VO-2, and VO-3, respectively. Number of subjects (n) varies due to missing values in either VO-1, VO-2, or VO-3. CV, coefficient of variation. *P ≤ 0.05; †P ≤ 0.01; NS, not significant.
The blood flow at rest measured with plethysmography was more than twice \((P < 0.01)\) the flow measured with NIRS (Table 4). From rest to exercise, both FBF_{pleth} and FBF_{NIRS} increased significantly with a factor of 1.4, and the difference between FBF_{NIRS} and FBF_{pleth} that was found in rest was, therefore, maintained during exercise \((P < 0.01)\).

**DISCUSSION**

This study was performed to investigate the performance of NIRS for the quantitative measurement of local muscVO_2 and blood flow in the human forearm. Two independently operating identical NIRS systems were used simultaneously to study local differences based on the activity level of the muscle as well as on the measurement depth. Furthermore, local differences were compared with the more established, though global, Fick method.

**Methodological Considerations**

Because the penetration depth of the near-infrared light is limited to roughly half the distance between source and detector, ATT can be a substantial confounder in the measurement of muscle oxygenation \((5, 20, 31, 46)\). However, in this study, there was no correlation between ATT and muscVO_2 and the results are, therefore, not biased by ATT. This is probably due to the relatively narrow range of low values that we found for ATT in our subject group (Table 1). Although we did not find a correlation between ATT and muscVO_2 measurements, the individual differences in ATT might have increased to some extent the variability within the group.

NIRS is unable to distinguish between changes in O_2Hb and O_2Mb or in HHb and HMb because of identical absorption spectra of Hb and Mb. Although there is no consensus yet about whether the NIRS signal originates from Hb \((36, 43)\) or Mb \((33, 37)\), this does not affect our results because we were interested in the amount of O_2 consumed independent whether it came from Hb or Mb. Furthermore, we think that substantial desaturation of Mb is negligible in our study because the workload that we used was only 10% MVC.

The DPF for skeletal muscle has been measured by several investigators under different conditions and using different instrumentation \((8, 13, 15, 16, 41)\). The average values found for DPF in the human forearm lie between 3.59 and 4.57. We have chosen a DPF of 4.0

### Table 3. muscVO_2 values measured by NIRS during arterial vs. venous occlusion

<table>
<thead>
<tr>
<th></th>
<th>muscVO_2(NIRSAO)</th>
<th>muscVO_2(NIRSAO)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mlO_2 min^{-1} 100 g^{-1}</td>
<td>mlO_2 min^{-1} 100 g^{-1}</td>
<td></td>
</tr>
<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDS IO_{35}</td>
<td>0.11 ± 0.03</td>
<td>0.08 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>FDS IO_{50}</td>
<td>0.09 ± 0.03</td>
<td>0.14 ± 0.10</td>
<td>0.51</td>
</tr>
<tr>
<td>BR IO_{35}</td>
<td>0.13 ± 0.05</td>
<td>0.21 ± 0.12</td>
<td>* P &lt; 0.01</td>
</tr>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDS IO_{35}</td>
<td>0.58 ± 0.27</td>
<td>0.24 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>FDS IO_{50}</td>
<td>0.55 ± 0.22</td>
<td>0.24 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>BR IO_{35}</td>
<td>0.20 ± 0.09</td>
<td>0.21 ± 0.21</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n = 22\) for FDS IO_{35}; \(n = 26\) for FDS IO_{50} and BR IO_{35}. No P value was calculated for FDS IO_{35} during rest because muscVO_2 measured by venous occlusion (muscVO_2(NIRSAO)) was not reproducible. muscVO_2 measured by arterial occlusion. To protect against a type I error, an \(\alpha\) of 0.01 was chosen. *P < 0.01.

The fivefold increase in muscVO_2. The difference in FBF_{NIRS} between IO_{35} and IO_{50} found in rest was still present during exercise \((P < 0.01)\).

**Influence of Place**

No significant difference in muscVO_2(NIRSAO) was found between FDS and BR muscle during rest (Table 4). During exercise at 10% MVC, muscVO_2(NIRSAO) in the BR muscle increased significantly \((P < 0.01)\) with a factor of 1.6 but did not match the increase in the FDS muscle (Fig. 2). This resulted in a significantly higher \((P < 0.01)\) consumption during exercise in the FDS compared with the consumption in the BR muscle.

Although no difference in resting muscVO_2 was found between the two muscles, FBF_{NIRS} was significantly higher in the BR \((P < 0.01)\) compared with the FDS muscle. At the transition from rest to exercise, FBF_{NIRS} increased significantly \((P < 0.01)\) in both muscles, and the relative increase was the same for both muscles.

**Comparison of Fick and NIRS Methods**

muscVO_2 during rest was significantly higher \((P < 0.01)\) for the Fick method compared with the NIRS measurement at FDS IO_{35} (Table 4). During exercise, muscVO_2 of both methods increased, but the increase in muscVO_2(NIRSAO) was much larger than the increase in muscVO_2(Fick) and resulted in a significantly higher \((P < 0.01)\) muscVO_2(NIRSAO).

### Table 4. muscVO_2 and FBF values during rest and sustained isometric handgrip exercise at 10% maximum voluntary contraction

<table>
<thead>
<tr>
<th></th>
<th>muscVO_2, mlO_2 min^{-1} 100 g^{-1}</th>
<th>FBF, ml min^{-1} 100 ml^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>NIRS FDS IO_{35}</td>
<td>0.11 ± 0.03*</td>
<td>0.59 ± 0.27†</td>
</tr>
<tr>
<td>NIRS FDS IO_{50}</td>
<td>0.09 ± 0.03*</td>
<td>0.55 ± 0.22†</td>
</tr>
<tr>
<td>NIRS BR IO_{35}</td>
<td>0.13 ± 0.05</td>
<td>0.20 ± 0.09∗</td>
</tr>
<tr>
<td>Fick/plethysmography</td>
<td>0.15 ± 0.06*</td>
<td>0.30 ± 0.12†</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different \((P < 0.01)\) from FDS IO_{35}; †significant difference \((P < 0.01)\), rest vs. exercise.
significant differences are shown in Table 4. Resting values, and triangles represent values during exercise. Several variables during the occlusion, and the third the reproducibility. The first one concerns technical conditions of the protocol, the second a change of physiological variables during the occlusion, and the third the NIRS method during venous occlusion not being stable enough to provide a reliable \( \text{muscVO}_2 \) value.

Concerning the chosen protocol variables, it is our opinion that there were no differences among the three occlusions given that we applied the occlusions at distinct time periods and all with the same duration for both occlusion and recovery. The nonreproducibility was not caused by an insufficient recovery time between the determination of the MVC force and the first venous occlusion either because this effect was not present in the other measurements that were simultaneously performed.

Physiological changes affecting optical properties of the tissue caused by the venous occlusion itself are not very likely because a minor decrease (7%) of the optical path length has been detected after 3 min of venous occlusion (16), whereas we applied only 20 s of occlusion.

Venous occlusion has recently been used to calculate \( \text{muscVO}_2 \) by use of NIRS (9, 11, 19). This method is thought to be preferable to arterial occlusion because the procedure is less inconvenient for the subject and can be repeated at short time intervals (9, 19). However, venous occlusion is also more prone to ever-occurring variations in flow within the arm due to changes in blood pressure and local vasoreactivity, whereas these influences are negligible during arterial occlusion because of the closed compartment, temporarily cut off from centrally mediated variations. The arterial occlusion method to determine NIRS \( \text{muscVO}_2 \) measurements proved to be reproducible (7), but no data about the reproducibility of NIRS \( \text{muscVO}_2 \) measurement during venous occlusion are available.

Reproducibility of NIRS Measurements

\( \text{O}_2 \) consumption. \( \text{muscVO}_2(\text{NIRS} \_\text{VO}) \), was not uniformly reproducible over the three consecutive venous occlusions because \( \text{muscVO}_2 \) appeared to decrease over time when measured repeatedly at FDS IO35. We did not expect to find differences between the three occlusions, and we have three possible explanations for this nonreproducibility. The first one concerns technical conditions of the protocol, the second a change of physiological variables during the occlusion, and the third the NIRS method during venous occlusion not being stable enough to provide a reliable \( \text{muscVO}_2 \) value.

Concerning the chosen protocol variables, it is our opinion that there were no differences among the three occlusions given that we applied the occlusions at distinct time periods and all with the same duration for both occlusion and recovery. The nonreproducibility was not caused by an insufficient recovery time between the determination of the MVC force and the first venous occlusion either because this effect was not present in the other measurements that were simultaneously performed.

Physiological changes affecting optical properties of the tissue caused by the venous occlusion itself are not very likely because a minor decrease (7%) of the optical path length has been detected after 3 min of venous occlusion (16), whereas we applied only 20 s of occlusion.

Venous occlusion has recently been used to calculate \( \text{muscVO}_2 \) by use of NIRS (9, 11, 19). This method is thought to be preferable to arterial occlusion because the procedure is less inconvenient for the subject and can be repeated at short time intervals (9, 19). However, venous occlusion is also more prone to ever-occurring variations in flow within the arm due to changes in blood pressure and local vasoreactivity, whereas these influences are negligible during arterial occlusion because of the closed compartment, temporarily cut off from centrally mediated variations. The arterial occlusion method to determine NIRS \( \text{muscVO}_2 \) measurements proved to be reproducible (7), but no data about the reproducibility of NIRS \( \text{muscVO}_2 \) measurement during venous occlusion are available.

The relative variability within our group, when looking at the SD in relation to the mean, was consistently higher for \( \text{muscVO}_2(NIRS \_\text{VO}) \) compared with \( \text{muscVO}_2(\text{NIRS} \_\text{AO}) \), both in rest and during exercise (Table 3). In an unpublished study that we performed in healthy subjects (\( n = 78 \)), it was found that the arterial occlusion method had a substantially lower coefficient of variation (16.2%) than the venous occlusion method (32.6%), whereas the absolute values were roughly the same compared with this study. Moreover, no differences were found between the active FDS and the inactive BR muscle during light-intensity work, whereas differentiation in oxygenation pattern was expected based on elementary physiological principles of agonistic and synergistic muscles. On the basis of the above-mentioned points and the lack of data from the literature, we have to conclude that the venous occlusion method does not provide a reliable quantitative value for \( \text{muscVO}_2 \).

\( \text{FBF} \). The reproducibility for the measurement of \( \text{FBF} \) obtained both by plethysmography (\( \text{FBF} \_\text{pleth} \)) and by NIRS during venous occlusion (\( \text{FBF} \_\text{NIRS} \)) was good (Table 2). Although we found a higher coefficient of variation, our results are supported by De Blasi et al. (11) who studied the reproducibility of \( \text{FBF} \_\text{NIRS} \). They applied three to five repetitive venous occlusions with a 30-s interval between each measurement and found a coefficient of variation of 10.0 ± 5.5% for \( \text{FBF} \_\text{NIRS} \) and 6.2 ± 4.1% for \( \text{FBF} \_\text{pleth} \). Therefore, we conclude that \( \text{FBF} \_\text{NIRS} \) is a valid method to measure local flow.

Influence of Depth \( [\text{muscVO}_2(\text{NIRS} \_\text{AO}) \text{ and } \text{FBF} \_\text{NIRS}] \)

Table 4 shows a consistent difference between both depths, present in both \( \text{muscVO}_2 \) and \( \text{FBF} \) during rest and in \( \text{FBF} \) during exercise. During rest, \( \text{muscVO}_2 \) and \( \text{FBF} \) were slightly higher (\( P \leq 0.01 \)) in the superficial region of the FDS compared with the deeper region. \( \text{muscVO}_2 \) during exercise increased more than fivefold for both measurements, and this eliminated the difference in \( \text{muscVO}_2 \) between superficial and deep. The difference between superficial flow and deep flow that was present in rest was maintained during exercise. The flow increase was the same for both depths, but flow did not increase with the same factor as the \( \text{muscVO}_2 \). The high demand for \( \text{O}_2 \) must, therefore, be partly met by an increase of \( \text{O}_2 \) extraction from the blood.

---

**Fig. 2.** Average and SD for NIRS \( \text{muscVO}_2 \) vs. NIRS FBF showing an increased \( \text{muscVO}_2 \) in the active flexor digitorum superficialis muscle (FDS) during isometric handgrip exercise with interoptode distances of 35 and 50 mm (IO 35 and IO 50, respectively) compared with the relatively inactive brachioradialis muscle (BR). Circles represent resting values, and triangles represent values during exercise. Significant differences are shown in Table 4.
The reason for the difference in O$_2$ consumption at rest in relation to the depth of the measurement is unclear. It might be related to local and/or temporary differences in relation to the activity level of that specific part of the muscle.

**Influence of Place [muscVO$_2$(NIRS,wo) and FBF$_{NIRS}$]**

Concerning the measurement place, no significant difference was found in muscVO$_2$ between FDS and BR during rest. At the transition from rest to exercise, muscVO$_2$ in the BR did not increase as much as that in the FDS. Although this difference in muscVO$_2$ during exercise was expected because we localized the FDS as the most active muscle during handgrip exercise (unpublished 128-channel surface electromyogram data) and because the function of the BR is not directly related to handgrip exercise, it is the first time that these local differences in muscVO$_2$ are actually quantified. The flow increase was roughly the same for both muscles. In the case of the BR, the increase in O$_2$ consumption is equally matched by an increase in delivery. A possible explanation for the lag in delivery in relation to the consumption in the FDS might be an impaired flow within the muscle due to the increased intramuscular pressure enforced by the contracting muscle. It is known that the capillaries within the exercising muscle will be compressed when exercise exceeds 25–30% MVC, which will lead to obstruction of the blood flow (3, 22, 26). These findings, however, give an estimation of the flow in the total limb whereas NIRS is focused on the local flow within one muscle. If the flow in the total arm becomes obstructed at 25–30% MVC, it might be reasonable to assume that the local flow in the active muscle will be impeded at lower work intensities. This is supported by Barcroft and Millen (3), who hypothesized that ischemia and hyperemia might both be present in the limb as a result of considerable differences in contraction strength from one muscle to another.

**Comparison of Fick and NIRS Arterial Occlusion Methods**

According to Table 4, we found a consistent difference between both methods, present in both muscVO$_2$ and FBF. During rest, muscVO$_2$ and FBF were higher according to the Fick method compared with the NIRS measurements. FBF$_{pleth}$ during exercise was also higher than FBF$_{NIRS}$. The difference in muscVO$_2$ between both methods reversed during exercise, resulting in a high muscVO$_2$ measured by NIRS. As for the local muscVO$_2$ measured by NIRS (FDS), it might be expected to find a higher value during exercise compared with the Fick method because the Fick method will reflect an average value of muscVO$_2$ in the forearm. The exercise performed was light-intensity work and was mainly generated by the FDS muscle, probably without much support from other forearm muscles. Therefore, local muscVO$_2$ can increase more than fivefold, whereas the increase of muscVO$_2$ in the total forearm is only twofold.

The lower NIRS muscVO$_2$ during rest compared with Fick muscVO$_2$ is less clear. On the basis of the hypothesis of local vs. global measurement, no difference in resting muscVO$_2$ between both methods was expected. It implies a higher muscVO$_2$ elsewhere in the forearm, but we did not find this higher muscVO$_2$ either in the deeper region of the FDS or in the BR muscle. This higher muscVO$_2$ will probably not been found in skin tissue or bone tissue either. Therefore, we conclude that the difference between NIRS and Fick is not physiological but must have its origin somewhere else. A methodological explanation might be found in a systematic discrepancy between FBF$_{pleth}$ and FBF$_{NIRS}$: FBF$_{pleth}$ triples the flow that is measured with NIRS. This difference was present both in rest and during exercise. Our values for blood flow obtained by plethysmography were comparable with previous observations of blood flow in resting muscle (11, 14, 21, 34, 35, 45). The discrepancy that we found between FBF$_{pleth}$ and FBF$_{NIRS}$ is in agreement with De Blasi et al. (11), who found that FBF$_{pleth}$ was almost twice as high as FBF$_{NIRS}$ measured on top of the BR muscle and correlation between both methods was good.

Variations in flow measurements might be due to a heterogeneous distribution of flow (14) or fluctuations of blood flow over time, but there are also some methodological differences between plethysmography and NIRS. Plethysmographic flow reflects the total flow of the forearm. Apart from blood flowing through skeletal muscle, it contains blood coming from cutaneous tissues, bone, and tendons and might thus lead to a higher FBF$_{pleth}$. NIRS flow reflects only the local flow in the NIRS region of interest. Furthermore, NIRS is limited to monitoring capillaries that have a diameter smaller than ~1 mm because of the absorption of light in vessels with larger diameters (28). During rest, only part of the capillaries are perfused, and most of the blood flows through metarterioles or arteriovenous anastomoses. This blood will bypass the capillaries on its way from the arterial to the venous side of the circulation and will only partly contribute to the NIRS signal. Compared with FBF$_{pleth}$, the FBF$_{NIRS}$ will be underestimated and the muscVO$_2$ calculated from Fick will be overestimated. In addition, because of the lower hematocrit in capillaries, the FBF$_{NIRS}$ will also be underestimated (11, 25).

**Overall Observations**

Overall, we see that, at the transition from rest to sustained isometric handgrip exercise at a workload of 10% MVC, the blood flow increased homogeneously despite the difference in flow between different muscles and between different methods. This is not the case for O$_2$ consumption because the increase during exercise depends on the muscle that is monitored as well as on the method used. Local O$_2$ consumption is high in the active muscle and much lower in the relatively inactive muscle. This is in accordance with basic exercise physiology and is directly and noninvasively detectable by NIRS. The value for muscVO$_2$ as mea-
sured by the combination of blood samples, POX, and plethysmography lies in between the consumption value of the active and inactive muscle that we measured. This is in agreement with the assumption that the Fick method represents the average value for O$_2$ consumption in the total forearm as determined by blood sampling from mixed venous blood and the measurement of total blood flow.

The increase in FBF at the transition from rest to low-intensity work was roughly 1.4, whereas the average (Fick method) increase in muscV˙O$_2$ was 2.0. Thus the increase in O$_2$ consumption was higher than the increase in flow and, apparently, the increased demand for O$_2$ is met by an increase in extraction of O$_2$ from the blood. When we look at the NIRS data during exercise in the FDS compared with the BR, we see that in the active FDS the increased demand for O$_2$ is mostly met by an increase in extraction, whereas in the relatively inactive BR it is almost completely met by the increase in flow. This is in agreement with De Blasi et al. (11), who also found an increase in muscV˙O$_2$ that was many times greater than the increase in flow after a period of ischemic exercise.

In conclusion, NIRS is a suitable tool to give new insight into the heterogeneity of local muscle metabolism. We have shown that NIRS is able to discriminate between the resting and exercising states of the muscle. With the use of two independent simultaneously operating NIRS systems, the technique also discrimi- nates between physically active and less active muscle. O$_2$ consumption measured by the global Fick method during exercise lies in between our NIRS results measuring local O$_2$ consumption in the active FDS and the less active BR muscle. Furthermore, it is shown that the increase in blood flow during exercise is much more homogeneous compared with the local increase in muscV˙O$_2$

We thank Jos Evers, Maria Hopman, Berend Oeseburg, and Marjo van de Ven for their assistance in the blood sampling, Gea Drost for electromagnetic measurements, and Ruurd de Graaf and Sabine van der Bosch for statistical assistance.

This research was supported in part by European Union contract BMH4-CT96.1658.

REFERENCES
