Near-infrared spectroscopy and indocyanine green derived blood flow index for noninvasive measurement of muscle perfusion during exercise

Helmut Habazettl,1,2 Dimitris Athanassopoulos,3,4 Wolfgang M. Kuebler,1,2 Harrieth Wagner,5 Charis Roussos,3 Peter D. Wagner,5 Juergen Ungruhe,1 Spyros Zakynthinos,3 and Ioannis Vogiatzis3,4

1Institute of Physiology, Charité Campus Benjamin Franklin, Berlin; 2Institute of Anesthesiology, German Heart Institute, Berlin, Germany; 3Department of Critical Care Medicine and Pulmonary Services, Evangelismos Hospital, “M. Simou, and G.P. Livanos Laboratories,” National and Kapodistrian University of Athens; 4Department of Physical Education and Sport Sciences, National and Kapodistrian University of Athens, Greece; and 5Department of Medicine, University of California San Diego, La Jolla, California

Submitted 10 November 2009; accepted in final form 25 January 2010

Near-infrared spectroscopy and indocyanine green derived blood flow index (MBF) in humans is an important tool for exercise physiology studies. The traditional indicator-dilution method, which relies on Fick’s principle after arterial (or antecubital venous) injection of a tracer bolus into a limb and subsequent monitoring of muscle effect venous tracer efflux, has the disadvantages of requiring arterial and muscle venous cannulation and measures perfusion of the whole limb without differentiating between limb tissues, such as working and nonworking muscle, skin, or fat (2).

Near-infrared spectroscopy (NIRS) combined with the tracer indocyanine green (ICG) has recently been validated for measurement of regional MBF during exercise (2). This technique makes use of the characteristics of near-infrared light (λ = 700–1,000 nm) to penetrate deeply into biological tissue and measure concentrations of light-absorbing chromophores. For perfusion measurements the passage of ICG through the tissue is monitored noninvasively by NIRS probes taped to the skin overlying the muscles of interest. The most commonly used algorithm to analyze the tracer requires simultaneous recording of the ICG concentration curve in arterial blood as the input function (2). Thus, although NIRS itself is noninvasive, the measurement requires arterial cannulation and continuous withdrawal of blood through a photodensitometer for several seconds after injection. Alternatively, a fiber optic catheter may be introduced into the artery. Recently a noninvasive pulse dye densitometry technique for measuring arterial ICG concentration has been introduced (7) and applied for measurement of cerebral blood flow in anesthetized piglets (3, 13) or anesthetized humans (5). However, the reliability of this technique has recently been challenged (12). Considering that arterial cannulation, which is associated with the potential risks of bleeding, vascular perforation, or clotting, is not feasible in all situations or because the equipment for constant rate blood withdrawal and measurement of ICG may not be available, an alternative algorithm has been proposed to calculate tissue perfusion from the NIRS data, namely the blood flow index (BFI). This index is calculated by dividing the ICG peak concentration by the time for ICG to reach peak concentration. Kuebler et al. (10) validated the BFI for determining cerebral perfusion in pigs against simultaneously measured regional blood flow derived from the radioactive microsphere technique. Although the BFI does not provide absolute blood flow values, it has been shown to sensitively detect perfusion differences between cerebral hemispheres after an acute ischemic stroke (14), to be well reproducible, and to be suited to detect intraindividual changes in brain blood flow (9, 16). Thus the BFI is well established as a reliable and reproducible noninvasive algorithm for the analysis of NIRS ICG data to detect changes in cerebral perfusion, but its applicability to resting and working muscle remains unknown.

We therefore analyzed a set of MBF data previously obtained during exercise (15) to determine whether the NIRS-ICG derived BFI reflects intraindividual changes in muscle...
perfusion as reliably as blood flow calculated by the Fick principle, 2) correlate individual BFI against blood flow values, and 3) assess interobserver variability in blood flow measurements obtained by the two techniques.

METHODS

Subjects. Ten competitive male cyclists (subject characteristics are given in Table 1) participated in the study, which was approved by the authors’ University Hospital Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. Prior to participation in the study all subjects were informed of any risks and discomforts associated with the experiments and gave written, informed consent.

Study design. Experiments were conducted in two visits. In visit 1, subjects underwent an incremental exercise test to the limit of tolerance (WRmax; for data see Table 1). In visit 2 subjects undertook a graded exercise test. During the graded exercise test subjects completed six bouts of constant-load exercise corresponding to the following targeted intensities: 1) 30% WRmax for 5 min, 2) 60% for 5 min, 3) 70% WRmax for 5 min, 4) 80% WRmax for 5 min, 5) 90% WRmax for 3–4 min, and 6) 100% WRmax for 2–3 min. Between exercise bouts at 30 to 80% WRmax, subjects cycled at a constant work rate of 100 W for 10 min, whereas after completion of exercise bouts at 80 and 90% WRmax subjects rested for 60 min (15).

Catheterization. Subjects were prepared first with arterial and venous catheters for blood flow measurements and blood sampling. With use of local anesthesia (2% lidocaine) and sterile technique, identical catheters oriented in the proximal direction were introduced into the left seventh intercostal space and the other over the left vastus lateralis muscle. The catheters were secured in place by adhesive tape. The cardiac perfusion as reliably as blood flow calculated by the Fick principle, 2) correlate individual BFI against blood flow values, and 3) assess interobserver variability in blood flow measurements obtained by the two techniques.

Table 1. Pulmonary function and maximal exercise data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35 ± 10 (23–45)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 5 (168–183)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 8 (58–83)</td>
</tr>
<tr>
<td>WRmax, W</td>
<td>361 ± 31 (302–413)</td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>62 ± 8 (51–71)</td>
</tr>
<tr>
<td>HRmax, beats/min</td>
<td>179 ± 8 (170–192)</td>
</tr>
<tr>
<td>RER at WRmax</td>
<td>1.18 ± 0.05 (1.12–1.26)</td>
</tr>
<tr>
<td>Vtmax, l/min</td>
<td>159 ± 26 (126–183)</td>
</tr>
<tr>
<td>Vtmax, l/min</td>
<td>3.0 ± 0.3 (2.5–3.4)</td>
</tr>
<tr>
<td>fmax, breaths/min</td>
<td>53 ± 10 (36–65)</td>
</tr>
</tbody>
</table>

Values are means ± SD (range) for 10 subjects. Exercise data depict the results of the 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. WRmax, maximal work rate; VO2max, maximal oxygen uptake; HRmax, maximal heart rate; RER, respiratory exchange ratio; Vtmax, maximal minute ventilation; Vtmax, maximal tidal volume; fmax, maximal breathing frequency.

Data analyses. BFI was obtained as previously described by Kuebler et al. (10) by dividing the NIRS-derived muscle ICG concentration difference (peak height) by the rise time from 10 to 90% of peak (Fig. 1). For measurement of MBF according to the Fick principle (2), muscle ICG concentration difference at time t (ICGm) during dye accumulation in muscle tissue (between 10 and 70% of peak concentration) was divided by the integral under the arterial ICG concentration (ICGa) curve until time t and multiplied by t and a factor k for unit conversion:

\[ \text{MBF} = \frac{k \times \text{ICG}_m \times t}{\int_0^t \text{ICG}_a \, dt} \]

MBF was calculated twice, for two different time points t of the identical ICG concentration curve, and the mean of both values was used. To estimate the interobserver variability of both techniques, BFI and MBF were reanalyzed independently by a second observer who was blinded to the original results.

![Fig. 1. Typical example of a quadriceps muscle indocyanine green (ICG) concentration curve recorded by near-infrared spectroscopy (NIRS) during exercise at 30% limit of tolerance (WRmax). The original tracing (gray line) appears with marked oscillations (at a frequency of 84/min; 1.4 Hz) owing to muscle contraction and relaxation during cycling. Low-pass filtering with a cutoff frequency of 0.5 Hz produced the smoothed curve (black line) that was used for blood flow index (BFI) calculation. Data points at 10 and 90% of ΔICG concentration peak are indicated, and an example of BFI calculation is given.](https://jap.physiology.org/doi/10.1210/jap.2010-0765)
Statistics. Pearson’s correlation coefficient was used to establish associations between variables. The interobserver variability in the BFI and MBF data was analyzed by Bland-Altman plots (1). Data are presented as means ± SE. The level of statistical significance was set at \( P < 0.05 \).

RESULTS

The responses of MBF to exercise have been published and discussed previously (15). They are repeated here to allow for direct comparison with the respective responses of BFI. During exercise mean intercostal MBF and BFI increased with increasing exercise work rate up to 60% \( \text{WR}_{\text{max}} \) reaching a plateau between 60 to 80% \( \text{WR}_{\text{max}} \) (Fig. 2A). At maximal exercise intercostal MBF decreased (\( P = 0.002 \)) compared with exercise at 80% \( \text{WR}_{\text{max}} \) (Fig. 2A). Similarly, quadriceps MBF and BFI increased with increasing exercise intensity up to 60% \( \text{WR}_{\text{max}} \) but then plateaued at higher intensities (Fig. 2B).

Correlation of mean BFI with MBF was excellent, for both intercostal (\( r = 0.98, P < 0.001 \)) and quadriceps muscle groups (\( r = 0.96, P < 0.001 \), Fig. 3) Correlation analyses of individual BFI vs. MBF values showed reasonable agreement for both intercostal and quadriceps muscles (\( r = 0.73 \) and 0.72, \( P < 0.001 \)), albeit with considerable scattering of data points (Fig. 4).

Mean interobserver difference for intercostal muscles MBF was 6.9 ml·min\(^{-1}\)·100 g\(^{-1}\) at a data range from ~8 to 75 ml·min\(^{-1}\)·100 g\(^{-1}\). For quadriceps, muscle mean difference was 6.6 ml·min\(^{-1}\)·100 g\(^{-1}\) at a data range from ~2 to 135 ml·min\(^{-1}\)·100 g\(^{-1}\) (Fig. 5). Wide areas of agreement (±36 and ±56 ml·min\(^{-1}\)·100 g\(^{-1}\) for intercostal and quadriceps muscles, respectively) indicated considerable differences between individual blood flow values determined by both observers.

Mean interobserver differences for BFI (Fig. 6) were small at 1.3 nM/s for both intercostal (data range: 0.5 to 26 nM/s) and quadriceps muscles (data range: 0.2 to 54 nM/s). Individual measurements agreed well with narrow areas of agreement (±3.4 nM/s for quadriceps and ±4.2 nM/s for intercostal muscles). Visual inspection of the Bland-Altman plots indicated that for the quadriceps muscle agreement between observers was better for BFI than MBF values up to 25 nM/s. One observer systematically overestimated BFI compared with the other observer, only at very high blood flow.

DISCUSSION

The main findings of the present analyses are the following: First, mean exercise-induced changes in blood flow to quadriceps and intercostal muscles were equally well reflected by the BFI and by the blood flow measurements calculated according to Fick’s principle. Second, in both muscle groups, individual values for the BFI may differ considerably from the corresponding MBF values. Third, the interobserver variability is considerably smaller for the BFI than for the MBF analysis based on Fick’s principle.

For a balanced comparison of BFI and MBF, the individual methodological strengths and pitfalls of each individual technique need to be taken into account. For both, BFI and MBF, ICG is an exogenous tracer with an almost ideal profile for NIRS because of the following reasons: 1) It is already in clinical use with minimal rates of adverse reactions, 2) it has an absorption peak of 805 nm within the range of the near-infrared spectrum, 3) it follows Lambert-Beer’s law at the concentrations used in vivo, 4) it remains largely within the vasculature due to its binding to plasma proteins, and 5) it can be injected.
up to 50 times before the maximal daily dosage is reached (9).
As in all indicator dilution techniques, homogeneous mixing of ICG with blood is a necessary prerequisite for valid measurements of MBF and BFI. In the present study this was achieved by venous injection of the dye. The turbulent flow patterns in both the right and subsequently the left heart can be considered to guarantee homogeneous distribution of ICG in the blood.

The major advantage of BFI over MBF is that arterial cannulation and measurement of the arterial ICG concentration curve are not necessary because it is calculated solely from the tissue concentration curves which can be obtained noninvasively by NIRS. However, this principle may render BFI particularly vulnerable to variations in the arterial ICG bolus height and kinetics. These may be influenced by the kinetics of dye injection, by the subjects’ blood volume, and by blood flow kinetics in large vessels (2). In the present study the mode of dye injection was highly standardized in that the same person performed all ICG injections. To account for different blood volumes in different subjects, the ICG dose may be adjusted to body weight (10). We used a fixed dose of 5 mg ICG, but body weights were reasonably similar among the lean and fit athletes participating in this study (mean 73.4 kg, range 58 to 83 kg). Normalization of BFI for mean body weight had no major effect on the mean values or on the correlation with MBF (data not shown). In a less homogeneous study populations, however, adjustment of ICG dose to body weight may not be sufficient if major differences in body composition exist. Fat tissue is poorly perfused and does not contribute much to circulating blood volume. However, errors resulting from different body weight or composition would only affect the absolute BFI value but not the exercise-induced changes within the same person.

A possible alternative to arterial cannulation to record the arterial ICG concentration would be the use of a pulse dye densitometer, which may measure arterial dye concentration by employing the same principle as clinically used pulse oximeters, providing one measurement per heart beat (7). This technique has been used to record the arterial input function for NIRS measurement of cerebral blood flow in anesthetized piglets (3, 13) and resting humans (5). However, it has not been validated for use in exercise. In the present study the rapid transit of the dye through muscle tissue at 60% of maximal exercise already required to obtain the area under the arterial ICG concentration curve during the first 1–1.5 s of the arterial dye peak. Since heart rate at this time was ~140 beats per

![Fig. 4. Regression analyses of individual BFI vs. MBF values for intercostal muscles (top) and quadriceps muscle (bottom). Linear regression equations, regression coefficients, and significance levels are given in the figure.](http://www.jap.org/jap108/i03/i03fig04.jpg)

![Fig. 5. Interobserver variability for analyses of MBF by the Fick principle for intercostal (top) and quadriceps muscles (bottom). Mean differences between the 2 observers are represented by the continuous horizontal lines, the areas of agreement (mean ± 1.96 SD) by the dotted lines.](http://www.jap.org/jap108/i03/i03fig05.jpg)
in Innovative Methodology, only 2–3.5 data points would be available to estimate the area under the curve. In addition, Reekers et al. (12) recently validated the precision of the pulse dye densitometry method to obtain cardiac output measurements in patients using the finger probe of the device, which was highly sensitive to motion artifacts, which would pose a serious problem when used during strenuous exercise. We conclude that pulse dye densitometry may be an alternative to the Fick principle. The major advantage of using BFI over the Fick principle is the additional benefit of a lesser variability between observers. Although mean differences between observers were reasonably small for both methods, MBF and BFI, the limits of agreement are considerably wider for MBF compared to BFI. The source of greater individual MBF data variability may lie in the above-mentioned random errors resulting from aligning times of peak onset. This procedure may be a source of random errors that may contribute to the variability of individual MBF values, especially when oscillations of the ICG concentration curve associated with muscle contraction obscure the onset of the peak. In contrast, BFI calculation uses time interval between 10 and 90% of peak maximum and ICG tissue concentration difference, which are less prone to reading errors.

An important quality feature of measurement and data analyses techniques is a low interobserver variability. We assessed this with Bland-Altman plots, which were originally developed to assess the agreement between two clinical measurement techniques (1) but may also be used to assess interobserver variability (11). Although mean differences between observers were reasonably small for both methods, MBF and BFI, the limits of agreement are considerably wider for MBF compared with BFI. The source of greater individual MBF data variability may lie in the above-mentioned random errors resulting from aligning times of peak onset of the arterial and muscle ICG concentration curves.

In conclusion, BFI derived from NIRS using ICG faithfully reflects exercise-induced mean changes in muscle perfusion within a group of subjects, but individual values may vary considerably vs. the reference technique of MBF calculation. Hence, BFI may present a valid substitute for the NIRS measurement of MBF by the Fick principle in studies focusing on mean changes in subject or patient cohorts of sufficient size with the advantage of a considerably reduced invasiveness and the additional benefit of a lesser variability between observers.
Yet caution is required when BFI is used for diagnostic or prognostic purposes in individual patients.

ACKNOWLEDGMENTS

We are grateful to Ludwig Schleinkofer from Hamamatsu Photonics Deutschland GmbH and John Pastelas from Bio Pro L. T. D. Hellas for loaning us the NIRO 200 spectrophotometer (Hamamatsu Photonics KK, Hamamatsu, Japan).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES