BLOOD FLOW INDEX USING NEAR INFRARED SPECTROSCOPY AND INDOCYANINE GREEN AS A MINIMALLY INVASIVE TOOL TO ASSESS RESPIRATORY MUSCLE BLOOD FLOW IN HUMANS

Jordan A. Guenette¹, William R. Henderson¹,², Paolo B. Dominelli¹, Jordan S. Querido¹, Penelope M. Brasher³, Donald E.G. Griesdale²,⁴, Robert Boushel⁵ and A. William Sheel¹

¹ School of Human Kinetics, University of British Columbia, Vancouver, BC, Canada
² UBC Program of Critical Care Medicine, University of British Columbia, Vancouver, BC, Canada
³ Centre for Clinical Epidemiology and Evaluation, VCH Research Institute, University of British Columbia, Vancouver, BC, Canada
⁴ Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada
⁵ Center for Healthy Aging, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

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Correspondence:

Jordan A. Guenette, Ph.D.
Respiratory Investigation Unit
Department of Medicine
Queen’s University and Kingston General Hospital
76 Stuart Street
Kingston, ON, K7L-2V7
Tel: 613.549.6666 ext. 4033
Fax: 613.548.1307
Email: guenette@queensu.ca

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ABSTRACT

Near infrared spectroscopy (NIRS) in combination with indocyanine green (ICG) dye has recently been used to measure respiratory muscle blood flow (RMBF) in humans. This method is based on the Fick principle and is determined by measuring ICG in the respiratory muscles using transcutaneous NIRS in relation to the [ICG] in arterial blood as measured using photodensitometry. This method is invasive since it requires arterial cannulation, repeated blood withdrawals and re-infusions. A less invasive alternative is to calculate a relative measure of blood flow known as the blood flow index (BFI), which is based solely on the NIRS ICG curve, thus negating the need for arterial cannulation. Accordingly, the purpose of this study was to determine if BFI can be used to measure RMBF at rest and during voluntary isocapnic hyperpnea at 25, 40, 55 and 70% of maximal voluntary ventilation in 7 healthy humans. BFI was calculated as the change in maximal ICG concentration divided by the rise time of the NIRS derived ICG curve. Intercostal and sternocleidomastoid muscle BFI were correlated with simultaneously measured work of breathing and electromyography (EMG) data from the same muscles. BFI showed strong relationships with the work of breathing and EMG for both respiratory muscles. The coefficients of determination (R²) comparing BFI vs. the work of breathing for the intercostal and sternocleidomastoid muscles were 0.887 (P < 0.001) and 0.863 (P < 0.001), respectively, whereas the R² for BFI vs. EMG for the intercostal and sternocleidomastoid muscles were 0.879 (P < 0.001) and 0.930 (P < 0.001), respectively. These data suggest that the BFI closely reflects RMBF in conscious humans across a wide range of ventilations and provides a less invasive and less technically demanding alternative to measuring RMBF.
INTRODUCTION

The ability to measure human respiratory muscle blood flow (RMBF) has the potential to provide insight into a number of physiological and pathophysiological conditions. Unfortunately, the complex anatomical arrangement and vascular network of the respiratory muscles imposes major challenges to measuring RMBF. One of the only measurements of human RMBF required highly invasive methods and iatrogenic sedation (3). In this study of three patients with respiratory failure, the effects of dopamine on diaphragmatic blood flow was determined by timed volume collection of left inferior phrenic venous effluent. While demonstrating that diaphragm blood flow can be altered pharmacologically, this study provides little insight into RMBF regulation during physiological stresses such as exercise. Accordingly, much of what we know about RMBF during exercise or high ventilations stems from animal investigations (17, 20, 23, 31, 37, 38). To overcome these narrow experimental conditions, a more versatile method was needed to quantify RMBF in humans. The first study to measure RMBF in conscious humans utilized the light absorbing tracer indocyanine green (ICG) in combination with transcutaneous near-infrared spectroscopy (NIRS) of the intercostal muscles (12). Guenette et al. (12) measured the accumulation of ICG in the respiratory muscle using NIRS while the concentration of ICG in the arterial blood was measured using photodensitometry. This technique has the advantage of being able to quantify absolute muscle blood flow with simultaneous measures of cardiac output. This method has been used previously to assess brain and skeletal muscle hemodynamics and oxygenation in humans and animals (6, 32, 34, 36). However, the practicality and safety of this technique is limited due to the need for arterial cannulation, blood withdrawals and blood re-infusions. Complications associated with arterial cannulation include hemorrhage, infection, ischemia, vascular insufficiency, embolization, thrombosis and injury to adjacent nerves (7).
The blood flow index (BFI) method is a minimally invasive alternative and was first developed for rapid and repeated bedside estimation of cerebral blood flow (18). The BFI is derived only from the transcutaneously measured NIRS ICG curve and not from the arterial ICG curve. Thus, the only invasive component of this technique is venous cannulation for bolus injection of the ICG tracer. It should be noted however, that the BFI is a relative measure of blood flow as absolute flow cannot be determined unless arterial ICG concentration is measured. BFI has traditionally been used for the assessment of cerebral blood flow (4, 27, 41, 45) but has recently been applied to skeletal muscles (13). In a retrospective analysis, Habazettl et al. (13) compared BFI values in the vastus lateralis and 7th intercostal space against absolute muscle blood flow determined using NIRS and ICG. These authors demonstrated excellent agreement between BFI and NIRS derived absolute muscle blood flow values. However, these results must be interpreted cautiously because the BFI and absolute muscle blood flow values are both derived, either partially or entirely from the NIRS-ICG concentration curves. Thus, these statistical correlations may be confounded due to mathematical coupling between variables (29, 46). Mathematical coupling in correlation analysis occurs when one variable either directly or indirectly contains the whole or components of the second variable (1). Nevertheless, the study by Habazettl et al. (13) provides an important first step for using BFI as a minimally invasive tool to assess skeletal muscle blood flow in humans. The purpose of the present study was to extend these observations to determine if BFI can be used to specifically assess blood flow to respiratory muscles in conscious humans using a prospective experimental design which avoids issues of mathematical coupling. BFI from the intercostal and the sternocleidomastoid muscles were measured during voluntary isocapnic hyperpnea across a wide range of ventilations (i.e., from rest to levels commonly experienced during strenuous exercise) with simultaneous measurements of electromyography (EMG) and the work of breathing. We hypothesized that respiratory muscle BFI
values would be linearly related to the work of breathing and EMG and would therefore demonstrate that the BFI method is a suitable and sensitive method for assessing RMBF in humans.

**METHODS**

**Subjects:** Eight healthy and physically active subjects (7 males and 1 female) were recruited to participate in this study. One male subject was excluded from the analysis because of technical problems with the NIRS device during experimental testing. Therefore, the data reported throughout the manuscript comes from seven subjects (6 males and 1 female). Subjects were excluded from participating if they were smokers or had a history of cardiopulmonary disease. Subjects with nasal septum deviation, esophageal ulcers or allergies to local anaesthetics, penicillin, sulfa drugs, iodine and radiology contrast dye were also excluded from participating. The subjects gave informed written consent and all experimental procedures received institutional ethical approval and conformed to the Declaration of Helsinki.

**Protocol:** Subjects were seated comfortably in a chair while breathing through a two-way non-rebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO). They then performed three 12 second maximal voluntary ventilation (MVV) manoeuvres with the highest value used to calculate the target ventilations, which corresponded to 25, 40, 55 and 70% of MVV. We used these target ventilations in addition to resting measurements in order to have evenly spaced conditions across the physiological range (i.e., from rest to levels that would be encountered during strenuous exercise). Following 10 minutes of resting breathing, subjects then randomly performed voluntary isocapnic hyperpnea at the 4 target ventilations for 4 minutes separated by 10 minutes of recovery between bouts. Subjects were required to maintain the target minute ventilation but were not required to adopt
a specific breathing pattern. Subjects received continuous real-time feedback of their minute ventilation by viewing a computer screen placed directly in front of them. Ventilatory parameters, esophageal pressure, EMG and NIRS were continuously monitored throughout each hyperpnea bout and are described in detail below.

**Flow, volume, pressure and CO₂:** Inspiratory and expiratory flow were monitored using separate pneumotachographs (model 3813, Hans Rudolph, Kansas City, MO) connected to each end of a two-way non-rebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO). The flow signals were then summed to obtain bi-directional flow and then integrated to obtain volume. Mouth pressure was monitored at a port located in the mouthpiece while esophageal pressure was measured using a balloon-tipped catheter (no. 47-9005, Ackrad Laboratory, Cranford, NJ), both of which were attached to calibrated piezoelectric pressure transducers (± 100 cmH₂O; Raytech Instruments, Vancouver, BC, Canada). Esophageal pressure measurements were performed according to standard techniques and are described elsewhere (11). Trans-pulmonary pressure was calculated as the difference between esophageal and mouth pressure. Breath-by-breath CO₂ was continuously monitored throughout the experiment through a port located in the mouth piece. Partial pressure of end-tidal CO₂ (PETCO₂) was maintained by the manual addition of 100% CO₂ into the inspiratory circuit.

**Electromyography:** EMG of the sternocleidomastoid and intercostal muscles was measured using surface electrodes (Soft-E H59P: Kendall-LTP, Chicopee, MA, USA). Sternocleidomastoid EMG electrodes were placed between the mastoid process and the medial end of the clavicle and intercostal EMG electrodes were placed on the 7th intercostal space along the anterior axillary line on the right side of the body. EMG signals were amplified, band-pass filtered and the analog signals were A/D converted.
**Near Infrared Spectroscopy:** Dual-channel laser diodes with emitting and receiving optodes were carefully placed over the sternocleidomastoid and intercostal muscles on the left side of the body. Position of the NIRS optodes corresponded to the same position as the EMG electrodes on the right side of the body. The optode separation distance for both muscles was 4 cm; corresponding to a penetration depth of ~ 2 cm. Optodes were connected to a NIRO 300 spectrophotometer (Hamamatsu Photonics KK, Hamamatsu, Japan) which was used to measure ICG (Pulsion ICG, ViCare Medical) concentration following a bolus injection of ICG into a forearm vein through a 16 gauge catheter that was previously inserted using sterile technique. A bolus injection consisted of 5mg of ICG (4mg for the female subject) followed by a 20ml flush of 0.9% normal saline through an inline port into a standard 20cm length of intravenous tubing. NIRS detected the ICG by measuring light attenuation at four wavelengths and was analyzed using an algorithm incorporating the modified Beer-Lambert law (6). Since the measured light attenuation in the tissue is influenced by ICG and oxy- and deoxyhemoglobin, the independent contribution of ICG to the light-absorption signal was isolated using a matrix operation that incorporated pathlength-specific extinction coefficients for each of the light-absorbing chromophores (hemoglobin + myoglobin and ICG) at each wavelength employed by the NIRS machine (Hamamatsu Photonics KK) using a similar approach as previously described by Kuebler et al. (18). ICG injections were made at the end of the 10 minute resting period and in the final minute of each 4 minute bout of isocapnic hyperpnea.

**Data Processing:** All ventilatory and respiratory pressure data were collected using a 16-channel data acquisition system (PowerLab/16SP model ML 795, ADI, Colorado Springs, CO) and recorded simultaneously (Chart v6.1.3, ADInstruments, Colorado Springs, CO) at 1000Hz. NIRS data was sampled at 6 Hz and time synchronized to the ventilatory and respiratory pressure data.
**Data Analysis:** The work of breathing was measured as the area within the trans-pulmonary pressure-volume loop with the addition of that portion of a triangle describing the work that fell outside the loop representing part of the elastic work of breathing (26). The work of breathing was then multiplied by breathing frequency and converted into joules per minute and separated into the inspiratory and total (inspiratory + expiratory) work of breathing. EMG data was rectified, low pass filtered (30-50Hz) and integrated. The intercostal EMG was integrated for each inspiration and expiration whereas the sternocleidomastoid EMG was only integrated during inspiration. The integrated EMG (iEMG) signals were averaged over 30 seconds and then multiplied by the breathing frequency. EMG was multiplied by breathing frequency in order to provide an overall index of total muscle activation during a given bout of hyperpnea (i.e., muscle activity for a given contraction multiplied by the number of contractions per minute). The EMG data were then expressed as a percentage of the maximal iEMG values obtained during the MVV manoeuvres. BFI was calculated as the maximal change in ICG ($\Delta$ICG$_{\text{max}}$) divided by the rise time (Figure 1). The $\Delta$ICG$_{\text{max}}$ was calculated as the peak ICG value minus the baseline value (mean of 15 seconds prior to the rise) and the rise time was defined as the time interval between 10 and 90% of the $\Delta$ICG$_{\text{max}}$ (33), thus eliminating the need for exact temporal definition of the start and end of the ICG washin. NIRS derived ICG curves were filtered using a Butterworth filter prior to calculating the BFI. The breaths used for the work of breathing and EMG analysis corresponded to the identical 30 second window of data in which the bolus of ICG was injected.

**Statistical Analysis:** Random-coefficients regression was used to model the relationship between BFI and each of EMG and work of breathing for the intercostal and sternocleidomastoid muscles and also for modelling the relationship between EMG and the work of breathing for each muscle. This approach accounts for the within-subject correlation arising from the repeated measurements on an
individual. To characterize the strength of the relationships we calculated $R^2$ according to the method of Xu (47). We assessed the assumptions underlying the regression models using residual plots. No departures from the assumed models were evident. The total work of breathing (inspiratory + expiratory work) was used in the regression analysis of the intercostal muscles as we assumed that intercostal blood flow reflected both inspiratory and expiratory intercostal muscles (12). We used inspiratory work of breathing in the regression analysis for the sternocleidomastoid muscle as the sternocleidomastoid is an inspiratory muscle. Data are presented as means ± SD.
RESULTS

Subject Characteristics: The subjects were all young, healthy, lean and physically active. The age, height, mass and BMI of the subjects were 28 ± 6 (range: 22-40 yrs), 176 ± 11 (156-187 cm), 69 ± 11 (51-85 kg) and 22.2 ± 1.6 (19.7-24.8 kg·m⁻²) respectively. The procedures and protocols were well tolerated by all subjects and there were no adverse reactions to ICG.

Hyperpnea Protocol: The MVV of the subjects was 164 ± 25 l·min⁻¹ (range: 120 – 194 l·min⁻¹). Resting minute ventilation was 9.4 ± 3.7 l·min⁻¹. Actual ventilations corresponding to the target values of 25, 40, 55 and 70% MVV were 41.5 ± 5.0, 65.0 ± 9.7, 87.9 ± 13.4 and 110.4 ± 15.3 l·min⁻¹, respectively. Throughout the protocol, the PETCO₂ was maintained within 2-3 mmHg of resting values (43.3 ± 2.3 mmHg). PETCO₂ at 25, 40, 55 and 70% of MVV were 41.1 ± 2.7, 41.2 ± 2.7, 40.8 ± 2.8 and 41.8 ± 3.8 mmHg, respectively.

Blood Flow, Work of Breathing and EMG Responses: The total work of breathing and BFI responses for the intercostal and sternocleidomastoid muscles are shown in Figure 2. Figure 3 shows filtered ICG traces and corresponding raw EMG data for the sternocleidomastoid muscle in an individual subject at rest and during the different ventilatory conditions. The characteristics of these ICG curves (i.e., ΔICG_max and rise time) remained relatively constant at rest and during the first two bouts of isocapnic hyperpnea when EMG activity remained minimal. However, as EMG activity of the sternocleidomastoid increased during the last two hyperpnea bouts, there was a considerable change in ICG curve characteristics, consistent with increases in sternocleidomastoid blood flow. Figure 4 shows the individual responses and the average fitted curve for the intercostal and sternocleidomastoid BFI vs. iEMG and the work of breathing. The R² relating BFI vs. the work of
breathing for the intercostal and sternocleidomastoid muscles were 0.887 ($P < 0.001$) and 0.863 ($P < 0.001$), respectively, whereas the $R^2$ for BFI vs. EMG for the intercostal and sternocleidomastoid muscles were 0.879 ($P < 0.001$) and 0.930 ($P < 0.001$), respectively. The $R^2$ relating EMG vs. the work of breathing for the intercostal and sternocleidomastoid muscles were 0.925 ($P < 0.001$) and 0.683 ($P < 0.001$), respectively. Individual $R^2$ values are shown in Table 1. The low $R^2$ value for subject 5 occurred because respiratory muscle recruitment strategy changed during the 55%MVV hyperpnea bout. He was able to maintain the target ventilation resulting in a progressive and typical work of breathing response (Figure 5 panel A). This unusual change in respiratory muscle recruitment underestimated the relationship between BFI and the inspiratory work of breathing in this subject because BFI spiked at the 55%MVV condition (Figure 5 panel B). This did not influence the EMG regression because the EMG detected the increased recruitment of the sternocleidomastoid muscle (Figure 5 panel C). Another outlier in Table 1 for the intercostal muscle was due to a large spike in blood flow at the 40%MVV condition in subject 6. We are unable to explain this single outlier but it may have been due to an experimental error given the fact that there was still a strong linear relationship between the work of breathing and intercostal EMG in this subject. There was no visible intercostal or sternocleidomastoid EMG activation at rest. Sternocleidomastoid EMG always occurred during inspiration but intercostal EMG had variable responses with some subjects showing EMG activity on inspiration, some on expiration and some with a combination of EMG activity on inspiration and expiration. Figure 6 shows individual examples of the variable intercostal EMG response. Panel A shows intercostal EMG activity during the expiratory phase and Panel B shows intercostal EMG activity on inspiration and expiration in the same subject at a higher ventilation. Panel C is an individual example of intercostal EMG activity during inspiration with slight activation on expiration.
DISCUSSION

This is the first study to examine the inter-relationship between muscular recruitment, perfusion and mechanical work produced by human respiratory muscles across a wide range of physiologically-relevant ventilations. We found that progressive increases in the mechanical work of breathing and EMG result in linear increases in blood flow to the sternocleidomastoid and intercostal muscles. The present study demonstrates that RMBF can be measured using the BFI method and is the first to measure blood flow to an accessory respiratory muscle (i.e., sternocleidomastoid) in humans. The BFI method is a sensitive and attractive alternative to more invasive techniques for measuring RMBF and has the potential to answer a number of novel questions pertaining to RMBF regulation in both health and disease.

Respiratory Muscle Blood Flow Distribution. Under resting conditions we observed little EMG in the seventh intercostal space (see Figure 6) and this corresponded with a low work of breathing and blood flow index. This observation is in agreement with the findings of De Troyer et al. (8) who used intramuscular electrodes to compare the neural drive to the dorsal portion of the external intercostal muscle in the third, fifth and seventh interspaces during resting breathing. All subjects demonstrated inspiratory activity in the third and fifth interspaces. EMG activity was largely absent in the seventh interspace although there was consistent phasic inspiratory activity in one subject. They also report inspiratory discharges from the dorsal external intercostal during large voluntary inspirations. These findings suggest that there is some between-subject variation in the neural drive to the external intercostals but it is generally distributed along a rostro-caudal gradient. Our findings of progressive and parallel increases in intercostal muscle blood flow index and EMG activity extend the concept of a rostro-caudal gradient. As ventilation increases to levels seen with dynamic exercise, there is greater electrical activity and blood flow in the seventh intercostal space. These increases likely reflect an
increased contribution of the ribcage muscles to the generation of intrathoracic pressure. Like the intercostal muscles, the sternocleidomastoid muscle was also inactive at rest but was recruited progressively with increasing ventilation and work of breathing.

Determining the absolute amount of blood flow directed to the muscles of respiration in humans is difficult because the musculature is largely inaccessible, the active muscle mass can’t be determined accurately and the muscles cannot be studied in isolation. However, detailed measures of respiratory muscle blood flow under dynamic exercising conditions in the pony (21-25) show that whole-body oxygen uptake is increased 38-fold and is accompanied by a 22-fold increase in costal diaphragmatic blood flow. The studies in exercising ponies demonstrate that the respiratory muscles receive approximately 15% of the total cardiac output during maximal exercise, with 8% of the cardiac output directed to the inspiratory muscles and 6% to the expiratory muscles. There is reasonably good agreement amongst studies that have quantified respiratory muscle blood flow in different species (19, 21, 30). The findings from animal studies are also consistent with available estimates in human studies. For example, by reducing or increasing the work of breathing while simultaneously measuring cardiac output and leg blood flow, Harms et al. (15, 16) showed that up to 14-16% of the total cardiac output is directed to the human respiratory muscles to support their metabolic requirements during strenuous exercise. Further study across a range of ventilations with simultaneous measures of multiple respiratory muscles and cardiac output is required to provide a comprehensive quantification of blood flow distribution. The BFI method used in the present study is capable of characterizing relative changes in perfusion to multiple superficial respiratory muscles with a single bolus of ICG. The data from the present study provides the first attempt at measuring RMBF in two regions during the course of voluntary hyperpnea at ventilations commonly experienced during exercise. We demonstrated that blood flow to the sternocleidomastoid and the intercostal muscles increases linearly with increases in minute ventilation. However, it is important to note that these
responses may not be linear during whole-body exercise when the respiratory and locomotor muscles are competing for a limited cardiac output. Further studies are required to characterize patterns and distribution of RMBF in multiple respiratory muscles in humans during dynamic exercise.

**Current methods of measuring human RMBF:** Aubier et al. (3) estimated diaphragm blood flow by timed volume collections of left inferior phrenic venous effluent. Three patients who were hospitalized for acute respiratory failure were studied to determine the effects of dopamine on diaphragmatic blood flow. Experimental animal studies have shown that under physiologically relevant conditions, such as dynamic exercise, there is heterogeneous distribution of blood flow where large quantities of blood are directed to the respiratory musculature (9, 23). Until recently, our understanding of RMBF in humans has been limited to patient-based studies (3), indirect estimates (16) and inference from animal investigations (17, 20, 31, 35, 37, 38). The first attempt to measure RMBF in conscious humans utilized NIRS and the tracer ICG in combination with photodensitometry to quantify absolute RMBF and was subsequently used for experimental studies examining RMBF regulation during exercise (2, 42, 44). This technique has previously been validated for skeletal muscle blood flow measurements in humans using dye dilution in combination with magnetic resonance imaging measures of muscle volume (6) and has the major advantage of quantifying absolute muscle blood flow. Unfortunately, this method has not been widely adopted due to its invasiveness. Moreover, the photodensimetric method requires considerable technical expertise and it involves specialized equipment that is not widely available. BFI is an alternative method that avoids the need for arterial cannulation and does not require specialized equipment.

**Validation of BFI:** The BFI method is an algorithm based solely on the NIRS derived ICG concentration curves (Figure 1). BFI was first developed by Perbeck et al. (33) using sodium
fluorescein as an indicator substance to determine relative capillary blood flow in the intestine. The BFI was later validated for cerebral blood flow determination using ICG against the gold standard microspheres technique by Kuebler et al. (18). This method has been shown to be a safe and reliable method for repeated bedside measurements of cerebral blood flow (41, 45). However, the reliability of BFI for cerebral blood flow determination remains controversial (40). Validating BFI for RMBF is difficult because a gold standard method does not exist for measuring RMBF in conscious humans. The only available method, utilizes NIRS and ICG in combination with photodensitometry, as previously described. Therefore, validating BFI of the respiratory muscles against absolute blood flow derived from NIRS and ICG may yield artificially strong correlations because both methods are intimately linked mathematically. This was an approach recently used by Habazettl et al. (13) in order to retrospectively validate the BFI method for assessing skeletal muscle blood flow in humans during cycling exercise. In the present study, we chose to use an alternative approach for determining the sensitivity of BFI for measuring blood flow to respiratory muscles. Specifically, we determined the relationship between flow values and physiological parameters that would predictably increase with increasing levels of blood flow. This was the strategy used by Guenette et al. (12) when assessing the utility of NIRS-ICG for initially measuring absolute blood flow to the respiratory muscles. It is well established that blood flow increases in close relation to increases in muscular work, thus reflecting a close match between metabolic demand and oxygen delivery (39). The findings of the present study suggest that BFI is sensitive in detecting changes in respiratory muscle work and muscular activation in association with increasing levels of ventilation. Unique to this study is the fact that we measured respiratory muscle EMG with simultaneous esophageal-pressure derived work of breathing measurements. The work of breathing provides an index of overall respiratory muscle work but is not specific to any one respiratory muscle. Thus, the work of breathing may increase in a predictable fashion as subjects generate the required pressures and volumes to attain their target minute
ventilation. However, the respiratory muscle recruitment strategies used by individual subjects can change which will ultimately influence the perfusion to specific muscles. These changes in respiratory muscle recruitment would not be detectable with the work of breathing measurements alone. This was demonstrated in an individual subject as shown in Figure 5. This individual successfully maintained his target ventilation resulting in a progressive work of breathing response (panel A). However, the large spike in blood flow (panel B) was directly attributable to his unusual change in respiratory muscle recruitment/activation for the 55%MVV hyperpnea bout as shown in the EMG response (panel C). This individual example, coupled with the strong relationships in Figure 4 and Table 1 suggest that the BFI is sensitive in detecting changes in RMBF.

**Advantages of BFI:** BFI has a number of distinct advantages for measuring RMBF in humans over the method described previously for measuring absolute RMBF. The most important advantage is that arterial cannulation is not required and therefore blood is not withdrawn or re-infused into the subject for arterial ICG concentration measurements. This reduces risk to the patient/subject and makes the technique less technically demanding on the experimenters. The algorithm for calculating BFI (Figure 1) is highly reproducible. This was shown by Habazettl et al. (13) who demonstrated that interobserver variability was considerably less for BFI than the method for calculating absolute flow. There are a number of additional advantages of using NIRS and ICG for measuring RMBF. For example, respiratory muscle tissue oxygenation can be monitored in close time sequence to blood flow measurements and if multiple NIRS optodes are available, then blood flow to several muscles can be measured simultaneously with a single intravenous bolus injection of ICG. In addition, ICG is nontoxic and serious adverse reactions are rare (5, 28) and has been reported as approximately 1:250,000 (10). ICG is also completely excreted by the liver and is cleared with a half-time of 3.2 to 3.4 minutes (14). In humans without impaired hepatic function, it is possible to have up to 50
injections of ICG in a day at the given dosage of 0.1mg/kg (18). The physiological importance of being able to measure RMBF in humans has already been demonstrated in initial studies that have applied this technique to study mechanisms of diaphragm fatigue and to assess RMBF limitations during exercise in athletic humans (42, 44) and in patients with chronic obstructive pulmonary disease (43).

**Disadvantages of BFI:** The most notable disadvantage of using BFI for measuring RMBF is that it provides a relative rather than an absolute measure of flow. BFI is proportional to flow but it has an unknown factor of proportionality (18). Therefore, absolute BFI values are comparable on an intraindividual basis but not an interindividuated basis. The reason that the within-subject comparisons can be viewed as quantitative is that, for a given subject, all of the flows would be compared to the same theoretical arterial ICG curve. This is not the case when comparing flows across subjects. Finally, while BFI appears reproducible for the measurement of cerebral blood flow (45), it’s reproducibility for measurements of RMBF has not yet been established.

**Limitations of the present study:** We measured RMBF over the left 7th intercostal space which has been the site of choice for those studies measuring RMBF in humans using the NIRS-ICG approach (2, 12, 13, 42-44). We used this site for two reasons. Firstly, because of its location in relation to the costal diaphragm and because it encompasses several respiratory muscles which may provide an overall assessment of RMBF. Secondly, we used this site to allow for comparisons with the existing literature. Unique to this study is the fact that we are the first to measure RMBF to the sternocleidomastoid muscle which is an accessory inspiratory muscle activated during high ventilatory loads. We can be reasonably confident that our blood flow values are primarily reflecting the sternocleidomastoid given the thickness and superficial nature of this particular muscle. On the other
hand, muscles within the intercostal space impose interpretive challenges due to the complexity of muscles within view of the NIRS optodes over the 7th intercostal space. It is unknown whether blood flow values reflect the internal or external intercostals or if there is a contribution from the costal diaphragm. We observed variable EMG responses for the intercostal muscles during different bouts of hyperpnea between and within subjects. For example, in some trials we observed intercostal EMG activity on both inspiration and expiration and in some subjects we observed EMG activity during inspiration or expiration alone (Figure 6). Therefore, RMBF over the 7th intercostal space likely reflects a combination of flow from the intercostals and perhaps the costal diaphragm. Thus, RMBF measured at the 7th intercostal space most likely represents a global measure of RMBF. For simplicity, we have referred to this as intercostal muscle blood flow throughout the manuscript but acknowledge that additional muscles may also be contributing to the blood flow response.

**Perspectives and Significance:** The present study is the first to simultaneously examine the inter-relationship between muscular recruitment, perfusion and pressure generation from two respiratory muscles in conscious humans. We have shown that intercostal and sternocleidomastoid blood flow increases linearly with increases in both electrical activation and the mechanical work of breathing across a range of ventilations during voluntary hyperpnea in humans. The findings from the present study suggest that NIRS derived BFI is a sensitive and minimally invasive tool for assessing relative changes in blood flow to human respiratory muscles. This method has the potential to further our understanding of respiratory muscle blood flow regulation under various physiological and pathophysiological conditions.
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REFERENCES


### Table 1. Regression analysis for individual subjects

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R² values relating blood flow index (BFI) with integrated EMG (iEMG), inspiratory work of breathing (WOB₁₈₁₉₆) and the total WOB (WOB₉₀₇) for the sternocleidomastoid (SCM) and intercostal (IC) muscles. Due to technical issues, we were unable to obtain IC blood flow values in subject 5.
FIGURE LEGEND

**Figure 1.** Example of a sternocleidomastoid ICG curve in an individual subject. The raw curve (thin line) is filtered using a Butterworth filter. BFI is calculated as the change in maximal ICG concentration ($\Delta \text{ICG}_{\text{max}}$) divided by the rise time. $\Delta \text{ICG}_{\text{max}}$ is the difference between peak ICG and baseline. Baseline is calculated as a 15 second average prior to the rise in ICG. The rise time is determined as the time from 10% to 90% of the $\Delta \text{ICG}_{\text{max}}$.

**Figure 2.** Group mean responses of the total work of breathing (WOB$_{\text{tot}}$), intercostal blood flow index (IC - BFI) and sternocleidomastoid blood flow index (SCM - BFI) during rest and 4 bouts of increasing levels of voluntary isocapnic hyperpnea. Values are mean ± SD.

**Figure 3.** Raw EMG and ICG traces for the sternocleidomastoid muscle in an individual subject at rest and during 4 bouts of voluntary isocapnic hyperpnea. This subject’s sternocleidomastoid blood flow data is also highlighted in Figure 4.

**Figure 4.** Panels A and B show intercostal blood flow index (IC - BFI) vs. intercostal integrated EMG (IC - iEMG) and the total work of breathing (WOB$_{\text{tot}}$), respectively. Panels C and D show sternocleidomastoid blood flow index (SCM - BFI) vs. sternocleidomastoid integrated EMG (SCM - iEMG) and the inspiratory work of breathing (WOB$_{\text{insp}}$), respectively. Thin lines represent individual subjects. Thick line represents the fitted averaged curve. $R^2$ values are from regression analysis which accounts for the within-subject correlation arising from the repeated measurements on an individual. Small arrows represent the individual subject shown in Figure 3.

**Figure 5.** Inspiratory work of breathing (WOB$_{\text{insp}}$), sternocleidomastoid blood flow index (SCM - BFI) and sternocleidomastoid integrated EMG (SCM - iEMG) response in an individual subject during voluntary isocapnic hyperpnea. This subject had an unusual spike in blood flow during a single bout of hyperpnea which was attributable to a significant alteration in SCM recruitment as shown by the EMG response.

**Figure 6.** Individual examples of flow and raw intercostal (IC) EMG responses under various levels of ventilation. This figure demonstrates inspiratory and expiratory recruitment heterogeneity in the 7th
IC space between and within individual subjects. Panel A demonstrates IC EMG activity during the expiratory cycle at 40% MVV and Panel B shows IC EMG activity on inspiration and expiration in the same subject at 55%MVV. Panel C is an individual example of IC EMG activity during inspiration with slight activation on expiration. Positive flow represents expiration; negative flow represents inspiration and grey EMG trace represents resting levels.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.