Near-infrared spectroscopy using indocyanine green dye for minimally invasive measurement of respiratory and leg muscle blood flow in patients with COPD

Zafeiris Louvaris[1,2,3], Helmut Habazettl[4,5], Harrieth Wagner[6], Spyros Zakynthinos[1], Peter Wagner[6] and Ioannis Vogiatzis[1,2,7]

[1]st Department of Critical Care Medicine and Pulmonary Services, GP Livanos and M Simou Laboratories, Medical School of Athens University, Evangelismos Hospital, Athens, Greece.

[2]National and Kapodistrian University of Athens, Department of Physical Education and Sports Sciences. Athens, Greece.

[3]Faculty of Kinesiology and Rehabilitation Sciences, Division of Respiratory Rehabilitation, Department Rehabilitation Sciences KU Leuven, University Hospitals Leuven, Leuven, Belgium

[4]Institute of Physiology, Charite - University Medicine Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Germany.

[5]Institute of Anesthesiology, German Heart Institute Berlin, Berlin, Germany.

[6]Department of Medicine, University of California San Diego, La Jolla, California.

[7]Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University Newcastle, UK.

Running Head: Validation of NIRS-ICG derived BFI in COPD

Corresponding author: Dr. Louvaris Zafeiris (zafeiris.louvaris@kuleuven.be) Rehabilitation and Respiratory Division, UZ Gasthuisberg, Herestraat 49, O&N4 Building, Bus 1510, Leuven 3000, Belgium.

Abstract

Near-infrared spectroscopy (NIRS), measuring indocyanine green (ICG) after its peripheral venous injection, has been validated for minimally invasive measurement of relative muscle blood flow in fit young individuals, but not in COPD. Here we ask whether it could be used to evaluate respiratory and locomotor muscle perfusion in COPD patients. Vastus lateralis muscle blood flow (MBF, the reference method calculated from arterial and muscle ICG concentration curves) and a blood flow index (BFI, calculated using only the (same) muscle ICG concentration curves) were compared in 10 patients (FEV_1:51±6%predicted) at rest and during cycling at 25%, 50%, 75% and 100% of WRpeak. Intercostal muscle MBF and BFI were also compared during isocapnic hyperpnea at rest, reproducing ventilation levels up to those at WRpeak. Intercostal and vastus lateralis BFI increased with increasing ventilation during hyperpnea (from 2.5±0.3 to 4.5±0.7nM/s) and cycling load (from 1.0±0.2 to 12.8±1.9nM/s), respectively. There were strong correlations between BFI and MBF for both intercostal (r=0.993 group mean data, r=0.872 individual data) and vastus lateralis (r=0.994 group mean data, r=0.895 individual data). Fold changes from rest in BFI and MBF did not differ for either the intercostal muscles or the vastus lateralis. Group mean BFI data showed strong interrelationships with respiratory and cycling workload, and whole body metabolic demand (r ranged from 0.913 to 0.989) simultaneously recorded during exercise. We conclude that BFI is a valid and minimally invasive tool for evaluating relative changes in respiratory and locomotor muscle perfusion from rest to peak exercise in COPD patient groups.

News and Noteworthy

We show that non-invasive near-infrared spectroscopic (NIRS) detection of indocyanine green dye (ICG) after peripheral venous injection adequately reflects respiratory and locomotor muscle perfusion during exercise and hyperpnea in COPD patients. Mean, individual, and fold-change responses from rest to exercise or hyperpnea correlated closely with the reference method, which requires arterial sampling. NIRS-ICG is a valid, robust and essentially non-invasive tool for assessing relative changes in respiratory and locomotor muscle perfusion in COPD patient groups.

Keywords: NIRS, Indocyanine Green dye, muscle perfusion, respiratory muscles, exercise, COPD, validation, BFI

Introduction

In patients with Chronic Obstructive Pulmonary Disease (COPD) restrictions in central hemodynamics owing to abnormal lung mechanics (18) can limit perfusion to the working muscles thereby exacerbating the competition between respiratory and locomotor muscles for the available blood flow (1, 5, 13). Measurement of muscle blood flow (MBF) in patients with COPD therefore provides an important tool for investigating the pathophysiological mechanisms involved in exercise intolerance and for assessing the effectiveness of pharmacological or non-pharmacological interventions.

Methods for measuring MBF have been developed over the past 120 years (2). However, the traditional techniques for assessing MBF are highly invasive exposing the individuals to risks and posing difficulties to differentiate working and non working muscles (3). For example, by using NIRS after an intravenous injection of the light-absorbing tracer indocyanine green (ICG), it is possible to measure absolute values of MBF by applying the law of conservation of mass (3, 6). Specifically, the rate of accumulation of a tracer in a given tissue is equal to its rate of inflow minus its rate of outflow. If a tracer is introduced rapidly and its rate of accumulation is measured over time, blood flow can be measured as a ratio of the tracer accumulated to the quantity of tracer introduced over a given time. However, to measure the quantity introduced requires continuous arterial blood sampling over the time of tracer introduction, and thus the need for an indwelling arterial cannula.

Recent studies indicate that in young, fit subjects, relative values of muscle blood flow can be determined by NIRS after intravenous ICG injection, measuring only the accumulation rate of tracer without arterial sampling (7, 8). The result, termed the blood flow index (BFI), was found to reliably reflect local (i.e., within the sampling volume of each NIRS optode) changes in both respiratory and locomotor MBF from rest to maximal ventilation (i.e., ~120 liters/minute) during (isocapnic) hyperpnea or during graded cycling exercise up to maximal levels (as high as 360 watts) (7, 8). However, the validity of this

method has not been investigated in a clinical population. Indeed, due to hemodynamic and muscular differences between healthy subjects and COPD patients (11, 18), the results obtained in healthy, fit subjects may not be transferrable to this patient population.

To this end, we retrospectively analyzed data obtained in a representative population of COPD patients across a wide range of exercise intensities and rates of minute ventilation during cycling and subsequently during isocapnic hyperpnea (16). Accordingly, the purpose of the present study was to examine in COPD patients whether NIRS-ICG derived BFI reflects changes in respiratory and locomotor muscle perfusions as reliably as simultaneously determined MBF calculated by Fick principle based on the law of conservation of mass.

Materials and methods

Subjects

We retrospectively analyzed data from ten clinically stable patients with COPD (FEV_1:51±6% predicted) classified by the Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2016) as stages II (n=4), III (n=3), and IV (n=3) from our previously published work (16). In the original study (16), vastus lateralis and intercostal muscle blood flow (MBF) were simultaneously measured in patients with COPD with the aim to investigate whether during exercise, intercostal MBF competes with locomotor muscles for the available blood flow (16). In the original study, only MBF data were analyzed and presented. In the present analysis, intercostal and vastus lateralis MBF are compared with simultaneously measured blood flow index (BFI) in the same locations. Measurements were made under two separate conditions: from rest to near-peak cycle exercise, and from resting to peak (isocapnic) hyperpnea.

Study Design

The exercise protocols and isocapnic hyperpnea tests during which MBF and BFI were compared, as well as the methods used for assessing the physiologically relevant variables presented in this study, have been previously described (16), and are thus not repeated here.

Intercostal and quadriceps MBF and BFI

Absolute values of intercostal and vastus lateralis MBF during exercise were measured based on the principle of conservation of mass using an equation developed by (3). In brief, for both intercostal and vastus lateralis muscle, ICG concentration difference (measured by NIRS) at time t ($[ICG]_m$) during dye accumulation in muscle tissue (between 10 and 70% of peak concentration) was divided by the integral under the arterial ICG concentration ($[ICG]_a$) curve (measured by linear photodensitometer) until time t and multiplied by t and a factor k for unit conversion as previously described (16):

$$\text{Blood flow (ml/100ml/min}^{-1}) = \frac{k \cdot [ICG]_m \cdot t}{\int_o^t [ICG]_a dt}$$

Relative perfusion (BFI) of intercostal and vastus lateralis muscles during exercise was also obtained. This was done by dividing the NIRS-derived muscle ICG concentration difference (peak height of the ICG concentration curve) by the rise time from 10 to 90% of peak as previously described (7-9). NIRS-derived muscle ICG concentration was expressed in nanomoles per liter (nM). After dividing by the rise time (in seconds) the final unit of measurements of BFI was nM/s (7-9). Representative examples for calculating BFI for intercostal and vastus lateralis muscle from one individual are shown in Figure 1a and Figure 1b, respectively. ICG concentration curves were analyzed by using Chart5 v.5.4.2 (ADInstruments) program (8). NIRS data were sampled at 6 Hz and time was synchronized to the metabolic, ventilatory, mechanical work and respiratory pressure data (16).

Statistical analysis

Data are presented as means ± SEM. Pearson's correlation coefficient (r) was used to establish associations between intercostal and vastus lateralis muscle BFI with MBF and other physiologically related variables. ANOVA with repeated measures and post-hoc comparisons (Tukey's test) were used to identify statistically significant differences across the cycling exercise and isocapnic hyperpnea trials. Analysis of agreement between BFI and MBF measurements was performed by using Bland-Altman analysis. A satisfactory agreement between BFI and MBF was considered when the difference between BFI and MBF measurements did not significantly vary from 0 (zero). For this purpose one sample t-test with testing variable the difference between BFI and MBF measurements and testing value 0 (zero) was performed. For Bland-Altman analysis purposes, both BFI in nM/s and MBF in ml/min/100g were expressed as fold changes from rest in order to allow a comparison between the two methods. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using the SPSS statistical software (v. 20 IBM SPSS Statistics, Chicago, IL, USA).

Results

Patient characteristics

Detailed subject characteristics in terms of demographics, pulmonary function, and peak exercise performance data have been published and discussed previously (16). Not reported previously, patients exhibited impaired functional capacity in terms of the six minute walking distance test (389±31meters, 59±8% predicted), quadriceps muscle strength (28±5kg, 61±6% predicted), and quadriceps muscle endurance, (43±8 seconds 54±5% predicted, respectively).

Perfusion responses to exercise

During the resting isocapnic hyperpnea trial, both intercostal muscle blood flow (MBF) and blood flow index (BFI) increased from: 7.0±0.6 to 11.4±1.3 ml/min/100gr (MBF) and from 2.5±0.3 to 4.5±0.7 nM/s, (BFI). Moreover, both MBF and BFI reached a plateau at 75% of peak minute ventilation (Figure 2a). During the cycling trial, both vastus lateralis MBF and BFI increased progressively with increasing power output: from 2.8±0.4 to 39.7±7.0 ml/min/100gr (MBF) and from 1.0±0.2 to 12.8±1.9 nM/s, (BFI) up to 100% of WRpeak (Figure 2c). When MBF and BFI data were expressed as changes relative to flows at rest, the pattern of increase in muscle perfusion with cycle exercise or with resting hyperpnea did not significantly differ (between MBF and BFI) either for the intercostal or the vastus lateralis muscle groups (Figures 2b and 2d)

Comparison of BFI and MBF

During isocapnic hyperpnea, there was a highly significant and close correlation ($r=0.993$, $P=0.001$) between mean values of intercostal muscle BFI and MBF (Figure 3a). Furthermore, the individual values of intercostal muscle BFI and MBF showed a close linear relationship ($r=0.872$, $P<0.001$, Figure 3b). Normalization of BFI for body weight (BW), body mass index (BMI) and fat free mass index (FFMI) did not meaningfully change the correlation with MBF ($r= 0.829$, 0.856 and 0.837, $P<0.001$, respectively).

During cycling exercise, the correlation between mean vastus lateralis muscle BFI and MBF was also close and highly significant ($r=0.994$, $P=0.001$, Figure 3c). Again, correlation analysis of individual patient vastus lateralis muscle BFI and MBF values showed close association between the two methods ($r=0.895$, $P<0.001$, Figure 3d). Normalization of BFI for BW, BMI and FFMI again did not meaningfully change the individual correlations with MBF ($r=0.798$, 0.843 and 0.809, $P<0.001$, respectively). Individual relationships between intercostal and vastus lateralis muscle BFI with intercostal and vastus lateralis MBF are shown in Figures 4 and 5, respectively. In addition, individual regression line slopes of the

individual relationships between intercostal and vastus lateralis muscle BFI and MBF are shown in Figure 6.

Agreement between BFI and MBF

Figure 7 shows the results of Bland-Altman analysis between MBF and BFI data expressed as fold change from rest for intercostal (Figure 7a) and vastus lateralis muscle groups (Figure 7b). Specifically, for both muscle groups there was a satisfactory agreement between MBF and BFI as the difference between the two methods in fold change from rest in muscle perfusion did not vary statistically significantly from 0 (zero) (intercostal muscles: mean difference: -0.05 fold change from rest, 95%CI: 0.07 to -0.17, p=0.424 and vastus lateralis: mean difference: -1.51 fold change from rest, 95%CI: -4.2 to 1.2, p=0.268, respectively).

Associations between BFI and physiologically relevant variables

During the isocapnic hyperpnea trial the mean change from rest in intercostal muscle BFI was linear with respect to the mean changes in whole body oxygen uptake (r=0.913, P=0.030), minute ventilation (r=0.954, P=0.012), the power of breathing (r=0.922, P=0.026), and tidal excursion in transdiaphragmatic pressure (r=0.962, P=0.009) (Figure 8). Similarly, the mean change from rest in vastus lateralis muscle BFI was linear with respect to mean change in whole body oxygen uptake (r=0.989, P=0.001, and mean cycling load (r=0.984, P=0.002) during graded cycling exercise (Figure 9). Individual r values for intercostal and vastus lateralis muscle between BFI and the aforementioned physiologically variables are shown in Table 1. Specifically, for the majority of patients, BFI increased linearly with physiological relevant variables, but the slope of the regression line varied considerably between subjects.

Discussion

Main findings

This study examined the validity of the essentially non-invasive NIRS-derived blood flow index (BFI) in reflecting respiratory and quadriceps muscle blood flow (MBF, the reference method) in a representative COPD population over a wide range of ventilation and exercise workloads. Under all conditions examined, individual, mean and fold change responses of BFI tracked MBF closely (Figures 2-5 and 7). Furthermore, while the slope of individual patient relationships between BFI and MBF may differ (Figure 6) individual correlations between the two methods for both intercostal and vastus lateralis muscle groups remained highly significant (Figure 3b and d and Figures 4 and 5). In addition, relative mean and individual changes in intercostal and vastus lateralis muscle BFI from rest to peak exercise or peak minute ventilation were strongly associated with measures of respiratory load, mechanical workload, and whole body metabolic demand across a wide range of physiologically relevant rates of minute ventilation and exercise intensities (Figures 8-9 and Table 1).

The findings of the present study extend previous observations in young, fit individuals (6, 7) by demonstrating that BFI is able to detect changes in respiratory and leg MBF during exercise over a range of ventilation rates and exercise workloads typically experienced by patients with COPD. However, the BFI method does not allow absolute blood flow to be determined – it provides relative flow rates between conditions, but with the major advantage of avoiding arterial catheterization.

Comparison of MBF to BFI

This study provides strong support for the use of BFI in place of MBF as the data show. A difficulty in their comparison however is their very different units as explained in the Methods section. To compare side by side, we chose to calculate fold changes (from rest) in MBF and BFI, and these also indicated close similarity as shown (Figure 2b and

Figure 2d). However, fold changes are very sensitive to small variations in the resting values (which form the denominator for fold changes). This explains the behavior noted in the Bland-Altman plots (Figure 7) of some points with huge differences between the two methods - the points identified with asterisks towards the right side of Figure 7a and 7b. Specifically, for intercostal muscles (Figure 7a) one point lies at ~ +1-fold difference and two points at ~ -1 -fold difference whist for vastus lateralis muscle (Figure 7b) two points lie at ~ +20-fold difference and three at ~ -20 -fold difference, which seem to indicate very high variance. In Figures 4 and 5 we identify the same eight points, and it is apparent that the flows on exercise fit very well with the regression line in each case, reinforcing the sensitivity of fold-change to the resting data. The Bland-Altman plot therefore distorts the comparison of the MBF and BFI data because of the reliance on fold changes as the comparison variable. However, that plot remains useful as an indicator that mean values were not different from zero over the range encountered.

Advantages of BFI for measuring muscle blood flow in COPD.

BFI measures muscle blood flow by dividing the muscle ICG peak concentration (assessed noninvasively by NIRS-ICG curve) by the rise time from 10 to 90% of peak (Figure 1). The use of these two cutoff points eliminates the need for exact temporal definition of the start and end of the ICG washin, which are somewhat observer-dependent (7). The only invasive component of this technique - compared to traditional methods for assessing MBF - is peripheral venous bolus injection of the ICG tracer. BFI requires only the determination of the muscle ICG peak concentration and the times at 10% and 90% of the rise in ICG concentration (Figure 1) thus reducing the possibility of observer errors. Indeed, the study by Habazettl et al., (8) in normal subjects reported that the reproducibility of BFI is high as there is less inter-observer variability for analyses of intercostal and quadriceps muscle BFI compared to the variability observed during MBF calculation measured by NIRS-ICG relying on the Fick principle. Taking into consideration the

practical and methodological advantages of BFI as well as the findings of the present study showing that mean and individual values of BFI and MBF were strongly associated over a wide range of ventilatory rates and exercise intensities (Figures 2-5) we suggest that BFI can be considered a reliable measure of relative blood flow changes from rest to exercise in patients with COPD.

Prior studies of the validation of the NIRS-ICG derived BFI

In clinical populations NIRS-ICG derived BFI has been shown to be sensitive and reproducible in detecting relative perfusion differences in cerebral blood flow during bedside assessment in patients with acute ischemic stroke (9, 14, 19). Furthermore, a validation study in young, fit subjects by Habazettl et al. (8) compared BFI values within the vastus lateralis and 7^{th} intercostal space against NIRS–ICG derived absolute MBF during cycling exercise up to a maximal level (~360 watt). The results indicated a strong group mean and individual agreement between BFI and MBF measured across both respiratory and quadriceps muscles. In addition, the study by Guenette et al. (7) extends the observation by Habazettl et al. (8) by investigating the sensitivity of BFI in intercostal and sternocleidomastoid muscles across a wide range of ventilatory rates up to maximal levels (i.e., ~120 liters/min) during an isocapnic hyperpnea trial with simultaneous measurements of electromyography and the work of breathing produced by these muscles. The results of that study showed a strong correlation with the work of breathing and electromyography (EMG) data for both aforementioned respiratory muscles (7).

Relationship between BFI and other physiological variables

In addition to the data comparing BFI to MBF, BFI was related to a number of physiologically relevant variables that were simultaneously recorded during the two protocols (i.e., cycling exercise and isocapnic hyperpnea trials). The strong group mean and individual associations that we found between BFI and those variables (Figures 8-9, Table 1) further support the physiological value of BFI as an index proportional to exercise-related

variables. In addition, these associations are also useful because considering the comparisons between BFI and MBF, one could argue that a bias may occur owing to the mathematical coupling between BFI and MBF variables (12, 20). This is because calculation of both BFI and MBF use the same muscle ICG-concentration curve. Therefore, relating BFI to independent relevant variables provides additional support for its validity. There were strong linear relationships between intercostal and vastus lateralis BFI and metabolic requirement (VO_2), ventilation, and mechanical loading (Figures 8-9 and Table 1) – relationships which avoid any mathematical coupling.

BFI application to individual patients and to groups of patients

The data show excellent reliability when average values for groups are to be compared (Figures 2, 3a and b). However it is more difficult to know whether BFI can be used to estimate MBF on a single patient basis. This is because while BFI is an index proportional to MBF, the slope of the BFI/MBF relationship varies across individuals (Figure 6). Therefore, one cannot convert BFI to actual MBF in all patients by a single assumed average proportionality constant (Figure 6). Figure 4 (Intercostals, hyperpnea trial), Figure 5 (Quadriceps, cycling trial), and Figure 6 address this point by plotting for each of the 10 patients the individual relationships (Figures 4-5) and the regression line slopes (Figure 6) between BFI and MBF. While in each case the relationships are closely linear for the intercostal muscles, for two patients (i.e., subjects 6 and 7, Figure 4) the slopes between BFI and MBF relationship differ considerably from the other 8 subjects (Figure 6a). It is of interest that for the same two subjects, the correlations between BFI and other physiologically relevant variables are also found to be weak (Table 1), suggesting a physiological rather than technical explanation. For the quadriceps (Figure 6b) the regression line slopes varied less as compared to intercostal muscles. In summary, intercostal and quadriceps BFI cannot be used for conversion to MBF in all individual

subjects under different conditions, although use of an average proportionality constant would be satisfactory for the majority of patients.

Methodological considerations

The present study assessed respiratory muscle BFI in COPD patients at the level of the 7th intercostal space as this has been previously proposed for measuring absolute MBF by studies using the NIRS-ICG approach (6, 7, 10, 15-17). This site of measurement (i.e., 7th intercostal space), while easily accessible by NIRS, reflects both internal and external intercostals muscles, thus providing an overall assessment of respiratory MBF. However, it does not reflect diaphragm blood flow. Nevertheless, in COPD patients, as the degree of dynamic lung hyperinflation gradually increases during exercise, the pressure generated by the diaphragm decreases and the act of inspiration is more dependent on the rib cage inspiratory muscles such as the intercostal muscles (4). Indeed, in our study we did not find any impact of dynamic lung hyperinflation on intercostal muscle BFI responses as both progressively increased, leveling off at 75% of WRpeak during isocapnic hyperpnea (actual increase in end-expiratory lung volume at 25, 50, 75, 100% of WRpeak was 60±90, 240±50, 550±110, 480±180 ml, respectively). This further supports the use of 7th intercostal space for assessing respiratory MBF in clinical populations irrespective of the occurrence of exercise-induced dynamic hyperinflation.

We found stronger associations between individual values of BFI and MBF (Figures 3b and d) in both respiratory (r=0.872 and locomotor muscles (r=0.895) compared to those reported in young, fit subjects by Habazettl et al. (8) (i.e., r=0.730 and r=0.720, respectively). In fact, the study by Habazettl et al. (8) reported substantial scattering of individual data of BFI and MBF mostly exhibited during high levels of exercise. In our study, scattering of individual data of BFI and MBF appears much less with increasing exercise intensity (Figures 3b and d). This may be attributed to the lower peak ICG values that COPD patients exhibited compared to healthy subjects (both in muscle and arterial

blood) - as response to lower exercise intensity - and thus making easier to calculate the peak ICG concentrations. Indeed, determination of peak ICG concentrations may be a source of random errors that may contribute to the variability of individual BFI vs. MBF values (9), especially when oscillations of the ICG concentration curve due to strong muscle contraction dynamics during forced ventilation or high workloads require smoothing of the original ICG concentration curve (for example see figure 1).

Another methodological consideration is the normalization of the BFI values for body mass/composition when major variability in body mass (BM) and/or composition exists (Habazettl et al., 2010). In our study we did not normalize as we were most interested in BFI/MBF relationships within each patient. However, we did analyze normalization of both intercostal and vastus lateralis muscle BFI to BM, BMI and fat-free mass index (FFMI). Although we found that BM, BMI and FFMI varied considerably among patients (i.e., BM: from 52 kg to 110 kg, BMI: from 18.4 to 34.7 kg/m^2 and FFMI: from 17.5 to 23.1 kg/m^2), this did not affect the level of association between the BFI and MBF in both muscle groups.

Conclusions

In a small population of patients with COPD, the essentially non-invasively derived BFI showed close agreement with MBF (determined from the same signals combined with arterial ICG concentration/time curves) when applied to group data. This was found for changes in intercostal and locomotor MBF across a wide range of ventilatory rates and exercise workloads up to peak levels. The findings support the quantitative validity of this method, thereby allowing for minimally invasive assessment of relative muscle blood flow in this population. While individual patient values of BFI and MBF were also generally similar in both muscle groups, occasional patients displayed variances which give rise to caution in application to individual patients.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article and have no relevant financial disclosures.

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Table 1. Regression analysis for individual subjects between BFI and physiologically relevant variables

	Intercostal muscles (hyperpnea trial)				Quadriceps muscle (cycling trial)	
Patients	BFI vs.VO_2	BFI vs.V_E	BFI vs. PoB	BFI vs. ΔPdi	BFI vs.VO_2	BFI vs. Work
1	0.909	0.953	0.962	0.961	0.937	0.958
2	0.900	0.973	0.955	0.953	0.969	0.912
3	0.915	0.949	0.891	0.893	0.895	0.937
4	0.893	0.895	0.906	0.915	0.921	0.938
5	0.710	0.713	0.568	0.559	0.698	0.541
6	0.260	0.273	0.330	0.313	0.829	0.729
7	0.686	0.493	0.289	0.328	0.903	0.927
8	0.907	0.901	0.904	0.931	0.890	0.990
9	0.925	0.942	0.958	0.959	0.989	0.970
10	0.852	0.789	0.796	0.836	0.875	0.938

Individual Pearson r values relating intercostal muscles blood flow index (BFI) oxygen uptake (VO_2), minute ventilation (V_E), power of breathing (PoB) and transdiaphragmatic pressure (ΔPdi) recorded from rest to peak minute ventilation during the isocapnic hyperpnea trial and quadriceps muscle blood flow index (BFI) with quadriceps muscle blood flow (MBF), oxygen uptake (VO_2) and work (watts) recorded from rest to WRpeak during cycling exercise.

Figure legends

Figure 1. Representative examples of an intercostal (a) and quadriceps (b) muscle indocyanine green (ICG) concentration curve recorded by near-infrared spectroscopy (NIRS) during isocapnic hyperpnea and cycling trials at 75% of WRpeak in an individual subject. Isocapnic hyperpnea was sustained at the level of minute ventilation (i.e., 40 l/min) similar to that recorded during cycling exercise at 75% of WRpeak (i.e., 58 watts). The original tracing (gray line) appears with marked oscillations owing to muscle contraction and relaxation during exercise. 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. ICG concentrations expressed in nanomoles/liter (nM) and the rise time expressed in seconds between 10% and 90% of ICG concentration peak are indicated, and intercostal and quadriceps muscle BFI calculations and results are shown.

Figure 2. Group mean responses of intercostal (a) and quadriceps (c) muscle blood flow (MBF) and blood flow index (BFI) during isocapnic hyperpnea and cycling trials respectively. Relative changes from rest of intercostal (b) and quadriceps (d) muscle blood flow (MBF) and blood flow index (BFI) are also shown. Data are presented as mean ± SEM. Asterisks denote significant differences from 100% of WRpeak, $P<0.05$. (MBF data were reproduced from reference 16).

Figure 3. Comparison of mean and individual blood flow index (BFI) and muscle blood flow (MBF) values for intercostal (a) and (b) and quadriceps (c) and (d) muscles during isocapnic hyperpnea and cycling trials, respectively. Linear regression equations, regression coefficients, and significance levels are presented in each figure. (MBF data were reproduced from reference 16).

Figure 4. Individual patient correlations between intercostal muscle blood flow index (BFI) and intercostal muscle blood flow (MBF). Linear regression equations and regression coefficients are presented in each figure. (MBF data were reproduced from reference 16).

Figure 5. Individual correlations between vastus lateralis muscle blood flow index (BFI) with vastus lateralis muscle blood flow (MBF). Linear regression equations and regression coefficients are presented in each figure. (MBF data were reproduced from reference 16).

Figure 6. Individual regression line slopes between (a) intercostal muscle blood flow index (BFI) and intercostal muscle blood flow (MBF) and (b) vastus lateralis muscle blood flow index (BFI) with vastus lateralis muscle blood flow (MBF). Mean regression line slope and equation is presented in each figure (MBF data were reproduced from reference 16).

Figure 7. Bland-Altman plots comparing fold changes form rest of intercostal (a) and quadriceps (b) muscle blood flow (MBF) and blood flow index (BFI) during isocapnic hyperpnea and cycling trials, respectively (MBF data were reproduced from reference 16).

Figure 8. Regression analyses of mean intercostal muscle blood flow index (BFI) and (a) oxygen uptake, (b) minute ventilation, (c) power of breathing, and (d) tidal excursion in transdiaphragmatic pressure during the isocapnic hyperpnea trial. Regression coefficients and significance levels are presented in each figure. (physiologically relevant data were reproduced from reference 16).

Figure 9. Regression analyses of mean quadriceps muscle blood flow index (BFI) and (a) oxygen uptake and (b) work rate recorded at rest and during the cycling trial. Regression

643 coefficients and significance levels are presented in each figure. (physiologically relevant
644 data were reproduced from reference 16).
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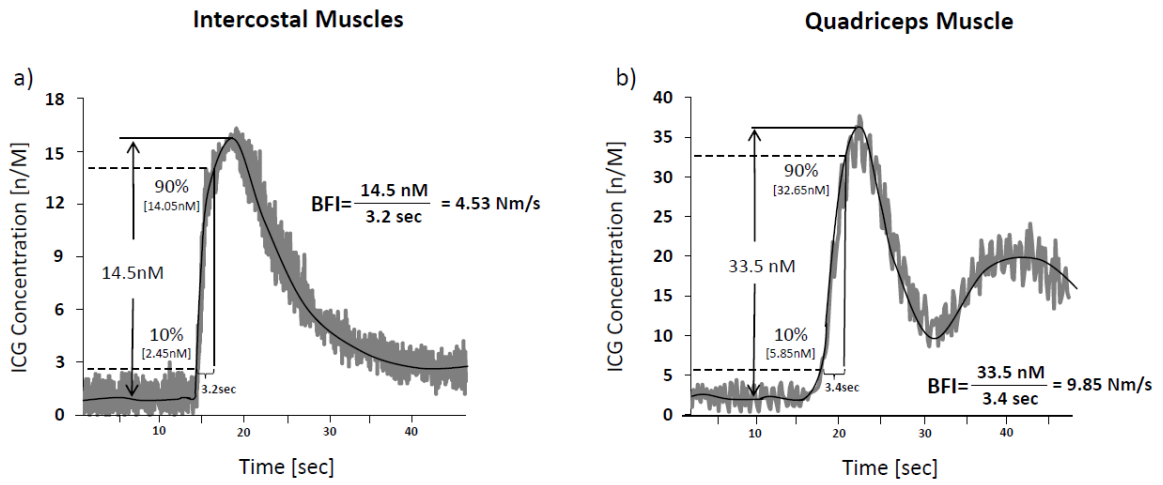
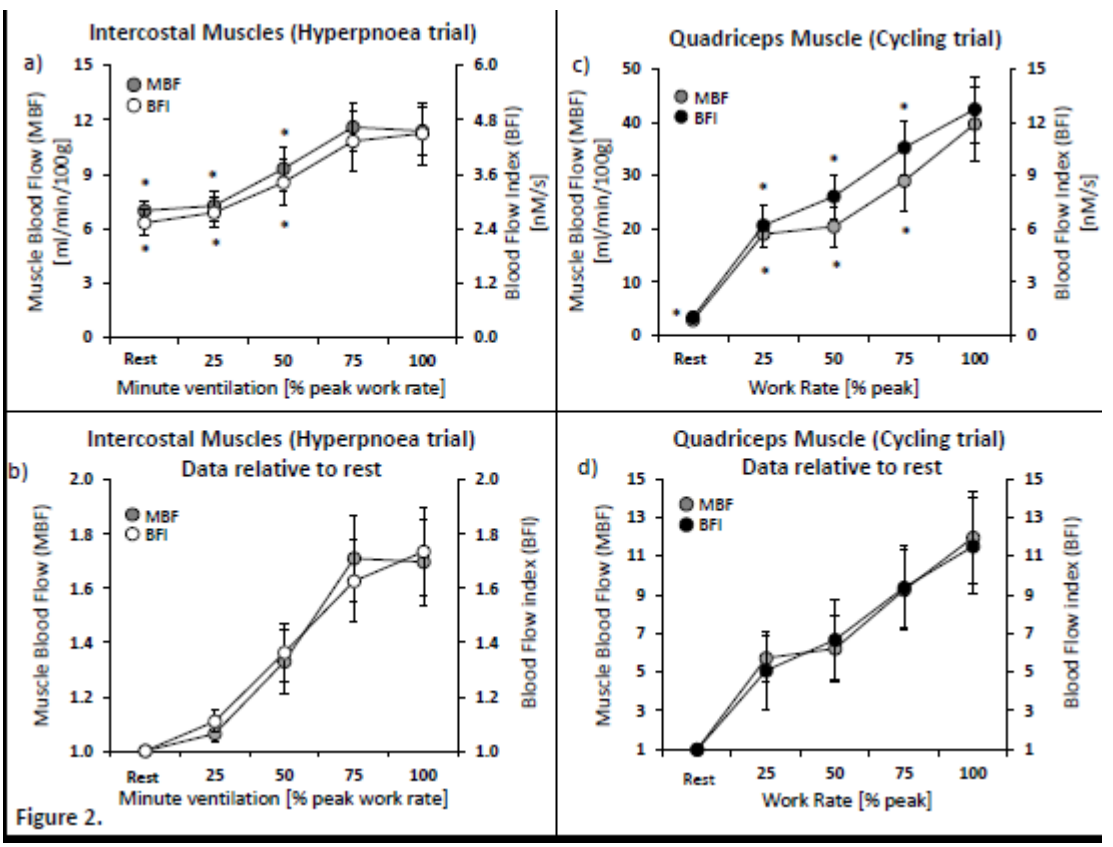


Figure 1.



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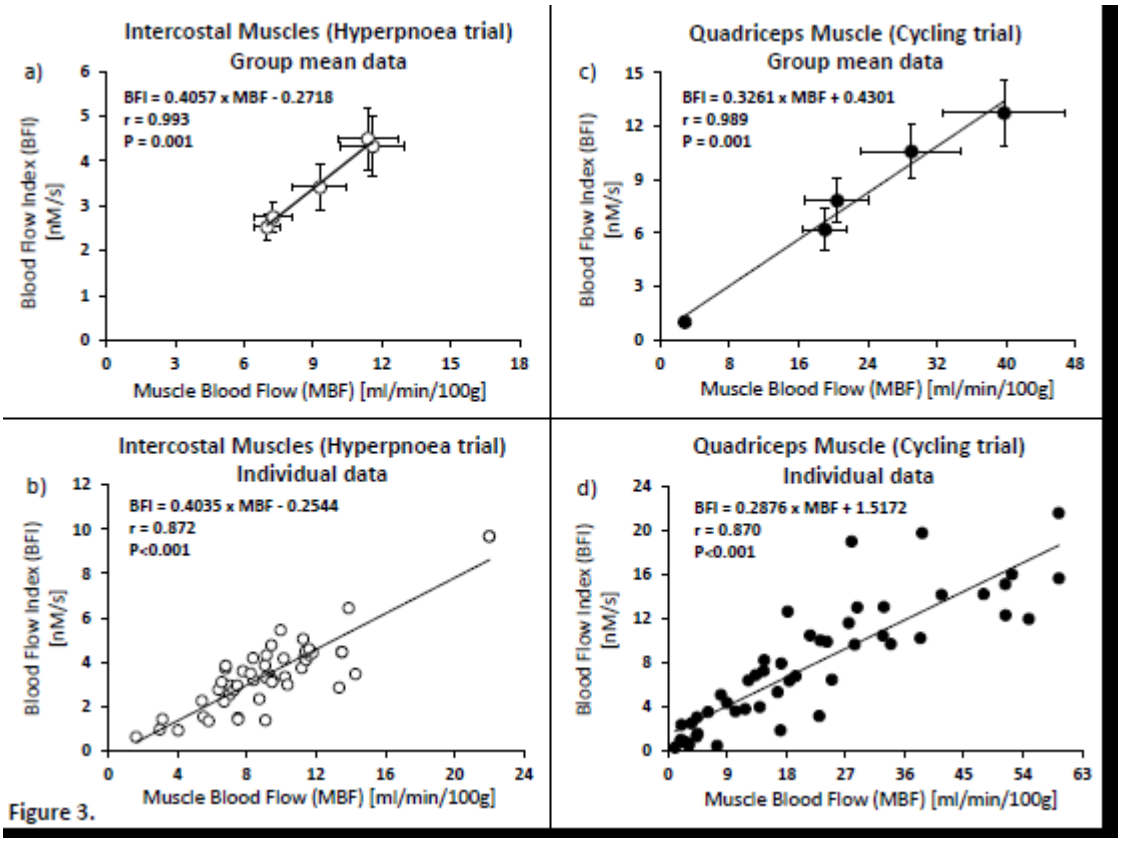

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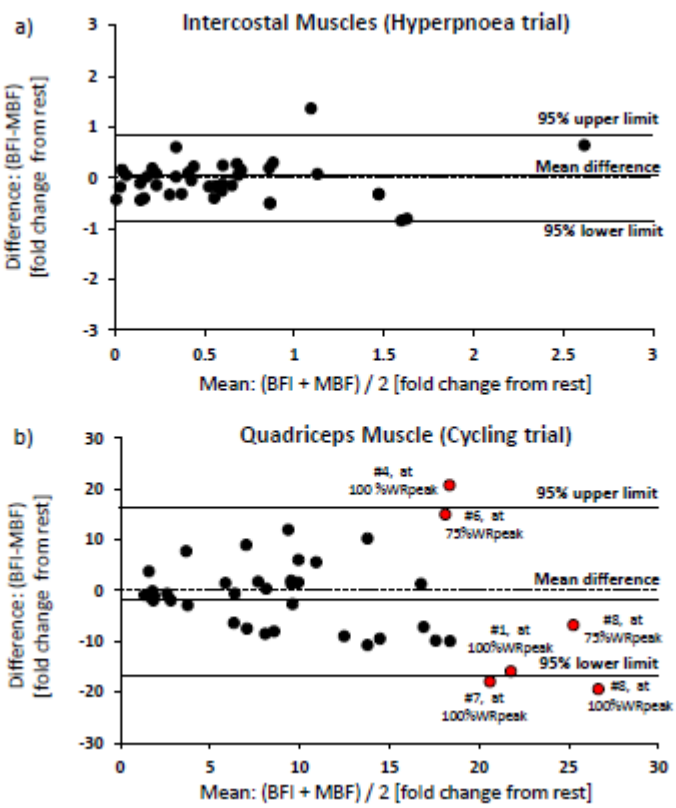

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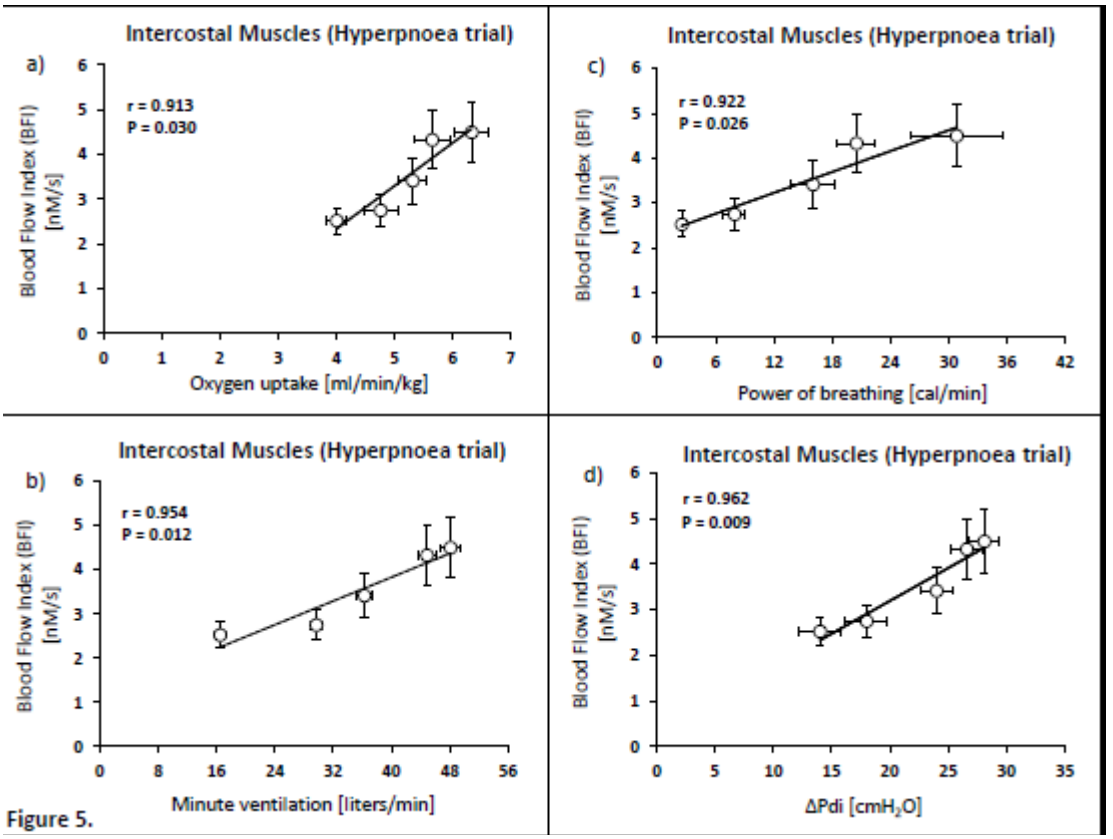

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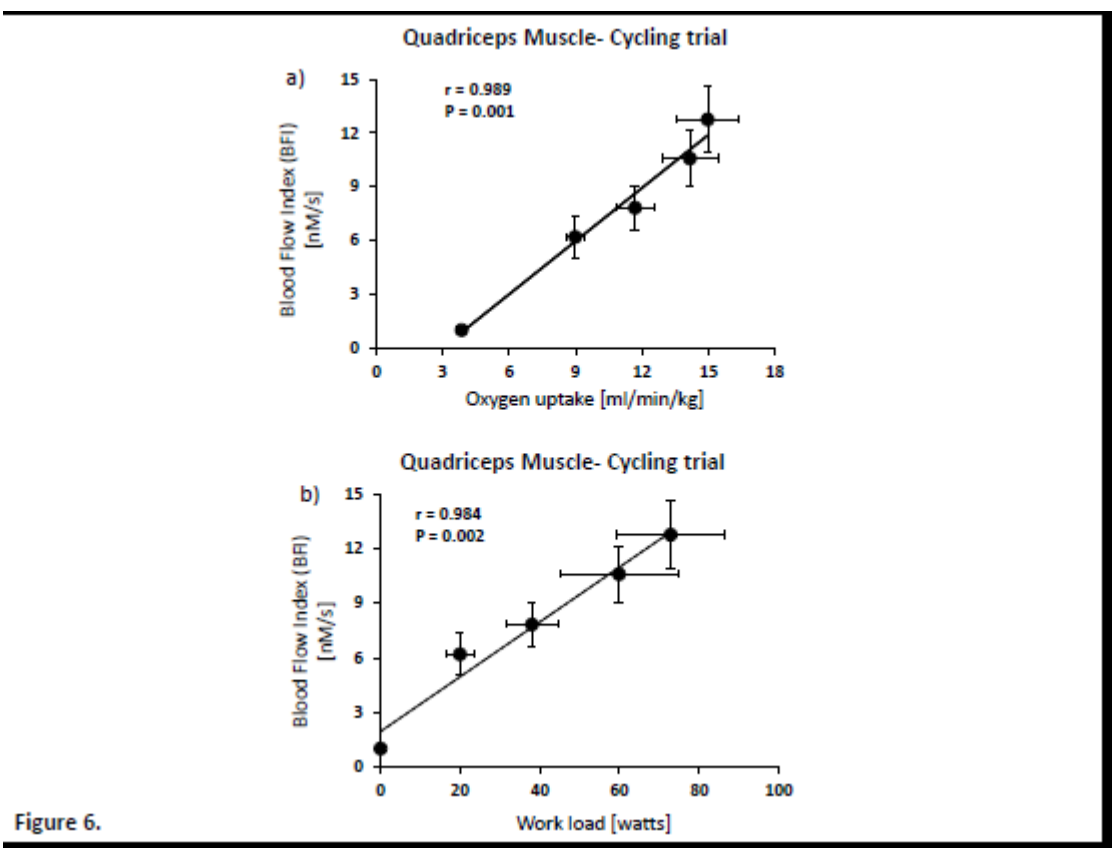

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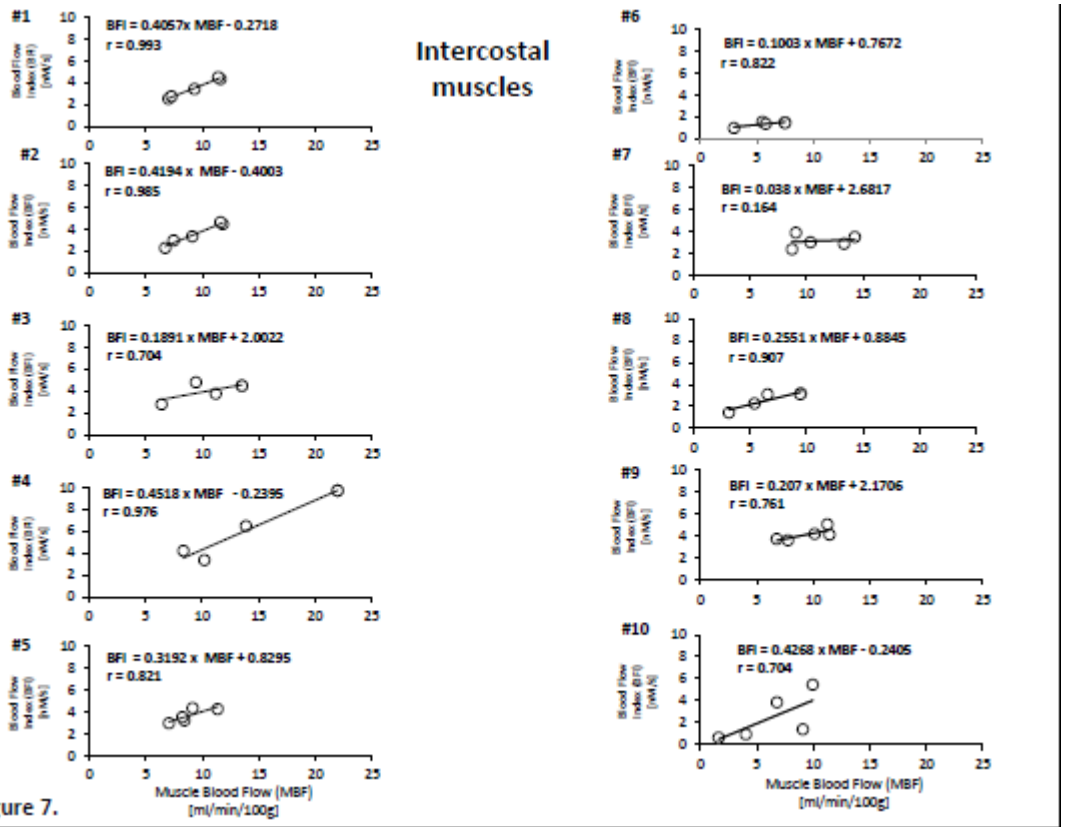

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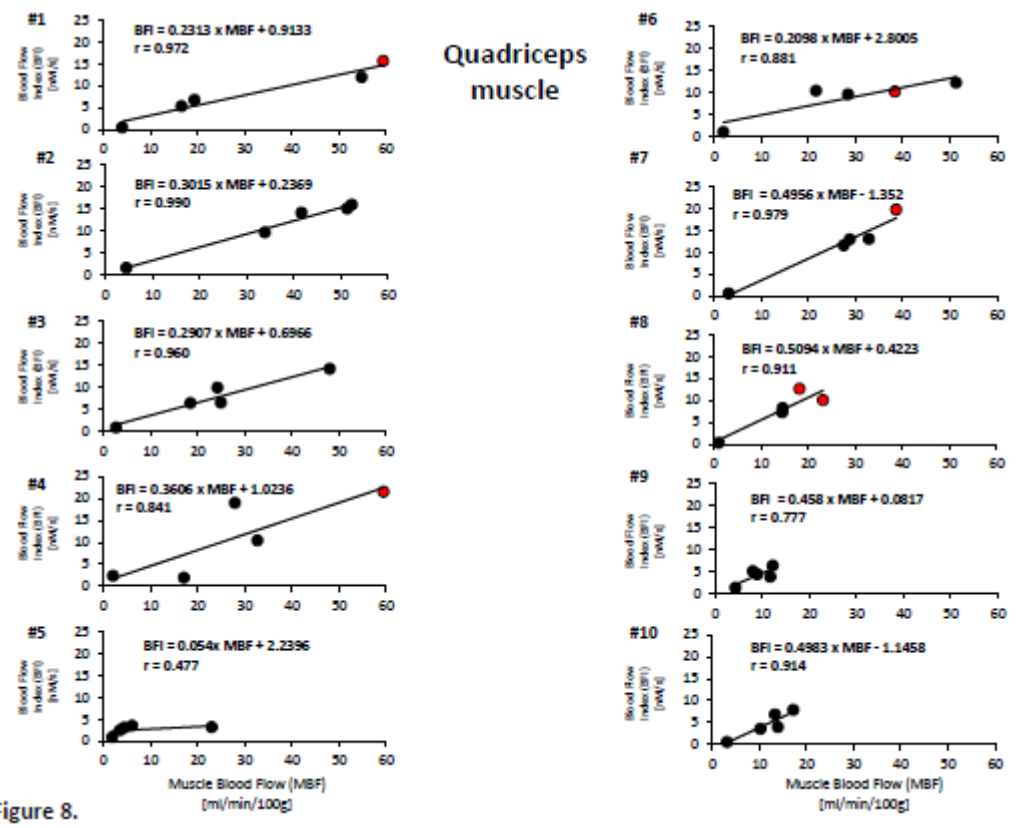

Figure 8.