

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 Wagner⁶, Spyros Zakyntinos¹, Peter Wagner⁶ and Ioannis Vogiatzis^{1,2,7}

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

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

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

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

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

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

⁷Department 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 ($FEV_1:51\pm6\%$ predicted) at rest and during cycling at 25%, 50%, 75% and 100% of WR_{peak} . Intercostal muscle MBF and BFI were also compared during isocapnic hyperpnea at rest, reproducing ventilation levels up to those at WR_{peak} . Intercostal and vastus lateralis BFI increased with increasing ventilation during hyperpnea (from 2.5 ± 0.3 to 4.5 ± 0.7 nM/s) and cycling load (from 1.0 ± 0.2 to 12.8 ± 1.9 nM/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.

33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78

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.

79 **Keywords:** NIRS, Indocyanine Green dye, muscle perfusion, respiratory muscles, exercise,
80 COPD, validation, BFI
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119

Introduction

120

121

122

123

124

125

126

127

128

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.

129

130

131

132

133

134

135

136

137

138

139

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.

140

141

142

143

144

145

146

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

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

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

157 **Materials and methods**

158 **Subjects**

159 We retrospectively analyzed data from ten clinically stable patients with COPD
160 ($FEV_1:51\pm6\%$ predicted) classified by the Global Initiative for Chronic Obstructive Lung
161 Disease (GOLD, 2016) as stages II (n=4), III (n=3), and IV (n=3) from our previously
162 published work (16). In the original study (16), vastus lateralis and intercostal muscle blood
163 flow (MBF) were simultaneously measured in patients with COPD with the aim to
164 investigate whether during exercise, intercostal MBF competes with locomotor muscles for
165 the available blood flow (16). In the original study, only MBF data were analyzed and
166 presented. In the present analysis, intercostal and vastus lateralis MBF are compared with
167 simultaneously measured blood flow index (BFI) in the same locations. Measurements were
168 made under two separate conditions: from rest to near-peak cycle exercise, and from resting
169 to peak (isocapnic) hyperpnea.

170

171

172 **Study Design**

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

176 **Intercostal and quadriceps MBF and BFI**

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

184
$$\text{Blood flow (ml/100ml/min}^{-1}\text{)} = \frac{k \cdot [ICG]_m \cdot t}{\int_0^t [ICG]_a dt}$$

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

195

196

197 **Statistical analysis**

198 Data are presented as means \pm SEM. Pearson's correlation coefficient (r) was used to
199 establish associations between intercostal and vastus lateralis muscle BFI with MBF and
200 other physiologically related variables. ANOVA with repeated measures and post-hoc
201 comparisons (Tukey's test) were used to identify statistically significant differences across
202 the cycling exercise and isocapnic hyperpnea trials. Analysis of agreement between BFI and
203 MBF measurements was performed by using Bland-Altman analysis. A satisfactory
204 agreement between BFI and MBF was considered when the difference between BFI and
205 MBF measurements did not significantly vary from 0 (zero). For this purpose one sample t-
206 test with testing variable the difference between BFI and MBF measurements and testing
207 value 0 (zero) was performed. For Bland-Altman analysis purposes, both BFI in nM/s and
208 MBF in ml/min/100g were expressed as fold changes from rest in order to allow a
209 comparison between the two methods. The level of statistical significance was set at $P <$
210 0.05. All statistical analyses were performed using the SPSS statistical software (v. 20 IBM
211 SPSS Statistics, Chicago, IL, USA).

212

213

Results

214 **Patient characteristics**

215 Detailed subject characteristics in terms of demographics, pulmonary function, and
216 peak exercise performance data have been published and discussed previously (16). Not
217 reported previously, patients exhibited impaired functional capacity in terms of the six
218 minute walking distance test (389 ± 31 meters, $59 \pm 8\%$ predicted), quadriceps muscle strength
219 (28 ± 5 kg, $61 \pm 6\%$ predicted), and quadriceps muscle endurance, (43 ± 8 seconds $54 \pm 5\%$
220 predicted, respectively).

221

222

223 **Perfusion responses to exercise**

224 During the resting isocapnic hyperpnea trial, both intercostal muscle blood flow
225 (MBF) and blood flow index (BFI) increased from: 7.0 ± 0.6 to 11.4 ± 1.3 ml/min/100gr
226 (MBF) and from 2.5 ± 0.3 to 4.5 ± 0.7 nM/s, (BFI). Moreover, both MBF and BFI reached a
227 plateau at 75% of peak minute ventilation (Figure 2a). During the cycling trial, both vastus
228 lateralis MBF and BFI increased progressively with increasing power output: from 2.8 ± 0.4
229 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
230 WRpeak (Figure 2c). When MBF and BFI data were expressed as changes relative to flows
231 at rest, the pattern of increase in muscle perfusion with cycle exercise or with resting
232 hyperpnea did not significantly differ (between MBF and BFI) either for the intercostal or
233 the vastus lateralis muscle groups (Figures 2b and 2d)

234 **Comparison of BFI and MBF**

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

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

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

251 **Agreement between BFI and MBF**

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

260 **Associations between BFI and physiologically relevant variables**

261 During the isocapnic hyperpnea trial the mean change from rest in intercostal muscle
262 BFI was linear with respect to the mean changes in whole body oxygen uptake ($r=0.913$,
263 $P=0.030$), minute ventilation ($r=0.954$, $P=0.012$), the power of breathing ($r=0.922$, $P=0.026$),
264 and tidal excursion in transdiaphragmatic pressure ($r=0.962$, $P=0.009$) (Figure 8). Similarly,
265 the mean change from rest in vastus lateralis muscle BFI was linear with respect to mean
266 change in whole body oxygen uptake ($r=0.989$, $P=0.001$, and mean cycling load ($r=0.984$,
267 $P=0.002$) during graded cycling exercise (Figure 9). Individual r values for intercostal and
268 vastus lateralis muscle between BFI and the aforementioned physiologically variables are
269 shown in Table 1. Specifically, for the majority of patients, BFI increased linearly with
270 physiological relevant variables, but the slope of the regression line varied considerably
271 between subjects.

272

273

274

Discussion

275

276 **Main findings**

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

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

296 **Comparison of MBF to BFI**

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

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

314 **Advantages of BFI for measuring muscle blood flow in COPD.**

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

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

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

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

347 **Relationship between BFI and other physiological variables**

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

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

361 **BFI application to individual patients and to groups of patients**

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

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

380 **Methodological considerations**

381 The present study assessed respiratory muscle BFI in COPD patients at the level of the
382 7th intercostal space as this has been previously proposed for measuring absolute MBF by
383 studies using the NIRS-ICG approach (6, 7, 10, 15-17). This site of measurement (i.e., 7th
384 intercostal space), while easily accessible by NIRS, reflects both internal and external
385 intercostals muscles, thus providing an overall assessment of respiratory MBF. However, it
386 does not reflect diaphragm blood flow. Nevertheless, in COPD patients, as the degree of
387 dynamic lung hyperinflation gradually increases during exercise, the pressure generated by
388 the diaphragm decreases and the act of inspiration is more dependent on the rib cage
389 inspiratory muscles such as the intercostal muscles (4). Indeed, in our study we did not find
390 any impact of dynamic lung hyperinflation on intercostal muscle BFI responses as both
391 progressively increased, leveling off at 75% of WR_{peak} during isocapnic hyperpnea (actual
392 increase in end-expiratory lung volume at 25, 50, 75, 100% of WR_{peak} was 60±90, 240±50,
393 550±110, 480±180 ml, respectively). This further supports the use of 7th intercostal space for
394 assessing respiratory MBF in clinical populations irrespective of the occurrence of exercise-
395 induced dynamic hyperinflation.

396 We found stronger associations between individual values of BFI and MBF (Figures
397 3b and d) in both respiratory ($r=0.872$) and locomotor muscles ($r=0.895$) compared to those
398 reported in young, fit subjects by Habazettl et al. (8) (i.e., $r=0.730$ and $r=0.720$,
399 respectively). In fact, the study by Habazettl et al. (8) reported substantial scattering of
400 individual data of BFI and MBF mostly exhibited during high levels of exercise. In our
401 study, scattering of individual data of BFI and MBF appears much less with increasing
402 exercise intensity (Figures 3b and d). This may be attributed to the lower peak ICG values
403 that COPD patients exhibited compared to healthy subjects (both in muscle and arterial

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

410 Another methodological consideration is the normalization of the BFI values for body
411 mass/composition when major variability in body mass (BM) and/or composition exists
412 (Habazettl et al., 2010). In our study we did not normalize as we were most interested in
413 BFI/MBF relationships within each patient. However, we did analyze normalization of both
414 intercostal and vastus lateralis muscle BFI to BM, BMI and fat-free mass index (FFMI).
415 Although we found that BM, BMI and FFMI varied considerably among patients (i.e., BM:
416 from 52 kg to 110 kg, BMI: from 18.4 to 34.7 kg/m² and FFMI: from 17.5 to 23.1 kg/m²),
417 this did not affect the level of association between the BFI and MBF in both muscle groups.

418 **Conclusions**

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

428

429

430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448

449

450

451

452

453

454

455

456

457

458

459

460

461

462

463

Acknowledgements

We thank our subjects for their considerable patience during this intensive and exhausting investigation.

This work was supported by Thorax Foundation and by grants from the “A. Perotti” visiting Professorship fund of the Thorax Foundation.

Dr. Louvaris Zafeiris is the recipient of an ERS Long-Term Research fellowship number LTRF 2016-6686 and is a post-doctoral research fellow of the FWO-Flanders (12U5618N)

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.

- 465 1. **Aliverti A, Macklem P.** The major limitation to exercise performance in COPD is
466 inadequate energy supply to the respiratory and locomotor muscles. *J Appl Physiol* 105:
467 749–751, 2008.
- 468
- 469 2. **Barrett EJ, Rattigan S.** Muscle perfusion: its measurement and role in metabolic
470 regulation. *Diabetes* 61: 2661-8, 2012.
- 471
- 472 3. **Boushel R, Langberg H, Olesen J, Nowak M, Simonsen L, Bulow J, Kjaer M.**
473 Regional blood flow during exercise in humans measured by near-infrared spectroscopy
474 and indocyanine green. *J Appl Physiol* 89: 1868-1878, 2000.
- 475
- 476 4. **De Troyer A, Pride NB.** The chest wall and respiratory muscles in chronic obstructive
477 pulmonary disease. In: Roussos C, editor. *The Thorax*. New York: Dekker. (1995); pp.
478 1975–2006.
- 479
- 480 5. **Debigare R, Maltais F.** The major limitation to exercise performance in COPD is
481 lower limb muscle dysfunction. *J Appl Physiol* 105: 751–753, 2008.
- 482
- 483 6. **Guenette JA, Vogiatzis I, Zakyntinos S, Athanasopoulos D, Koskolou M,**
484 **Golemati S, Vasilopoulou M, Wagner HE, Roussos C, Wagner PD, Boushel R.**
485 Human respiratory muscle blood flow measured by near-infrared spectroscopy and
486 indocyanine green. *J Appl Physiol* 104: 1202-1210, 2008.
- 487
- 488 7. **Guenette JA, Henderson WR, Dominelli PB, Querido JS, Brasher PM, Griesdale**
489 **DE, Boushel R, Sheel AW.** Blood flow index using near-infrared spectroscopy and
490 indocyanine green as a minimally invasive tool to assess respiratory muscle blood flow
491 in humans. *Am J Physiol Regul Integr Comp Physiol* 300: 984-92, 2011.
- 492
- 493 8. **Habazettl H, Athanasopoulos D, Kuebler WM, Wagner H, Roussos C, Wagner PD,**
494 **Ungruhe J, Zakyntinos S, Vogiatzis I.** Near-infrared spectroscopy and indocyanine
495 green derived blood flow index for noninvasive measurement of muscle perfusion
496 during exercise. *J App Physiol* 108: 962-967, 2010.
- 497
- 498 9. **Kuebler WM, Sckell A, Habler O, Kleen M, Kuhnle GE, Welte M, Messmer K,**
499 **Goetz AE.** Noninvasive measurement of regional cerebral blood flow by near-infrared
500 spectroscopy and indocyanine green. *J Cereb Blood Flow Metab* 18: 445–456, 1998.
- 501
- 502 10. **Louvaris Z, Vogiatzis I, Aliverti A, Habazettl H, Wagner H, Wagner P,**
503 **Zakyntinos S.** Blood flow does not redistribute from respiratory to leg muscles during
504 exercise breathing heliox or oxygen in COPD. *J Appl Physiol* 117: 267-76, 2014.
- 505
- 506 11. **Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigaré R,**
507 **Dekhuijzen PN, Franssen F, Gayan-Ramirez G, Gea J, Gosker HR, Gosselink R,**
508 **Hayot M, Hussain SN, Janssens W, Polkey MI, Roca J, Saey D, Schols AM, Spruit**
509 **MA, Steiner M, Taivassalo T, Troosters T, Vogiatzis I, Wagner PD; ATS/ERS Ad**
510 **Hoc Committee on Limb Muscle Dysfunction in COPD.** An official American Th
511 oracic Society/European Respiratory Society statement: update on limb muscle
512 dysfunction in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 189:
513 e15-e62, 2014.

514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564

12. **Moreno LF, Stratton HH, Newell JC, Feustel PJ.** Mathematical coupling of data: correction of a common error for linear calculations. *J Appl Physiol* 60: 335–343, 1986.
13. **O'Donnell D, Webb K.** The major limitation to exercise performance in COPD is dynamic hyperinflation. *J Appl Physiol* 105: 753–755, 2008.
14. **Terborg C, Bramer S, Harscher S, Simon M, Witte OW.** Bedside assessment of cerebral perfusion reductions in patients with acute ischaemic stroke by near-infrared spectroscopy and indocyanine green. *J Neurol Neurosurg Psychiatry* 75: 38-42, 2004.
15. **Vogiatzis I, Athanasopoulos D, Habazettl H, Kuebler WM, Wagner H, Roussos C, Wagner PD, Zakynthinos S.** Intercostal muscle blood flow limitation in athletes during maximal exercise. *J Physiol* 15: 3665-77, 2009.
16. **Vogiatzis I, Athanasopoulos D, Habazettl H, Aliverti A, Louvaris Z, Cherouveim E, Wagner H, Roussos C, Wagner PD, Zakynthinos S.** Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 182: 1105-13, 2010.
17. **Vogiatzis I, Habazettl H, Aliverti A, Athanasopoulos D, Louvaris Z, Lo Mauro A, Wagner H, Roussos C, Wagner PD, Zakynthinos S.** Effect of helium breathing on intercostals and quadriceps muscle blood flow during exercise in COPD patients. *Am J Physiol Regul Intergr Comp Physiol* 300: 1549-1559, 2011.
18. **Vogiatzis I, Zakynthinos S.** Factors Limiting Exercise Tolerance in Chronic Lung Diseases. *Comprehensive Physiology* 2: 1779-1817, 2012.
19. **Wagner BP, Gertsch S, Ammann RA, Pfenninger J.** Reproducibility of the blood flow index as noninvasive, bedside estimation of cerebral blood flow. *Intensive Care Med* 29: 196-200, 2003.
20. **Walsh TS, Lee A.** Mathematical coupling in medical research: lessons from studies of oxygen kinetics. *Br J Anaesth* 81: 118-120, 1998.

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

Patients	Intercostal muscles (hyperpnea trial)				Quadriceps muscle (cycling trial)	
	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

565

566

567 Individual Pearson r values relating intercostal muscles blood flow index (BFI) oxygen
 568 uptake (VO₂), minute ventilation (V_E), power of breathing (PoB) and transdiaphragmatic
 569 pressure (ΔPdi) recorded from rest to peak minute ventilation during the isocapnic
 570 hyperpnea trial and quadriceps muscle blood flow index (BFI) with quadriceps muscle
 571 blood flow (MBF), oxygen uptake (VO₂) and work (watts) recorded from rest to WRpeak
 572 during cycling exercise.

573

574

575

576

577

578

579

580

581

582

583

584

585

586

587

588

589

590

Figure legends

591

592

593 **Figure 1.** Representative examples of an intercostal (a) and quadriceps (b) muscle
594 indocyanine green (ICG) concentration curve recorded by near-infrared spectroscopy
595 (NIRS) during isocapnic hyperpnea and cycling trials at 75% of WRpeak in an individual
596 subject. Isocapnic hyperpnea was sustained at the level of minute ventilation (i.e., 40 l/min)
597 similar to that recorded during cycling exercise at 75% of WRpeak (i.e., 58 watts). The
598 original tracing (gray line) appears with marked oscillations owing to muscle contraction
599 and relaxation during exercise. Low-pass filtering with a cutoff frequency of 0.5 Hz
600 produced the smoothed curve (black line) that was used for blood flow index (BFI)
601 calculation. ICG concentrations expressed in nanomoles/liter (nM) and the rise time
602 expressed in seconds between 10% and 90% of ICG concentration peak are indicated, and
603 intercostal and quadriceps muscle BFI calculations and results are shown.

604

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

611

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

617

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

621

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

625

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

630

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

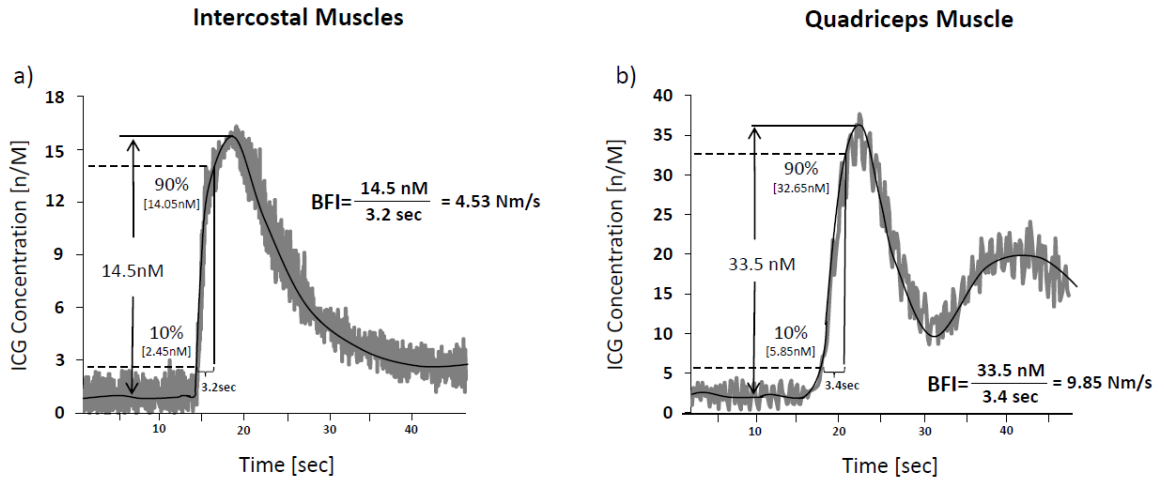
634

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

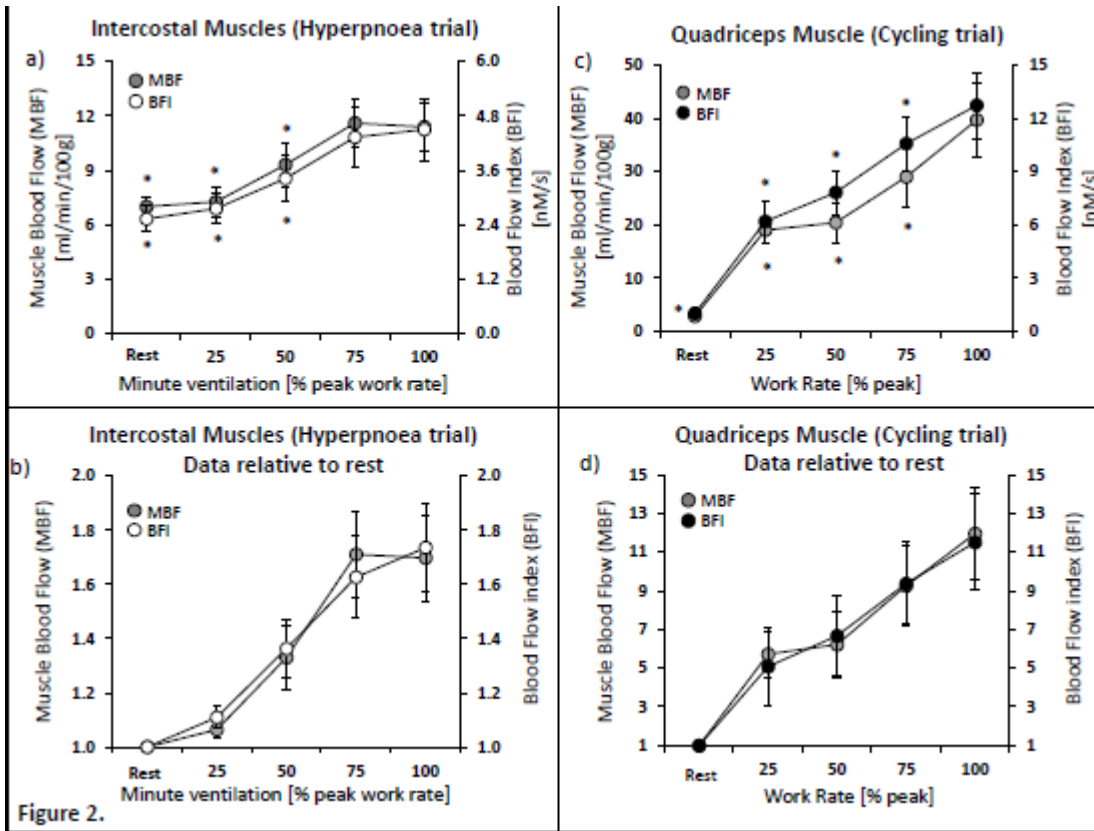
640

641 **Figure 9.** Regression analyses of mean quadriceps muscle blood flow index (BFI) and (a)
642 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).
645
646



647 Figure 1.
648



649 Figure 2.
650
651
652
653

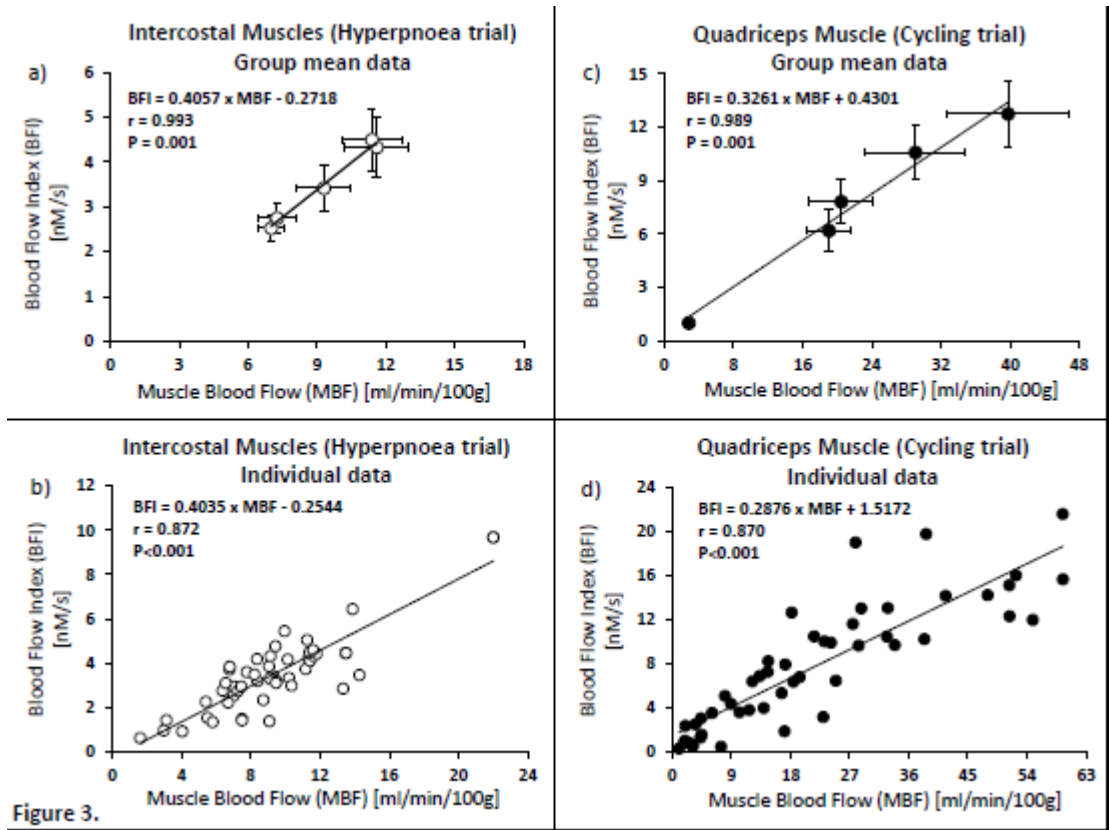


Figure 3.

654
 655
 656
 657

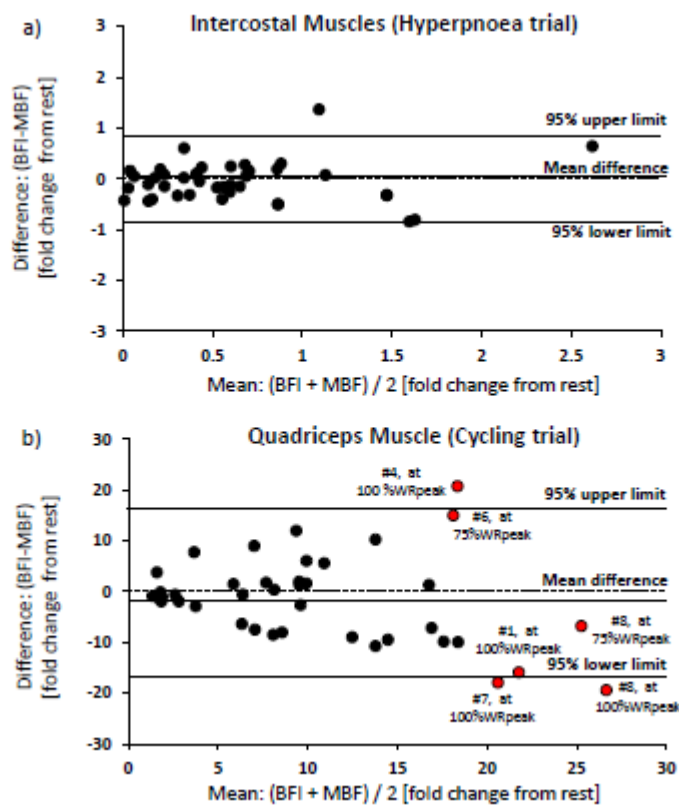
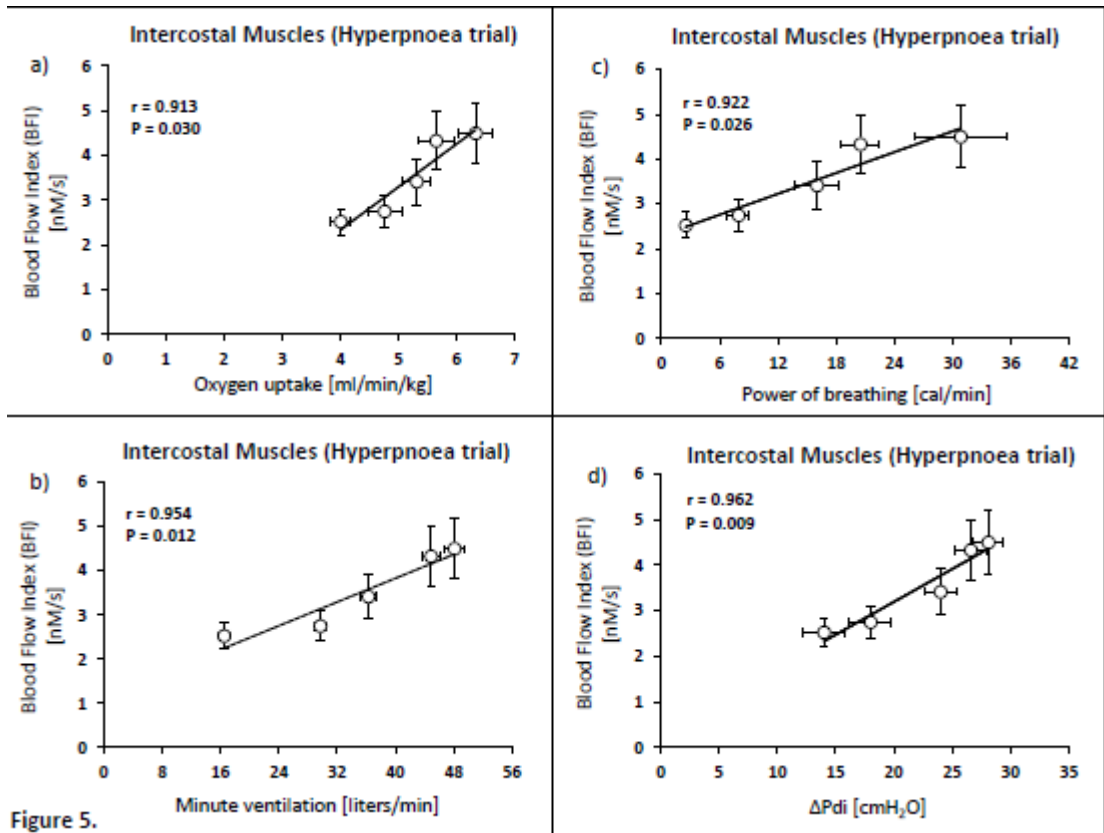


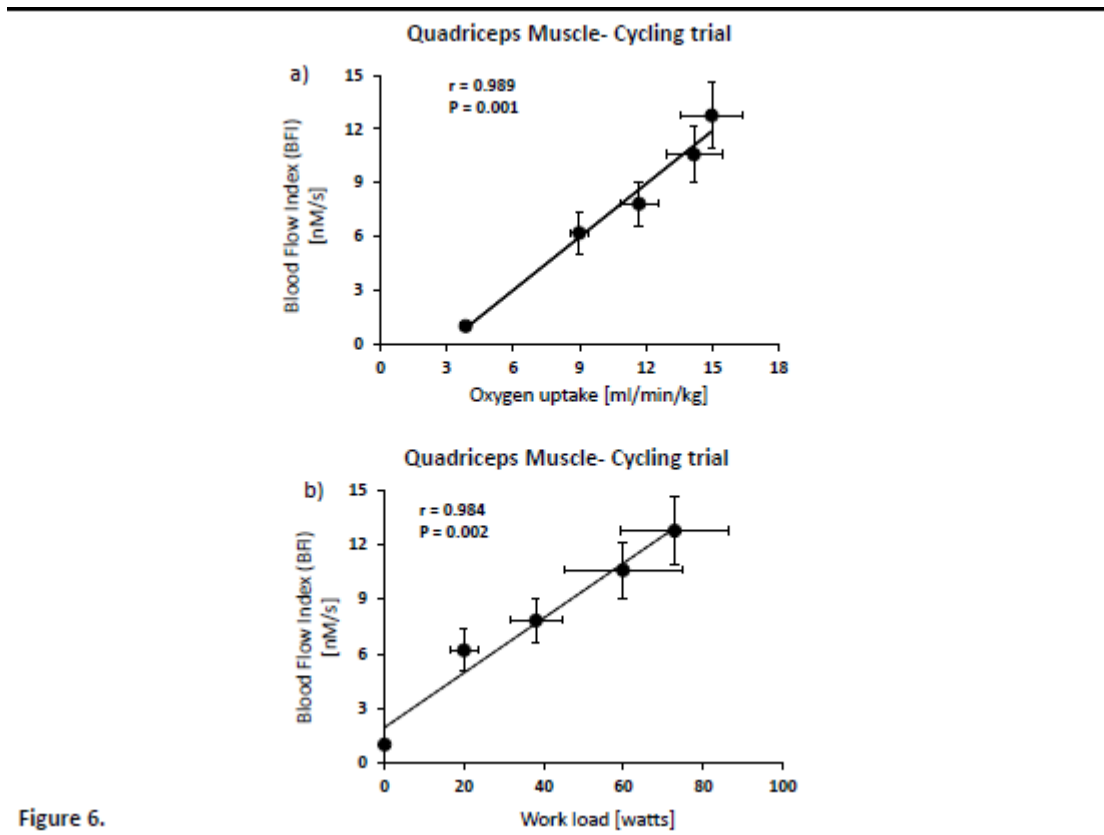
Figure 4.

658
 659
 660
 661

662
663



664
665
666



667
668
669

Intercostal muscles

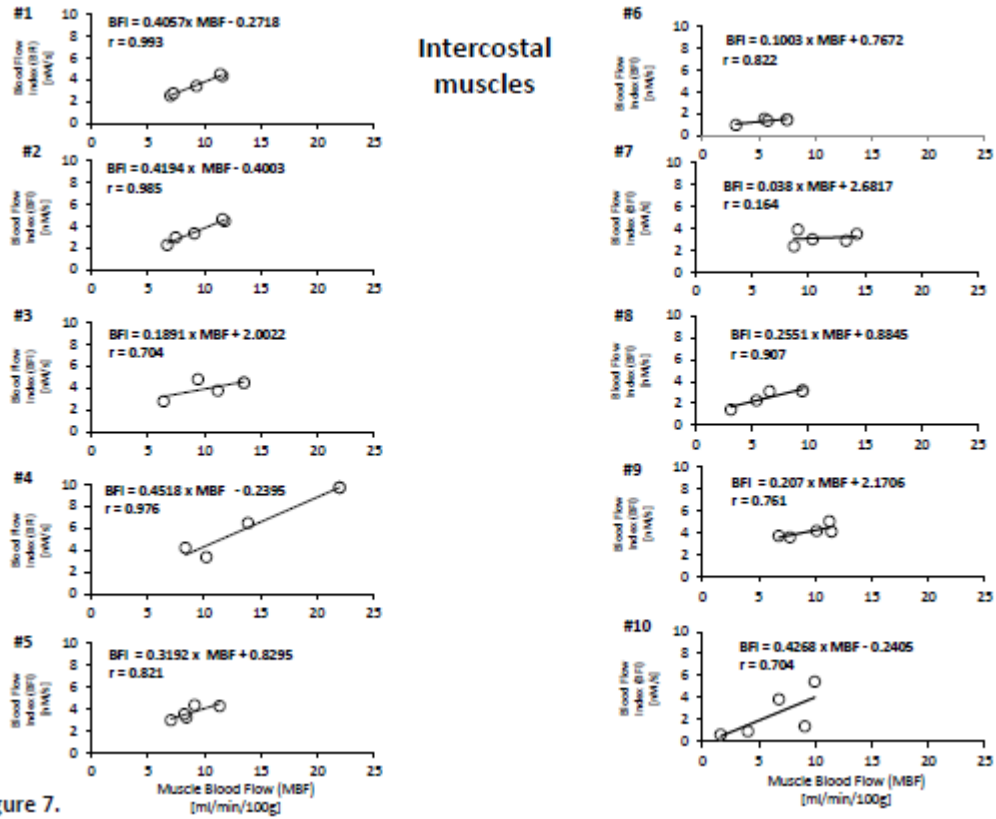


Figure 7.

670
671
672

Quadriceps muscle

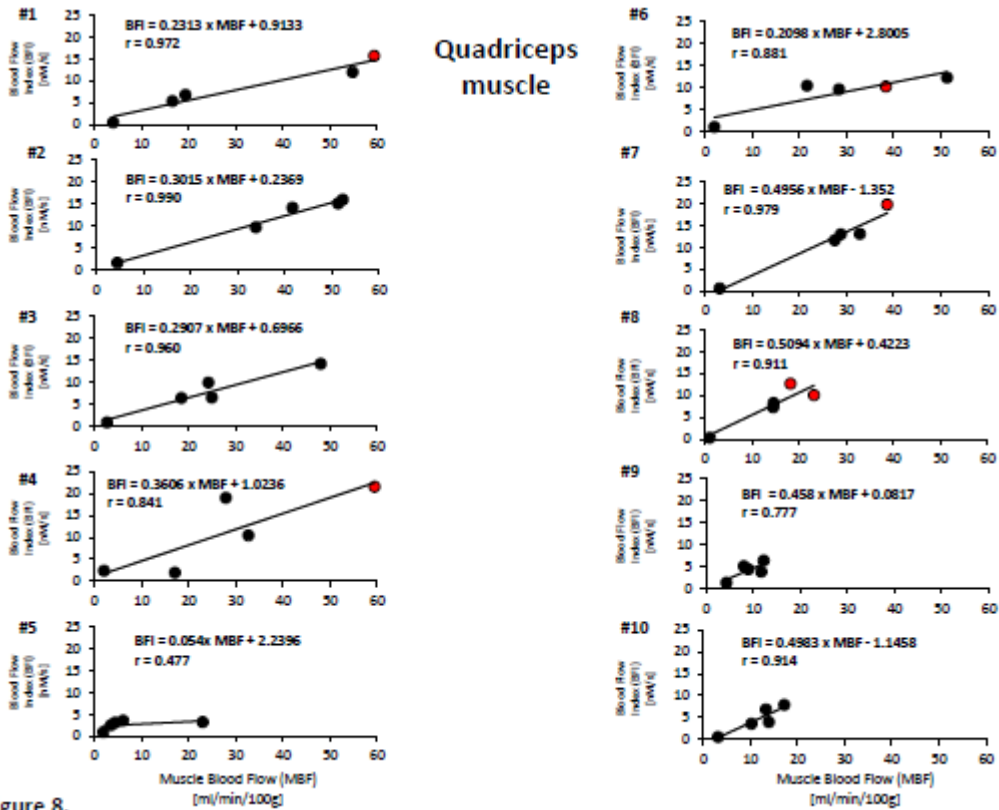


Figure 8.

673