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Panagiotou, Marios; Vogiatzis, Ioannis; Louvaris, Zafeiris; Jayasekera, Geeshath; MacKenzie, Alison; Mcglinchey, Neil; Baker, Julien; Church, Alistair; Peacock, Andrew; Johnson, Martin

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Near-infrared spectroscopy for the assessment of peripheral tissue oxygenation in pulmonary arterial hypertension.

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Complete List of Authors:	<p>Panagiotou, Marios; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit Vogiatzis, Ioannis; National and Kapodistrian University of Athens, Department of Physical Education and Sports Sciences Louvaris, Zafeiris; National and Kapodistrian University of Athens, Department of Physical Education and Sports Sciences Jayasekera, Geeshath; Golden Jubilee National Hospital, Scottish Pulmonary Vascular unit MacKenzie, Alison; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit McGlinchey, Neil; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit Baker, Julien; University of the West of Scotland, Institute of Clinical Exercise and Health Science Church, Alistair; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit Peacock, Andrew; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit Johnson, Martin; Golden Jubilee National Hospital, Scottish Pulmonary Vascular Unit</p>
Key Words:	near infrared spectroscopy, pulmonary arterial hypertension, oxygen delivery and consumption, skeletal muscle

May 2016

To the Editors,

On behalf of all the authors, I would like to thank you for extending us the possibility to publish our work as a Research letter in the *European Respiratory Journal*.

We also want to extend our appreciation to the editor and reviewers for taking the time and effort to provide us with valuable comments that we used to revise our manuscript. Please find a detailed response to the reviewers' comments in the manuscript files.

Sincerely,

Marios Panagiotou, MD.

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7 **Near infrared spectroscopy for the assessment of peripheral tissue oxygenation in**
8 **pulmonary arterial hypertension.**
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12 **Authors**
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15 Marios Panagiotou¹, Ioannis Vogiatzis², Zafeiris Louvaris², Geeshath Jayasekera¹, Alison
16 McKenzie¹, Neil Mcglinchey¹, Julien S. Baker³, Alistair C. Church¹, Andrew J. Peacock¹,
17
18 Martin K. Johnson¹.
19
20

21
22
23 **Affiliations**
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25
26 ¹Scottish Pulmonary Vascular Unit, Golden Jubilee National Hospital, Glasgow, UK;
27
28 ²National and Kapodistrian University of Athens, Department of Physical Education and
29 Sports Sciences, Athens, Greece; ³Institute of Clinical Exercise and Health Science,
30 University of the West of Scotland, Hamilton, UK.
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32
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34
35 **Corresponding author:**
36

37 Marios Panagiotou: Scottish Pulmonary Vascular Unit, Golden Jubilee National Hospital,
38
39 Agamemnon Street, Glasgow, G81 4DY, UK; mariopanag@gmail.com;
40
41
42 +447478664894.
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49 n° LTRF 2014-3106 supported by an unrestricted grant by GSK.
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54 **“take home” message:** Near infrared spectroscopy offers a qualitative, noninvasive
55 indication of mixed venous oxygen saturation in PAH.
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3 Pulmonary arterial hypertension (PAH) is characterised by increased pulmonary vascular
4 resistance and results in increased morbidity and mortality due to right heart failure and a
5
6 progressive decline in cardiac output (CO) [1]. The latter disturbs oxygen delivery to the
7
8 periphery and may lead to pathological changes in tissue oxygenation. The balance
9
10 between global oxygen supply and demand is reflected in mixed venous oxygen
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12 saturation (SvO₂), an index that is generally reduced in patients with PAH [2]. SvO₂ at
13
14 baseline is one of the strongest predictors of survival in PAH [3-5]; this is also true for
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16 changes in SvO₂ during follow-up [4]. Cut-off values of 60% [6] and 65% [4] have been
17
18 used to distinguish between prognostic groups suggesting that these may be suitable
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20 treatment goals. SvO₂ is measured invasively in the pulmonary artery, where venous
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22 blood mixes after circulating through the superior and inferior vena cava, coronary
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24 sinuses and the right-heart chambers.
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32 Spatially resolved near infrared spectroscopy (NIRS) offers a noninvasive, rapidly
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34 responsive method for measuring skeletal muscle oxygenation by examining absorption
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36 differences in the near infrared spectrum of light between oxy- and deoxy- haemoglobin
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38 and myoglobin molecules in the microvasculature. The *tissue oxygenation index* (StO₂) is
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40 commonly adopted as an index of the dynamic balance between local tissue oxygen
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42 supply (availability) and utilization (extraction) in both health and disease [7, 8]. Because
43
44 the contribution of the myoglobin to the NIRS signal is not critical, StO₂ is largely
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46 considered as the ratio of oxygenated to total tissue hemoglobin concentration expressed
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48 as [oxyhemoglobin/ (oxyhemoglobin + deoxyhemoglobin)] × 100 (%). To evaluate NIRS
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50 in PAH, we correlated measurement of vastus lateralis StO₂ with SvO₂ and venous
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52 oxygen saturation in the inferior vena cava (SivcO₂) during right heart catheterisation.
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3 To measure StO₂, one transcutaneous sensor (S-Type Probe; NIRO-200NX spatially
4 resolved spectrophotometer, Hamamatsu Photonics KK, Japan) was placed over each
5 vastus lateralis muscle, 10-12 cm above the lateral epicondyle. StO₂ values shown are the
6 average values obtained from both legs at the time of SvO₂ and S_{icv}O₂ single-point
7 measurements. SvO₂ was measured from the distal port of the Swan–Ganz catheter.
8 Resting S_{ivc}O₂ was measured with a pigtail catheter advanced through the right internal
9 jugular vein sheath to the level of S1 vertebra.
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21 Concurrent, single-point measurements of SvO₂ and StO₂ were repeated during supine
22 exercise in consecutive patients who consented to this task. One patient performed
23 straight leg raise and nine patients exercised on an electronically braked lower limb cycle
24 ergometer secured to the catheterization table. Subjects cycled at 60 revolutions/min for 6
25 minutes at a constant workload set at 50% of peak work rate achieved during an upright
26 cycle cardiopulmonary exercise test the previous day. SvO₂ and StO₂ were measured
27 during the sixth minute. Supplementary oxygen was provided as required to maintain
28 normoxia.
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40 Twenty-five subjects with PAH were studied at rest, 10 of whom also exercised.

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42 The main patient characteristics are presented in figure 1. Combining all the resting and
43 exercise data points (n=35), StO₂ showed a good correlation with SvO₂ (r=.703, p<.001).
44 This level of correlation persisted when looking separately at rest (r=.701, p<.001) and
45 exercise data (r=.863, p=.001) (figure 1) but also the change from rest to exercise in StO₂
46 and S_vO₂ (r=.669, p=.034). A significant reduction (p<.001) was observed in StO₂ during
47 exercise. Resting values of StO₂ exhibited similar level of correlation with S_{ivc}O₂
48 (r=.655, p=.001). A good correlation (r=.703, p<.001) was observed between S_{ivc}O₂ and
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3 SvO₂, whereas the resting correlations of StO₂ with S_vO₂ and S_{ivc}O₂ were not statistically
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5 different ($Z=0.3$, $p=.76$).
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10 Resting StO₂ correlated with age ($r=-.416$, $p=.038$) but also with indices of disease
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12 severity including the six-minute walk distance ($r=.528$, $p=.008$), N-terminal pro-brain
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14 natriuretic peptide ($r=-.395$, $p=.05$) and diffusing lung capacity for carbon monoxide
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16 percent predicted ($r=.398$, $p=.049$).
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22 To our knowledge, this is the first study to report on the association between StO₂ and
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24 SvO₂. Good correlation between vastus lateralis StO₂ and femoral venous oxygen
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26 saturation has also been reported in healthy trained subjects [9] albeit earlier studies did
27
28 not confirm such correlation [10, 11]. However, comparisons should be made with
29
30 caution as responses of StO₂ depend highly on the mode, intensity and duration of
31
32 exercise and neither of those studies is matched in design to our resting and steady-state
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34 exercise protocol. Instead, they report on measurements either during incremental
35
36 exercise protocol. Instead, they report on measurements either during incremental
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38 exercise [9] or over time during constant load exercise [10, 11].
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44 Nonetheless, the correlation between StO₂ and S_vO₂ or S_{ivc}O₂ is not absolute and results
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46 of relating StO₂ to venous blood oxygenation should not be interpreted in a quantitative
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48 sense. This may be because the specific tissue volume investigated by NIRS is not fully
49
50 representative of the oxygen status of the body segment (lower limb) or global tissue
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52 oxygenation as measured by S_{ivc}O₂ and S_vO₂, respectively [7]. It is not surprising that
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54 the highest correlations between StO₂ and venous oxygen saturation were shown when
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3 the sampled venous effluent was specific for the interrogated tissue volume such as that
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5 obtained from a deep forearm vein that drained the exercising muscle ($r=.92$) [8] or from
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7 a vein that drained only the electrically simulated muscle of dogs ($r=.97$) [12].
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10 Accordingly, $SivcO_2$ would be expected to exhibit a higher correlation with StO_2 than
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12 SvO_2 . However, we observed similar correlation between StO_2 and SvO_2 . This seeming
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14 paradox may be due to venous return from the lower limbs being the major determinant
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16 of SvO_2 in the supine leg exercise.
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21 The design of the present study does not allow for reliable conclusions on the tissue
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23 oxygen status *per se*; the presented measurements should be interpreted within the
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25 context of oxygen supplementation to maintain resting normoxia and the absence of a
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27 matched control group. However, our findings provide support for the use of NIRS in the
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29 investigation of the pathophysiological abnormalities in PAH. Also, taken together,
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31 findings cannot exclude a role of the periphery in the pathophysiology of PAH since
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33 skeletal muscle tissue microenvironment, an important factor of the local oxygen status,
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35 is disturbed in PAH [13]. Perhaps, combination of NIRS with other techniques such as
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37 vascular occlusion, sidestream dark field imaging and histological examination could
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39 enable further exploration.
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47 Limited experience from application of NIRS in PAH showed significantly lower resting
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49 thenar muscle oxygen saturation in PAH patients compared with matched healthy
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51 subjects and patients with CHF [14]. Also, study of the kinetics of the vastus lateralis
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53 fractional oxygen extraction ($\% \Delta$ deoxyhemoglobin/myoglobin) relative to oxygen
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55 uptake at the beginning of heavy-intensity exercise, suggests that patients with PAH have
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3 greater microvascular oxygen delivery-to-utilization inequalities compared to healthy
4 control, which contribute to slow adaptation rate of aerobic metabolism [15].
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10 In summary, skeletal muscle StO₂ in PAH subjects correlated significantly with SvO₂
11 under both resting and exercise conditions. Also, StO₂ correlated significantly, albeit
12 weakly, with indices of disease severity. These novel findings suggest that StO₂ may
13 serve as a clinical and research tool for the qualitative, noninvasive assessment of the
14 dynamic balance between oxygen supply and utilization in PAH. Further studies are
15 warranted to explore the value of NIRS in the assessment and prognosis of PAH.
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3 **Figure legends**
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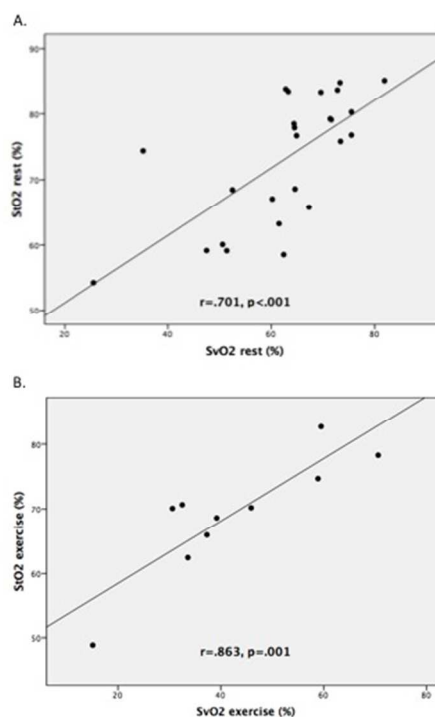
5 **Figure 1:** Patient characteristics and correlations between StO₂ and SvO₂ at rest (A) and
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8 during exercise (B).
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Patient characteristic	Rest (n=25)	Exercise (n=10; rest/exercise)
Age, y	61.9 ± 11.6	55.6 ± 8.4
Sex, male: female	11: 14	4: 6
IPAH; FPAH; CTD; CHD; PoPH	13;1;7;1;3	4;1;3;1;1
6MWD, m	256 ± 134	344 ± 144
NTproBNP, pg/ml	1101 (153, 2928)	263 (42, 1461)
mPAP, mm Hg	43 ± 11	40.7 ± 10.2/66.1 ± 13.8*
CO, L/min	4.6 ± 1.7	5.5 ± 1.96/8.6 ± 3.3*
PVR, Wood units	9.5 ± 5.9	7.3 ± 4.3/7.9 ± 4.5
SvO ₂ , %	62.5 ± 12.9	70.3 ± 8.6/42.3 ± 16.6*
SivcO ₂ , %	64.2 ± 14.3	N/A
StO ₂ , %	73.1 ± 9.8	77.62 ± 6.9/69.2 ± 9.2*



Data are presented as mean ± SD or median and quartiles. 6MWD: six-minute walk distance; IPAH: idiopathic pulmonary arterial hypertension; FPAH: familial pulmonary arterial hypertension; CTD: connective tissue disease-associated PAH; CHD: congenital heart disease-associated pulmonary arterial hypertension; PoPH: portopulmonary arterial hypertension; mPAP: mean pulmonary artery pressure; CO: cardiac output; PVR: pulmonary vascular resistance; SvO₂: mixed venous oxygen saturation; SivcO₂: inferior vena cava oxygen saturation; StO₂: quadriceps tissue oxygen saturation. * Statistically significant difference between values at rest and during exercise, $p<0.01$.

Legend: Figure 1: Patient characteristics and correlations between StO₂ and SvO₂ at rest (A) and during exercise (B).

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ERJ-00608-2016

" Near infrared spectroscopy for the assessment of peripheral tissue oxygenation in pulmonary arterial hypertension."

Responses to Editors' and reviewers' comments

Reviewer: 1

1. Although these results in a small group seem clinically important, I would like to see the sensitivity and specificity of StO₂ according to the prognostic relevant cut of SvO₂ values of 60% and or 64% as mentioned by the authors in the introduction.

Response: We appreciate this comment. However, the limited available space of the Research letter does not allow further elaboration.

2. Ad 1. This lines can be shown in the graphs from Figure 1.as well.

Response: We regret we are unable to understand this comment.

3. Figure 1. I would prefer only Figure B (rest) and C (exercise) separately. This is also statistically more sound. Only one data point for each measured subject in one correlation graph.

Response: The recommendation has been applied in the new figure.

4. From the tables 1 and 2. From the SD values (NTproBNP, CO (exercise SD= 10,9??, typing error?) and SvO₂ it seems that the data is not normally distributed and therefore the variance should be given as a range instead of SD

Response: NTproBNP is now presented as median and quartiles and the typing error has been corrected. The rest of the values are normally distributed.

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5. Table 3 shows only the correlations of resting StO₂, I would expect also a correlation between StO₂ during exercise and 6MWD (although n=10), please show these values

Response: We did not observe a correlation between StO₂ during exercise and 6MWD.

Reviewer: 2

Comments to the Author

1. *It is mentioned that “The exercised subjects showed a significant reduction in StO₂ during exercise ($p < .001$)”. Is this “abnormal”? As the author recognize, lack of a control group make it difficult to grasp a consistent view on what this measurement actually tell us.*

Response: We agree that in the absence of a control group we cannot draw any safe conclusion. We have now understated this finding and simply report it in the Results paragraph to demonstrate sensitivity of the NIRS method in peaking up changes in oxygenation during exercise.

2. *StO₂ interrogates the lower limbs. Thus, better correlations with inferior cava SO₂ (compared to SvO₂) could be anticipated. This, however, was not the case. This reviewer found it hard to logically conciliate this apparent paradox.*

Response: This appear paradoxical at first sight. However, seen from a different perspective, this suggests that the venous return from (or the oxygenation status of) lower limb is the major determinant of SvO₂ in the supine exercise. We have added this comment in the text.

3. *Correlations were found between resting (not exercise) StO₂ with six-minute walk distance (6MWD) ($r = .528$, $p = .008$) less consistently with NTproBNP ($r = -.395$, $p = .05$), DLCO% pred. ($r = .398$, $p = .049$) and age ($r = -.416$, $p = .038$). They are not particularly impressive and the small number of observations and several confounders make them difficult to interpret.*

Response: We have understated these observations.

4. *Absolute StO₂-NIRS is likely to be strongly dependent on the work rate performed. Subjects cycled at 50% of their peak work rate. Thus, less impaired patients likely exercised at higher work rates. Inter-subject variations in StO₂ might reflect this rather than true physiological impairment.*

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4 **Response:** We agree on the validity of this comment but cannot be applied in the Research letter.
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7 *5. I am really not sure if the resting correlation (StO₂ x SvO₂) would remain significant if the single*
8 *subject on the far left were excluded.*
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12 **Response:** The correlation at rest remains significant after removing the subject on the far left: $r=.602$,
13 $p=.002$
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17 *6. The authors mention that “This finding extends previous experience from application of NIRS in*
18 *PAH (limited to a single study), showing significantly lower resting thenar muscle oxygen saturation*
19 *in PAH patients compared with matched healthy subjects but also, patients with CHF [29].” A quick*
20 *search on PUBMED revealed a study for Barbosa et al. (Eur J Appl Physiol. 2011 111(8):1851-61) in*
21 *PAH using similar methodology. The reviewer had no access to this paper but, based on the Abstract,*
22 *it seems to address a similar topic in PAH.*
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29 **Response:** The study by Barbosa et al. is now cited in the Research letter.
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32 *6. Was there any correlation with SaO₂? Cardiac output (CO)? DO₂ (CO x CaO₂)?*
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35 **Response:** We did not perform measurement of SaO₂.
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39 *7. Due to the cross-sectional nature of the study, it remains unclear whether NIRS would be sensitive*
40 *enough to detect positive “peripheral” consequences of improved central hemodynamics.*
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43 **Response:** We appreciate the concern of this reviewer. However, this is not applicable to the Research letter.
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