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Peripheral muscle microcirculatory alterations in patients with pulmonary arterial hypertension: A pilot study

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Abstract

Background:Pulmonary microcirculation abnormalities are the main determinants of pulmonary arterial hypertension (PAH) pathophysiology. We hypothesized that PAH patients have peripheral tissue microcirculation alterations that might benefit from hyperoxic breathing. The aim of this study was to evaluate peripheral muscle microcirculation before and after hyperoxic breathing by Near Infra-Red Spectroscopy (NIRS).

Methods: Eight PAH patients, eight healthy subjects (controls) matched for age, gender and BMI as well as 16 patients with chronic heart failure (CHF) matched for functional capacity with PAH patients underwent NIRS evaluation. Tissue O_2 saturation (StO_2,%), defined as the percentage of hemoglobin saturation in the microvasculature compartments, was measured on the thenar muscle. Subsequently, 3-min brachial artery occlusion technique was applied before, during, and after 15 min of 100% of O_2-breathing. Main measurements included oxygen consumption rate (OCR,%/min), the reactive hyperemia time (RHT,min), the time needed for StO_2 to reach its baseline values after the release of the occlusion.

Results:PAH patients had a significantly lower resting StO_2 (65.8±14.9 versus 82.1±4.0,P=0.005), a trend to a decreased OCR (35.3±9.1 versus 43.4±19.7,P=0.6) and a significant higher RHT (3.0±0.6 versus 2.0±0.3,P<0.001) comparing to controls. PAH patients had also lower StO_2 (P=0.08) and peripheral arterial oxygen saturation (P=0.01) values, and higher RHT (P=0.016) compared to CHF patients. After hyperoxic breathing in PAH, there was an increase in StO2 (65.8±14.9 to 71.4±14.5,P<0.05), while OCR was reduced (35.3±9.1 to 25.1±6.6,P<0.05) and RHT had a further increase (3.0±0.6 to 4.2±0.7,P<0.01).

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Conclusions:PAH patients exhibit significant impairments of peripheral muscle microcirculation during evaluation with NIRS VOT. Specifically, PAH patients exhibit decreased StO_2, possibly due to hypoxemia and slower RHT, possibly due to endothelium dysfunction and peripheral systemic vasoconstriction. Acute hyperoxic breathing improves resting StO_2 as an expression of higher oxygen delivery, whilst it deteriorates OCR and RHT during reperfusion possibly due to increased oxidative stress and evoked vasoconstriction.

Keywords: endothelium; microcirculation; Near Infra-Red Spectroscopy; oxygen breathing; pulmonary arterial hypertension

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Introduction

Pulmonary arterial hypertension is (PAH) a life-threatening disease that it is associated with poor prognosis[1]. PAH patients are characterized by exercise intolerance with marked fatigue and dyspnea during exercise limiting their daily life activity. The profound cardiopulmonary abnormalities in PAH lead to chronic hypoxemia and right ventricular failure[2].

The endothelial cell dysfunction plays a significant role in the pulmonary vascular "remodeling" of PAH [3]. The increased levels of endothelin-1, thromboxane and the decreased nitric oxide synthase and prostacyclin lead to increased vessel tone, cell proliferation and vasoconstriction in the pulmonary microcirculation with progressive increase in pulmonary vascular resistance[4-6].

Although "remodeling" of small pulmonary arteries and pulmonary microcirculation abnormalities represent the main key elements of PAH pathophysiology, the role of the peripheral systemic microcirculation has been less investigated. Recent interesting observational studies have shown that PAH may be associated with peripheral endothelial dysfunction[7-9].

Current treatment for PAH is mainly based on restoring the imbalance between vaso-active mediators and correcting the status of hypoxemia[10]. Oxygen therapy has some beneficial effects in the tissue hypoxia and the hypoxic pulmonary vasoconstriction; however, the effect in peripheral microcirculation of PAH patients, is not clearly defined yet.

Near Infra-Red Spectroscopy (NIRS) vascular occlusion technique has been recently shown to be a valid, non-invasive technique that can evaluate peripheral tissue microcirculation in various population studies[11-18].

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In the present study we hypothesized that PAH patients have systemic peripheral tissue microcirculation alterations monitored by NIRS that might benefit from hyperoxic breathing.

The aim of this study is:

1. to evaluate peripheral muscle microcirculation of PAH patients
2. and to investigate the effects of hyperoxic breathing on peripheral muscle microcirculation in these patients.

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Materials and Methods

Study population

The study population consisted of 8 patients with stable PAH (2 men and 6 women), 16 patients with stable systolic chronic heart failure (CHF) and 8 healthy subjects. The diagnosis of PAH was confirmed by a right heart catheterization (RHC) that showed a moderate to severe pulmonary arterial hypertension with a normal pulmonary capillary wedge pressure and an echocardiogram with evidence of normal left ventricular ejection fraction (LVEF), (Table 1). RHC in PAH patients was performed within 1 month from NIRS evaluation as part of their PAH follow-up in our Institution. None of the PAH patients had history/evidence of thrombo-embolic pulmonary disease on isotope perfusion scanning of the lungs or other secondary causes of pulmonary hypertension. All CHF patients (8 ischemic / 8 dilated cardiomyopathy) were at stable optimal medical treatment including beta-blockers, angiotensin-converting-enzyme inhibitors, aldosterone-antagonists, furosemide for at least 3 months. The CHF group was matched with PAH group for age, gender, body mass index and functional exercise capacity (New York Heart Association class).

In the PAH group, four patients had a diagnosis of PAH associated to congenital cardiac disease (two had an atrial septal defect surgically corrected, one had a common ventricle with transposition of the great vessels and one had an atrial septal defect not surgically corrected); two patients had a diagnosis of PAH associated with connective tissue disease (one systemic lupus erythematosus and one systemic scleroderma); one patient had a diagnosis of PAH associated with hereditary spherocytosis [without haemolytic anaemia (Hb:16.6g/dl) and without thrombocytosis] and history of splenectomy; and one had diagnosis of PAH associated with chronic drug abuse. One of the patients with atrial septal

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defect had a permanent pacemaker VVI, due to complete atrio-ventricular block implanted 5 years before enrolment in the study. Seven PAH patients had a clinical functional capacity of New York Heart Association (NYHA) class II and one patient had a NYHA class III. Seven PAH patients were under treatment with diuretics, six with sildenafil, five patients with bosentan, three with digoxin, two with nifedipine, and six with anticoagulants. PAH patients and controls with a history of smoking, alcohol, diabetes mellitus, systemic arterial hypertension, dyslipidemias or obesity were excluded from the study.

The control group consisted of 8 healthy subjects individually matched for age, gender and body mass index with PAH patients. None of the healthy subjects was receiving medication. The baseline characteristics of all patients and controls are listed in Table 1. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the Human Study Committee of our institution. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing[19].

Study design

All PAH patients, CHF patients and healthy subjects were evaluated under the same conditions (in the morning, in a seated position, at rest for at least 20 minutes, and without a meal for at least 8 hours). Evaluation of peripheral microcirculation was performed by the NIRS 3-min vascular occlusion technique as previously described [12-14,17,18]. Healthy subjects and CHF patients were evaluated with the NIRS 3-min vascular occlusion technique at room air conditions (21% O_2), while PAH patients were additionally assessed after O_2 inhalation with the following procedure: the first NIRS 3-min occlusion assessment was performed at room air conditions (21% O_2), the 2^{nd} one after 15 min of 100%

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percentage of oxygen inhalation applying a non re-breathing mask and the 3rd one 15 minutes after stopping the oxygen inhalation.

Peripheral arterial O_2 saturation (SpO2, %) and heart rate were continuously monitored during NIRS assessment by using pulse arterial oxymetry.

NIRS measurements

The use of NIRS in humans has been previously validated [20]. With a probe placed on the surface of the skin, NIRS uses the principles of light spectroscopy to measure StO_2, the percentage of hemoglobin saturation in all tissue vascular compartments (arterial, capillary, venous). The light transmitted from the probe has a penetration depth of approximately 12.5 mm, thus enabling the measurement in the respective muscle.

In our study, StO_2 was measured by NIRS (InSpectra, Hutchinson Technology, Minnesota) at the thenar muscle. The StO_2, (%) was monitored continuously before, during, and after upper limb ischemia. Vascular occlusion was applied by a pneumatic cuff that was placed over the elbow and inflated to a pressure 50mmHg above the patients' systolic blood pressure. The occlusion was retained for 3 minutes, and the StO_2 was continuously recorded. Movement of the upper limb was not allowed during measurements. StO_2 curves were analyzed by the Inspectra Analysis Program version 2.0 (Hutchinson Technology, Minn) running in Mat Lab 7.0 (Math Works Inc, Natick, Mass). The first degree slope of the desaturation of hemoglobin during stagnant limb ischemia was used to extrapolate the oxygen consumption rate (OCR) (%/min), and the reperfusion rate (RR), (%/min) as the first degree slope of the increase of StO_2 after the release of the brachial artery occlusion. We also estimated the endothelial function by the time interval (TI), (min) that was needed for StO_2 to reach the highest from the lowest value at the end of the occlusion period and the tissue oxygen restoration rate of the respective tissue microcirculation by the reactive

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hyperemia time (RHT), (min), which was the time needed for StO_2 to reach its baseline values after the release of the occlusion.

Statistical analysis

All continuous variables are presented as mean ± SD. Prior to analysis, all continuous variables were tested by Kolmogorov-Smirnov test. Group means of continuous variables that showed a normal distribution were compared by unpaired Student's t-test and continuous variables that did not show a normal distribution were compared by a non parametric Mann-Whitney U test. Analysis of variance (ANOVA) with post hoc Bonferroni multiple comparisons was performed in continuous variables for between group differences (PAH vs CHF vs Controls). Repeated measures ANOVA were used for the statistical evaluation of the NIRS measurements within PAH group before, during and after hyperoxic breathing. The level of significance was set at $p<0.05$. The statistical analysis was performed by using the SPSS 14.0 (Software Package for Social Sciences).

Results

No statistical differences were observed in age, gender and body mass index between patients and controls. CHF patients had lower LVEF and higher left ventricular end diastolic diameter compared to PAH patients ($P<0.001$).

PAH versus healthy subjects (controls)

Patients with PAH had a significant lower values of StO_2 and SpO_2 compared to controls ($P=0.005$ and $P=0.01$, respectively). RHT was significantly higher in PAH patients compared to controls ($P<0.001$), while no statistical difference was found in OCR compared to the control group ($p=0.615$). A trend to lower values was found in TI and RR, ($P=0.11$ and $P=0.06$, respectively) in PAH compared to controls, (Table 2).

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PAH versus CHF patients

PAH patients had lower StO_2 (P=0.08) and SpO_2 (P=0.01) values, and higher RHT (P=0.02) compared to CHF patients (Table 2). There was not a significant difference in OCR between PAH and CHF patients.

A descriptive scheme of NIRS occlusion technique in a PAH patient and a healthy control subject is illustrated in figure 1.

PAH patients before, during and after 100 % O_2 breathing

After 15 min of 100 % O_2 breathing, there was a significant increase in StO_2, (Table 3), and RHT, (figure 2), ($P<0.05$, $P<0.01$; respectively) and a decrease in OCR, (Table 3), ($P<0.05$) in PAH patients compared to baseline values. All measurements had a trend to return to baseline values after discontinuation of oxygen supplementation, (Table 3).

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Discussion

This study shows that PAH patients are characterized by several skeletal muscle tissue microcirculation alterations as assessed by the Near-Infrared Spectroscopy vascular occlusion technique. More specifically, PAH patients have a lower resting skeletal muscle tissue oxygenation, a trend to a decreased oxygen consumption rate and a slower tissue oxygen restoration rate during reperfusion comparing to controls. PAH patients present also with a slower tissue oxygen restoration rate during reperfusion and a trend to a lower resting skeletal muscle tissue oxygenation comparing to CHF patients matched with PAH patients for exercise capacity.

By applying the NIRS occlusion technique, we have assessed several novel aspects of peripheral tissue microcirculation in PAH patients, monitoring in this way tissue microcirculation changes during hyperoxic breathing. To our knowledge, this study is the first to evaluate skeletal muscle microcirculation by NIRS vascular occlusion technique in PAH patients and to monitor systemic microcirculation changes after chemo-reflex deactivation.

Recent studies have observed an impaired peripheral endothelial function in pulmonary arterial hypertension in relation to disease severity[7-9]. The evaluation of endothelium function was done by using an ultrasound measurement of brachial artery flow-mediated vasodilatation (FMD) during reactive hyperemia[7] and by a non-invasive plethysmograph assessing post-occlusion brachial artery FMD[8,9]. Our study confirmed these findings related to endothelium dysfunction in PAH patients by using NIRS occlusion technique.

In this study, resting tissue oxygen saturation differences between PAH patients and healthy subjects can be mainly explained from the hypoxemia observed in PAH patients leading to a decreased oxygen delivery and thus low tissue oxygen saturation. The

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concomitant low cardiac output might also be a contributing factor. It has been previously shown that low tissue oxygen saturation is related to severity of patients with chronic left ventricular dysfunction that can be improved by the infusion of inotropic agents[13] and by exercise training[14]. In our study, PAH patients had even lower tissue oxygen saturation than CHF patients. However, PAH patients of the present study did not present low cardiac output making it less likely the implication of this mechanism. Another possible explanatory mechanism might be the increased sympathetic tone found in PAH patients that leads to poor tissue perfusion Interestingly, recent studies have shown an increased sympathetic nerve activity[21] with an impaired autonomic nervous activity that is related to disease severity[22,23] in PAH patients.

During the vascular occlusion technique we demonstrate a trend to a decreased oxygen consumption rate in PAH patients comparing to controls indicating a relatively low tissue oxygen diffusion rate possibly due to skeletal muscle abnormalities (reduction of oxidative muscle fibers, depletion of mitochondria). This might be explained by the presence of skeletal muscle atrophy due to chronic low tissue perfusion and the de-conditioning status of PAH patients. Recent studies have shown significant skeletal muscle dysfunction in PAH patients[24,25].Imbalance between vasoconstrictor and vasodilator micro-vascular tone regulated mainly by local (endothelial function) and systemic neuro-humoral factors seem to be the main reason for the slower tissue oxygen restoration rate during reperfusion in PAH patients. It has been previously shown that there is an imbalance between the excretion of thromboxane and prostacycline metabolites in pulmonary hypertension[6], with presence of decreased nitric oxide synthases[5] and high plasma levels of endothelin-1[4]. Endothelium dysfunction plays a central role in the initiation and progression of pulmonary hypertension[3]. In a recent study, Gabrielly et al.[26] have also demonstrated

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that PAH adult patients present with an increased oxidative stress and endothelial dysfunction markers, while in another study Friedman et al.[27] have shown that idiopathic PAH children have a significant systemic endothelial dysfunction in association with disease severity. However, whether these abnormalities found in systemic endothelium function affect the pulmonary hypertension disease process or represent a consequence of the disease is not yet known. Further longitudinal studies are needed to investigate the exact mechanism.

Another significant finding emerged from this study was that hyperoxic breathing improves resting skeletal muscle tissue oxygenation, while induces a slower oxygen consumption rate and a more pronounced delay in tissue oxygen restoration rate in PAH patients.

The acute changes of peripheral tissue microcirculation after hyperoxic supplementation in PAH patients consisted of improving resting tissue oxygen saturation mainly as an expression of higher oxygen delivery due to improved hypoxemia and less possibly to higher cardiac output. The acute correction of hypoxemia induces a slight reduction in sympathetic activity of PAH patients[21,28] and thus, indirectly reduces the stiffness of the main pulmonary artery[28], lowering right ventricular after-load. However, previous historical and recent studies in healthy subjects have shown that high concentrations of oxygen breathing induce a vagus-dependent decrease in heart rate and a rate-dependent decrease in cardiac index with an increase of peripheral systemic resistance[29-32].

Interestingly, oxygen consumption rate was slowed and there was a more pronounced delay in tissue oxygen restoration rate after hyperoxic breathing suggesting deterioration in peripheral tissue microcirculation in PAH patients. Even though the exact mechanism of these changes is not known, a possible explanation might be the increased oxidative stress, the formation of reactive oxygen species and the evoked peripheral vasoconstriction

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induced by high fraction of oxygen supplementation[29]. The latter mechanism has also been proposed by recent investigators using FMD assessment in conductance arteries and in the microvasculature in healthy adults[30] as well as in patients of high cardiovascular risk[31]. In a previous important study it was shown that pulmonary vascular resistance decreased and systemic vascular resistance increased after hyperoxic breathing in patients with severe pulmonary vascular obstructive disease; the effect being more pronounced on the systemic than on the pulmonary vascular bed[33]. Furthermore, as recent systemic reviews have shown, hyperoxia from high-concentration oxygen therapy causes a marked reduction in coronary blood flow and myocardial oxygen consumption[34], while there is limited evidence of routine use of oxygen therapy in uncomplicated myocardial infarction[35]. Interestingly, in a recent investigational study the detrimental effects of oxygen therapy were noted in patients with heart failure[36]. However the risk of detrimental consequences of hyperoxic breathing on peripheral tissue microcirculation must be the aim of future studies in order to establish the exact benefits and the safety limits of oxygen supplementation in PAH patients; NIRS occlusion technique might be a useful clinical tool in the assessment of this issue.

Clinical implications

PAH patients present with several abnormalities in peripheral tissue microcirculation that can be detected by NIRS occlusion technique. Acute oxygen supplementation induces changes in peripheral tissue microcirculation that can be evaluated and monitored by NIRS occlusion technique.

The NIRS occlusion technique is a promising and relatively new method that has been applied in healthy subjects[11], smokers[12], patients with sepsis[16] and in patients with chronic heart failure[13-15]. This simple, non-invasive technique contains valuable

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information regarding peripheral tissue microcirculation and might be used as a monitoring and prognostic tool. This technique may detect those PAH patients with more pronounced peripheral tissue microcirculation abnormalities that might benefit more by certain medical treatments such as endothelin antagonists, prostanoid analogs, phospho-diesterase inhibitors, ACE inhibitors, oxygen therapy, and exercise training programs.

Limitations

The small number of PAH patients did not allow us to perform a correlation with disease severity. While we did not directly evaluate endothelium function, we used NIRS occlusion technique as an indirect evaluation of peripheral tissue microcirculation. A lower OCR was found in PAH patients compared to controls, but there was only a trend to a statistical significant result. This was possibly due to the small number of PAH patients. We do not report cardiac output data for CHF patients as only a minority of these patients had a recent right heart catheterization. However, it could be hypothesized that this CHF group has a rather normal to low cardiac output at difference to PAH group that has a normal and not a decreased cardiac output as Table 2 reports. PAH patients were also under specific medical treatment (endothelin antagonists, sildenafil) that might alter the significance of the results. However these drugs are potent vasodilators that improve endothelial function. For these reasons this treatment enhances rather than favors the negative findings observed in PAH patients of our study. In this study we identified systemic microcirculation changes in PAH patients by comparing their results to those of healthy subjects after hyperoxic breathing; however, the exact mechanisms explaining these findings cannot be confirmed by our study. Future studies are required to confirm these mechanisms.

Conclusions

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PAH patients are characterized by several alterations in peripheral tissue microcirculation as assessed by NIRS occlusion technique. Specifically, PAH patients exhibit decreased tissue oxygen saturation, possibly due to hypoxemia and slower tissue oxygen restoration rate, possibly due to endothelium dysfunction and peripheral vasoconstriction in systemic microcirculation. Acute hyperoxic breathing improves resting tissue oxygen saturation as an expression of higher oxygen delivery, whilst it deteriorates tissue oxygen consumption rate and tissue oxygen restoration rate during reperfusion possibly due to increased oxidative stress and evoked vasoconstriction.

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Abbreviations

FMD: Flow-mediated vasodilatation

NIRS: Near Infra-Red Spectroscopy

NYHA: New York Heart Association

OCR: Oxygen consumption rate

PAH: Pulmonary arterial hypertension

RHT: Reactive hyperemia time

StO_2: Tissue O_2 saturation

TI: Time interval

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References

1. Thenappan T, Shah SJ, Rich S, Gomberg-Maitland M. A USA-based registry for pulmonary arterial hypertension 1982-2006. Eur Respir J 2007;30(6):1103-1110.
2. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR et al. ACCF/AHA. ACCF/AHA. ACCF/AHA 2009 expert consensus document on pulmonary hypertension. Circulation 2009;119(16):2250-2294.
3. Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. Circulation 2004;109(2):159-165.
4. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? Ann Intern Med 1991;114(6):464-469.
5. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med 1995;333(4):214-221.
6. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992;327(2):70-75.
7. Wolff B, Lodziewski S, Bollmann T, Opitz CF, Ewert R. Impaired peripheral endothelial function in severe idiopathic pulmonary hypertension correlates with the pulmonary vascular response to inhaled iloprost. Am Heart J 2007;153(6):1088.e1-7
8. Peled N, Bendayan D, Shitrit D, Fox B, Yehoshua L, Kramer MR. Peripheral endothelial dysfunction in patients with pulmonary arterial hypertension. Respir Med 2008;102(12):1791-1796.

9. Peled N, Bendayan D, Shitrit D, Fox B, Yehoshua L, Kramer MR. Peripheral arterial stiffness and endothelial dysfunction in idiopathic and scleroderma associated pulmonary arterial hypertension. J Rheumatol 2009;36(5):970-975.
10. Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest 2007;131(6):1917-1928.
11. De Blasi RA, Cope M, Elwell C, Safoue F, Ferrari M. Non-invasive measurement of human forearm oxygen consumption by near infrared spectroscopy. Eur J Appl Physiol Occup Physiol 1993;67(1):20–25.
12. Siafaka A, Angelopoulos E, Kritikos K, Poriazi M, Basios N, Gerovasili V et al. Acute effects of smoking on skeletal muscle microcirculation monitored by near-infrared spectroscopy. Chest 2007;131(5):1479-1485.
13. Nanas S, Gerovasili V, Dimopoulos S, Pierrakos C, Kourtidou S, Kaldara E et al. Inotropic agents improve the peripheral microcirculation of patients with end-stage chronic heart failure. J Card Fail 2008;14(5):400-406.
14. Gerovasili V, Drakos S, Kravari M, Malliaras K, Karatzanos E, Dimopoulos S et al. Physical exercise improves the peripheral microcirculation of patients with chronic heart failure. J Cardiopulm Rehabil Prev 2009;29(6):385-391.
15. Abozguia K, Phan TT, Shivu GN, Maher AR, Ahmed I, Wagenmakers A, Frenneaux MP. Reduced in vivo skeletal muscle oxygen consumption in patients with chronic heart failure--a study using Near Infrared Spectrophotometry (NIRS). Eur J Heart Fail 2008;10(7):652-657.

16. Nanas S, Gerovasili V, Renieris P, Angelopoulos E, Poriazi M, Kritikos K et al. Non invasive assessment of the microcirculation in critically ill patients. Anaesth Intensive care 2009;37(5):700-702.

17. Gerovasili V, Dimopoulos S, Tzanis G, Anastasiou-Nana M, Nanas S. Utilizing the vascular occlusion technique with NIRS technology. Int J Ind Ergon 2010;40(2):218-222.

18. Manetos C, Dimopoulos S, Tzanis G, Vakrou S, Tasoulis A, Kapelios C et al. Skeletal muscle microcirculatory abnormalities are associated with exercise intolerance, ventilatory inefficiency, and impaired autonomic control in heart failure. J Heart Lung Transplant 2011;30(12):1403-1408.

19. Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2010;144(1):1-2.

20. Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near infrared spectroscopy in humans. J Appl Physiol 1994;77(6):2740–2747.

21. Velez-Roa S, Ciarka A, Najem B, Vachiery JL, Naeije R, van de Borne P. Increased sympathetic nerve activity in pulmonary artery hypertension. Circulation 2004;110(10):1308-1312.

22. Dimopoulos S, Anastasiou-Nana M, Katsaros F, Papazachou O, Tzanis G, Gerovasili V et al. Impairment of autonomic nervous system activity in patients with pulmonary arterial hypertension – a case control study. J Cardiac Failure 2009;15(10):882-889.

23. Wensel R, Jilek C, Dörr M, Francis DP, Stadler H, Lange T et al. Impaired cardiac autonomic control relates to disease severity in pulmonary hypertension. Eur Respir J 2009;34(4):792-794.

24. Bauer R, Dehnert C, Schoene P, Filusch A, Bärtsch P, Borst MM et al. Skeletal muscle dysfunction in patients with idiopathic pulmonary arterial hypertension. Respir Med 2007;101(11):2366-2369.
25. Mainguy V, Maltais F, Saey D, Gagnon P, Martel S, Simon M, Provencher S. Peripheral Muscle Dysfunction in Idiopathic Pulmonary Arterial Hypertension. Thorax 2010;65(2):113-117.
26. Gabrielli LA, Castro PF, Godoy I, Mellado R, Bourge RC, Alcaino H, Chiong M, Greig D, Verdejo HE, Navarro M, Lopez R, Toro B, Quiroga C, Díaz-Araya G, Lavandero S, Garcia L. Systemic oxidative stress and endothelial dysfunction is associated with an attenuated acute vascular response to inhaled prostanoid in pulmonary artery hypertension patients. J Card Fail. 2011 Dec;17(12):1012-7
27. Friedman D, Szmuszkovicz J, Rabai M, Detterich JA, Menteer J, Wood JC Systemic endothelial dysfunction in children with idiopathic pulmonary arterial hypertension correlates with disease severity. J Heart Lung Transplant. 2012 Jun;31(6):642-7
28. Haneda T, Nakajima T, Shirato K, Onodera S, Takishima T. Effects of oxygen breathing on pulmonary vascular input impedance in patients with pulmonary hypertension. Chest 1983;83(3):520-527.
29. Daly WJ, Bondurant S. Effects of oxygen breathing on the heart rate, blood pressure, and cardiac index of normal men--resting, with reactive hyperemia, and after atropine. J Clin Invest 1962;41:126-132.
30. Waring WS, Thomson AJ, Adwani SH, Rosseel AJ, Potter JF, Webb DJ, Maxwell SR. Cardiovascular effects of acute oxygen administration in healthy adults. J Cardiovasc Pharmacol 2003;42(2):245-250.

31. Frøbert O, Holmager P, Jensen KM, Schmidt EB, Simonsen U. Effect of acute changes in oxygen tension on flow-mediated dilation. Relation to cardiovascular risk. Scand Cardiovasc J 2008;42(1):38-47.

32. Gole Y, Gargne O, Coulange M, Steinberg JG, Bouhaddi M, Jammes Y et al. Hyperoxia-induced alterations in cardiovascular function and autonomic control during return to normoxic breathing. Eur J Appl Physiol. 2011;111(6):937-946.

33. Krongrad E, Helmholz HF, Ritter DG. Effect of breathing oxygen in patients with severe pulmonary vascular obstructive disease. Circulation 1973;47(1):94-100.

34. Wijesinghe M, Perrin K, Ranchord A, Simmonds M, Weatherall M, Beasley R. Routine use of oxygen in the treatment of myocardial infarction: systematic review. Heart 2009;95(3):198–202.

35. Farquhar H, Weatherall M, Wijesinghe M, Perrin K, Ranchord A, Simmonds M, Beasley R. Systematic review of studies of the effect of hyperoxia on coronary blood flow. Am Heart J 2009;158(3):371-377.

36. Park JH, Balmain S, Berry C, Morton JJ, McMurray JJ. Potentially detrimental cardiovascular effects of oxygen in patients with chronic left ventricular systolic dysfunction. Heart 2010;96(7):533-538.

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Figure Legends

Figure 1. An illustrative scheme describing the 3-min Near Infrared Spectroscopy (NIRS) occlusion technique in a healthy control subject (upper plan) and a patient with pulmonary arterial hypertension (PAH), (lower plan).

Figure 2**.** Reactive hyperemia time (RHT, min) before, during and after hyperoxic breathing in PAH patients ($P < 0.01$: comparison between RHT at baseline and after 15-min of 100% O_2 measurements, $P < 0.05$: comparison between RHT after 15-min of 100% O_2 and after O_2 discontinuation measurements). Horizontal bars represent median values; boxes represent interquartile ranges; and whiskers show ranges.

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Table 1. Baseline characteristics in patients with pulmonary arterial hypertension (PAH), chronic heart failure (CHF) and controls

Characteristics	PAH (N=8)	CHF (N=16)	Controls (N=8)
Age, years	53±15[52]	57±12[58]	55±14[51]
Male/Female	2/6	6/10	2/6
BMI, kg/m^2	23±4[23]	26±4[24]	24±3[23]
NYHA class	7 II/1 III	14 II/2 III	-
Haemoglobin, g/dl	14±4[16]	13±2[12]	-
Creatinine, mg/dl	1.0±0.3[1.05]	1.1±0.5[1.05]	-
LVEF, %	61±5[60] †	32±10[29]	-
LVEDD, mm	48±9[48] †	66±7[67]	-
PCWP, mmHg	12±4[12]	-	-
sPAP, mmHg	77±41[67]	-	-
dPAP, mmHg	34±18[26]	-	-
mPAP, mmHg	56±27[48]	-	-
CO, L/min	5.8±3.0[4.8]	-	-
CI, L/min/m^2	3.4±1.8[2.5]	-	-

Continuous variables are presented as Mean±Standard Deviation[Median]. BMI=Body mass index; NYHA=New York Heart Association; LVEF=Left ventricular ejection fraction, LVEDD=Left ventricular end diastolic diameter, PCWP=Pulmonary capillary wedge pressure, sPAP=Systolic pulmonary artery pressure, dPAP=Diastolic pulmonary artery pressure, mPAP=Mean pulmonary artery pressure, CO=Cardiac output, CI=Cardiac index

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†P<0.001: Comparison between patients with PAH and CHF

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Table 2. Near infrared spectroscopy measurements combined with vascular occlusion technique in patients with pulmonary arterial hypertension (PAH), chronic heart failure (CHF) and controls

Variables	PAH patients (N=8)	CHF patients (N=16)	Controls (N=8)	ANOVA p value
StO_2, (%)	65.8±14.9[69] †	75±7[76] **	82.1±4.0[82]	0.006
OCR, (%/min)	35.3±9.1[33]	32±9[29]	43.4±19.7[44]	0.133
RR, (%/min)	535±176[573]	548±137[558] ***	702±166[720]	0.056
TI, (min)	0.4±0.1[0.35]	0.4±0.1[0.35] #	0.28±0.05[0.23]	0.05
RHT, (min)	3.0±0.6[3.0] ††	2.4±0.5[2.5] *	2.0±0.3[2.0]	0.001
SpO_2, (%)	90±7[94] †††	97±1[97]	98±1[98]	<0.001
HR, (bpm)	74±13[73]	67±9[65]	69±2[69]	0.692

Values are presented as Mean±Standard Deviation [Median]. StO_2=Tissue oxygen saturation; OCR=Oxygen consumption rate; RR=reperfusion rate; TI=Time interval needed the StO_2 to reach the highest from the lowest value at the end of the occlusion period; RHT=Reactive hyperemia time; SpO_2= Oxygen peripheral arterial saturation; HR=heart rate

†P =0.005, comparison between PAH and controls

††P<0.001, comparison between PAH and controls

†††P=0.01, comparison between PAH and CHF, PAH and controls

*P=0.016, comparison between PAH and CHF

**P=0.08 comparison between PAH and CHF

***P=0.09 comparison between PAH and CHF

#P=0.06, comparison between CHF and controls

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Table 3. Near infrared spectroscopy measurements combined with the 3-min vascular occlusion technique in patients with pulmonary arterial hypertension (PAH) at baseline (21% O_2), after 15-min of 100% O_2 inhalation and 15-min after discontinuation of hyperoxic breathing

Variables	Baseline 21% O_2	After 15-min 100% O_2	After 15-min of O_2 supply discontinuation
StO_2, (%)	65.8±14.9[69]	71.4±14.5[74]†	63.9±14.8[68]**
OCR, (%/min)	35.3±9.1[33]	25.1±6.6[25]†	31.3±13.3[27]
RR, (%/min)	535±176[573]	601±162[654]	619±209[609]
TI, (min)	0.4±0.1[0.35]	0.35±0.12[0.28]	0.36±0.16[0.29]
RHT, (min)	3.0±0.6[3.0]	4.2±0.7[4.2]††	3.1±0.2[3.1]*
SpO_2, (%)	90±7[94]	95±6††[98]	91±8*[95]
HR, (bpm)	74±13[73]	68±10[67]†	72±11[70]***

Values are expressed as Mean±SD [Median]

StO_2=Tissue oxygen saturation; OCR=Oxygen consumption rate; RR=reperfusion rate; TI=Time interval needed the StO_2 to reach the highest from the lowest value at the end of the occlusion period; RHT=Reactive hyperemia time; SpO_2= Oxygen peripheral arterial saturation; HR=heart rate

$^{\dagger}P$ <0.05, comparing baseline and after 15-min 100% O_2 measurements,

$^{\dagger\dagger}P$ <0.01 comparing baseline and after 15-min 100% O_2 measurements,

$^{*}P$ <0.05, comparing after 15-min 100% O_2 and after O_2 discontinuation measurements,

**P <0.01, comparing after 15-min 100% O_2 and after O_2 discontinuation measurements

***P=0.06, comparing after 15-min 100% O_2 and after O_2 discontinuation measurements

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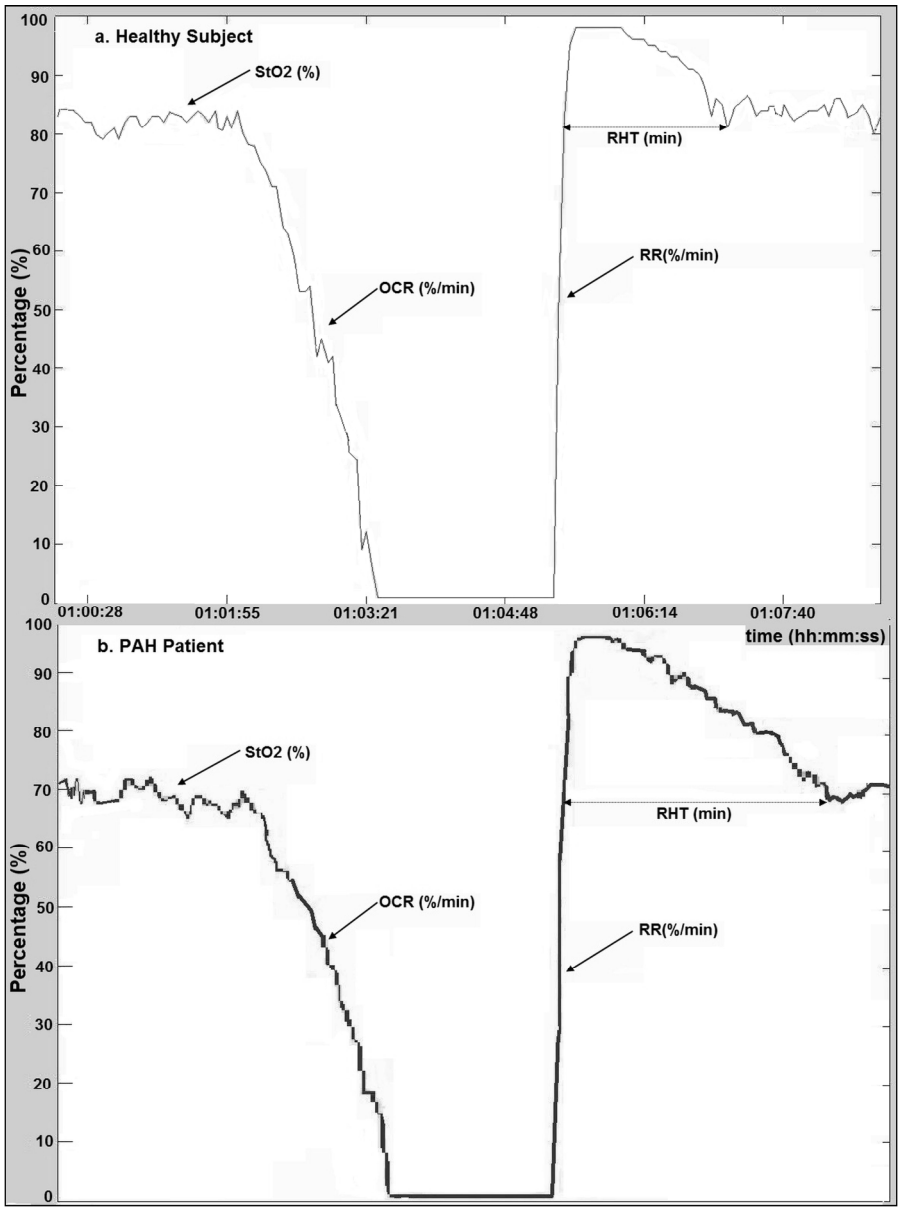

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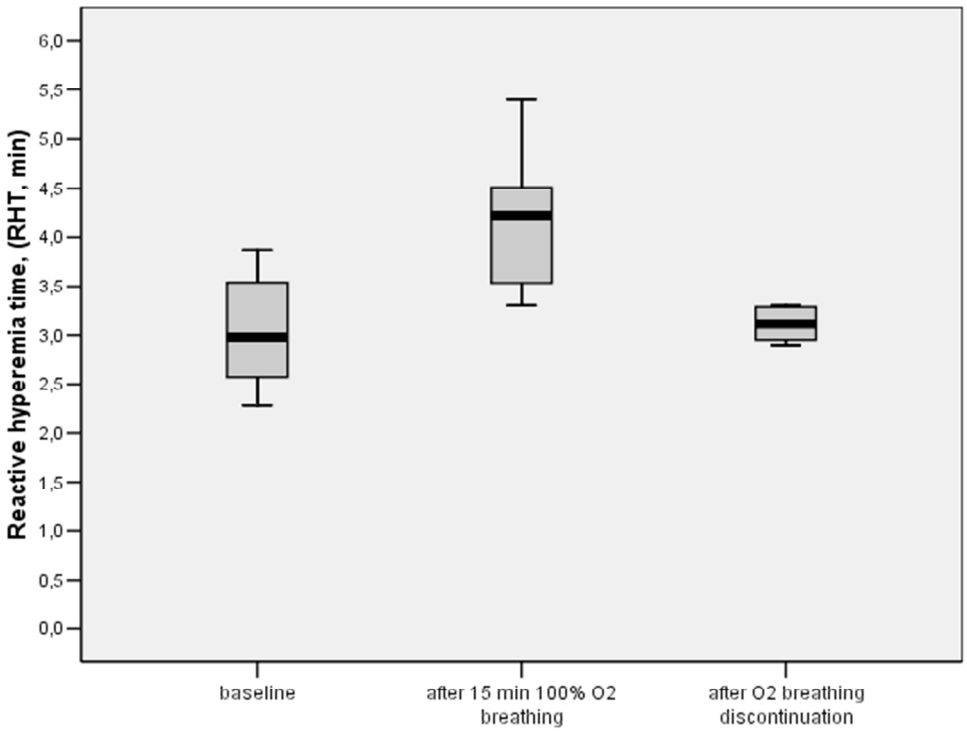

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