ON-LINE SUPPLEMENT

Cerebral cortex oxygen delivery and exercise limitation in patients with COPD

Ioannis Vogiatzis[1,2], Zafeiris Louvaris[1,2], Helmut Habazettl,[3,4] Vasileios Andrianopoulos [1], Harrieth Wagner[5], Charis Roussos[1], Peter D. Wagner[5] and Spyros Zakynthinos[1]

[1] Department of Critical Care Medicine and Pulmonary Services, Evangelismos Hospital, "M. Simou, and G.P. Livanos Laboratories", National and Kapodistrian University of Athens, Greece

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

[3] Institute of Physiology, Charité Campus Benjamin Franklin, Berlin, Germany

[4] Institute of Anesthesiology, German Heart Institute, Berlin, Germany

[5] Department of Medicine, University of California San Diego, La Jolla, CA, USA

Methods

Subjects

Twelve patients (2 females) with clinically stable COPD participated in the study according to the following inclusion criteria: 1) a post-bronchodilator forced expiratory volume in one second (FEV_1) < 60% predicted without significant reversibility (<12% change of the initial FEV_1 value or <200 ml); 2) optimal medical therapy according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) (1) and 3) moderate hypoxemia (arterial blood oxygen saturation < 93% and > 84%) in room air during preliminary exercise testing. Exclusion criteria included clinically manifest cor pulmonale, cardiovascular illness, musculoskeletal abnormalities or other diseases that could contribute to exercise limitation. The study was approved by the University Hospital Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. Prior to participation in the study, all patients were informed of any risks and discomforts associated with the experiments and gave written, signed, informed consent.

Experimental Design

Experiments were conducted in two visits. In visit 1, patients underwent an incremental preliminary exercise test to the limit of tolerance [peak work rate (WR_{peak})]. In visit 2, patients undertook three constant-load exercise tests at 75% WR_{peak} initially breathing room air and afterwards breathing in a balanced ordering sequence either pure oxygen or normoxic heliox. Protocols were separated by 120 minutes of rest. During exercise protocols in room air, normoxic heliox and pure oxygen, patients were asked to pedal for as long as possible against the constant load to the limit of tolerance (Tlim). Tlim was defined as the time point in which the patients signaled to stop exercising or could not maintain the required pedaling rate for 10 seconds despite being encouraged by the investigators. Prior to imposing the target load on the bicycle ergometer, patients were asked to perform unloaded cycling for 60 seconds reaching and maintaining a cadence of approximately 50 revolutions/min. Pure oxygen (100% oxygen) and normoxic heliox (21% oxygen and 79% helium) breathing was achieved by having subjects inspire from a Douglas bag,

containing either pure oxygen or normoxic heliox, that was connected to the inspiratory port of a nonrebreathing two-way valve by a piece of tubing. The same apparatus was utilized during room air breathing in order to ensure a blinding strategy of administration of the inspired gas mixture. During protocols, frontal cerebral cortex blood flow [assessed by near-infrared spectroscopy (NIRS) and the light-absorbing tracer indocyanine green (ICG) dye], as well as cardiac output (assessed by the dye dilution method again using ICG), were measured at rest and during the final minute of each of the exercise protocols. As endurance time was expected to be significantly prolonged whilst breathing pure oxygen or normoxic heliox, measurements during these trials were also performed at the time point where exercise in room air was terminated (i.e.: at isotime) in order to detect whether potential differences in frontal cerebral cortex oxygen delivery at isotime could, in part, explain differences in endurance time whilst breathing oxygen or heliox. NIRS was also used to continuously record frontal cerebral cortex oxygen saturation (StO_2), which is commonly adopted as an index of tissue oxygen availability, reflecting the balance between oxygen supply and demand (2-4).

Preliminary Testing

In visit 1, the incremental exercise tests were performed on an electromagnetically braked cycle ergometer (Ergoline 800; Sensor Medics, Anaheim, CA, USA) with a ramp increase of load increments of 5 or 10 W/min to the limit of tolerance (the point at which the work rate could not be tolerated due to severe sensation of dyspnea and/or leg fatigue; peak exercise data are included in Table 2 of the main manuscript). Throughout the exercise tests, patients were encouraged to maintain a pedaling frequency of 40-50 revolutions/min. Tests were preceded by a 3-min rest period, followed by 3 min of unloaded pedaling. The following pulmonary gas exchange and ventilatory variables were recorded breath by breath (Vmax 229; Sensor Medics, Anaheim, CA, USA): oxygen uptake, carbon dioxide elimination, minute ventilation, tidal volume, breathing frequency, and respiratory exchange ratio. Heart rate and percentage of oxygen saturation (% SpO_2) were determined using the R-R interval from a 12-lead on-line electrocardiogram (Marquette Max;

Marquette Hellige, Freiburg, Germany) and a pulse oximeter (Nonin 8600; Nonin Medical, North Plymouth, MN, USA), respectively (5).

Subject Preparation

Subjects were prepared with arterial and venous catheters. Using local anaesthesia (2% lidocaine) and sterile technique, identical catheters were introduced percutaneously into the right forearm vein and the right radial artery, both oriented in the proximal direction. The catheters were used to collect arterial and venous blood samples and to inject ICG dye into the venous line and sample blood after each injection from the arterial line for cardiac output measurement and cerebral blood flow calculation. The catheters were kept patent throughout the experiment by periodic flushing with heparinized (1 unit/ml) saline.

Cardiac Output

Cardiac output was determined by the dye dilution method (6), using known volumes of ICG (1.0 ml at 5 mg/ml) injected into the right forearm vein followed by a rapid 10-ml flush of isotonic saline. Blood was withdrawn from the right radial artery using an automated pump (Harvard Apparatus, Holliston, MA, USA) at 20 ml/min through a linear photodensitometer (Pulsion ICG; ViCare Medical, Denmark) connected to a cardiac output computer (Waters CO-10; Rochester, MN, USA) through a closed loop, sterile tubing system, as previously described (7-8). The blood was re-infused into the forearm vein immediately upon completion of the measurements. The cardiac output computer was connected to a data acquisition system (DI-720; Dataq, OH, USA). Data were sampled at 100 Hz and stored on a computer for subsequent analysis. To remove the influence of dye recirculation, the downslopes of the dye concentration curves were linearly extrapolated using a semi-logarithmic scale in the conventional manner. Cardiac output was calculated as the ratio of ICG mass injected to the mean arterial ICG concentration over the time interval of the curve and expressed as L/min. ICG calibration curves were obtained following each experiment by measuring the raw voltage deflection from three 20 ml blood samples containing

various concentrations of ICG. Calibrations at each concentration were performed 2-3 times to ensure linearity and consistency, as previously described (7).

Frontal Cerebral Cortex Blood Flow by NIRS

To measure frontal cerebral cortex blood flow, one set of NIRS optodes was placed on the skin over the left frontal cortex region of the forehead, secured using double-sided adhesive tape. A special net headset was used to hold the NIRS probe firmly attached over the frontal cortex region. The optode separation distance was 4 cm, corresponding to a penetration depth of ~2 cm. Optodes were connected to a NIRO 200 spectrophotometer (Hamamatsu Photonics KK, Hamamatsu, Japan), which was used to measure ICG concentration following the same 5-mg bolus injection of ICG in the right forearm vein used for cardiac output assessment, as previously described (8). Tissue microcirculatory ICG was detected transcutaneously by measuring light attenuation with NIRS at 775-, 813- and 850-nm wavelengths and analyzed using an algorithm incorporating the Modified Beer-Lambert Law (9-12). Since the measured light attenuation in the tissue is influenced by ICG and oxy- and deoxyhaemoglobin, the independent contribution of ICG to the light absorption signal was isolated using a matrix operation (MATLAB). The matrix operation incorporates path length-specific extinction coefficients for each of the light absorbing chromophores [haemoglobin + myoglobin (Hb+Mb) and ICG] at each wavelength employed by the NIRS machine (Hamamatsu Photonics KK).

Blood flow was calculated from the rate of tissue ICG accumulation over time measured by NIRS according to the Sapirstein principle (13). Accordingly, for any time interval less than the time to reach peak tissue accumulation of tracer, the tissue receives the same fraction of the ICG bolus as quantified in arterial blood (input function). Two separate time points within the first half of the curve were used to calculate flow, and the average value was taken to represent the tissue ICG accumulation. Therefore total blood flow was calculated using the following equation:

$$\text{blood flow}(\text{ml} \cdot 100\ \text{ml}^{-1} \cdot \text{min}^{-1}) = \frac{k \cdot [\text{ICG}]_m \cdot t}{\int_0^t [\text{ICG}]_\text{a} \text{d}t}$$

where k is the molecular weight of ICG for the conversion of ICG in moles to grams per litre; $[ICG]_m$ is the accumulation of ICG in tissue over time t expressed in micromoles; and $\int 0t[ICG]_a dt$ is the time integral of the arterial ICG concentration expressed in milligrams per litre (11). The ICG calibration procedure, as described for cardiac output, was also used to quantify the input function for calculation of the regional tissue blood flow with NIRS.

Systemic and frontal cerebral cortex oxygen delivery was calculated as the product of the cardiac output and the frontal cerebral cortex blood flow, and arterial oxygen content, respectively.

Frontal Cortex Cerebrovascular Oxygenation by NIRS

Frontal cortex cerebrovascular oxygenation was assessed continuously throughout the whole testing period by the same NIRO 200 spectrophotometer as used for the measurement of frontal cerebral cortex blood flow. High ICG tissue concentrations during the passage of the dye bolus through the brain may interfere with hemoglobin (Hb) results. Therefore, to avoid any interference between ICG and Hb wavelengths, oxygenation data were averaged over 10 seconds immediately prior to occurrence of the ICG peak. A derived parameter from NIRS studies in humans is the ratio of oxygenated Hb to total Hb, an index of changes in cerebrovascular oxygen saturation (StO_2) relative to rest (2-4, 12, 13).

Blood Analysis and Calculations

Arterial tensions of O_2 (PaO_2) and CO_2 ($PaCO_2$), pH, Hb concentration, and percentage of arterial oxygen saturation (SaO_2) were measured from 2-ml blood samples using a blood gas analyzer combined with a cooximeter (ABL 625; Radiometer, Copenhagen, Denmark) within few seconds of collection. Arterial O_2 content (CaO_2) was computed [$CaO_2 = (1.34 \times Hb \times SaO_2) + (0.003 \times PaO_2)$]. The blood gas analyser was auto-calibrated every 4 hours throughout the day and calibrating gases of known concentrations were run before each set of measurements. Blood gas measurements were corrected for patient's tympanic temperature taken during withdrawal of each arterial blood gas sample.

Assessment of fat free mass index (FFMI)

Body composition was assessed by single-frequency bioelectrical impedance (Maltron BF 907 body fat analyzer, UK). FFM was calculated using the following equation which has been specifically validated for patients with respiratory diseases (17):

FFM = -6.06 + (height x 0.283) + (weight x 0.207) - (resistance x 0.024) + (sex (males=1, females=0) x 4.036)

FFMI was calculated by dividing body mass (in kg) of FFM by height (in m) squared in order to adjust for body surface area (18). In healthy male adults (age range: 55 to 74 years), FFMI is ranged between 17.0-22.1 kg/m^2 (5^{th}-95th percentile, respectively). In healthy female adults (age range: 55-74 years), FFMI is ranged between 14.1-19.0 kg/m^2 (5^{th}-95th percentile, respectively).

References

1. Global Initiative for Chronic Obstructive Lung Disease, *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Executive summary, 2005*. Available from: www.goldcopd.com/guidelineitem.aspl1 52&l251&intId51816.

2. DeLorey DS, Kowalchuk JM, Paterson DH. Effects of prior heavy intensity exercise on pulmonary O_2 uptake and muscle deoxygenation kinetics in young and older adult humans. *J Appl Physiol* 2004: *97*, 998-1005.

3. DeLorey DS, Kowalchuk JM, Paterson DH. Adaptation of pulmonary O_2 uptake kinetics and muscle deoxygenation at the onset of heavy intensity exercise in young and older adults. *J Appl Physiol* 2005: *98*: 1697-1704.

4. Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on – transitions in humans. *J Appl Physiol* 2003: *95*, 149 – 158.

5. Vogiatzis I, Aliverti A, Golemati S, Georgiadou O, LoMauro A, Kosmas E, Kastanakis E, Roussos C. Respiratory kinematics by optoelectronic plethysmography during exercise in men and women. *Eur J Appl Physiol* 2005:93,581-587.

6. Dow P. Estimations of cardiac output and central blood volume by dye dilution. *Physiol Rev* 1956: 36, 77-102.

7. Vogiatzis I, Athanasopoulos D, Boushel R, Guenette JA, Koskolou M, Vasilopoulou M, Wagner H, Roussos C, Wagner PD, Zakynthinos S. Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. *J Physiol* 2008:586, 5575-5587.

8. Guenette JA, Vogiatzis I, Zakynthinos S, Athanasopoulos D, Koskolou M, Golemati S, Vasilopoulou M, Wagner HE, Roussos C, Wagner PD, Boushel R. Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol* 2008:104,1202-1210.

9. van der Zee P, Cope M, Arridge SR, Essenpreis M, Potter LA, Edwards AD, Wyatt JS, McCormick DC, Roth SC, Reynolds EO. Experimentally measured optical pathlengths for the adult head, calf and forearm and the head of the newborn infant as a function of inter optode spacing. *Adv Exp Med Biol* 1992:316,143-153.

10. Duncan A, Meek JH, Clemence M, Elwell CE, Tyszczuk L, Cope M, Delpy DT. Optical pathlength measurements on adult head, calf and forearm and the head of the newborn infant using phase resolved optical spectroscopy. *Phys Med Biol* 1995:40, 295-304.

11. Boushel R, Langberg H, Olesen J, Nowak M, Simonsen L, Bulow J, Kjaer M. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol* 2000: 89,1868-1878.

12. Kalliokoski KK, Scheede-Bergdahl C, Kjaer M, Boushel R. Muscle perfusion and metabolic heterogeneity: insights from non-invasive imaging techniques. *Exerc Sport Sci Rev* 2006: 34,164-170.

13. Sapirstein LA. Fractionation of the cardiac output of rats with isotopic potassium. *Circ Res* 1956: 4, 689-692.

14. Chance B, Dait MT, Zhang C, Hamaoka T, Hagerman F. Recovery from exercise – induced desaturation in the quadriceps muscles of elite competitive rowers. *Am J Physiol Cell Physiol* 1992: *262*, C766-C775.

15. DeLorey DS, Kowalchuk JM, Paterson DH. Relationship between pulmonary O_2 uptake kinetics and muscle deoxygenation during moderate – intensity exercise. *J Appl Physiol* 2003: *95*, 113-120.

16. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr* 2004; 23: 1226-1243.

17. Kyle UG, Pichard C, Rochat T, Slosman DO, Fitting JW, Thiebaud D. New bioelectrical impedance formula for patients with respiratory insufficiency: comparison to dual-energy X-ray absorptiometry. *Eur Respir J* 1998;12:960-6.

18. VanItallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. *Am J Clin Nutr* 1990;52:953-9.