ON-LINE DATA SUPPLEMENT

Intercostal muscle blood flow limitation during exercise in chronic obstructive pulmonary disease

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Methods

Subjects

Ten patients (1 female) 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) <80% predicted without significant reversibility (<12% change of the initial FEV_1 value or <200 ml); 2) optimal medical therapy according to GOLD (E1); and 3) the absence of other significant 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 (WR_{peak}). In visit 2, patients initially undertook a graded exercise test (protocol 1), which was followed by resting isocapnic hyperpnoea trials (protocol 2). During the graded exercise test patients completed four bouts of constant-load exercise corresponding to the following targeted intensities: (i) 25% WR_{peak} for 5 min; (ii) 50% WR_{peak} for 5 min; (iii) 75% WR_{peak} for 3-4 min and (iv) 100% WR_{peak} for 2-3 min. 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 rpm. Between exercise bouts at 25 and 50% WR_{peak} patients rested for 15 min, whereas after completion of exercise bouts at 50 and 75% WR_{peak} patients rested for 15 and 30 min, respectively. Two hours after completion of the exercise tests, patients performed five 5-min bouts of isocapnic hyperpnoea at the same tidal volume, breathing frequency and thus minute ventilation recorded at rest and during exercise at 25, 50, 75 and 100% WR_{peak}.

Blood flow over the 7th intercostal space and over the vastus lateralis muscles as well as cardiac output were measured during the final minute of each of the exercise and the hyperpnoea bouts (Figure 1).

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^{-1}. Throughout the exercise tests, patients were encouraged to maintain a pedaling frequency of 40-50 revolutions.min^{-1}. 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 (VO_2), carbon dioxide elimination (VCO_2), minute ventilation (VE), tidal volume (VT), breathing frequency (f), and respiratory exchange ratio (RER). Heart rate (HR) and percentage oxygen saturation (% SpO_2) were determined using the R-R interval from a 12-lead on-line electrocardiogram (Marquette Max; Marquette Hellige GmbH, Germany) and a pulse oximeter (Nonin 8600; Nonin Medical, North Plymouth, MN, USA), respectively.

Subject preparation

Subjects were prepared first with arterial and venous catheters for blood flow measurements, and then with oesophageal and gastric balloons for assessment of esophageal and gastric pressures and of the power of breathing. Using local anaesthesia (2% lidocaine) and sterile technique, identical catheters were introduced percutaneously into the left femoral vein and the right radial artery, both oriented in the proximal direction. The catheters were used to collect arterial and venous blood samples and also to inject indocyanine green dye (ICG) into the venous line and sample blood after each injection from the arterial line for

cardiac output measurement and muscle blood flow calculation. The catheters were kept patent throughout the experiment by periodic flushing with heparinized (1 unit.ml^{-1}) saline.

Esophageal (Pes) and gastric (Pga) pressures were assessed by thin-walled balloon catheters (Ackrad Laboratories, Inc., Crandford, NJ, USA) coupled to differential pressure transducers (MP-45, ±250 cmH_2O; Validyne Corp., Northridge, CA, USA). The balloons were inserted by nasal intubation following the application of 2% lidocaine anaesthetic gel to the nose and with the assistance of continuous pressure monitoring. The balloons were positioned in the middle third of the oesophagus and the stomach respectively.

Protocol 1: graded exercise

During these tests recordings of pulmonary gas exchange and ventilatory variables were performed as mentioned above, whereas arterial blood pressure was measured by a standard sphygmomanometer during the final minute of exercise. Pes and Pga were continuously monitored. Flow was measured with a hot wire pneumotachograph (Vmax 229; Sensor Medics) near the mouthpiece, and tidal volume changes were obtained by integrating the flow signal. Esophageal and gastric pressures and air flow rates were displayed on a computer screen and digitized at 60 Hz using an analog-to-digital converter connected to the same computer used for optoelectronic plethysmography (OEP system, BTS, Milan, Italy). End-inspiratory and end-expiratory compartmental (rib cage and abdominal) chest wall volume changes during exercise were determined by OEP (E2). In brief, the movement of 89 retro-reflective markers placed front and back over the chest wall from clavicles to pubis was recorded. Markers were tracked by six video cameras, three in front of the subject and three behind. Dedicated software recognized in real time the markers on each camera, reconstructed their 3D co-ordinates by stereophotogrametry and calculated volume changes.

Pes and Pga were averaged over 30 s breath samples in every minute of the exercise tests. The mechanical power of breathing over a typical breath was determined at each

exercise or hyperpnoea level by ensemble-averaging several breaths to integrate the average tidal volume - esophageal pressure loop multiplied by the breathing frequency and expressed in $Cal.min^{-1}$ (E3). More specifically, respiratory muscle power was calculated as the area enclosed by the tidal chest wall volume change - esophageal pressure dynamic loops multiplied by respiratory frequency. We considered the entire loops (i.e., both inspiration and expiration), therefore our respiratory muscle power represents the total power developed by all (both inspiratory and expiratory) respiratory muscle groups. Pes was used as an index of pleural pressure (Ppl). Transdiaphragmatic pressure (Pdi) was obtained by subtracting Pes from Pga. Tidal excursion in Pdi (ΔPdi) was taken as peak Pdi during inspiration minus baseline Pdi. Tidal excursion in pleural pressure (ΔPpl) was obtained as peak Ppl during inspiration minus Ppl at the beginning of inspiratory effort as determined by the start of inspiratory Pdi swing. In this way, the decrease in Ppl at the onset of inspiratory effort caused by the relaxation of the expiratory muscles (E3a) was taken into account. Tidal excursion in gastric pressure (ΔPga) was obtained as peak Pga during inspiration minus mean end-expiratory Pga during quiet breathing. The ventilatory recruitment pattern index (ΔPga/ΔPpl) was calculated as expressing the relative contribution of the diaphragm to the pressure generated by the whole inspiratory muscles. The pressure-time products for the diaphragm (PTPdi) and expiratory abdominal muscles (PTPab) were obtained by multiplying the area subtended by each trace (i.e., the integral of Pdi and Pga, respectively, over time) by the respiratory frequency and had units of $cmH_2O \bullet s \bullet min^{-1}$ (E3b). The baseline for PTPdi and PTPab was determined for each breath as the level observed at the start of inspiration and expiration, respectively.

.Protocol 2: resting isocapnic hyperpnoea trials

Patients were asked to maintain targeted ventilation equal to their own mean ventilation recorded at rest and during exercise at 25, 50, 75 and 100% WR_{peak}. Experimenters

provided verbal guidance to adjust the rate and depth of their breathing such that the target ventilation was obtained and held constant to within 5%. Isocapnia was maintained by having subjects inspire from a Douglas bag containing 5% CO_2, 21% O_2, balance N_2 that was connected to a two-way non-rebreathing valve (model 2700, Hans Rudolph) by a piece of tubing. Blood flow over the 7th intercostal space and over the vastus lateralis muscle as well as cardiac output and arterial blood pressure were measured during the final minute of each hyperpnoea bout. Compartmental chest wall volume regulation and the power of breathing were determined as described above for protocol 1, whereas recordings of all ventilatory variables were performed throughout each hyperpnoea bout.

Cardiac output

Cardiac output was determined by the dye dilution method (*E4*), using known volumes of ICG (ICG, 1.0 ml at 5 mg.ml^{-1}) injected into the left femoral 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, USA) at 20 ml.min^{-1} 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. The blood was re-infused into the femoral 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 litres per minute. 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 (*E5).*

Intercostal and quadriceps muscle blood flow by NIRS

In order to measure intercostals and quadriceps muscle blood flow, two sets of near-infrared spectroscopy (NIRS) optodes were placed, one on the skin over the left 7th intercostal space and the other over the left vastus lateralis muscle 10-12 cm above the knee, both secured using double sided adhesive tape. The optode separation distance was 4 cm, corresponding to a penetration depth of ~2 cm. The left intercostal space was used in order to avoid potential blood flow contributions from the liver on the right side of the body. Optodes were connected to a NIRO 200 spectrophotometer (Hamamatsu Photonics KK, Hamamatsu, Japan), which was used to measure ICG concentration following the same 4-5 mg bolus injection of ICG in the right antecubital forearm vein as used for cardiac output assessment as previously described (*E6).*

Tissue microcirculatory ICG was detected transcutaneously by measuring light attenuation with NIRS at 775, 813 and 850 nm wavelengths and analysed using an algorithm incorporating the Modified Beer-Lambert Law (*E7-E10*). 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).

Furthermore, muscle oxygenation was assessed by the same NIRO 200 spectrophotometer as used for the measurement of regional muscle blood flow. High ICG tissue concentrations during the passage of the dye bolus through the muscle may interfere with Hb results. Therefore, to avoid any interference between ICG and Hb wavelengths tissue

oxygenation data were averaged over 10 s immediately prior to ICG injection. The variables assessed by NIRS were the concentration changes of oxygenated, deoxygenated and total Hb. A commonly derived parameter from NIRS studies in humans is tissue O_2 saturation (StO_2) i.e.: the ratio of oxygenated Hb to total Hb, that is commonly adopted as an index of tissue oxygen availability reflecting the balance between muscle oxygen supply and demand (E11)

Blood flow was calculated from the rate of tissue ICG accumulation over time measured by NIRS according to the Sapirstein principle *(*E12*).* 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:

Eq [1]

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

where k is the molecular weight of ICG for the conversion of ICG in moles to grams per liter; $[ICG]_m$ 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 liter (E9). 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, quadriceps and intercostal muscle vascular conductance was calculated by dividing the cardiac output, the quadriceps and intercostal muscle blood flow by the mean arterial blood pressure.

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