ONLINE DATA SUPPLEMENT

Helium-Hyperoxia versus Hyperoxia During Exercise for Hypoxaemic Patients with COPD

Fernando Queiroga Jr[1], MD
Marcos Nunes[1], MSc
Ethiane Meda[1], MSc
Gaspar Chiappa[1], PhD
Maria Christina Machado[1], MD
Luiz Eduardo Nery[1], MD
J Alberto Neder[1,2], MD

[1]Pulmonary Function and Clinical Exercise Physiology Unit (SEFICE), Division of Respiratory Diseases, Department of Medicine, Federal University of Sao Paulo (UNIFESP), São Paulo, SP, Brazil.
[2] Division of Respiratory and Critical Care Medicine, Department of Medicine, Queen´s University, Kingston, Ontario, Canada

Corresponding Author: J.A. Neder, MD, PhD. Coordinator, Pulmonary Function and Clinical Exercise Physiology Unit (SEFICE); Respiratory Division, Department of Medicine; Federal University of São Paulo – Paulista School of Medicine (UNIFESP-EPM). Rua Professor Francisco de Castro 54, Vila Clementino, CEP: 04020-050, São Paulo, Brazil. Phone: (+55-11) 5082-4420. FAX: (+55-11) 5575-2843. E-mail: nederalb@gmail.com

METHODS

Study Population

Fifty-one sedentary males under long-term oxygen therapy (LTOT) with severe COPD (ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) < 70%, post-bronchodilator FEV_1 < 50% predicted) (1) were invited for study participation. Only males were considered for inclusion in this study for two reasons: i) they constitute the great majority (more than 80%) of the patients under LTOT in our Institution and ii) previous experience with the impedance cardiography system indicated a lower signal-to-noise ratio in females (probably by changes in electrodes positioning due to the breasts). Patients were recruited from the LTOT outpatient clinic of the Division of Respiratory Division of the Sao Paulo Hospital, Federal University of Sao Paulo, Brazil. Patients were required to be in stable clinical condition without recent exacerbation or change in the medication status (within 1 month). In addition, no patient was enrolled or had been enrolled in pulmonary rehabilitation in the year preceding the study. Twenty-seven patients were excluded due to severe cardiovascular co-morbidity, tracheostomy, osteomuscular limitation to cycling, acute exacerbation just before the tests or lack of interest in exercise studies. The remaining 24 patients had no evidences of ischaemic heart disease, left ventricular dysfunction (ejection fraction <60% assessed by Doppler echocardiography), or severe pulmonary hypertension (estimated systolic pulmonary arterial pressure <40 mm Hg). All patients were taking inhaled long-acting β2-agonists, 24 were on inhaled steroids, 18 were on inhaled long-acting inhaled anticholinergics and 8 on theophylline. No patient was on systemic steroids. Patients were told to continue their medications throughout the study. They were informed that the aim of the study was to compare the efficacy of different gas mixtures in improving exercise tolerance but they were not told the specific study hypothesis (i.e., that HeHOx would be superior to HOx on this regard). All subjects had signed a written informed consent and the study protocol was approved by the medical ethics committee of Federal University of Sao Paulo.

Study Protocol

This was a randomized, crossover, and double-blinded study. On the first visit, patients underwent pulmonary function tests and anthropometric measurements. They were then randomly assigned to receive HeHOx or HOx during incremental and constant work rate tests (IWR and CWR tests on visits 2 and 3, respectively). On a given day, the tests with each mixture were separated by a 60-min resting interval. The tests were repeated in the same day for the following reasons: i) near-infrared spectroscopy probes were used to evaluate muscle oxygenation (data not shown) and minimal day-today variations in optodes positioning can interfere with signal reproducibility; ii) most patients were housebound with limited access to private or public transport thereby limiting their access to multiple visits to the laboratory, and c) the Ethical Committee asked the investigators to reduce the number of experimental visits to a minimum to avoid further discomfort to these frail patients. Although tests repetition in the same day could impair patients´ performance on the second test, we ascertained that the resting level of patients symptoms were identical before each test (see below). Moreover, tests sequence was randomized thereby avoiding the bias to systematically interfere with the performance on a give intervention.

Measurements

Body composition assessment

Fat-free mass (kg) was determined by a tetrapolar electrical bioimpedance device (BIA 450 Bioimpedance analyser, Biodynamics, Seattle, WA, USA) following standard recommendations (2).

Pulmonary function tests

The pneumotachometer was calibrated with the experimental gas mixture (HeHOx or HOx) before each spirometry. Spirometry, maximal voluntary ventilation (MVV, L/min) and inspiratory capacity (IC, L) maneuvers (to estimate end-expiratory lung volume (EELV, L) were performed with the flow-module of a metabolic cart (CardiO$_2$ System, MGC, MN, USA). Static lung volumes (total lung capacity (TLC, L) and residual volume (RV, L)) by constant-volume body plethysmography (CPF System, MGC) and arterial blood gases (ABL 330™, Radiometer, Copenhagen, Denmark) were measured with the patients breathing room air.

Cardiopulmonary exercise tests

Application of experimental gas mixtures

The gas mixture (HeHOx or HOx) was directed via a closed circuit to a 120-L latex-neoprene balloon (Douglas bag) and thereafter to the inspiratory port of a low-resistance two-way valve (2700 series, Hans-Rudolph Inc. MO, USA). The pneumotachometer (PreVent Pneumotach, Medical Graphics Corporation (MGC), St. Paul, MN, USA*)* was attached in series to the valve and a face mask. In order to blind the mixture under study for the accompanying physician and the patient, a screen was placed in front of the gas cylinders and the patient was instructed to avoid talking during the whole experiment (i.e., to avoid the characteristic changes in voice tone when helium is breathed). The subjects breathed the experimental mixtures for at least 15 minutes before each test to maximize intra-pulmonary distribution.

Incremental work rate test (IWR)

The tests were performed on an electronically-braked cycle ergometer (Corival, Lode, Groningen, Germany) at 50 ± 5 rpm which was controlled by the CardiO$_2$

System. After two minutes of unloaded cycling at 0 W, the WR increased 5 W per minute following a ramp-pattern of increment. The test ceased when the patients indicated that he could not cycle any longer despite strong verbal encouragement. Patients graded their sense of breathing and leg effort every 2 min before the IC maneuvers using the modified 0-10 Borg Scale with "0" being the resting level and "10" the most severe breathing and leg effort ever experienced by the patient. Patients were specifically asked *"how hard is your breathing effort now"* and *"how hard is your leg effort now"* every minute during exercise and at exercise cessation.

Assuming that resting TLC remains constant during exercise, changes in IC were taken to reflect variations in EELV (TLC – IC) [3]. Development of exercise-induced dynamic hyperinflation was defined as progressive reduction in IC during exercise. Patients were fully familiarized with the technique at rest and during a practice session. After a few breaths warning, patients were given a prompt for the maneuver ("*at the end of the next breath out, take a deep breath all the way in*") with strong verbal encouragement to reach a maximal inspiratory level. When maximal inspiratory has been reached, patients were told to relax and continue breathing normally. If anticipatory variations in breathing pattern were noticed before an IC maneuver, the patient was instructed not to perform the maneuver but to continue breathing normally. Only after some additional breaths, the patient was asked to perform the maneuver. Each test was individually reviewed for the correct positioning of the line corresponding to the end-expiratory level of the few breaths which preceded the maneuver.

Constant work rate test (CWR)

The CWR tests were performed to the limit of tolerance (T*lim*, s) at 70–80% peak work rate (WR) obtained under HOx. In patients with very low exercise capacity (i.e., peak WR < 40 W), the test was performed at 30 W to secure $\dot{V}O_2$ amplitude which was sufficiently high for the kinetics analysis [4].

Central hemodynamics.

Haemodynamic variables (CO, L/min) were measured non-invasively throughout the CPET using the *PhysioFlow PF-05™* (Manatec Biomedical, France). The *PhysioFlow™* principle is based on the assumption that variations in the impedance to a high-frequency (75 kHz) low-magnitude (1.8 mA) alternating current across the thorax during cardiac ejection result in a waveform from which SV can be calculated. The system methodology is different from previously used impedance systems as its algorithm does not require basal thoracic impedance measurement (Z0) making the position of the electrodes not critical for the accuracy of the measurements. In addition, estimation of blood resistivity is not needed as the system does not measure changes in blood volume.[5]

Initially, a SV index (SVi, mL/m^2= SV / BSA (body surface area= 0.024265 x $weight^{0.5378}$ x $height^{0.3964}$) is calculated at rest by evaluating 24 consecutive heart beats (autocalibration procedure) and recording the largest impedance variation during systole ($Z_{max} - Z_{min}$) and the largest rate of variation of the impedance signal (dZ/dt_{max} or the "contractility index", CTI). In addition, the system calculates the time interval between the first impedance and the first nadir after the peak of the CTI thereby estimating the ventricular ejection time (called "thoracic flow inversion time" or TFIT). TFIT is further weighted (W) by an algorithm which takes into consideration the pulse pressure (systolic – diastolic) and HR [15,16]:

$$SVi_{cal} = k \times [(dZ/dt_{max}) / (Z_{max} - Z_{min}) \times W (TFIT_{cal}) \qquad \text{[Eq. 1]}$$

Variations of the parameters are then recorded and compared to those obtained during the calibration procedure, using the following approach:

$$SVi = SVi_{cal} \times \sqrt[3]{CTI/CTI_{cal}} \times TFIT_{cal}/TFIT \qquad \text{[Eq. 2]}$$

CO is calculated as:

$CO = SVi \times HR \times BSA$ [Eq. 3]

After shaving and cleaning the skin, two pairs of electrodes were firmly positioned at the left base of the neck and over the dorsal column at the xiphoid level for transmitting and receiving electrical currents. Two electrodes were also placed on the chest (V1/V6 position) for the ECG signal. Adhesive tape was used in order secure an optimal contact with the skin. Before each exercise test, the system was auto-calibrated after a period of at least 5 min in which patients were sitting immobile on the cycling ergometer. This procedure took into account age, sex, weight, height, body mass and blood pressure values: verification of the correct signal quality was performed by visualizing the ECG tracing and its first derivative (dECG/dt) and the impedance waveform (ΔZ) with its first derivative (dZ/dt).[5] The autocalibration procedure was started. SV, CO and CI values were stored as average of X beats. The coefficient of variation (CV) for changes in CO and SV at peak exercise was 3.3% and 4.1% for replicate measurements, respectively [6}.

Data analysis

Due to the extreme intolerance to IWR exercise of some patients, physiological data were analyzed both at the lowest sub-maximal WR that elicited response amplitudes amenable to inter-subject comparisons for most patients (iso-WR) and at peak WR. In the CWR test, responses were analyzed both at isotime (the shortest length of time that a patient tolerated the test) and at Tlim. For the kinetics analyses, breath-by-breath $\dot{V}O_2$ data were interpolated each second (*SigmaPlot 10.0; Systat Software Inc., San Jose, CA, USA*) and fitted by the following monoexponential equation [257]:

$[Y]_{(t)} = [Y]_{(b)} + Ap\ (1 – e^{-(t\text{-}TDp)/\tau p})$

where b and p refer to baseline unloaded cycling and primary component, respectively, and A, TD, and τ are the amplitude, time delay, and time constant of the exponential response, respectively. The overall kinetics were determined by the mean response time ($MRT = \boldsymbol{\tau} + TD$). Since values for haemodynamic data did not follow a mono-exponential pattern of response in all patients, the half time ($t_{1/2}$, s) was calculated.

Statistical analysis

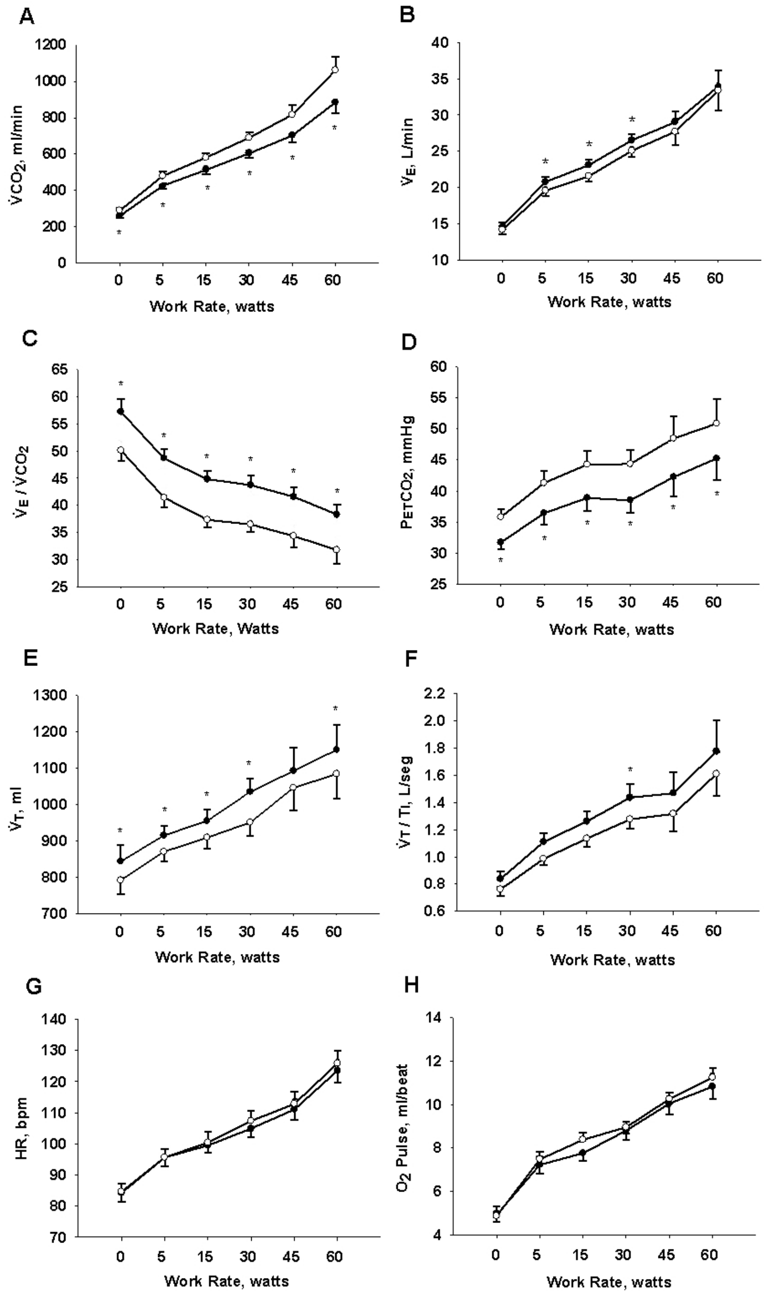
The SPSS version 15.0 statistical software was used for data analysis (*SPSS, Chicago, IL, USA*). Results are presented as mean ±SD or median (range). In order to contrast exercise responses with HOx and HeHOx, paired *t* (or Wilcoxon for non-parametric data) tests were used. Mixed-design ANOVA model (Split-plot ANOVA (SPANOVA)) contrasted the responses over time and between-interventions. Population variances of the repeated measurements and the population correlations among all pairs of measures equality (sphericity) were tested by Mauchly's test and the homogeneity of inter-correlations were tested by Box´s M. Pearson’s product moment correlation was used to assess the level of association between continuous variables. Stepwise backward regression analysis was used to establish the independent predictors of improvement in exercise tolerance with HeHOx. The level of statistical significance was set at $p<0.05$ for all tests.

Variables	HOx	HeHOx (n=24)
Pulmonary function		
FEV_1, L [% predicted]	0.91 ± 0.30 [32.5 ± 10.1]	1.02 ± 0.30 [36.4 ± 10.3]*
FVC, L [% predicted]	2.68 ± 0.63 [73.4 ± 15.2]	3.00 ± 0.77 [82.7 ± 19.3]*
FEV_1/FVC	0.34 ± 0.09	0.35 ± 0.09
MVV , L/min	34.1 ± 11.2	38.2 ± 11.2*
IC , L	1.98 ± 0.41	2.07 ± 0.43*
EELV, L	5.13 ± 1.13	5.04 ± 1.07*
Ventilatory		
$\dot{V}_E$, L/min	13.9 ± 2.6	14.3 ± 2.3
VT, mL	778 ± 172	827 ± 213*

Table E1. Resting pulmonary-ventilatory responses under hyperoxia (HOx) and helium-hyperoxia (HeHOx) (N= 24).

Variables are mean ± SD. *Definition of abbreviations*: FEV_1= forced expiratory volume in one second, FVC= forced vital capacity, MVV= maximal ventilatory ventilation; IC= inspiratory capacity, EELV= end-expiratory lung volume, $\dot{V}_E$= minute ventilation, VT = tidal volume. * $p < 0.05$

FIGURE E1. Physiological effects of hyperoxia (*open circles*) and helium-hyperoxia (*closed circles*) during incremental exercise (N= 24).



Footnotes: variables are mean ± SE. *Definition of abbreviations*: $\dot{V}CO_2$= carbon dioxide output, $\dot{V}_E$= minute ventilation, P_{ET}= end-tidal partial pressure, V_T = tidal

volume, T_I= inspiratory time, HR= heart rate. * $p < 0.05$ for between-intervention differences at a given time point.

References

1. ***Global Strategy for the Diagnosis, Management and Prevention of COPD,*** Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2011. Available from: http://www.goldcopd.org/.
2. Walter-Kroker A, Kroker A, Mattiucci-Guehlke M, Glaab T. A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. *Nutr J* 2011; 10:35.
3. O'Donnell DE, Lam M, Webb KA. Measurement of symptoms, lung hyperinflation, and endurance during exercise in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; 158: 1557-1565.
4. Puente-Maestu L, Sánz ML, Sánz P, Nuñez A, González F, Whipp BJ. Reproducibility of the parameters of the on-transient cardiopulmonary responses during moderate exercise in patients with chronic obstructive pulmonary disease. *Eur J Appl Physiol* 2001; 85: 434-441.
5. Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammosser M, Mettauer B, Geny B, Lonsdorfer J. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the direct Fick method. *Eur. J. Appl. Physiol* 2000; 82: 313-320.
6. Borghi-Silva A, Oliveira CC, Carrascosa C, Maia J, Berton DC, Queiroga F Jr, Ferreira EM, Almeida DR, Nery LE, Neder JA. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. *Thorax* 2008; 63: 910-915.
7. Whipp BJ, Ward SA. Physiological determinants of pulmonary gas exchange kinetics during exercise. *Med Sci Sports Exerc* 1990; 22: 62-71.