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The Effect of Acute Exposure to Hyperbaric Oxygen on Respiratory System Mechanics in the Rat

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Abstract
Purpose This study was designed to investigate the possible effects of acute hyperbaric hyperoxia on respiratory mechanics of anaesthetised, positive-pressure ventilated rats.
Methods We measured respiratory mechanics by the end-inflation occlusion method in nine rats previously acutely exposed to hyperbaric hyperoxia in a standard fashion. The method allows the measurements of respiratory system elastance and of both the "ohmic" and of the viscoelastic components of airway resistance, which respectively depend on the newtonian pressure dissipation due to the ohmic airway resistance to air flow, and on the viscoelastic pressure dissipation caused by respiratory system tissues stress–relaxation. The activities of inducible and endothelial NO-synthase in the lung's tissues (iNOS and eNOS respectively) also were investigated. Data were compared with those obtained in control animals.
Results We found that the exposure to hyperbaric hyperoxia increased respiratory system elastance and both the "ohmic" and viscoelastic components of inspiratory resistances. These changes were accompanied by increased iNOS but not eNOS activities.
Conclusions Hyperbaric hyperoxia was shown to acutely induce detrimental effects on respiratory mechanics. A possible causative role was suggested for increased nitrogen reactive species production because of increased iNOS activity.

Keywords End-inflation occlusion method · Hyperbaric hyperoxia · NO-synthase · Rat · Respiratory mechanics

Introduction

The exposure to hyperbaric oxygen (HBO) is a widely applied therapeutical approach used for the treatment of diseases, such as ischemia–reperfusion injury, necrotizing vascular diseases, chronic non-healing wounds, gas gangrene, and others. However, lung injury has been found to develop frequently [1, 2].

Experiments specifically designed to investigate the possible effects of acute HBO on respiratory system mechanics have not been recently described in the literature. In an old paper [3], HBO was described to have insignificant effects on respiratory system elastic properties and alveolar surfactant, whereas a reduction in the airway resistance to air flow was detected. The same absence of effects on respiratory system compliance has been more recently confirmed [4]. Nevertheless, more recent findings suggested that HBO may affect respiratory mechanics: for example, prolonged HBO has been described to reduce exhaled nitric oxide concentration [5], possibly inducing bronchoconstriction [6] and a related increment in airway resistance. This increment, which has never been directly investigated, also is suggested by data

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showing that prolonged HBO affects spirometric performance of healthy individuals, inducing a decrement in the value of the maximum expiratory flow at 50 % of the vital capacity (MEF50) [7] or of MEF50 and MEF25 [8]. Indeed, Adamiec [9] for prolonged and Kirsteen et al. [10] for acute HBO exposure were able to demonstrate increased values of airway resistance in humans. A role for oxygen radicals in causing this effect was proposed later by Katsumata et al. [11].

We designed specific experiments to directly investigate the possible acute HBO effects on the elastic and resistive characteristics of the rat respiratory system.

The end-inflation occlusion method was applied, which has been extensively validated both in humans [12, 13] and rat [6, 14–18] studies. Based on a theoretical approach describing the respiratory system on the basis of a two-compartment model, the end-inflation occlusion method allows the definition of the elastic and resistive properties of the respiratory system; the latter includes both the Newtonian pressure dissipation due to the ohmic airway resistance to air flow and the viscoelastic pressure dissipation caused by respiratory system tissues stress–relaxation. The effects of HBO on the latter component of airway resistance have never been investigated before.

In addition, we also researched for possible activities changes of nitric oxide synthase in the lung's tissue after HBO exposure, because nitric oxide has recently been shown to be importantly involved in causing HBO-linked lung injury [1, 2].

Thus, the purposes of our study were (1) to describe the possible effects of acute HBO on respiratory mechanics parameters, such as respiratory system elastance, and ohmic and viscoelastic resistances, and (2) to search for NO-synthase activity changes involved in possible cause-effects relationships.

Materials and Methods

Animals

The experiments were performed on nine albino rats of both sexes (mean weight 330 ± 29 g, 5 males). The rats were studied to investigate the effects of HBO on respiratory mechanics after the below described exposition in the pressurised chamber, and the results were compared with data collected from other 22 rats with similar characteristics (mean weight 309 ± 29 g, 11 males), which were previously studied in the same way (see below). The animals were housed and treated in accordance with Italian law on animal experimentation (L. 116/92) and with the European Council (EC) provision 86/609/EEC.

HBO Exposure

A 420-L research chamber for HBO (Costruzioni Riunite Moro, C.R.M., Treviso, Italy) was used. Before HBO administration on animals, the chamber was flushed twice with pure oxygen up to 2 atmospheres absolute (atm abs) pressure for 10 min to vent the air inside before the compression. The HBO therapy session consisted of 100 % oxygen at 2.5 atm abs (where 1 atm = 101.3 kN/m^2) for 90 min. After the pressure of 2.5 atm abs was reached, continuous O_2 flow of 0.5 L/min was administrated to prevent accumulation of CO_2 in the chamber. The temperature inside the chamber was kept at 24 ± 2 °C by a heated water blanket. Compression and decompression of the chamber was completed gradually in 15 min. All administrations were started at the same hour in the morning (10:00 A.M.) to equalize possible effects of the circadian rhythm. During HBO, animals were visually observed through an artificial light on the chamber and all changes in their behaviour were recorded. The time separating the end of HBO exposure from the respiratory mechanics measurement was approximately 15 min.

Experimental Procedure

Each rat was anaesthetized with 50 mg/100 g intraperitoneal chloralose and laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2-mm ID 5-cm long) was inserted through an incision in the second tracheal ring and firmly secured in place. Limb electrocardiogram (ECG) probes were placed and the rat was paralyzed (cisatracurium 1 mg/100 g intraperitoneally injected).

Positive pressure ventilation using a 10 mL/kg tidal volume (VT) and a 60/min breathing frequency (PEEP 3 cmH_2O) (Rodent Ventilator 7025, Basile, Italy) was begun and maintained constantly throughout the experiment (apart from the short time necessary to measure respiratory mechanics, see below). Positive-pressure ventilation was maintained for 5 min, and respiratory mechanics were measured at that time using the end-inflation occlusion method [6, 12–18].

The ventilator was disconnected, PEEP was discontinued, and the tracheal cannula was connected to a constant flow pump (SP 2000 Series Syringe Pump sp210iw, World Precision Instruments, USA) set to deliver a VT of 3 mL with a square wave flow (F) of 4 mL/sec. The rise and the fall flow time was approximately 30 ms. The pump setting was carefully checked during trial runs carried out before the experiments were begun. To avoid determinant arterial blood gas changes, the time the ventilator remained disconnected for each inflation procedure was approximately 20 s [19].

The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d, Honeywell, USA) and continually recorded (1326 Econo Recorder, Biorad, Italy). The frequency response of the transducer and the pressure measuring system were tested using sinusoidal forcing and found to be flat up to 20 Hz. In accordance with data reported in the literature [13, 14], the frequency response was considered adequate to avoid mechanical artefacts in pressure signal records.

Data Calculation

The end-inflation occlusion method was utilised to determine respiratory mechanics parameters. The static elastic pressure of the respiratory system (Pel,rs) and the sudden Newtonian resistive pressure drop at flow interruption (Pmin,rs) were measured on adequately magnified tracings (Fig. 1). Pmin,rs was calculated as the difference between Pdyn,max, the maximum value of pressure at end inflation, and P1, the pressure value immediately after the flow was interrupted (Fig. 1). The sum of Pmin,rs and of the slower, nearly exponential, pressure drop following flow interruption due to respiratory system visco-elastic behaviour, i.e., stress relaxation [6, 12, 13], was termed Pmax,rs. Our tracings made it possible to identify P1 (Fig. 1), which separates the pressure drop due to friction related to the movement of airflow in the airway (Pmin,rs) from the successive viscoelastic pressure drop.

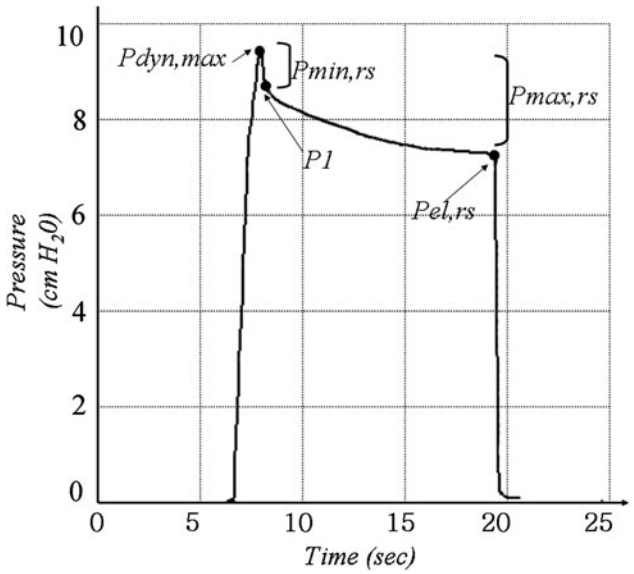


Fig. 1 Example of tracheal pressure tracing recorded upon constant flow inflation arrest. The maximum pressure achieved at end inflation (Pdyn,max), the pressure drop due to frictional forces in the airway (Pmin,rs) and the overall resistive pressure drop (Pmax,rs), including Pmin,rs and the nearly exponential pressure dissipation due to viscoelasticity, are shown. *P1* pressure value immediately after flow arrest

To avoid a viscoelastic pressure component in Pmin,rs, P1 values were identified by extrapolating the pressure tracings to the time the flow stopped [20]. The Newtonian, "ohmic," component of airway resistance may be thought as the airway resistance occurring at infinite breathing frequency, when the time allowed for the pressure drop due to viscoelastic phenomena approaches zero. Instead, the additional viscoelastic component defines a higher resistance value, which depends on a complete stress relaxation-linked pressure drop, theoretically occurring at zero breathing frequency. Accordingly, in real conditions in vivo, the actual resistance value will be a function of the breathing frequency, depending on the viscoelastic phenomena slow time course [13].

The mean pressure data obtained from 3 to 5 inflations for each rat were used to calculate the respiratory system static elastance (Est,rs = Pel,rs/VT), and the total inspiratory resistance (Rmax,rs = Pmax,rs/F), including the "ohmic" inspiratory resistance to airflow offered by the airways and the movement of respiratory system tissues (Rmin,rs = Pmin,rs/F), and the viscoelastic inspiratory resistance due to stress relaxation (Rvisc,rs = Rmax,rs − Rmin,rs).

The equipment resistance, including the tracheal cannula and the standard three-way stopcock, was measured separately at a flow rate of 4 mL/sec and amounted to 0.0575 $cmH_2O/mL/s$ (Req). All inflations were performed at a fixed flow rate of 4 mL/sec, and Req was subtracted from the results, which thus represent intrinsic values. For each rat, the experimental procedure lasted less than 1 h.

Morphological Analysis

Tissues have been fixed in 10 % phosphate-buffered formalin for 72 h, dehydrated through ascending alcohols and xylene, and then paraffin embedded. Samples have been then dewaxed (progressively lower concentrations of xylene and alcohol) and the tissue sections, 5-μm thick, have been processed for haematoxylin–eosin staining. Samples have been then observed by means of Leica DM 4000 light microscopy (Leica Cambridge Ltd, Cambridge, UK) equipped with a Leica DFC 320 camera (Leica Cambridge Ltd) for computerized images.

Western Blot

Lung lysates (20 μg) have been electrophoresed and transferred to nitrocellulose membrane. Nitrocellulose membranes, blocked in 5 % non-fat milk, 10 mmol/L Tris pH 7.5, 100 mmol/L NaCl, 0.1 % Tween-20, have been probed with mouse anti β-tubulin antibody (Sigma, USA) (primary antibodies dilution 1:1,000), and rabbit anti-iNOS and anti-eNOS antibodies (Santa Cruz, Santa Cruz Biotechnology,

CA) (primary antibodies dilution 1:200) and then incubated in the presence of specific enzyme conjugated IgG horseradish peroxidase. Immunoreactive bands have been detected by ECL detection system (Amersham Intl., Buckinghamshire, UK) and analysed by densitometry. Densitometric values, expressed as Integrated Optical Intensity (I.O.I.), have been estimated in a CHEMIDOC XRS system by the QuantiOne 1-D analysis software (BIORAD, Richmond, CA). Values obtained have been normalized basing on densitometric values of internal β tubulin.

Statistics

All data are expressed as mean values ± SE. A Student's *t* test for unpaired data was applied to investigate the statistical differences in the mean values between experimental and control animals. Due to the relatively small sample size, the statistical analysis of the differences in the mean values also was confirmed by applying nonparametric tests (Mann–Whitney). The level of statistical significance was set at $p < 0.05$, and the statistical tests yielded essentially the same result.

Results

The results comparing the mean values of respiratory system mechanics parameters observed in the experimental and control animals are reported in Table 1, which shows that HBO-induced respiratory system elastance and resistances increments. In Table 2 are reported the individual data from all of the animals. The heart rate mean values measured in the experimental and control groups immediately before the determination of respiratory mechanics parameters resulted 318 ± 44 and 294 ± 37 b/min respectively, not significantly different.

In rats exposed to hyperoxia histopathological analyses of lung samples showed perivascular and parenchymal infiltrates, composed both of polymorphonuclear cells and lymphocytes. Alveolar septa did not show other pathologic changes (Fig. 2).

Table 1 Mean values (±SE) of respiratory mechanics parameters measured in control rats and after acute hyperbaric oxygen exposure

	HBO	Control
Est,rs (cmH_2O/mL)	3.1 ± 0.29**	2.01 ± 0.17
Rmax,rs (cmH_2O/mL/sec)	0.85 ± 0.08*	0.55 ± 0.05
Rmin,rs (cmH_2O/mL/sec)	0.26 ± 0.04**	0.13 ± 0.02
Rvisc,rs (cmH_2O/mL/sec)	0.59 ± 0.08*	0.42 ± 0.04

* $p < 0.05$; ** $p < 0.01$

Western blot analysis showed higher expression of iNOS in exposed animals with respect to controls (0.28 ± 0.02 vs. 0.17 ± 0.01, $p < 0.05$). Conversely, statistically significant differences were not found between controls and exposed animals for eNOS expression (0.52 ± 0.05 and 0.6 ± 0.06, respectively) (Fig. 3).

Discussion

Experimental Procedure

Modelling the respiratory system as consisting of two distinct compartments, the end-inflation occlusion method has been widely used to study respiratory mechanics in experimental animals [6, 14–18] and in humans [12, 13]. Ideally, the inflation flow should stop instantaneously, but this is practically impossible to achieve. However, a procedure has been proposed to correct for this technical limitation [20].

In this procedure, pressure tracings are manually extrapolated to account for the time that is necessary to halt the inspiratory flow completely, thereby minimizing the error [6, 14]. This procedure was employed to analyse the inflation pressure tracings in the current study and, similarly to what previously reported [14, 21], the corrections resulted almost negligible.

The mechanical ventilation settings used in these experiments were the same as those described as "noninjurious" in the literature [6, 21]. In particular, "noninjurious" ventilation that lasts 1 h has been shown to induce no alterations of respiratory system mechanics [6, 21]. The results reported, therefore, were not influenced by the injurious effects that longer-term mechanical ventilation per se might exert.

The mean heart rate values we observed are similar to those previously reported in anaesthetised rats [22, 23] and suggest that the conditions of the animals during the experimental procedure were generally stable. However, a trend suggesting heart rate reductions in HBO was seen, likely due to a baroreceptor reflex activity elicited by the effects of HBO on systemic arterial pressure [1]. The mean values of respiratory system mechanics parameters here reported for control animals are comprised in the range of those previously measured by the same technique in rats by various authors working in different laboratories [6, 14–18, 21].

Effects of HBO on Respiratory Mechanics

Presently described experiments for the first time systematically investigated the acute effects of HBO on respiratory mechanics, including measurements of the effects of

Table 2 Individual data of respiratory mechanics parameters measured in control animals and in animals exposed to HBO

Rat no.	Est,rs (cmH_2O/mL)		Rmax,rs ($cmH_2O/mL/sec$)		Rmin,rs ($cmH_2O/mL/sec$)		Rvisc,rs ($cmH_2O/mL/sec$)	
	CTR	HBO	CTR	HBO	CTR	HBO	CTR	HBO
1	1.43	1.785	0.34	0.53	0.055	0.074	0.285	0.46
2	1.56	2.07	0.43	0.6	0.038	0.15	0.392	0.45
3	1.35	2.1	0.42	0.55	0.084	0.11	0.336	0.44
4	1.97	3.25	0.43	0.87	0.047	0.24	0.383	0.63
5	2.4	3.74	0.70	1.26	0.08	0.3	0.62	0.96
6	2.33	4.12	0.54	1.03	0.09	0.3	0.45	0.73
7	4.30	3.52	1.23	0.98	0.27	0.44	0.96	0.54
8	3.44	3.465	1.07	0.85	0.33	0.355	0.74	0.5
9	3.52	3.7	0.97	0.96	0.27	0.35	0.7	0.61
10	1.61		0.4		0.15		0.25	
11	1.68		0.36		0.13		0.23	
12	1.92		0.52		0.15		0.37	
13	1.68		0.36		0.17		0.19	
14	1.68		0.36		0.05		0.31	
15	1.96		0.57		0.19		0.38	
16	1.43		0.34		0.055		0.287	
17	1.56		0.48		0.09		0.39	
18	1.39		0.41		0.084		0.33	
19	1.97		0.47		0.084		0.39	
20	2.4		0.58		0.083		0.5	
21	1.5		0.59		0.14		0.45	
22	1.2		0.56		0.15		0.41	

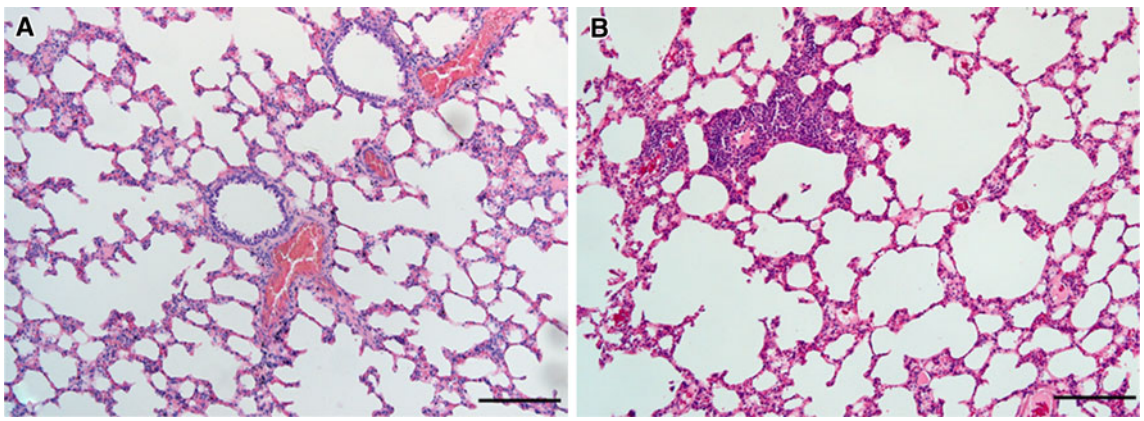
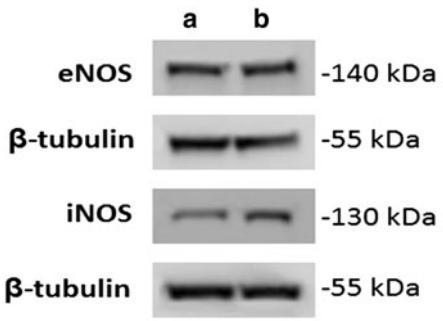


Fig. 2 Lung sections showing perivascular and parenchymal inflammatory infiltrates in rats exposed to hyperbaric hyperoxia (**b**) and not in rats exposed to normoxia (**a**)

HBO on the ohmic component of airway resistance, due to frictional forces in the airway, and on the additional viscoelastic component of resistance, due to pressure dissipation because of stress–relaxation effects. As shown in Table 1, HBO caused increments of both the respiratory system elastance and the different components of airway resistance with respect to control rats.

Present results are in agreement with data previously obtained in humans [9, 10], although for more prolonged HBO exposure. This observed increment in airway resistance allows to confirm directly previously reported data, which indirectly only suggested this effect: for example, prolonged HBO has been described to reduce exhaled nitric oxide concentration [5], possibly inducing bronchoconstriction [6],



	eNOS	iNOS
a	0.52 ± 0.05	0.17 ± 0.01
b	0.60 ± 0.06	0.28 ± 0.02*

Fig. 3 Western blot analysis of eNOS and iNOS expression. Each membrane has been probed with β-tubulin antibody to verify loading evenness. Western blotting is the most representative out of three different consistent experiments. Data are the densitometric measurements of protein bands expressed as Integrated Optical Intensity (IOI) mean (±SE) of three separate experiments. (*a*) normoxia, (*b*) hyperoxia. * Hyperoxia vs. normoxia $p < 0.05$

and to affect spirometric performance of healthy individuals, inducing a decrement in the value of the maximum expiratory flow at 50 % of the vital capacity [7] or of MEF50 and MEF25 [8].

The mechanisms responsible for the presently observed increment in airway resistance include the following: (a) an effect of oxygen radicals, which are known to be produced during HBO, and which have been demonstrated to induce airway constriction and hyperresponsiveness in anesthetised cats [11]; (b) an extensive airway wall damage with inflammatory infiltration [1, 2], which was observed also in the present experimentation (Fig. 2). Even normobaric hyperoxia has been demonstrated to alter airway wall cells physiology, viability, and histology and to increase their secretion of inflammatory mediators, such as IL-6 and IL-8 [24]. The latter have been shown to increase airway resistance [21, 25, 26]; (c) the effect of HBO-induced increase in lung blood volume because of increased systemic vascular resistance and reduced cardiac output [1]: lung blood volume increments have been shown to increase both the measured components of respiratory system resistances [27].

We also observed significant increments on the visco-elastic component of respiratory system resistance (Table 1) in HBO-exposed animals with respect to controls. Visco-elasticity combines solid-like and liquid-like characteristics of the lung tissues, and the chief mechanism responsible for the macroscopic visco-elastic behaviour of the respiratory system is the microstructural rearrangement and interaction processes of collagen and elastin, such as reciprocal sliding, also in relation to interstitial liquid movements [28]. According to our results, these phenomena seem to be significantly affected by HBO and/or the HBO-induced proinflammatory changes in the lung's tissues [1, 2]. This suggestion may be confirmed by the previously shown effect on Rvisc,rs of inflammation mediators, such as IL-6 [20]. However, present data are the first published about this subject and, most of all, the structural basis and the exact mechanisms of viscoelastic tissues properties are still approximately only understood, so that further confirms are needed.

Previously reported results about the effects of HBO on respiratory system elastic properties are conflicting. In fact, although referring to longer than presently tested HBO exposure, data describing both no change [3, 4] and significant decrements [9] of lung compliance have been reported.

However, lung compliance decrements have been proposed as an even early detector of pulmonary oxygen-induced injury, at least after normobaric hyperoxia [29].

Presently observed HBO-induced impairment of respiratory system elastic properties may be explained because of the diffuse parenchymal damage and extensive inflammatory responses in lung's tissues [1, 2], which we also observed (Fig. 2), and of the above cited increment of lung pulmonary blood volume [27]. In addition, we do not exclude that HBO may alter alveolar surfactant function and metabolism, as already demonstrated as an effect of at least prolonged normobaric hyperoxia in rats [30].

Our results confirm that the mammalian lung is a primary target of the toxic effects of oxygen. Nitric oxide production pathways have been indicated to substantially contribute to the severity of HBO toxicity [1, 2]. The current prevailing opinion is that both normo- and hyperbaric hyperoxia cause lung damage by the same mechanism, i.e., the increased productions of reactive oxygen and nitrogen species, and hyperbaric conditions accelerate this process with respect to normobaric hyperoxia [1]. Accordingly, we tested the effects of acute HBO on inducible and endothelial NO-synthase expressions. Our results showed a significantly increased expression of inducible but not of endothelial NO-synthase expression as an effect of HBO, thus suggesting a causing role of respiratory system damage for the former. Demchenko et al. [2] suggested a major role of neural NO-synthase in HBO, whereas inducible NO-synthase effects seemed to predominate in normobaric hyperoxia-caused lung damage. However, they proved predominant, and not exclusive, roles. In fact, the protective effects of nonselective or selective NO-synthase inhibitors do not differ significantly [1]. In addition, neural and inducible NO-synthases have been proven to be functionally and quantitatively linked each other [31–34].

The effects on respiratory system resistance of even normobaric hyperoxia have been previously documented

[35]. In addition, normobaric hyperoxia was shown to increase airway reactivity in guinea pigs and rats [36–38].

Conclusions

Clinical HBO protocols are in use for several conditions. Treatments occur for just one or few days rather than weeks; they are performed at high O_2 partial pressures (2.5–3.0 ATA) and may occur multiple times in the same day, using a monoplace or multiplace hyperbaric chamber [39].

It is general knowledge that HBO possesses the potential risk of oxygen toxicity due to the increased formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). However, ROS and RNS also serve as signaling molecules in transduction cascades, or pathways, so that reactive species can generate positive or negative effects depending on their concentration and localization [40]. In fact, as suggested in animal models, HBO-mediated inhibition of neutrophil sequestration and inflammatory cascade (ROS mediated) has been shown to ameliorate reperfusion injuries of brain, skeletal muscle, and intestine [41–43].

In this study it has been adopted a standard protocol of HBO treatment used in current clinical practice (90 min of pure oxygen at 2.5 atm), and we focused on healthy rats to minimize pathophysiological variables such as ischemia–reperfusion injury.

The results demonstrate significant effects of HBO on respiratory mechanics. Our data pertain to animal experimentations, but it may be cautiously suggested that the same detrimental effects may ensue in humans too. On this basis, present study suggests that patients presenting respiratory system diseases per se affecting respiratory mechanics might risk ventilatory failure because of the presently documented HBO effects. Due to increased values of respiratory system elastance and resistance, an increment in the mechanical work of breathing the respiratory muscles have to develop to maintain alveolar ventilation will ensue, and the risk will be a ventilation failure because of muscles fatigue. Accordingly, an accurate clinical monitoring is indicated following HBO exposure, particularly in respiratory patients.

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Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Demchenko IT, Welty-Wolf KE, Allen BW, Piantadosi CA (2007) Similar but not the same:normobaric and hyperbaric pulmonary oxygen toxicity, the role of nitric oxide. Am J Physiol Lung Cell Mol Physiol 293:L229–L238
2. Demchenko IT, Atochin DN, Gutsaeva DR, Godfrey RR, Huang PL, Piantadosi CA, Allen BW (2008) Contributions of nitric oxide synthase isoforms to pulmonary oxygen toxicity, local vs. mediated effects. Am J Physiol Lung Cell Mol Physiol 294:L984–L990
3. Hurley RM, Rosenberg E (1970) The effect of high oxygen tensions on the mechanical properties of rat lungs. J Physiol 207:63–76
4. Amin HM, Cigada M, Hakim TS, Camporesi EM (1993) Pulmonary mechanical and vascular responses after acute hyperbaric oxygen exposure. Can J Physiol Pharmacol 71:592–596
5. Taraldsoy T, Bolann BJ, Thorsen E (2007) Reduced nitric oxide concentration in exhaled gas after exposure to hyperbaric hyperoxia. Undersea Hperb Med 34:321–327
6. Rubini A (2011) The effect of N-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, on respiratory mechanics in rats. Respiration 82:468–475
7. Mialon P, Barthèlèmy L, Michaud A, Lkacou JM (2001) Pulmonary function in men after repeated sessions of oxygen breathing at 0.25 MPa for 90 min. Aviat Space Environ Med 72:215–218
8. Tetzlaff K, Friege L, Reuter M, Haber J, Mutzbauer T, Neubaier B (1998) Expiratory flow limitation in compressed air divers and oxygen divers. Eur Respir J 12:895–899
9. Adamiec L (1977) Effect of hyperbaric oxygen therapy on some basic vital functions. Acta Physiol Pol 28:215–224
10. Dewar KMS, Smith G, Spence A, Ledingham ImcA (1972) Effect of hyperoxia on airway resistance in man. J Appl Physiol 32:486–490
11. Katsumata U, Miura M, Ichinose M, Kimura K, Takahashi T, Inoue H, Takishima T (1990) Oxygen radicals produce airway constriction and hyperresponsiveness in anesthetized cats. Am Rev Resp Dis 141:1158–1161
12. Bates JH, Rossi A, Milic-Emili J (1985) Analysis of the behavior of the respiratory system with constant inspiratory flow. J Appl Physiol 58:1840–1848
13. D'Angelo E, Calderini E, Torri G, Robatto FM, Bono D, Milic-Emili J (1989) Respiratory mechanics in anesthetized paralyzed humans: effects of flow, volume, and time. J Appl Physiol 67:2556–2564
14. Reta GS, Riva JA, Piriz H, Medeiros AS, Rocco PR, Zin WA (2000) Effects of halothane on respiratory mechanics and lung histopathology in normal rats. Br J Anaesth 84:372–377
15. Peratoner A, Nascimento CS, Santana MC, Cadete RA, Negri EM, Gullo A, Rocco PR, Zin WA (2004) Effects of propofol on respiratory mechanic and lung histology in normal rats. Br J Anaesth 92:737–740
16. Rubini A, Del Monte D, Catena V (2012) Effects of the pneumoperitoneum and Trendelenburg position on respiratory mechanics in the rats by the end-inflation occlusion method. Ann Thorac Med 7:205–209
17. Rubini A, Redaelli M, Parmagnani A (2012) The effect of angiotensin converting enzyme inhibition by captopril on respiratory mechanics in healthy rats. J Enz Inhibi Med Chem 27:854–860
18. Rubini A, Bondì M (2007) Effect of the oestral cycle on respiratory mechanics in the rat. Acta Physiol (Oxf) 189:379–383
19. Cutillo A, Bigler AH, Perondi R, Turiel M, Watanabe S, Renzetti AD Jr (1982) Hypocapnia and measurement of airflow resistance

and dynamic compliance as function of respiratory frequency. Thorax 37:212–217
20. Bates JH, Hunter IW, Sly PD, Okubo S, Filiatrault S, Milic-Emili J (1987) Effect of valve closure time on the determination of respiratory resistance by flow interruption. Med Biol Eng Comput 25:136–140
21. Rubini A (2010) IL-6 increases airway resistance in the rat. Cytokine 51:266–273
22. Rubini A, Bosco G (2013) The effect of body temperature on the dynamic respiratory system compliance-breathing frequency relationship in the rat. J Biol Phys. doi:10-1007/s10867-013-9298-8
23. Rubini A, Carniel EL, Natali AN (2012) The effect of body warming on respiratory system stress recovery in the rat. Acta Bioeng Biom 14:59–66
24. Zhu Y, Miller TL, Singhaus CJ, Shaffer TH, Chidekel A (2008) Effects of oxygen concentration and exposure time on cultured human airway epithelial cells. Pediatr Crit Care Med 9:224–229
25. Aihara K, Oga T, Chihara Y, Harada Y, Tanizawa K, Handa T, Hitomi T, Uno K, Mishima M, Chin K (2012) Analysis of systemic and airway inflammation in obstructive sleep apnea. Sleep Breath. doi:10.1007/s11325-012-0726-y
26. van de Kant KD, Jansen MA, Klaassen EM, van der Grinten CP, Rijkers GT, Muris JW, van Schayck OC, Jobsis Q, Dompeling E (2012) Elevated inflammatory markers at preschool age precede persistent wheezing at school age. Pediatr Allergy Immunol 23:259–264
27. Rubini A, Gasperetti A, Catena V, Del Monte D (2010) Effects of acute blood volume expansion on respiratory mechanics in the rat. Respiration 79:497–505
28. Suki B, Barabasi AL, Lutchen KR (1994) Lung tissue viscoelasticity: a mathematical framework and its molecular basis. J Appl Physiol 76:2749–2759
29. Arkovitz MS, Garcia VF, Szabò C, McConnell K, Bove K, Wispè JR (1997) Decreased pulmonary compliance is an early indicator of pulmonary oxygen injury. J Surg Res 67:193–198
30. Zenri H, Rodriquez-Capote K, McCaig L, Yao LJ, Brackenbury A, Possmayer F, Veldhuizen R, Lewis J (2004) Hyperoxia exposure impairs surfactant function and metabolism. Crit Care Med 32:1155–1160
31. Czapski GA, Cakala M, Chalimoniuk M, Gajkowska B, Strosznajder JB (2007) Role of nitric oxide in the brain during lipopolysaccharide-evoked systemic inflammation. J Neurosci Res 85:1694–1703
32. Khovryakov AV, Podrezova EP, Kruglyakov PP, Shikhanov MP, Balykova MN, Semibratova NV, Sosunov AA, Mc Khann G 2nd, Airapetyants MG (2010) Involvement of the NO synthase system in stress-mediated brain reactions. Neurosci Behav Physiol 40:333–337
33. Kubo S, Kurokawa Y, Doe I, Masuko T, Sekiguchi F, Kawabata A (2007) Hydrogen sulfide inhibits activity of three isoforms of recombinant nitric oxide synthase. Toxicology 241:92–97
34. Qu XW, Wang H, De Plaen IG, Rozenfeld RA, Hsueh W (2001) Neuronal nitric oxide synthase (NOS) regulates the expression of inducible NOS in rat small intestine via modulation of nuclear factor kappa B. FASEB J 15:439–446
35. Murchie P, Johnston PW, Ross JA, Godden DJ (1993) Effects of hyperoxia on bronchial wall dimensions and lung mechanics in rats. Acta Physiol Scand 148:363–370
36. Schulman SR, Canada AT, Fryer AD, Winsett DW, Costa DL (1997) Airway hyperactivity produced by short-term exposure to hyperoxia in neonatal guinea pigs. Am J Physiol 272:L1211–L1216
37. Szarek JL (1989) In vivo exposure to hyperoxia increases airway responsiveness in rats. Demonstration in vivo and in vitro. Am Rev Resp Dis 140:942–947
38. Szarek JL, Ramsay HL, Andringa A, Miller ML (1995) Time course of airway hyperresponsiveness and remodelling induced by hyperoxia in rats. Am J Physiol 269:L227–L233
39. Gesell LE (2008) Hyperbaric oxygen therapy indications, 12th edn. Undersea and Hyperbaric Medical Society, Durham
40. Thom SR (2009) Oxidative stress is fundamental to hyperbaric oxygen therapy. J Appl Physiol 106:988–995
41. Yang ZJ, Bosco G, Montante A, Ou XI, Camporesi EM (2001) Hyperbaric O_2 reduces intestinal ischemia-reperfusion-induced TNF-alpha production and lung neutrophil sequestration. Eur J Appl Physiol 85:96–103
42. Bosco G, Yang ZJ, Nandi J, Wang JP, Chen C, Camporesi EM (2007) Effects of hyperbaric oxygen on glucose, lactate, glycerol and anti-oxidant enzymes in the skeletal muscle of rats suring ischaemia and reperfusion. Clin Exp Pharmacol Physiol 34:70–76
43. Zj Yang, Bosco G, Xie Y, Chen Y, Camporesi EM (2010) Hyperbaric oxygenation alleviates MCAO-induced brain injury and reduces hydroxyl radical formation and glutamate release. Eur J Appl Physiol 108:513–522