Short isocapnic hyperoxia affects indices of vascular remodeling and intercellular adhesion molecules in healthy men

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Abstract

In preparation for tracheal intubation during induction of anesthesia, the patient may be ventilated with 100% oxygen. To investigate the impact of acute isocapnic hyperoxia on endothelial activation and vascular remodeling, ten healthy young men (24 ± 3 years) were exposed to 5-min normoxia (21% O₂) and 10-min hyperoxia trials (100% O₂). During hyperoxia, intercellular adhesion molecules (ICAM-1) (hyperoxia: 4.16 ± 0.85 vs normoxia: 3.51 ± 0.84 ng/mL, P=0.04) and tissue inhibitor matrix metalloproteinase 1 (TIMP-1) (hyperoxia: 8.40 ± 3.84 vs normoxia: 5.73 ± 2.15 pg/mL, P=0.04) increased, whereas matrix metalloproteinase (MMP-9) activity (hyperoxia: 0.53 ± 0.11 vs normoxia: 0.68 ± 0.18 A.U., P=0.03) decreased compared to the normoxia trial. We concluded that even short exposure to 100% oxygen may affect endothelial activation and vascular remodeling.

Key words: Endothelium; Hyperoxia; ICAM-1; Metalloproteinase-9; Vascular remodeling

Introduction

Prolonged exposure to high oxygen levels (hyperoxia) has been related to lung injury, increased myocardial infarct size, and mortality (1,2), whereas the effects of short exposure to hyperoxia are not fully understood. In preparation for tracheal intubation during induction of anesthesia, the patient may be ventilated with 100% oxygen and the vascular endothelium may be challenged by oxygen toxicity. In vitro, hyperoxia seems to provoke an extended pro-inflammatory endothelial cell phenotype by inducing oxidative stress and endothelial cell inflammation (3).

In vitro exposure of endothelial cells to hyperoxia leads to a pro-inflammatory state with an increase in endothelial expression of cell adhesion molecules (CAMs) (4). CAMs are involved in the binding of cells with other cells and with the extracellular matrix (ECM), mediating leukocyte adhesion to the vascular endothelium, which can increase the inflammatory response and vascular permeability and is associated with the early stages of atherosclerosis (3). Furthermore, hyperoxia is an important modulator of ECM, resulting in vascular remodeling (5,6). The regulation of ECM turnover is defined by the balance between the activity of matrix metalloproteinase (MMPs), zinc-dependent endopeptidases with the ability to degrade components of ECM, and their tissue inhibitors of matrix metalloproteinase (TIMPs) (7). Increased MMPs activity results in elastin degradation leading to decreased elasticity, and reduced TIMPs levels provoke accumulation of collagen (8). Among all MMPs, the matrix metalloproteinase-9 (MMP-9) exhibits a main MMP responsible for cardiovascular remodeling in humans (9) and its ability to degrade components of the ECM has been associated to structural and functional vascular alterations in both physiological and pathological conditions (8,9). Taken together, the potential acute effects of hyperoxia on endothelial activation and vascular remodeling need to be addressed in vivo.
In addition, hyperoxia induces hyperventilation accompanied by reduced arterial carbon dioxide tension (here expressed as the end-tidal value, PetCO2), and CO2 influences the release of nitric oxide (NO) from endothelial cells (10,11). Since NO affects adherence of leukocytes to the endothelium by inhibition of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion protein-1 (VCAM-1) expression (12), isocapnic hyperoxia could control the CO2 confounding effects. This study evaluated the impact of short-term exposure to isocapnic hyperoxia on cell adhesion molecules and vascular remodeling in healthy men. We hypothesized that exposure to hyperoxia provokes an increase in CAM expression and MMP-9 activity along with reducing TIMP-1 levels in healthy men.

Material and Methods

Ethical approval

After verbal explanation, all subjects signed a consent form with a detailed explanation of the experimental procedures before participation in the study. The study protocol was approved by the Ethical Committee of Fluminense Federal University (CAAE: 57077316.1.0000.5243) according to the standards set by the latest revision of the Declaration of Helsinki.

Subjects

The subjects were recruited through advertisements at the university campus and in local newspapers. Fourteen men were invited to join the study, of which 10 individuals (24 ± 3 years; body mass index of 24 ± 2 kg/m²; systolic/diastolic pressure 124 ± 9/71 ± 6 mmHg) were eligible to take part. Inclusion criteria were no history of smoking, no regular physical exercise (<150 min per week of moderate-intensity cardiorespiratory exercise training), no cardiovascular, metabolic, or neurological diseases, and no current pharmacological therapy or nutritional supplementation.

Instrumentation

Respiratory rate and depth were registered using a piezoelectric respiratory belt transducer (MLT1132, ADInstruments, Australia) positioned in the upper or lower quadrant of the abdomen. Oxygen saturation was assessed using a breath-by-breath circuit while the subjects breathed through a face mask or a mouthpiece with a nose clip connected to a gas analyzer system (Ultima CPX; Medgraphics, USA).

Experimental setup

The experimental sessions were in the morning and at least 48 h after a hyperoxia familiarization session. The subjects were instructed to abstain from caffeine, alcohol, and intense exercise for 24 h before the experimental session. After instrumentation, subjects remained in supine rest for 15 min in a dark, temperature-controlled (21–23°C) quiet room. Respiratory rate, VE, tidal volume, PetO2, and PetCO2 were monitored for 10 min while the subjects breathed normoxic gas (21% O2 and 79% N2) to establish the target PetCO2 for the normoxic and hyperoxic trials. Subsequently, the subjects breathed at a rate of 20 cycles per minute for a 5-min normoxic trial and a 10-min hyperoxic (100% O2) trial. The isocapnic breathing pattern was maintained using a metronome, verbal instructions to control breathing amplitude, and a rebreathing circuit to prevent poikilocapnic hyperoxia-induced hyperventilation (13). At the last minute of each trial, blood was taken from the antecubital vein (and replaced with NaCl 0.9%) to quantify cell adhesion molecules and vascular remodeling.

Matrix metalloproteinase-9 activity

The MMP-9 activity, a proxy of extracellular matrix turnover and vascular remodeling, was assessed by zymography in plasma samples (14). The gelatinolytic activity was detected as unstained bands against the dark blue background of the Coomassie blue-stained gelatin using an Epson digital scanner. An internal standard (control plasma sample) and a protein molecular weight marker (161-0375, Bio-Rad, USA) were used to allow inter-gel analysis and comparison. Scion Image software (Scion Corporation, USA) was used to quantify band intensities. The active form of MMP-9 was identified as a band at 87 kDa (see Supplementary Figure S1).

Tissue inhibitor of metalloproteinase concentration

Plasma TIMP-1 was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Human TIMP-1 Tissue Inhibitors of Metalloproteinase 1, Elabscience®, USA) following the manufacturer’s instructions. Plasma samples were not diluted and absorbance was read at 450 nm.

ICAM-1, VCAM-1, and P-selectin concentration

Cell adhesion molecules (ICAM-1, VCAM-1, and P-selectin) were measured in plasma by a commercial kit (Human Cardiovascular Disease Magnetic Bead Panel 2, Millipore Sigma, USA) according to manufacturer’s instructions. Quantification of the magnetic beads was performed with a BioPlex MAGPIX system (Biorad, US) and results were analyzed using Xponent software (Luminexcorp, USA). The analyses of the concentration of cell adhesion molecules involved only eight subjects because of technical problems with the plasma samples.

Statistics

Data are reported as means ± SD. Normal distribution and homogeneity of variance were verified using the Shapiro-Wilk test and Levene’s test, respectively. When appropriate, a two-tailed paired Student’s t-test or
Wilcoxon signed rank test was used to compare the variables between normoxic and hyperoxic trials. The index of net MMP-9 activity was calculated as the ratio between MMP-9 activity and TIMP-1 concentration. The effect size of hyperoxia was calculated using Cohen’s $d$. A sample size of 8 subjects was considered necessary to detect a 5% difference between trials to establish a $P$-value of 0.05 and power of 0.80. Significance was accepted at the 0.05 level and all analyses were carried out with Statistica software (StatSoft Inc., USA).

**Results**

Table 1 shows the respiratory responses to normoxia and hyperoxia. As expected, hyperoxia evoked an increase in PetO$_2$ ($P=0.01$ vs normoxia) and O$_2$ saturation ($P=0.008$ vs normoxia). As intended, there was no change in PetCO$_2$ or VE during hyperoxia ($P>0.05$).

The effect of hyperoxia on cell adhesion molecules is shown in Figure 1. During hyperoxia, the level of ICAM-1 increased (~18%) compared to the normoxia trial (hyperoxia: 4.16 ± 0.85 vs normoxia: 3.51 ± 0.84 ng/mL, $P=0.04$; Cohen’s $d=0.91$). In contrast, hyperoxia did not change the level of VCAM-1 (normoxia: 26.62 ± 8.78 vs hyperoxia: 25.79 ± 6.78 pg/mL, $P=0.81$) or P-selectin (normoxia: 6.07 ± 2.92 vs hyperoxia: 5.01 ± 1.72 pg/mL, $P=0.33$).

The effect of hyperoxia on MMP-9, TIMP-1, and net MMP-9 activity are shown in Figure 2. During hyperoxia, the activity of MMP-9 decreased (~22%) compared to normoxia trial (hyperoxia: 0.53 ± 0.11 vs normoxia: 0.68 ± 0.18 A.U., $P=0.03$; Cohen’s $d=1$). Along with MMP-9 activity, hyperoxia evoked a greater increase (~46%) in TIMP-1 levels (hyperoxia: 8.40 ± 3.84 vs normoxia: 5.73 ± 2.15 pg/mL, $P=0.04$; Cohen’s $d=0.73$). The MMP-9/TIMP-1 ratio was low (~41%) in response to hyperoxia (hyperoxia: 0.07 ± 0.04 vs normoxia: 0.12 ± 0.06 A.U., $P=0.04$).

**Discussion**

To secure tissue oxygenation during a difficult tracheal intubation, the patient may be exposed to “pre-oxygenation” using 100% oxygen, often changed to inhalation of, e.g. 30% oxygen and mild positive pressure ventilation in order to prevent alveolar collapse once the tube is in place (15). This study found that the ICAM-1 level is increased in response to even 10 min of hyperoxia without affecting VCAM-1 and P-selectin levels. In contrast to our hypothesis, exposure to hyperoxia reduced MMP-9 activity and increased TIMP-1 levels in healthy men (Figure 3), and these findings likely contributed to reduction of the MMP-9/TIMP-1 ratio.

Endothelial activation is a term used to characterize an increase in adhesion molecules on the endothelial surfaces including ICAM-1, VCAM-1, and P-selectin. Hyperoxia promoted an increase in ICAM-1 levels without

**Table 1. Respiratory variables at normoxia and during hyperoxia.**

|                      | Normoxia (21% O$_2$) | Hyperoxia (100% O$_2$) | $P$-value |
|----------------------|-----------------------|------------------------|-----------|
| PetO$_2$ (mmHg)      | 98.8 ± 3.9            | 480.2 ± 4.3            | 0.01      |
| PetCO$_2$ (mmHg)     | 40.6 ± 1.0            | 40.4 ± 1.0             | 0.80      |
| O$_2$ saturation (%)  | 98.8 ± 0.3            | 100 ± 0.0              | 0.01      |
| VE (L/min)           | 11.6 ± 4.5            | 11.5 ± 3.5             | 0.84      |

Data are reported as means ± SD. Two-tailed paired Student’s $t$-test. PetO$_2$: end-tidal oxygen arterial pressure; PetCO$_2$: end-tidal carbon dioxide arterial pressure; VE: ventilation.

![Figure 1](image1.png)

**Figure 1.** Venous plasma levels of ICAM-1 (A), VCAM-1 (B), and P-selectin (C) in healthy men (n=8) at normoxia (21% O$_2$) and during hyperoxia (100% O$_2$). Data are reported as means ± SD. *$P<0.05$ vs normoxia (Wilcoxon signed-rank test). ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1.
affecting VCAM-1 or P-selectin levels. Accordingly, ICAM-1 is upregulated in response to hyperoxia (90% O₂–5% CO₂, 48–72 h) in both cultured human umbilical vein endothelial cells (HUVEC) and pulmonary endothelial cells (HPAEC) (3,4). Also, E-selectin and P-selectin levels did not change after exposure of endothelial cells to hyperoxia (4). Taken together, findings indicate that hyperoxia selectively upregulates the expression of ICAM-1 in healthy men, indicating endothelial activation.

Healthy men presented a decreased MMP-9 activity and increased TIMP-1 levels, which was consistent with a reduced MMP-9/TIMP-1 ratio. Although some in vitro studies and studies with animal models have observed an increase in MMP-9 concentration (50%) and activity (85%) after exposure to hyperoxia (6,16); others demonstrated that hyperoxia (>95% O₂) provokes MMP-9 down-regulation (5,17) and increases levels of TIMP-1 (5,18). It is believed that hyperoxia regulation depends on time exposure, i.e., the up-regulation of MMP activity is associated with prolonged exposure (>24 h), whereas down-regulation seems to be associated with a short exposure (<12 h) (17). Therefore, these findings represent evidence that hyperoxia triggered disturbances in vascular remodeling in healthy men.

Finally, it is conceivable that hyperoxia may exacerbate endothelial dysfunction in subjects with cardiovascular risk factors and cardiovascular disease, leading to worse cardiovascular outcomes. Furthermore, it is important to highlight that oxygen is the most common treatment strategy used in hospitalized patients with COVID-19. Given the significantly prolonged exposure to hyperoxia in intubated COVID-19 patients, we speculate that hyperoxia would provoke a fierce vascular response, resulting in increased inflammation, oxidative stress, expression of adhesion molecules, and vascular remodeling (19). However, the effect of hyperoxia on endothelium in patients with COVID-19 needs to be investigated.

Some limitations should be considered. First, the effect of hyperoxia on endothelium needs to be investigated under a poikilocapnic condition. Second, the trial order (normoxia and isocapnic hyperoxia) was not randomized. Nevertheless, we believe that this methodological concern...
does not invalidate our conclusions. Third, the activity of other metalloproteinases related to cardiovascular remodeling was not measured (i.e., MMP-2 and ADAM17). Finally, the present study was conducted in healthy men because women seem to be less susceptible to changes in cardiovascular physiology under hyperoxia (20). However, further studies are necessary to understand the hyperoxic effects on cell adhesion molecules and vascular remodeling in women, older adults, and in chronic diseases.

In conclusion, acute isocapnic hyperoxia in healthy men induced endothelial activation through increasing ICAM-1 and altered vascular remodeling by decreasing the MMP-9 and TIMP-1 ratio. Thus, we suggest that even short exposure to 100% oxygen affects endothelial adhesion and vascular remodeling factors, and that preoxygenation before anesthesia should be carried out with an oxygen level lower than 100%, significantly reducing these effects on vasculature.

**Supplementary Material**

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**References**

1. Kilgannon JH, Jones AE, Parrillo JE, Dellinger RP, Milcarek B, Hunter K, et al. Relationship between supranormal oxygen tension and outcome after resuscitation from cardiac arrest. *Circulation* 2011; 123: 2717–2722, doi: 10.1161/CIRCULATIONAHA.110.001016.

2. Kalnet RH, Matthy MA. Hyperoxic acute lung injury. *Respir Care*. 2013 Jan; 58: 123–41, doi: 10.4187/respcare.01963.

3. Suzuki Y, Nishio K, Takeshita K, Takeuchi O, Watanabe K, Sato N, et al. Effect of steroid on hyperoxia-induced ICAM-1 expression in pulmonary endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2000; 278: L245–L252, doi: 10.1152/ajplung.2000.278.2.L245.

4. Suzuki Y, Aoki T, Takeuchi O, Nishio K, Suzuki K, Miyata A, et al. Effect of hyperoxia on adhesion molecule expression in human endothelial cells and neutrophils. *Am J Physiol* 1997; 272: L418–L425, doi: 10.1152/ajpcell.1997.272.1.C355.

5. Hosford GE, Fang X, Olson DM. Hyperoxia decreases matrix metalloproteinase-9 and increases tissue inhibitor of matrix metalloproteinase-1 protein in the newborn rat lung: association with arrested alveolarization. *Pediatr Res* 2004; 56: 26–34, doi: 10.1203/01.PDR.0000130658.45564.1F.

6. Vogel ER, Britt RD, Faksh A, Kuipers I, Pandya H, Prakash YS, et al. Moderate hyperoxia induces extracellular matrix remodeling by human fetal airway smooth muscle cells. *Pediatr Res* 2017; 81: 376–383, doi: 10.1038/pr.2016.218.

7. Garcia VP, Rocha HNM, Silva GM, Amaral TAG, Secher NH, Nóbrega ACL, et al. Exogenous L-arginine reduces matrix metalloproteinase-2 and -9 activities and oxidative stress in patients with hypertension. *Life Sci* 2016; 157: 125–130, doi: 10.1016/j.lfs.2016.06.006.

8. Onal IK, Altun B, Onal ED, Kirkpantur A, Gul Oz S, Turgan C. Serum levels of MMP-9 and TIMP-1 in primary hypertension and effect of antihypertensive treatment. *Eur J Intern Med* 2009; 20: 369–372, doi: 10.1016/j.ejim.2008.10.003.

9. Vandooren J, Van den Steen PE, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol* 2013; 48: 222–272, doi: 10.3109/10409238.2013.770819.

10. Dean JB, Mulkey DK, Henderson RA, Potter SJ, Putnam RW. Hyperoxia, reactive oxygen species, and hyperventilation: oxygen sensitivity of brain stem neurons. *J Appl Physiol* (1985) 2004; 96: 784–791, doi: 10.1152/japplphysiol.00892.2003.

11. Fathi AR, Yang C, Bakhtian KD, Qi M, Lonser RR, Pluta RM. Carbon dioxide influence on nitric oxide production in endothelial cells and astrocytes: cellular mechanisms. *Brain Res* 2011; 1386; 50–57, doi: 10.1016/j.brainres.2011.02.066.

12. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991; 88: 4651–4655, doi: 10.1073/pnas.88.11.4651.

13. Mattos JD, Campos MO, Rocha MP, Mansur DE, Rocha HNM, Garcia VP, et al. Human brain blood flow and metabolism during isocapnic hyperoxia: the role of reactive oxygen species. *J Physiol* 2019; 597: 741–755, doi: 10.11.13/JP277122.

14. Storch AS, Rocha HNM, Garcia VP, Batista GMS, Mattos JD, Campos MO, et al. Oscillatory shear stress induces hemostatic imbalance in healthy men. *Thromb Res* 2018; 170: 119–125, doi: 10.1016/j.thromres.2018.08.019.

15. Hedenstierna G. Oxygen and anesthesia: what lung do we deliver to the post-operative ward? *Acta Anaesthesiol Scand* 2012; 56: 675–685, doi: 10.1111/j.1399-6576.2012.02689.x.

16. Pardo A, Selman M, Ridge K, Barrios R, Sznejder JI. Increased expression of gelatinases and collagenase in rat lungs exposed to 100% oxygen. *Am J Respir Crit Care Med* 1996; 154: 1067–1075, doi: 10.1164/ajrccm.154.4.8887609.

17. Cimino F, Balestra C, Geronpré P, De Bels D, Tillmans F, Saija A, et al. Pulsed high oxygen induces a hypoxic-like response in human umbilical endothelial cells and in humans. *J Appl Physiol* (1985) 2012; 113: 1684–1689, doi: 10.1152/japplphysiol.00922.2012.
18. Horowitz S, Shapiro DL, Finkelstein JN, Notter RH, Johnston C.J, Quible DJ. Changes in gene expression in hyperoxia-induced neonatal lung injury. *Am J Physiol* 1990; 258: L107–L111, doi: 10.1152/ajplung.1990.258.2.L107.

19. Hanidziar D, Robson SC. Hyperoxia and modulation of pulmonary vascular and immune responses in COVID-19. *Am J Physiol Lung Cell Mol Physiol* 2021; 320: L12–L16, doi: 10.1152/ajplung.00304.2020.

20. Bojkovic K, Rodgers JL, Vichare R, Nandi A, Mansour H, Saleem F, et al. The implications of hyperoxia, type 1 diabetes and sex on cardiovascular physiology in mice. *Sci Rep* 2021; 11: 23086, doi: 10.1038/s41598-021-02550-2.