Exposure to hypobaric hypoxia results in higher oxidative stress compared to normobaric hypoxia


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A B S T R A C T
Sixteen healthy exercise trained participants underwent the following three, 10-h exposures in a randomized manner: (1) Hypobaric hypoxia (HH; 3450 m terrestrial altitude) (2) Normobaric hypoxia (NH; 3450 m simulated altitude) and (3) Normobaric normoxia (NN). Plasma oxidative stress (malondialdehyde, MDA; advanced oxidation protein products, AOPP) and antioxidant markers (superoxide dismutase, SOD; glutathione peroxidase, GPX; catalase; ferric reducing antioxidant power, FRAP) were measured before and after each exposure. MDA was significantly higher after HH compared to NN condition (+24%). SOD and GPX activities were increased (vs. before; +29% and +54%) while FRAP was decreased (vs. before; −34%) only after 10 h of HH. AOPP significantly increased after 10 h for NH (vs. before; +83%), and HH (vs. before; +99%) whereas it remained stable in NN.

These results provide evidence that prooxidant/antioxidant balance was impaired to a greater degree following acute exposure to terrestrial (HH) vs. simulated altitude (NN) and that the chamber confinement (NN) did likely not explain these differences.

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1. Introduction

Exposure of humans to environmental hypoxia can be achieved either by reducing the ambient pressure (hypobaric hypoxia; HH) or by reducing the fraction of inspired oxygen (normobaric hypoxia; NH). It was long thought that the reduction of the partial pressure of inspired O2 (P O2) was the unique modulator of hypoxia-induced physiological responses. However, a growing body of evidence suggests that the difference in barometric pressure (BP) between HH and NH at the same P O2 may induce different physiological responses regarding ventilation (Loepky et al., 1997), nitric oxide (NO) metabolism (Henningsson and Linnarsson, 2009) and acute mountain sickness (Fulco et al., 2011). Although the underlying mechanisms of the differences between HH and NH are not yet fully understood, it was hypothesized that oxidative stress could be one of the key biological parameters involved in these processes (Millet et al., 2013). In this context, we recently reported higher select plasmatic oxidative stress markers during 24-h exposure to HH (3000 m natural altitude) compared to a simulated NH equivalent to 3000 m altitude in a hypoxic chamber (Faiss et al., 2013). It could, however, be argued that the observed oxidative stress in NH may, at least partially, result from the confinement in the chamber per se. Especially, since no control group that would be submitted to normobaric normoxia (NN) was employed. Accordingly, the aim of this study was to evaluate the effects of different modes of hypoxia (HH & NH) as well as the potential effects of confinement per se on prooxidant/antioxidant balance changes.

2. Methods

2.1. Participants

Sixteen healthy, trained male volunteered for this study (mean ± SD: age 34.7 ± 9.5 years, body weight 75.2 ± 7.2 kg, height 179.7 ± 5.7 cm, peak oxygen consumption (VO2max) 60.2 ± 9.9 ml·kg−1·min−1). All participants gave written informed consent before participation. They were all non-smokers, and neither acclimatized nor recently exposed to altitude. The subjects were asked
Fig. 1. Plasma concentration of malondialdehyde (MDA) before (H0) and after (H10) in hypobaric hypoxia (HH), normobaric hypoxia (NH) and normobaric normoxia (NN). Data are presented as means ± SD. **p < 0.01 significant difference with corresponding NN; *p < 0.05 significant difference with corresponding H10.

2.2. Experimental design

The experimental design consisted of three testing sessions preceded by a preliminary visit. Each testing session consisted of 10 h of exposure to the designated experimental condition (NN, NH, HH). During the preliminary visit, the participants were familiarized to the laboratory and perform baseline anthropometric and exercise testing. The following three exposure sessions were separated by at least 10 days and were performed in a randomized order. The HH session was performed at Altitude Research Station in Jungfraujoch (3450 m) while the NH and NN exposure sessions were performed in a hypoxic chamber (ATS Altitude, Sydney, Australia) at an altitude of 485 m (Sion, Switzerland) in a blinded manner. The hypoxic chamber is a well-ventilated room (2.4 m × 5.0 m × 2.5 m) with transparent glass panels (Faiss et al., 2013). The time spent in hypoxia was exactly the same for each session because of the very precise schedule. In addition, the progressive increase in altitude to access the Jungfraujoch by train during the HH sessions was simulated during the NH and NN sessions: For 45 min before entering the hypoxic chamber, the subjects breathed either room air (for NN) or hypoxic air (for NH) by using a mask and an Altitrainer.

Temperature inside the chamber was maintained at 22º in average maintained by an internal air conditioning system. The FiO2 within the chamber was controlled regularly with an electronic device (GOX 100 oximeter, Greisinger, Regenstauf, Germany). In order to blind participants to either the NH or NN exposure, the system was also running normoxic airflow into the chamber during the NH sessions.

During all sessions, peripheral blood oxygen saturation (SpO2) was monitored continuously using a finger pulse oxymeter (WristOx2™, Model 3150, Nonin, Nonin Medical, Inc., Minnesota, USA).

The environmental conditions during the exposure sessions were the following are detailed in Table 1.

2.3. Plasma oxidative stress and antioxidants assays

A 5-mL blood sample was obtained at rest from the antecubital vein before (H0) and immediately after the 10 h exposure (H10). After centrifugation, 400 µL aliquots of plasma were frozen and stored at 80 ºC until blinded analysis performed less than 6 months after the experiment in the same laboratory.

<table>
<thead>
<tr>
<th>Environment conditions during the hypobaric hypoxia (HH), normobaric hypoxia (NH) and normobaric normoxia (NN) sessions.</th>
<th>FIO2 (%)</th>
<th>BP (mm Hg)</th>
<th>P02 (mm Hg)</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH (3450 m terrestrial altitude)</td>
<td>20.9</td>
<td>481.5 ± 4.7</td>
<td>90.9 ± 1.0</td>
<td>21.3 ± 0.6</td>
<td>42.8 ± 4.4</td>
</tr>
<tr>
<td>NH (3450 m simulated altitude)</td>
<td>13.6</td>
<td>715.8 ± 3.8</td>
<td>91.0 ± 0.6</td>
<td>22.7 ± 0.8</td>
<td>41.0 ± 4.8</td>
</tr>
<tr>
<td>NN (485 m terrestrial altitude)</td>
<td>20.9</td>
<td>718.1 ± 3.3</td>
<td>140.5 ± 0.8</td>
<td>23.0 ± 1.0</td>
<td>45.1 ± 8.3</td>
</tr>
</tbody>
</table>

Fraction of inspired oxygen: FIO2; barometric pressure: BP; partial pressure of inspired oxygen: P02.
Our research team routinely performs the following oxidative stress and antioxidants measurements.

Plasma advanced oxidation protein products (AOPP) were measured according to the semi-automated methods developed by Witko-Sarsat et al. (1996). The plasma concentrations were determined by spectrophotometry and were calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the reaction was read at 340 nm. AOPP concentrations were expressed as μmol L⁻¹ of chloramine-T equivalents.

Concentrations of plasma malondialdehyde (MDA), as thio-barbituric reactive substances, were determined as previously described (Pialoux et al., 2006). The pink chromogen was extracted with n-butanol and its absorbance was measured at 532 nm by spectrophotometry using 1,1,3,3-tetraethoxypropan as standard. The quantitative determination of the Superoxide dismutase (SOD) activity was performed using the method described by Oberley and Spitz (1984). SOD activity was determined by the degree of inhibition of the reaction between superoxide radicals, produced by a hypoxanthine-xanthine oxidase system, and nitroblue tetrazolium. Catalase activity in the plasma was determined by using hydrogen peroxide (H₂O₂) as a substrate, and formaldehyde as a standard. Catalase activity was determined by the formation rate of formaldehyde induced by the reaction of methanol and H₂O₂ using catalase as enzyme.

Glutathione Peroxidase (GPX) activity was determined by the modified method of Paglia and Valentine (1967) as the rate of oxidation of NADPH to NADP⁺ after addition of glutathione reductase (GR), reduced glutathione (GSH) and NADPH, using H₂O₂ as a substrate.

Ferric reducing antioxidant power (FRAP) plasma concentrations were measured at a controlled temperature (37°C) by spectrophotometry. FRAP concentrations were calculated using an aqueous solution of known Fe²⁺ concentration (FeSO₄, 7H₂O₂) as standard at a wavelength of 593 nm.

2.4. Data analysis and statistics

Statistical analyses were performed using Statistica (Version 8.0; StatSoft, Tulsa, OK). Data are reported as mean ± Standard Deviation (SD). The changes from baseline have been calculated as the mean of the individual percentage changes from baseline. After verifying normality with Kolmogorov–Smirnov test, a one-way analysis of variance (ANOVA) followed by Sidak test were used for multiple comparisons between the 3 conditions (HH, NH and NN) at different times (H0 and H10). The level of significance was set at p < 0.05.

3. Results

SpO₂ was significantly lower at H10 for both HH (84.4 ± 2.2%; p < 0.01) and NH (86.4 ± 1.6%; p < 0.01) compared to H0 (95.9 ± 1.7%). No changes were noted following NN (95.1 ± 1.1%). SpO₂ in HH was significantly lower compared to NH at H10 (−2.3%; p < 0.05).

AOPP significantly increased after 10 h of hypoxic exposure independently of barometric pressure (+8%, p < 0.05 for NH and +99%, p < 0.05 for HH; Table 2) with no changes following the NN.

At H10, the MDA was significantly higher in HH compared to NN condition (p < 0.01; Fig. 1). Between H0 and H10, MDA remained stable for NH and was decreased (−22%; p < 0.05; Fig. 1) for NN condition.

GPX and SOD activities were significantly higher at H10 (vs. H0) in the HH only (+54%, p < 0.01; +29% Fig. 2A and Table 2) with no significant changes observed following both NH and NN. Moreover, at H10 the catalase was significantly higher in HH than in NH (p < 0.05; Fig. 2B).
**Table 2**

Effect on markers of oxidative stress and enzymatic antioxidants before (H0) and after (H10) in hypobaric hypoxia (HH), normobaric hypoxia (NH) and normobaric normoxia (NN).

<table>
<thead>
<tr>
<th></th>
<th>AOPP (μmol L(^{-1}))</th>
<th>SOD (μmol L(^{-1}) min(^{-1}))</th>
<th>FRAP (μmol L(^{-1}))</th>
<th>UA (μmol L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH (3450 m terrestrial)</td>
<td>H0 30.35 ± 8.8</td>
<td>1.9 ± 0.9</td>
<td>3.9 ± 0.3</td>
<td>942 ± 168</td>
</tr>
<tr>
<td>NH (3450 m simulated)</td>
<td>H0 31.22 ± 8.9</td>
<td>5.1 ± 0.9</td>
<td>617 ± 153***</td>
<td>1116 ± 147</td>
</tr>
<tr>
<td>NN (485 m terrestrial)</td>
<td>H0 32.4 ± 10.5</td>
<td>7.1 ± 1.5</td>
<td>919 ± 309</td>
<td>1073 ± 374</td>
</tr>
</tbody>
</table>

Plasma advanced oxidation protein products (AOPP), superoxide dismutase (SOD), plasma ferric-reducing antioxidant power (FRAP) and uric acid (UA). Data are presented as means ± SD. "p < 0.01; "p < 0.05 significant difference with comparing NH; "p < 0.01; "p < 0.05 significant difference with comparing NH; "p < 0.01; "p < 0.05 significant difference with comparing NH.

FRAP was significantly decreased following the HH exposure (−34%; p < 0.01 vs. H0) but remained stable following the NH and NN (Table 2). Moreover, after 10 h, FRAP was significantly lower in HH condition compared to NN condition (p < 0.01; Table 2).

Uric acid significantly decreased after 10 h of hypoxic exposure independently of barometric pressure (−39%, p < 0.01 for NH and −25%, p < 0.05 for HH) with no changes during the NH (Table 2). In addition, uric acid was significantly lower at H10 for both HH and NH condition compared to NN.

4. Discussion

Recent studies suggest that hypobaric hypoxia results in higher oxidative stress than normobaric hypoxia (Millet et al., 2013). The present data clearly show that oxidative stress is significantly higher following 10 h of HH compared to a NH at similar PiO\(_2\) (i.e., ~91 mm Hg) and ambient temperature. These results are in agreement with previous observations done during slightly longer hypoxic exposures (24 vs. 10 h, Faiss et al., 2013). More specifically, this conclusion is supported by the higher plasma content in MDA and the activities of our 3 antioxidant enzymes associated with lower FRAP in HH compared with NH. The higher increase in SOD, GPX and catalase suggests that the generation of superoxide anion and hydrogen peroxide is likely enhanced in HH, compared in NH. It was previously speculated that the higher ventilatory levels observed during NH vs. HH (Savourey et al., 2003; Loepky et al., 1997) may lead to a reduction of the hypoxia-induced plasma ac-
dosis and could explain the higher plasma oxidative stress observed in HH. Moreover, the lower SpO\(_2\) resulting from HH compared to NH may also explain the higher oxidative stress observed in HH since it was previously shown that oxidative stress was negatively correlated with haemoglobin oxygen saturation (Bailey et al., 2001; Pialoux et al., 2009a).

The increase in AOPP during both NH and HH is in line with previous studies performed in NH reporting continuously increased AOPP during 12 h of hypoxic exposure to approximately 3000 m (Pialoux et al., 2009b). The difference observed between MDA and AOPP regarding the response to either NH or HH could results from different reactive oxygen species (ROS) production pathways. Briefly, the AOPP generation in the plasma results from the myeloperoxidase pathway activation via monocytes activation (Witko-Sarsat et al., 1996) whereas MDA being the end-product of the oxidation of polyunsaturated fatty acids (Lefèvre et al., 1996).

The likely ROS overproduction occurring during HH compared to NH may thus explain the higher MDA and lower FRAP measured in the plasma. Interestingly, plasma uric acid was decreased in both HH and NH. Since uric acid is, along with vitamin C and E, one of the main antioxidant modulator of FRAP in the plasma (Benzie and Strain, 1996), it is surprising that we did not observe higher uric acid decrease in HH. However, it could be hypothesized that vitamin E, which is principally located between the polyunsaturated fatty acids of the cell membrane, may have been preferentially oxidized by the excessive ROS produced in HH and therefore subsequently affect the antioxidant capacity in plasma measured by FRAP.

It could be argued that the chamber confinement may partially explain the difference observed between HH and NH since MDA was decreased in response to NH. However, neither SOD, GPX, catalase nor FRAP were modified during the 10 h of NH. This would confirm that the differences observed for these markers between HH and NH are most likely the result of the BP difference.

In this study we decided to use endurance exercise trained sub-
jects because this population is probable the most relevant in regard to difference between HH and NH. Indeed, endurance athletes used hypoxic training to improve performance and since more than ten years normobaric hypoxia is as much used than natural altitude as hypoxic paradigm. It should be however acknowledge that exercise trained subjects used to present higher antioxidant enzymes activities and lower oxidative stress markers in plasma than sedentary people (Ji, 1995; Leeuwenburgh and Heinecke, 2001). It can be thus hypothesized that exercise trained subjects’ likely present better capacity to buffer ROS produced by hypoxic stimulus than sedentary (Pialoux et al., 2009c) due to their improved antioxidant enzyme system and therefore to express lower oxidative stress markers in plasma in response to this stimulus. In this context, although our population might have better antioxidant capacity to fight against oxidative stress, our data reveals that lipid peroxidation and antioxidant enzymes activities were nonetheless significantly higher after HH compared to both, NH and NN. It is very likely that the differences observed were amplified with sedentary participants.

In conclusion, this study confirms that ROS is over-generated during HH compared to NH, leading to higher oxidative stress and antioxidant enzymes activities. This effect of barometric pressure per se on oxidative stress during exposure to hypoxia might have important medical and sports performance implications since oxidative stress pathway is known to regulate Hypoxia Inducible Factor 1 (Miyata et al., 2011), which has a pivotal role in broad number of pathologies (i.e., cardiovascular and respiratory dis-

eases, cancer) and is also known to modulate ventilatory and hematological adaptations to altitude training. Further studies are warranted to elucidate whether this barometric pressure-induced differences in oxidative stress might importantly modulate the effects of hypoxic methods/trainings used for both, treatment in cardiovascular pathologies (Burschel et al., 2010) and improvement of aerobic performance in athletes (Lundby et al., 2012).

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