Effects of acute and sub-acute hypobaric hypoxia on oxidative stress: a field study in the Alps

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Abstract

Purpose High altitude results in lower barometric pressure and hence partial pressure of O_2 decrease can lead to several molecular and cellular changes, such as generation of reactive oxygen species (ROS). Electron Paramagnetic Resonance technique was adopted in the field, to evaluate the effects of acute and sub-acute hypobaric hypoxia (HH) on ROS production by micro-invasive method. Biological biomarkers, indicators of oxidative stress, renal function and inflammation were investigated too.

Methods Fourteen lowlander subjects (mean age 27.3 ± 5.9 years) were exposed to HH at 3269 m s.l. ROS production, related oxidative damage to cellular components, systemic inflammatory response and renal function were determined through blood and urine profile performed at 1st, 2nd, 4th, 7th, and 14th days during sojourn.

Results Kinetics of changes during HH exposition showed out significant (range $p < 0.05$–0.0001) increases that at max corresponds to 38% for ROS production rate, 140% for protein carbonyl, 44% for lipid peroxidation, 42% for DNA damage, 200% for inflammatory cytokines and modifications in renal function (assessed by neopterin concentration: 48%). Conversely, antioxidant capacity significantly ($p < 0.0001$) decreased − 17% at max.

Conclusion This 14 days in-field study describes changes of oxidative-stress biomarkers during HH exposure in lowlanders. The results show an overproduction of ROS and consequent oxidative damage to protein, lipids and DNA with a decrease in antioxidant capacity and the involvement of inflammatory status and a transient renal dysfunction. Exposure at high altitude induces a hypoxic condition during acute and sub-acute phases accompanied by molecular adaptation mechanism indicating acclimatization.

Keywords Acclimatization · Electron paramagnetic resonance · Hypobaric hypoxia · Oxidative stress · Reactive oxygen species

Abbreviations

AMS	Acute mountain sickness
BL	Baseline
EPR	Electron paramagnetic resonance
HH	Hypobaric hypoxia
HPLC	High-performance liquid chromatography
IL-1β; IL-6	Interleukin-1β, -6
8-iso-PGF2α	8-Isoprostane
8-OH-dG	8-Hydroxy-2-deoxy guanosine
OxS	Oxidative stress
PC	Protein carbonyl
ROS	Reactive oxygen species
TAC	Total antioxidant capacity

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Introduction

The reduction of barometric pressure and the consequent fall in the partial pressure of oxygen at high altitude leads to a condition defined hypobaric hypoxia (HH). When lowlanders are acutely exposed to HH several functional changes occur in human body to cope with the decreased oxygen availability. Whereas some of these adjustments (increased ventilation, cardiac output and hemoglobin concentration) are well characterized, others are still poorly understood.

A growing body of evidence indicates that HH appears to decrease the activity and effectiveness of antioxidant enzymes system and can cause an increased production of reactive oxygen species (ROS) with a consequent increment of oxidative damage to lipids (Joanny et al. 2001; Schmidt et al. 2002; Areneda et al. 2005), proteins, and DNA (Moller et al. 2001; Schmidt et al. 2002) of cellular compartments (Jefferson et al. 2004; Strapazzon et al. 2016; Malacrida et al. 2019), and also an increase in systemic inflammation (Siervo et al. 2014).

Indeed, several studies have reported increased Oxidative Stress (OxS) levels following acute and sub-acute high-altitude exposure (Bakonyi and Radak 2004) in various ethnic groups (i.e., Indian soldiers, Caucasian workers) (Vij et al. 2005; Strapazzon et al. 2016). However, the experimental protocols adopted were very different, and the specific mechanisms underlying the increased OxS levels are still unclear. It seems, however, that the hypoxia exposure dose (related to the ascent profile and to the exposure duration) may affect both the ROS production level and the kinetics of the oxidative damage biomarkers (Joanny et al. 2001; Askew et al. 2002; Dosek et al. 2007; Pialoux et al. 2009; Debevec et al. 2014).

ROS are physiological modulators of cellular redox status, signaling processes and are also involved in the regulation of a wide range of patho-physiological processes (Bailey and Davies 2001; Jefferson et al. 2004; Magalhaes et al. 2004). The high reactivity of ROS makes them difficult to measure. Thus, it is usual that, from the accumulation of the end-products of ROS and lipids, proteins, and DNA interaction, the extent of oxidative stress (OxS) is assessed. The only technique capable to provide direct evidence as well as an absolute ROS quantification is Electron Paramagnetic Resonance (EPR) (Sajfutdinov et al. 2001; Mrakic-Sposta et al. 2012, 2017a) but, to the best of our knowledge, it has never been utilized at high altitude.

It is well known that, in humans exposed to high altitude, hypoxia stimulates inflammatory cytokines expression (Ghezzi et al. 1991; Hartmann et al. 2000) but also that kidneys play a crucial role in human adaption to high altitude during acclimatization through their roles in regulating body fluids, electrolytes and acid–base homeostasis (Goldfarb-Rumyantzev et al. 2014).

Thereafter exposure of healthy humans to natural HH provides an opportunity to explore associations between hypoxia, OxS, inflammation and renal function.

The aim of the present study was to evaluate the effects of two acclimatization phases: sub-acute (up to 72 h) and acute (short-term exposure) (Jefferson et al. 2004). A group of volunteers was exposed to high altitude in well-controlled experimental conditions, to avoid possible confounding factors (e.g. changes in altitude level, cold, physical exertion and altered energy balance). We hypothesized that hypoxia exposure would increase OxS by an augmented production of ROS measured, for the first time directly, by EPR technique. Inflammatory response and renal function were evaluated too.

Materials and methods

Participants

Fourteen Caucasian participants (3 females, 11 males, age 27.3 ± 5.9 years) were recruited for the study. All participants resided below an altitude of 500 m s.l. and did not regularly use antioxidant or anti-inflammatory substances or drugs. They had no signs or symptoms related to infectious, cardiovascular, cerebrovascular, or pulmonary diseases contraindicating the study participation and they did not previously experience symptoms related to high-altitude illnesses. Furthermore, at baseline, venous blood samples were drawn for the standardized clinical hematological analyses to confirm the absence of pathological condition. Parameters were determined using an automated hematology analyzer, according to the standard analysis methods of ASST Grande Ospedale Metropolitano Niguarda laboratories. Data are reported in Table 1. One of the 14 participants was a smoker.

The study was approved by the Local Ethics Committee (BESTA/IBFM, Report #27, 9/03/2016) and was carried out in accordance with the Declaration of Helsinki. All participants were informed of the experimental protocol and all associated risks before giving written informed consent to take part in the study.

Table 1 Hematological parameters determined at the baseline: hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW)

Biochemical-hematological parameters	
Hb (g/dL)	14.61 ± 1.08
Erythrocytes (10^{12}/L)	4.94 ± 0.42
Leucocytes (10^{9}/L)	5.78 ± 1.39
MCV (fL)	87.05 ± 3.92
MCH (pg)	29.60 ± 1.16
MCHC (g/dL)	34.02 ± 0.83
RDW (%)	13.03 ± 0.42
Platelets (10^{9}/L)	200.00 ± 32.14
Neutrophils (10^{9}/L)	3.04 ± 1.05
Eosinophils (10^{9}/L)	0.13 ± 0.08
Lymphocytes (10^{9}/L)	2.07 ± 0.39
Monocytes (10^{9}/L)	0.51 ± 0.11
Basophils (10^{9}/L)	0.01 ± 0.03

Data are reported as mean ± SD

Experimental design

Participants were evaluated before expedition at sea level (Milan, 122 m) and at the 1st, 2nd, 4th, 7th, and 14th day of the sojourn at high altitude. Participants traveled by car from Milan to Cedec's lakes (2830 m) continuing to ascend on foot to the Casati Hut (3269 m) where the subjects dwelled for 14 days. The ascent profile followed by the group is depicted in Fig. 1. During the sojourn, outdoor activities were allowed for no more than 1 h per day with positive/negative altitude changes less than 100 m. Participants were asked to follow a specific diet, with the proportions of macronutrients recommended in a high-altitude diet (i.e., increase in iron requirements, intake energy and glycogen use) (Stellingwerff et al. 2019). Subjects were asked to follow a diet providing 12–15% of total energy from proteins, 50–60% from carbohydrates and 22–25% from fats (Hill et al. 2011). Compliance with diet and activity instructions were verified using 24 h diet records.

Measurements

Anthropometric measures were performed with the participants in underwear and without shoes. Besides height and weight, anthropometric characterizations of subjects included also measurement of total body water (TBW) and body mass composition [Fat Mass (FM) and Free Fat Mass (FFM)], determined by bipolar bio-impedentiometry (TBF-400 Body Composition Analyzer; Tanita Corporation, Arlington Heights, IL, USA). Subjects' body mass index (BMI) were calculated according to the Quetelet index, based on the [WT·(HT) $^{-2}$, kg m $^{-2}$]. In the morning of the testing days resting heart rate (HR), blood pressure and peripheral arterial oxygen saturation (SpO_2) were assessed with subjects lying supine for at least 30 min. Heart rate and blood pressure were recorded in triplicate by an automatically inflating cuff around the upper arm (Omron M7 Intelli IT, Omron, Japan), SpO_2 was measured using a pulse oximeter (TuffSat, GE Datex Ohmeda, USA).

Thereafter, each participant completed the Lake Louise Questionnaire as validated score (LLS) for diagnosing and grading of Acute Mountain Sickness (AMS) (Roach et al. 2018). AMS was defined as LLS ≥ 3 with headache.

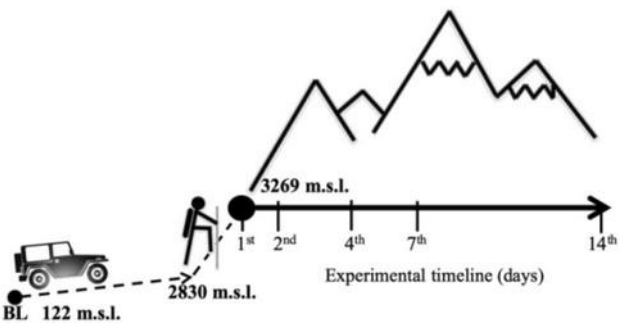

Fig. 1 Sketch of the experimental protocol indicating the type of ascent, the exposure profile and the timing of blood and urine sampling

Blood and urine samples collection

In the morning before breakfast, a 5-ml blood sample were drawn from an antecubital vein and collected in heparinized tubes (Becton Dickinson Company, UK), centrifuged (Centrifuge 5702 R, Eppendorf, Germany) at 4000 rpm for 10 min at 4 °C and separated. Urine samples were collected in a sterile container provided to the participants. Plasma and urine samples were frozen and stored in multiple aliquots in cryogenic dewars containing liquid nitrogen until analysis assayed.

ROS determination

ROS production rate was determined by means of a well-consolidated micro-invasive method (Mrakic-Sposta et al. 2012, 2014, 2015a, b, 2020). An X-band EPR instrument (E-scan-Bruker BioSpin GmbH, Billerica, MA, USA) was utilized for ROS detection. Shortly, 50 µL of capillary blood, taken from the fingertip, were immediately treated (1:1) with probe solution CMH (1-hydroxy- 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine). The adopted acquisition parameters were previously determined (Mrakic-Sposta et al. 2012, 2014, 2015a, b). A controller ''Bio III'' unit, interfaced to the spectrometer, was used to stabilize sample temperature at 37 °C. A stable radical CP· (3-Carboxy2,2,5,5-tetramethyl-1-pyrrolidinyloxy) was used as external reference to convert ROS determinations in absolute quantitative values (µmol min^{-1}).

Antioxidant capacity measurement

A sample of 10 µl of capillary blood was used to assess the blood antioxidant capacity (TAC) by means of the EDEL potentiostat electrochemical analyzer (Edel Therapeutics, Switzerland) equipped with a redox sensor (Liu et al. 2005), According to Tacchini et al. (2013), values were expressed in nW.

Oxidative damage assessment

Plasmatic content of Protein Carbonyls (PC), and urinary levels of 8 hydroxy-2-deoxy guanosine (8-OH-dG) and 8-isoprostane (8-iso-PGF2α), established markers of oxidized proteins, DNA damage and lipid peroxidation

respectively, were assessed by commercial enzyme immunoassay kits (Cayman Chemical, USA).

Inflammatory status

Interleukins: IL-6 and IL-1β plasmatic levels were determined by Human interleukins ELISA kits (Cayman Chemical, Michigan, USA) according to the manufacturer's instruction.

Creatinine and neopterin concentration

Creatinine and neopterin urinary concentrations were measured by high-pressure liquid chromatography (HPLC) method as previously described (Mrakic-Sposta et al. 2015b, 2017b). Also, blood creatinine concentration was assessed by commercial enzyme immunoassay kits (Cayman Chemical, USA) to evaluate muscle wasting at altitude.

Urine test strip

The Urine Test Strip (Siemens Healthcare S.r.l. 10sys Multistix, Italy) was used for semi-quantitative determinations of ketones, pH, blood, urobilinogen, bilirubin, proteins, specific gravity/density and leukocytes in urine. Test was immediately performed after sample collection in duplicate for each subject.

Statistical analysis

Data are expressed as mean ± SD and were analyzed using a software package (GraphPad Prism 8.3, Software Inc., San Diego, CA) and Statistical Package for the Social Science (IBM SPSS Statistics v. 25, Armonk, NY, USA) software.

The normality of the data distribution was tested with the Shapiro Wilk's test and data resulted normally distributed. Data were compared by one-way ANOVA for repeated measures, followed by Bonferroni's multiple comparison test to further check the among group significance. $p < 0.05$ statistical significance level was accepted. Percentage changes [((post value − pre value)/pre value) × 100] are also reported in the text.

Prospective calculation of power to determine subjects' number was made using G × Power software (Faul et al. 2007). The prospective calculation of the sample size for was determined choosing the ROS production as primary outcome.

Results

The highest values of LLS (2 ± 1) were recorded in the 1th day at altitude but no subject was AMS positive. During the remaining days, the average values of LLS were equal or less than 1. No drugs were administered.

Anthropometric and physiological parameters

Anthropometric and physiological resting parameters obtained at the different time-points are reported in Table 2. After 4–7 days spent at high-altitude weight decreased from sea level values reaching significant ($p < 0.05$) lower levels at 7th and 14th days. SaO_2 resulted significantly ($p < 0.0001$) reduced all over the HH exposition. A significant ($p < 0.05$) increase in HR at 1st day was observed too (see Table). Not significant differences over time in blood creatinine concentrations were observed.

Table 2 Anthropometric and physiological parameters from all subjects at the baseline (BL- sea level) and during the 2 weeks at 3260 m s.l

	Subjects (n = 14)					
	BL	1st day	2nd day	4th day	7th day	14th day
Weight (kg)	72.1 ± 11.1	72.6 ± 11.1	72.7 ± 11.1	71.1 ± 11.6*	70.4 ± 12.3*	70.4 ± 12.5*
BMI (kg m^{-2})	23.4 ± 1.9	23.1 ± 2.3	23.1 ± 2.2	22.9 ± 2.3	22.8 ± 2.4	22.6 ± 2.5
FM (kg)	10.2 ± 6.2	10.0 ± 5.9	10.0 ± 5.9	10.9 ± 5.2	9.7 ± 4.3	9.9 ± 4.5
FFM (kg)	62.1 ± 6.9	59.9 ± 8.6	59.9 ± 8.6	59.9 ± 8.6	61.7 ± 8.3	60.1 ± 12.3
TBW (%)	45.3 ± 7.3	44.1 ± 6.9#	44.0 ± 6.9	43.6 ± 7.3	43.6 ± 7.6	43.6 ± 7.7
HR (bpm)	67.4 ± 13.3	85.5 ± 18.0*	82.2 ± 20.7	80.3 ± 16.3	77.4 ± 9.1	75.6 ± 6.6
SaO2 (%)	99.0 ± 0.7	88.5 ± 3.0a	88.8 ± 3.4a	91.1 ± 1.9a	91.5 ± 1.8a	92.6 ± 1.3a
SBP (mmHg)	123.7 ± 11.6	130.1 ± 7.8	131.3 ± 9.5	132.1 ± 8.7	130.2 ± 9.3	131.3 ± 10.7
DBP (mmHg)	72.7 ± 13.1	79.6 ± 12.9	76.5 ± 9.9	77.4 ± 10.0	72.7 ± 7.5	74.1 ± 7.7

Data are displayed as mean ± SD

BMI body mass index, *FM* fat mass, *FFM* free fat mass, *TBW* total body water, *HR* heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *SaO2* arterial oxygen saturation

p values refer to different time points compared to the baseline and are represented by symbols as follows: *$p < 0.05$; #$p < 0.01$; §$p < 0.001$; a$p < 0.0001$

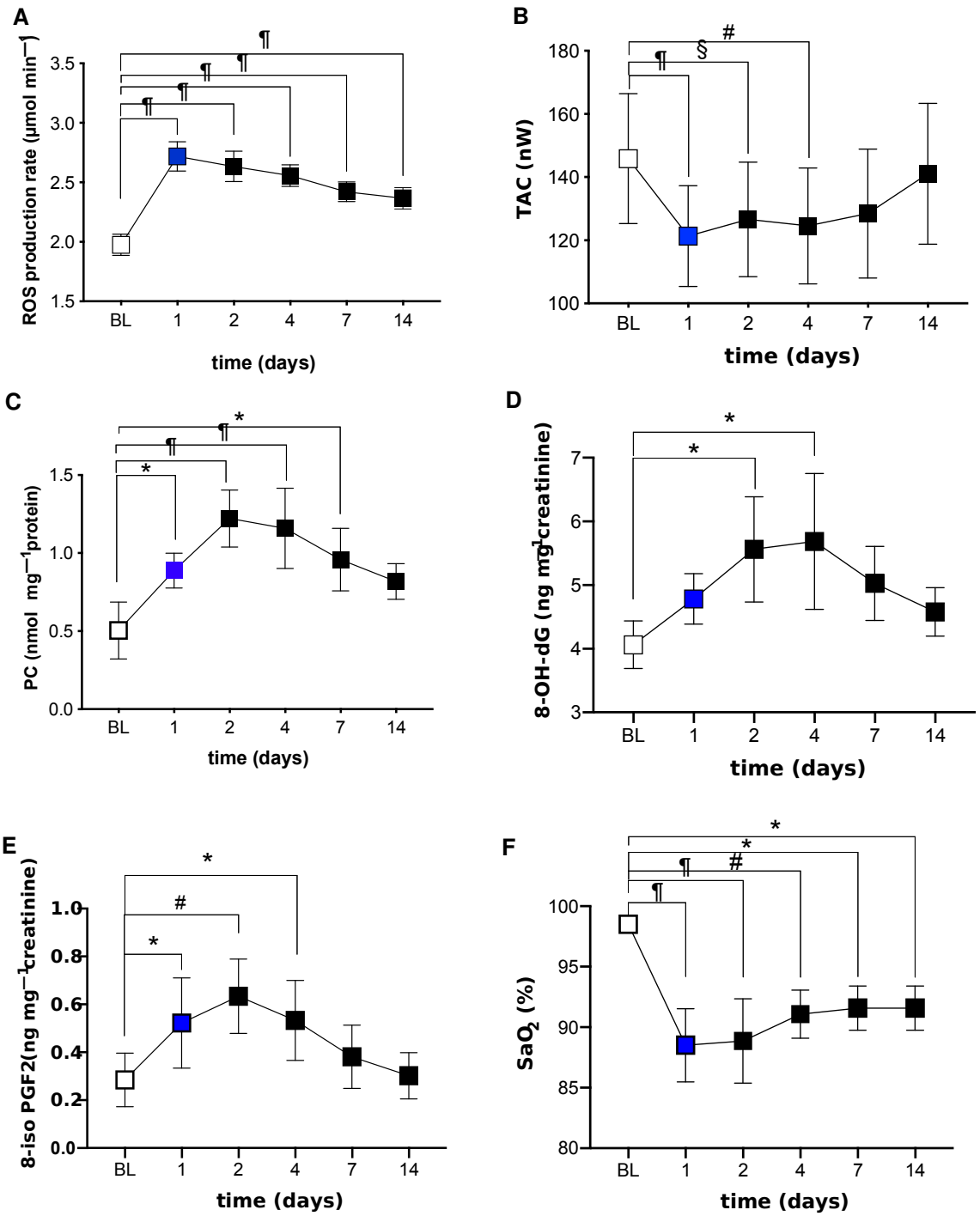

Fig. 2 Time course of: **a** Reactive Oxygen Species production rate (ROS, µmol min $^{-1}$), **b** Total Antioxidant Capacity (TAC, nW), **c** Carbonyls Protein (PC, nmol.mg-1 protein), **d** DNA damage (8-OH-dG, ng mg $^{-1}$ creatinine), **e** 8-isoprostanes (8-isoPGF2α, ng mg $^{-1}$ creatinine), and **f** arterial oxygen saturation (SpO2, %) at baseline (BL, 122 m) and after 1, 2, 4, 7, and 14 days at 3269 m. Data are reported as mean ± SD. *p* values refer to different times vs BL and are represented with symbols: *$p < 0.05$, #$p < 0.01$, §$p < 0.001$, and ¶$p < 0.0001$. Data of baseline sea level are represented in empty square, and data of 1st day of exposition are represented in blue full square

ROS production and oxidative damage assessments

ROS production rate measured in capillary blood and OxS biomarkers concentrations in plasma and urine are shown in Fig. 2. Acute and sub-acute HH exposure, led to significant changes in the OxS indices. In details: with respect to the baseline measurements at sea level (BL):

the capillary ROS production rate significantly ($p < 0.0001$) increased reaching a peak value at the 1st day and then remaining elevated from 1st to 14 days (1st: 2.72 ± 0.13; 2nd: 2.63 ± 0.13; 4th: 2.56 ± 0.09; 7th: 2.42 ± 0.08; 14th: 2.37 ± 0.09 µmol min $^{-1}$), (Fig. 2a);
plasmatic PC concentration significantly (range $p < 0.05$ to < 0.0001) increased from 1st to 7 days (1st: 0.89 ± 0.11; 2nd: 1.22 ± 0.18; 4th: 1.16 ± 0.26; 7th: and 0.95 ± 0.20 nmol mg^{-1}protein) showing a peak at the 2nd day (Fig. 2c);
urinary 8-OH-dG significantly ($p < 0.05$) increased at 2nd, and 4th day (5.56 ± 0.82 and 5.69 ± 1.07 ng mg^{-1} creatinine) with a peak at the 4th day, (Fig. 2d);
urinary 8-iso PGF2α significantly (range $p < 0.05$ to < 0.01) increased from 1st to 4 days (1st: 0.52 ± 0.18; 2nd: 0.63 ± 0.15; 4th: 0.53 ± 0.16 ng mg^{-1}creatinine) with a peak at 2nd day (Fig. 2e).
Conversely, capillary TAC significantly (range $p < 0.001$ to < 0.001) decreased at 1st, 2nd, 4th day (121.4 ± 15.9, 126.6 ± 18.1, 124.6 ± 18.3 nW respectively) respect to BL level (145.9 ± 20.5 nW) (Fig. 2b). Moreover, a significant (range $p < 0.05$ to < 0.0001) decrease of SaO_2 confirmed the reduction in inspired PO_2, with a peak at 1st (88.5 ± 3.0%) and 2nd (88.8 ± 3.5%) day respect to BL level (99.0 ± 7.0%) (Fig. 2f**).**

Inflammation status

During the sojourn at HH significant changes in the inflammatory status were observed. The IL-6 concentration significant (range $p < 0.05$–0.001) increased respect to BL level from the 1st to the 4th day (1st: 7.78 ± 2.17; 2nd: 11.98 ± 4.51; 4th: 11.99 ± 3.86 pg mL^{-1}) with a peak at the 2nd and 4th day (Fig. 3a).

By contrast, IL-1β did not reveal significant variations during hypoxia exposure respect to BL level (1.44 ± 0.41 pg mL^{-1}) see Fig. 3b.

Neopterin and urine standard parameters

The renal functional biomarker neopterin data are shown in Fig. 4. Neopterin concentration significantly ($p < 0.05$) increased respect to BL at 2nd and 4th days (2nd: 132.30 ± 49.55; 4th: 114.80 ± 28.70 µmol mol $^{-1}$creatinine) and then returned to the basal level.

Urine standard parameters are reported in Table 3. A significant increase of urine pH value was found from the 1st to the 7th day of HH exposure respect to sea level, with a peak at 4th day: 6.6 ± 0.5. Significant ketoacidosis and leukocytosis were found after 4 days of sojourn.

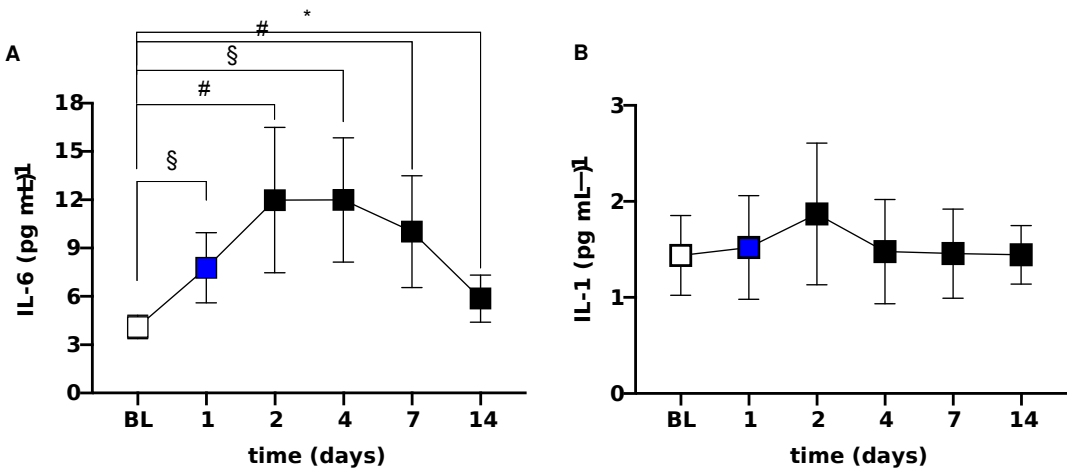

Fig. 3 Time course of pro-inflammatory cytokines: IL-6 and IL-1β. Data are reported as mean ± SD. *p* values refer to different time points compared to baseline and are represented with symbols: *$p < 0.05$, #$p < 0.01$, and §$p < 0.001$. Data of baseline sea level are represented in empty square, and data of 1st day of exposition are represented in blue full square

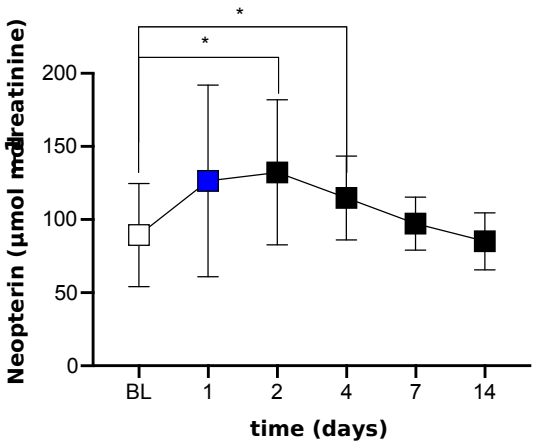

Fig. 4 Time course of Neopterin (µmol mol^{-1} creatinine) levels. Data are reported as mean ± SD. *p* values refer to different time points compared to BL and are represented by the symbol *$p < 0.05$. Data of baseline sea level are represented in empty square, and data of 1st day of exposition are represented in blue full square

Discussion

The time course of ROS production during acute and sub-acute hypobaric hypoxia exposure, measured 'just in the place' by means of an EPR micro-invasive method, has been here reported for the first time during 14 days of acclimatization. Changes of oxidative damage biomarkers levels, antioxidant capacity, inflammation and renal function over 14 days of exposure to constant hypobaric hypoxia (3269 m) were assessed too. Indeed, the observation length allowed us to span two acclimatization phases: (A) acute (up to 72 h) and (B) sub-acute (over 72 h) (Jefferson et al. 2004).

Previously reported data (Strapazzon et al. 2016; Irarrázaval et al. 2017) found herein confirmation: significant biomarkers' level changes during the first three days of acclimation were assessed.

In particular after 24 h, the imbalance between the ROS production rate (about + 40%) and the antioxidant scavenging (− 16%, see Fig. 1a, b) reflected the increase in the oxidative stress-related damage and the inflammation status. Such a prolonged unbalance induced, in turn, proteins, DNA, and lipids damage (see Fig. 1c, d, e).

After 3 days of sojourn at high altitude, recorded data showed that the redox-balance was returning to the baseline levels very slowly. In fact, after 14 days at HH, the ROS production still resulted very high (+ 20%), while the TAC value was tending to restore the balance. Nevertheless, such a prolonged enhanced ROS formation induced oxidative damage to proteins, lipids and DNA.

On the other hand, excessive ROS generation may become an essential stimulus to initiate adaptive responses to hypoxia and also an integral part of a series of signaling events (Bailey et al. 2001; Askew 2002) as ROS are involved in ROS-activated transcription factors and other proteins (Milkovic et al. 2019). Indeed, excessive ROS generation may become an essential stimulus to initiate adaptive responses. Furthermore, the inadequate antioxidant defense induces increased OxS that could lead to mal-adaptation and culminate into high altitude-related pathologies (Bailey et al. 2001; Askew 2002). To cope with elevated levels of ROS, cells in the first phase turn to antioxidative machinery (Fig. 1b). When the delicate redox balance is disturbed, the accumulation of ROS could become detrimental leading to oxidative damage.

It is well known that the lipid peroxidation level increases during hypoxia exposure (Radak et al. 2014). Data of the present study showed that after reaching a peak value at the second day (+ 125%), lipid peroxidation started to decrease at 4th day and after 2 weeks almost returned to the basal level (see Fig. 1e). Our data agree with many reports indicating the severity of oxidative reactions and increased levels of lipid peroxidation in people exposed to high-altitude environment (Pfeifeer et al. 1999; Joanny et al. 2001).

It is well known that, in humans exposed to high altitude, hypoxia stimulates inflammatory cytokines expression (Ghezzi et al. 1991; Hartmann et al. 2000). Herein

Table 3 Urine standard analysis. Urine test strip data values (mean ± SD) from all subjects

	Subjects (n = 14)					
	BL	1st day	2nd day	4th day	7th day	14th day
Bilirubin (µmol L^{-1})	7.2 ± 8.6	6.1 ± 8.5	3.8 ± 7.6	4.7 ± 7.8	5.9 ± 8.2	7.2 ± 8.6
Urobilinogen (µmol L^{-1})	0.3 ± 0.2	0.3 ± 0.3	0.4 ± 0.3	0.8 ± 0.3*	0.8 ± 0.3*	0.7 ± 0.3*
Ketones (mmol L^{-1})	1.4 ± 2.3	2.5 ± 2.4	5.3 ± 4.5*	5.2 ± 3.1*	4.6 ± 1.8#	4.3 ± 1.8*
Erytrocytes (Ery µL^{-1})	1.2 ± 3.1	2.1 ± 3.7	1.4 ± 3.6	3.3 ± 6.9	3.5 ± 13.3	0.5 ± 1.8
pH	5.8 ± 0.5	6.2 ± 0.5*	6.5 ± 0.5*	6.6 ± 0.5*	6.5 ± 0.5*	5.5 ± 0.03
Leucocytes (Leuko µL^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	7 ± 20.5	10.7 ± 27.2	1.7 ± 6.6
Specific gravity	1.02 ± 0.0	1.02 ± 0.0	1.02 ± 0.0	1.02 ± 0.0	1.02 ± 0.0	1.02 ± 0.00

Statistically significant differences symbols: *$p < 0.05$, #$p < 0.01$, §$p < 0.001$, and a$p < 0.0001$

obtained data demonstrated the presence of a moderate systemic inflammation in response to high altitude throughout the increase of pro-inflammatory cytokine IL-6. In well agreement with Klausen et al. (1997) IL-6 reached a peak level starting from the 2nd up to the 4th day then decreasing toward baseline during the following 10 days (Fig. 3a); inflammatory state is also reflected in the leukocytosis founded in the urine after 4 days of sojourn.

On the other hand, IL-1β did not significantly change in plasma during 2 weeks at high altitude. This result is in contrast with finding of another study, where the concentration of IL-1β circulating protein showed a slight transient increase after 24 h of HH exposure (Malacrida et al. 2019). Such a difference might be ascribed to the different age of the subjects participating in the two studies, and/or the method of ascent to high altitude.

Temporary impairment of renal function is suggested by the increase of neopterin level, identified marker of various conditions such as inflammatory and renal diseases (Unuvar and Aslanhan 2019). The impairment could also be considered as a likely physiological response to acute and sub-acute HH. Moreover, high neopterin production has been associated with immune cellular activation and increased production of oxidative stress too (Shao et al. 2014). Increase of urinary neopterin concentration was observed in the present study in the first 2–4 days of sojourn (+ 38%), associated with rising of ROS production and lowering of antioxidant concentration (see Figs. 1a, b and 4).

Others important parameters adopted to evaluate the high altitude-related stress on the renal response included changes in acid–base and electrolyte status. As reported in a simulated moderate altitude study by Ge et al. (2006), in the present study, the urine pH value significantly increased at the 1st day at 3269 m, but it did not immediately return to baseline level (Table 3). These different findings lead us to assume that at high altitude the renal contribution to acid–base compensation is slow.

Starting from the second day all over the 2 weeks of sojourn, an increase in urine ketone levels was observed (Table 3). As well known, ketones are the main anaplerotic substrates when muscle proteolysis is elevated and pyruvate oxidation is limited, and so may be an important energy source for skeletal muscle in high-altitude hypoxia (Murray and Montgomery 2014; Chicco et al 2018).

Limitations

The authors are aware that the current study suffers from certain limitations.

Even if the sample size (n = 14; 11 male and 3 female) was small, it was large enough to ensure an adequate study power, as determined by the Freeware G × Power software (see paragraph “Statistical analysis”). In fact, as already pointed out, at 80% power, the calculated sample size was 11 subjects. As already pointed out, the prospective calculation of the sample size was determined choosing the ROS production as primary outcome, and no other parameters were taken into account.

One of the participants was a smoker. We know that smokers usually have increased CO-Hb concentrations resulting in a leftward shift of the oxygen hemoglobin curve resulting in a better oxygen release, and that carbon monoxide has anti-inflammatory effects. However, we also want to point out that our subject was a light cigarette’s smoking: about 3–4/die.

Finally, there was no chance of calling back the participants, coming from all over Italy, to extend the study to the return to sea level.

Conclusions

This 14 days in-field study describes the ROS production rate, modification of OxS biomarkers and inflammation during HH exposure in lowlanders. Acute hypoxia and first part of acclimatization (sub-acute hypoxia) is accompanied by molecular adaptation mechanism where ROS occupy an essential role in the oxidative stress and related damage to cellular components.

We can conclude that lowlanders exposed to acute and sub-acute HH are highly susceptible to hypoxic insult, expressing high levels of OxS, despite the overall defense mechanisms. However, these metabolic changes are transient and not necessarily harmful to the body and may play a role in the hypoxia acclimatization process.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Araneda OF, García C, Lagos N et al (2005) Lung oxidative stress as related to exercise and altitude. Lipid peroxidation evidence in exhaled breath condensate: a possible predictor of acute mountain sickness. Eur J Appl Physiol 95:383–390. https//doi.org/10.1007/s0042 1-005-0047-y

Askew EW (2002) Work at high altitude and oxidative stress: antioxidant nutrients. Toxicology 180:107–119. https//doi.org/10.1016/s0300 -483x(02)00385-2

Bailey DM, Davies B (2001) Acute mountain sickness; prophylactic benefits of antioxidant vitamin supplementation at high altitude. High Alt Med Biol 2:21–29. https ://doi.org/10.1089/152702901750067 882

Bakonyi T, Radak Z (2004) High altitude and free radicals. J Sports Sci Med 3:64–69

Chicco AJ, Le CH, Gnaiger E et al (2018) Adaptive remodeling of skeletal muscle energy metabolism in high-altitude hypoxia: lessons from AltitudeOmics. J Biol Chem 293:6659–6671. https ://doi.org/10.1074/jbc.RA117 .000470

Debevec T, Pialoux V, Mekjavic IB et al (2014) Moderate exercise blunts oxidative stress induced by normobaric hypoxic confinement. Med Sci Sports Exerc 46:33–41. https ://doi.org/10.1249/MSS.0b013 e31829f87ef

Dosek A, Ohno H, Acs Z et al (2007) High altitude and oxidative stress. Respir Physiol Neurobiol 158:128–131. https//doi.org/10.1016/j.resp.2007.03.013

Faul F, Erdfelder E, Lang AG et al (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175–191. https ://doi.org/10.3758/bf03193146

Ge RL, Babb TG, Sivieri M et al (2006) Urine acid-base compensation at simulated moderate altitude. High Alt Med Biol 7:64–71. https ://doi.org/10.1089/ham.2006.7.64

Ghezzi P, Dinarello CA, Bianchi M (1991) Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. Cytokine 3:189–194. https//doi.org/10.1016/1043-4666(91)90015-6

Goldfarb-Rumyantzev AS, Alper SL (2014) Short-term responses of the kidney to high altitude in mountain climbers. Nephrol Dial Transplant 29:497–506. https//doi.org/10.1093/ndt/gft051

Hartmann G, Tschöp M, Fischer R et al (2000) High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. Cytokine 12:246–252. https//doi.org/10.1006/cyto.1999.0533

Hill NE, Stacey MJ, Woods DR (2011) Energy at high altitude. J R Army Med Corps 157:43–48. https ://doi.org/10.1136/jramc-157-01-08

Irarrázaval S, Allard C, Campodónico J et al (2017) Oxidative stress in acute hypobaric hypoxia. High Alt Med Biol 18:128–134. https//doi.org/10.1089/ham.2016.0119

Jefferson JA, Simoni J, Escudero E et al (2004) Increased oxidative stress following acute and chronic high altitude exposure. High Altitude Med Biol 5:61–69. https ://doi.org/10.1089/152702904322963 690

Joanny P, Steinberg J, Robach P et al (2001) Operation Everest III (Comex'97): the effect of simulated sever hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. Resuscitation 49:307–314. https//doi.org/10.1016/s0300-9572(00)00373-7

Klausen T, Olsen NV, Poulsen TD et al (1997) Hypoxemia increases serum interleukin-6 in humans. Eur J Appl Physiol 76(480–482):1089. https//doi.org/10.1007/s004210050278

Liu J, Roussel C, Lagger G et al (2005) Antioxidant sensors based on DNA-modified electrodes. Anal Chem 77:7687–7694. https//doi.org/10.1021/ac0509298

Magalhaes J, Ascensao A, Viscor G et al (2004) Oxidative stress in humans during and after 4 hours of hypoxia at a simulated altitude of 5500 m. Aviat Space Environ Med 75:16–22

Malacrida S, Giannella A, Ceolotto G et al (2019) Transcription factors regulation in human peripheral white blood cells during hypobaric hypoxia exposure: an in-vivo experimental study. Sci Rep 9:9901. https ://doi.org/10.1038/s41598-019-46391-6

Milkovic L, Cipak Gasparovic A, Cindric M, Mouthuy PA, Zarkovic N (2019) Short overview of ROS as cell function regulators and their implications in therapy concepts. Cells 8(8):793

Møller P, Loft S, Lundby C et al (2001) Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans. FASEB J 15:1181–1186. https//doi.org/10.1096/fj.00-0703c om

Mrakic-Sposta S, Gussoni M, Montorsi M et al (2012) Assessment of a standardized ROS production profile in humans by electron paramagnetic resonance. Oxid Med Cell Longev 2012:973927. https ://doi.org/10.1155/2012/973927

Mrakic-Sposta S, Gussoni M, Montorsi M et al (2014) A quantitative method to monitor reactive oxygen species production by electron paramagnetic resonance in physiological and pathological conditions. Oxid Med Cell Longev 2014:306179. https ://doi.org/10.1155/2014/306179

Mrakic-Sposta S, Gussoni M, Porcelli S et al (2015) Training effects on ROS production determined by electron paramagnetic resonance in master swimmers. Oxid Med Cell Longev 2015:804794. https ://doi.org/10.1155/2015/804794

Mrakic-Sposta S, Gussoni M, Moretti S et al (2015) Effects of mountain ultra-marathon running on ROS production and oxidative damage by micro-invasive analytic techniques. PLoS ONE 10:e0141780. https ://doi.org/10.1371/journal.pone.0141780

Mrakic-Sposta S, Vezzoli A, Malacrida S et al (2017) "Direct" and "indirect" methods to detect oxidative stress during acute or chronic high-altitude exposure. High Alt Med Biol 18:303–304. https ://doi.org/10.1089/ham.2017.0067

Mrakic-Sposta S, Gussoni M, Vezzoli A et al (2017) Acute effects of triathlon race on oxidative stress biomarkers. Oxid Med Cell Longev 2020:3062807. https//doi.org/10.1155/2020/3062807

Murray AJ, Montgomery HE (2014) How wasting is saving: weight loss at altitude might result from an evolutionary adaptation. BioEssays 3:721–729. https://doi.org/10.1002/bies.201400042

Pfeifeer JM, Askew EW, Roberts DE et al (1999) Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. Wilderness Environ Med 10:66–74. https//doi.org/10.1580/1080-6032(1999)010[0066:eoasou]2.3.co;2

Pialoux V, Mounier R, Rock E et al (2009) Effects of acute hypoxic exposure on prooxidant/antioxidant balance in elite endurance athletes. Int J Sports Med 30:87–93. https ://doi.org/10.1055/s-0028-1103284

Radak Z, Acs Z, Bori Z et al (2014) The effects of high-altitude exposure on reactive oxygen and nitrogen species. Syst Biol Free Radicals Antioxidants. https//doi.org/10.1007/978-3-642-30018-9_28

Roach RC, Hackett PH, Oelz O et al (2018) Lake louise ams scoring consensus committee. The 2018 lake louise acute mountain sickness score. High Altitude Med Biol 19(1):4–6. https ://doi.org/10.1089/ham.2017.0164

Sajfutdinov RG, Larina LI, Vakul'skaya TI et al (2001) Electron paramagnetic resonance in biochemistry and medicine. Kluwer Academic/Plenum Publishers, New York

Schmidt MC, Askew EW, Roberts DE et al (2002) Oxidative stress in humans training in a cold, moderate altitude environment and their response to a phytochemical antioxidant supplement. Wilderness Environ Med 13:94–105. https ://doi.org/10.1580/1080-6032(2002)013[0094:osiht i]2.0.co;2

Shao Z, Zhang R, Shrestha K et al (2014) Usefulness of elevated urine neopterin levels in assessing cardiac dysfunction and exercise ventilation inefficiency in patients with chronic systolic heart failure. Am J Cardiol 113:1839–1843. https ://doi.org/10.1016/j.amjcard.2014.03.016

Siervo M, Riley HL, Fernandez BO et al (2014) Effects of prolonged exposure to hypobaric hypoxia on oxidative stress, inflammation and gluco-insular regulation: the not-so-sweet price for good regulation. PLoS ONE 9:e94915. https ://doi.org/10.1371/journal.pone.0094915

Stellingwerff T, Peeling P, Garvican-Lewis LA et al (2019) Nutrition and altitude: strategies to enhance adaptation, improve performance and maintain health: a narrative review. Sports Med 49:169–184. https ://doi.org/10.1007/s4027 9-019-01159 -w

Strapazzon G, Malacrida S, Vezzoli A et al (2016) Oxidative stress response to acute hypobaric hypoxia and its association with indirect measurement of increased intracranial pressure: a field study. Sci Rep 6:32426. https//doi.org/10.1038/srep32426

Tacchini P, Lesch A, Neequaye A et al (2013) Electrochemical pseudotitration of water-soluble antioxidants. Electroanalysis 25:922–930

Ünüvar S, Aslanhan H (2019) Clinical significance of increased serum neopterin in chronic kidney failure as a biomarker of cell-mediated immunity. J Med Biochem 38:1–5

Vij AG, Dutta R, Satija NK (2005) Acclimatization to oxidative stress at high altitude. High Alt Med Biol 6:301–310. https://doi.org/10.1089/ham.2005.6.301

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