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Oxidative stress and nitric oxide metabolism responses during prolonged high-altitude exposure in preterm born adults

Marie Chambion-Diaz [a,b,†], Giorgio Manferdelli [c,†], Benjamin J. Narang [d,e,†], Guido Giardini [f], Tadej Debevec [e,f], Vincent Pialoux [a,g,*,#], Grégoire P. Millet [c,#]

[a] *Laboratoire Interuniversitaire de biologie de la motricité (LIBM), Université Lyon 1 Claude Bernard, Villeurbanne 69100, France*
[b] *Laboratoire de kinésiologie du PEPS, Département de kinésiologie, Faculté de médecine, Université Laval, Québec G1V 0A6, Canada*
[c] *Institute of Sport Sciences, University of Lausanne, Lausanne 1015, Switzerland*
[d] *Department of Automatics, Biocybernetics and Robotics, Jožef Stefan Institute, Ljubljana 1000, Slovenia*
[e] *Faculty for Sport, University of Ljubljana, Ljubljana 1600, Slovenia*
[f] *Mountain Medicine and Neurology Centre, Valle D'Aosta Regional Hospital, Aosta 11100, Italy*
[g] *Institut Universitaire de France, Paris 75231, France*

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Abstract

Background: Prematurely-born individuals tend to exhibit higher resting oxidative stress, although evidence suggests they may be more resistant to acute hypoxia-induced redox balance alterations. We aimed to investigate the redox balance changes across a 3-day hypobaric hypoxic exposure at 3375 m in healthy adults born preterm (gestational age ≤ 32 weeks) and their term-born (gestational age ≥ 38 weeks) counterparts.

Methods: Resting venous blood was obtained in normoxia (*prior to* altitude exposure), immediately upon arrival to altitude, and the following 3 mornings. Antioxidant (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and ferric reducing antioxidant power (FRAP)), pro-oxidant (xanthine oxidase (XO) and myeloperoxidase (MPO)) enzyme activity, oxidative stress markers (advanced oxidation protein product (AOPP) and malondialdehyde (MDA)), nitric oxide (NO) metabolites (nitrites, nitrates, and total nitrite and nitrate (NOx)), and nitrotyrosine were measured in plasma.

Results: SOD increased only in the preterm group ($p < 0.05$). Catalase increased at arrival in preterm group ($p < 0.05$). XO activity increased at Day 3 for the preterm group, while it increased acutely (arrival and Day 1) in control group. MPO increased in both groups throughout the 3 days ($p < 0.05$). AOPP only increased at arrival in the preterm ($p < 0.05$) whereas it decreased at arrival up to Day 3 ($p < 0.05$) for control. MDA decreased in control group from arrival onward. Nitrotyrosine decreased in both groups ($p < 0.05$). Nitrites increased on Day 3 ($p < 0.05$) in control group and decreased on Day 1 ($p < 0.05$) in preterm group.

Conclusion: These data indicate that antioxidant enzymes seem to increase immediately upon hypoxic exposure in preterm adults. Conversely, the blunted pro-oxidant enzyme response to prolonged hypoxia exposure suggests that these enzymes may be less sensitive in preterm individuals. These findings lend further support to the potential hypoxic preconditioning effect of preterm birth.

Keywords: Hypoxia; Premature birth; Antioxidant; Oxidative stress; Altitude

1. Introduction

According to the World Health Organization (WHO),[1] 15 million babies are born preterm each year (before 37 completed weeks of gestation). Very premature babies (<32 weeks of gestation) often present with cardiovascular and respiratory diseases or limitations.[2] These babies are also born with immature antioxidant defense systems, which may lead to further damage when they undergo oxygen therapy.[3]

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* Corresponding author.
E-mail address: vincent.pialoux@univ-lyon1.fr (V. Pialoux).
† These authors equally contributed to this work as first author.
# These authors share the last (senior) position.

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Oxidative stress is characterized by an imbalance between pro-oxidant and antioxidant enzyme activity, in favor of the former.[4] Oxidative stress can increase in response to several stimuli, including acute exercise and/or hypoxia. It is also known to be elevated in several acute and chronic diseases, as well as following premature birth.[5]

During premature birth, the newborn abruptly leaves the hypoxic intrauterine environment with an immature pulmonary system and a lack of antioxidant defenses.[6] Often, the baby then undergoes pure oxygen therapy, which increases oxidative stress via the hypoxia reoxygenation redox pathway.[5,6] However, it remains unclear whether this prematurity-related increase in oxidative stress observed at birth persists into adulthood. Recent findings indicate that adolescents born preterm exhibit higher oxidative stress, as measured by 8-isoprostane in exhaled breath, compared to age-matched adolescents born at term.[6] Consequently, the authors recommended limiting postnatal hyperoxic exposure as much as possible. On the contrary, Flahault et al.[7] observed no difference in oxidative stress biomarkers between young adults born preterm and their peers born at term. However, a lower birth weight for gestational age was associated with increased oxidative stress as measured by urinary 8-isoprostane, suggesting that low birth weight may affect oxidative stress status in adults born preterm.

Acute hypoxic exposure is known to increase oxidative stress[8] and elicit rapid physiological responses, including the hypoxic ventilatory response.[9,10] Many studies have reported an increase in oxidative stress markers after an acute hypoxic exposure in term-born individuals.[10,11] Usually, hypoxia-induced oxidative stress decreases nitric oxide (NO) bioavailability via NO inhibition by superoxide anion to form peroxynitrite ($ONOO^-$). However, prolonged hypoxia induces different physiological responses compared to acute hypoxia, in particular for oxidative stress and antioxidant capacity. At high altitude, hypoxia alters redox balance via overproduction of reactive oxygen species (ROS),[12] also evidenced by increased oxidative stress markers in blood during a high-altitude sojourn.[13] Prolonged hypoxia also increases NO production and NO metabolites, eliciting beneficial effects on blood flow in healthy adults,[14,15] which are not observed in acute hypoxia. Martin et al.[16] showed that adults born preterm present a greater resistance to oxidative stress during hypoxic exercise, suggesting specific adaptations to hypoxia in this population.

Since high altitude sojourns are becoming increasingly popular and accessible, improving our understanding of altitude-related oxidative stress modulation seems warranted. For full-term individuals, the oxidative stress responses to acute and prolonged hypoxic exposure are relatively well described. Conversely, for people born preterm, the acute oxidative stress response is beginning to be well characterized,[17,18] whereas the effects of prolonged or chronic exposure have not been investigated yet. Accordingly, we aimed to investigate the effects of prolonged exposure to hypobaric hypoxia on oxidative stress, antioxidant, and NO metabolite kinetics in prematurely born adults compared to a control group consisting of healthy, age- and aerobic capacity-matched full-term-born individuals.

2. Materials and methods

2.1. Participants

The experimental protocol was preregistered at Clinical Trials.gov (NCT04739904), and ethical approvals were obtained from both the University of Ljubljana Faculty of Sport Ethics Committee (8/2020-316) and the Aosta Hospital Ethical Committee (06/05/2021.0038781.I). The study protocol conformed to the Declaration of Helsinki. The preterm adult participants were recruited from the National Preterm Birth Register, managed by the University Clinical Centre in Ljubljana, Slovenia, using medical record screening and individual interviews. All the participants volunteered and gave written informed consent to participate in this study.

Preterm participants were required to fulfill the following inclusion criteria: gestational age $\leq$ 32 weeks, gestational mass $\leq$ 1500 g, hyperoxic treatment at birth, lack of pulmonary diseases (i.e., bronchopulmonary dysplasia). Full-term adults had a gestational age of at least 38 weeks and birth weight $\geq$ 2500 g. All participants were male and aged between 18 years and 30 years. Participants were free from medical risk factors related to exercise and/or altitude exposure and were disease free, non-smokers. Exclusion criteria for all participants included permanent residence at high altitude ($\geq$1000 m); cardiopulmonary, hematological, and/or kidney disorders; chronic medication use; smoking; and exposure to altitude or hypoxia ($\geq$2000 m) within the month *prior to* the study. The details of baseline characteristics of the participants given in Table 1 have been described elsewhere.[19,20]

2.2. Experimental procedure

All participants were exposed to 3 days of hypobaric hypoxia (Torino Hut, 3375 m, Mont Blanc massif, Italy).

Table 1
Characteristics of the participants (mean ± SD).

	Full-term born (control)	Preterm born	*p*
n	17	17	
Gestational age (week)	40 ± 0	29 ± 1	<0.001
Birth weight (g)	3621 ± 101	1132 ± 64	<0.001
Age at test (year)	21 ± 1	21 ± 1	0.066
Height (m)	1.82 ± 2.00	1.78 ± 2.00	0.210
Body mass (kg)	75.6 ± 1.7	72.4 ± 3.5	0.415
BMI (kg/m^2)	22.8 ± 0.4	22.5 ± 0.7	0.713
VO_{2peak} (mL/kg/min)	51.9 ± 1.9	48.5 ± 2.6	0.290
FVC (L)	5.67 ± 0.17	5.37 ± 0.31	0.285
FVC (%predicted)	98 ± 2	98 ± 3	0.809
FEV_1 (L)	4.63 ± 0.18	4.23 ± 0.13	0.082
FEV_1 (%predicted)	93 ± 2	92 ± 2	0.412

Abbreviations: BMI = body mass index; FEV_1 = forced expired volume in 1 s; FVC = forced vital capacity; MVV = maximal voluntary ventilation; PEF = peak expiratory flow; VO_{2peak} = peak oxygen uptake.

Blood samples were obtained from the antecubital vein at each of the following time points:

- Baseline (Normoxia): before traveling to altitude (resting values in normoxia);
- Arrival (A) : immediately upon arrival at altitude (2:00 p.m.−3:00 p.m.);
- Day 1 (D1): morning (fasting rested condition) sample following the first night at altitude;
- Day 2 (D2): morning (fasting rested condition) sample following the second night at altitude;
- Day 3 (D3): morning (fasting rested condition) sample following the third night at altitude.

Participants underwent various but standardized exercise sessions during the 3 days at altitude. Before each experimental session, participants were instructed to refrain from intense physical activity and avoid alcohol and caffeine consumption for 24 h. Additionally, they were required to avoid eating large meals within the 4 h preceding the tests. Each morning, oxygen saturation was recorded with a pulse oximeter (Xpod 3012LP; Nonin Medical, Plymouth, MN, USA) placed on the ear lobe (protocol detailed elsewhere).[20,21]

2.3. Blood processing and biochemical analyses

At sea-level and upon arrival at altitude, 6 mL of venous blood were obtained from the antecubital vein with participants in a seated position. Blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) blood collection tubes. Blood samples were immediately centrifuged (10 min at 3500 rotation per minute, 4°C) to separate the plasma, which was aliquoted into Eppendorf tubes (1.5 mL) and stored at −20°C and −80°C until subsequent analysis. The following oxidative stress markers, pro-/anti-oxidants enzymes, and NO metabolites were assessed: superoxide dismutase (SOD) and catalase activities, glutathione peroxidase (GPx) activity, the ferric reducing antioxidant power (FRAP), the xanthine oxidase (XO) activity, myeloperoxidase (MPO), the advanced oxidation protein product (AOPP), malondialdehyde (MDA), total nitrites and nitrates (NOx), and nitrotyrosine. All spectrophotometry measurements were performed with TECAN Infinite 2000 plate reader (Männedorf, Switzerland).

2.3.1. SOD

SOD is an antioxidant enzyme catalyzing the transformation of superoxide anion into hydrogen peroxide (H_2O_2). The activity of this enzyme is measured by the degree to which it inhibits the reaction between superoxides produced by the hypoxanthine-xanthine oxidase system and the nitrotetrazolium blue (NTB), forming the formazan blue at 450 nm.[21]

2.3.2. Catalase

Catalase allows the transformation of H_2O_2 into H_2O and O_2. Catalase activity was determined by measuring the kinetics (across 20 min) of formaldehyde apparition formed by the reaction between methanol and H_2O_2—a reaction also catalyzed by catalase. Absorbance is measured by a spectrophotometer at 540 nm and compared to the formaldehyde range.[22]

2.3.3. GPx

GPx activity was assessed by measuring nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) consumption, which is proportional to GPx activity to reduce H_2O_2 in the presence of glutathione reductase and reduced glutathione.[23] H_2O_2 is used as the initiator of the reaction. The kinetic was measured over 5 min by spectrophotometry at 340 nm.

2.3.4. FRAP

This antioxidant non-enzymatic marker reflects the FRAP. FRAP values were obtained by comparing the change in absorbance at 593 nm in samples after addition of ferric ion with ranges containing ferrous ions in known concentration.[24] An inverse correlation between FRAP and MDA has also been shown after hypoxic exposure,[25] highlighting the potential sensitivity of FRAP to oxidative stress.

2.3.5. XO

XO catalyzes the oxidation of hypoxanthine into xanthine and xanthine into uric acid (UA). XO uses oxygen to create UA but, above all, superoxide anion. The XO activity was calculated by measuring the kinetic of appearance of the complex superoxide anion and NTB by spectrophotometer at 560 nm.[26]

2.3.6. MPO

MPO transforms H_2O_2 into hypochlorous acid (HOCl). As it crosses cell membranes, it can cause protein fragmentation and aggregation. The assay of MPO activity is described by Suzuki et al.,[27] using tetramethylbenzidine (TMB) and H_2O_2 to initiate the reaction. The kinetics of appearance of peroxidized TMB was measured by absorbance at 653 nm.

2.3.7. Plasmatic AOPP

AOPP is determined by the Methods in Witko-Sarsat et al.,[28] using a spectrophotometer. The result is expressed in chloramine-T equivalent, which, in the presence of potassium iodide (KI), absorbs at 340 nm.

2.3.8. Plasmatic MDA

As the end-product of polyunsaturated fatty acid (PUFA) oxidation, MDA is often used as an indicator of lipid peroxidation. The assay determines the MDA concentration in a sample from its reaction with thiobarbituric acid (TBA).[29,30] A pink chromogen, formed by the MDA + 2TBA complex in an acidic and hot environment, is extracted using butanol. Its absorbance was measured at 532 nm with a spectrophotometer.

2.3.9. NOx/nitrites

NO metabolites, nitrite and nitrate, were measured using Griess's reagent—a mixture of sulfanilamide, naphthalene-ethylene-diamine-dihydrochloride (NED), and phosphoric acid—according to the modified method developed by Bratton and Marshall.[31] This reagent binds with the nitrite to form a dye, which absorbs at 550 nm. In a second measure, nitrate

reductase added to a plasma sample was used to convert nitrate into nitrite, and thereby measure the total amount of NOx.

2.3.10. Nitrotyrosine

The plasmatic concentration of nitrotyrosine was measured by enzyme-linked immunosorbent assay (ELISA) as previously described.[32] Nitrotyrosine is an end product of protein nitration by the peroxynitrite, which is itself formed by the bond of superoxide anion and NO.

2.4. Statistical analysis

Data are reported as mean ± SD. Statistical analyses were performed using GraphPad Prism Software (GraphPad Software, San Diego, CA, USA). Normality was assessed using the Shapiro-Wilk test. When normality was verified, a two-way mixed effects analysis of variance was performed to compare the means of each assayed marker across time (normoxia (Norm), A, D1, D2, and D3) and between groups (preterm and control (full term)). In the event of a statistically significant main effect of time or time × group interaction effect, a Fisher-LSD *post hoc* test was used to locate statistically significant differences. The significance level was set at $p < 0.05$.

3. Results

Data from A have been already published elsewhere.[19,20] For a full comprehension of the results, and to establish the complete time-course of the response to prolonged hypoxic exposure in our specific population, the data from A have also been included in the present study.

3.1. Antioxidant markers

SOD did not change in the control group, whereas an increase was observed at D1 (+24%, $p = 0.0028$) and D3 (+12%, $p = 0.0248$) compared to normoxia in the preterm group (Fig. 1A). Catalase did not change across the exposure in the control group (Fig. 1B). A transient increase was, however, observed at A, relative to normoxia, in the preterm group (+58%, $p < 0.0001$). GPx increased from A in both groups compared to normoxia (+6% for control group $p = 0.0011$; +12% for preterm group, $p = 0.0002$). Compared to normoxia, FRAP decreased in both the control group (−15%, $p = 0.0004$) and the preterm group (−8%, $p = 0.0082$) at D1.

3.2. Pro-oxidant markers

XO increased in A compared to Norm in the control group (+124% *vs.* normoxia, $p = 0.0028$) and remained elevated relative to normoxia until D3 (Fig. 2A). In the preterm group, XO increased only at D3 relative to normoxia (+65%, $p = 0.0235$). MPO was higher at D3 compared to normoxia in the control group (+31%, $p = 0.0051$) and was also higher in the preterm group through the 3 days compared to Norm (+27% at D3 *vs.* normoxia, $p = 0.0041$) (Fig. 2B). AOPP decreased at A in the control group (−25%, $p = 0.0218$) and remained lower until D2 (−11%, $p = 0.0277$). In preterm, AOPP was elevated only

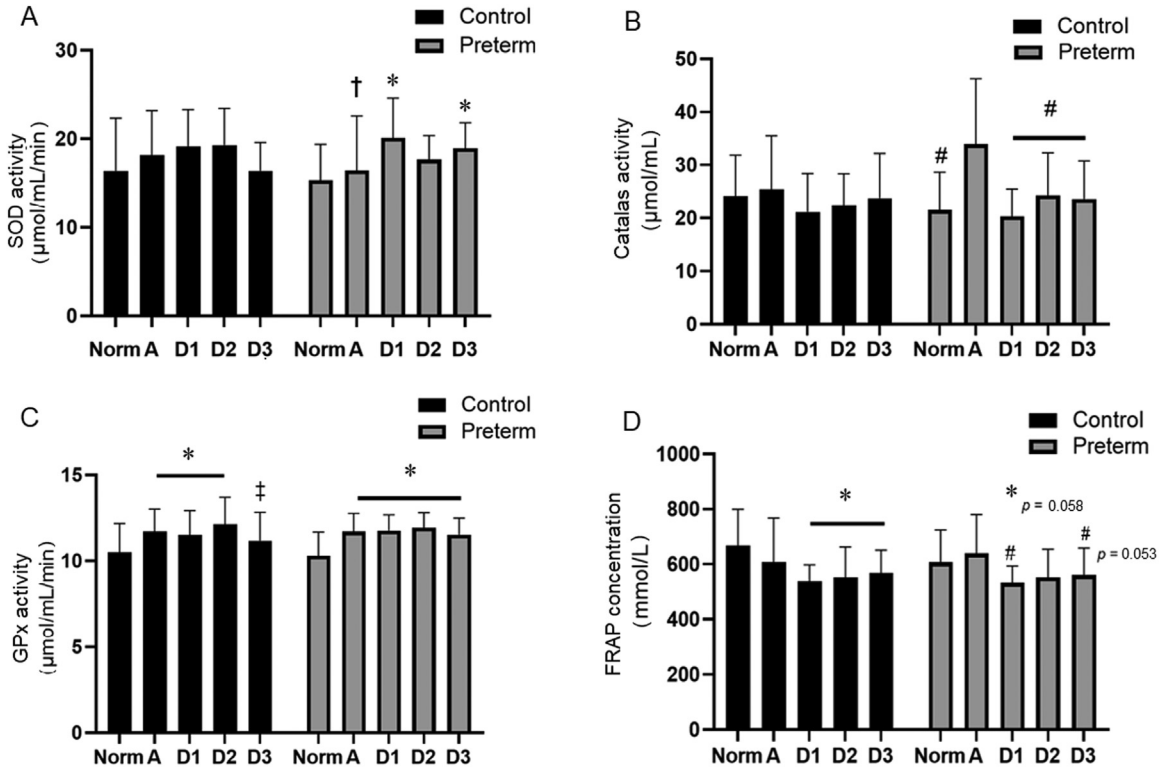

Fig. 1. Plasmatic activity of (A) SOD; (B) catalase; (C) GPx; (D) plasmatic concentration of FRAP in the preterm group (light gray) and the control group (dark) at the different time points. * $p < 0.05$ *vs.* Norm; # $p < 0.05$ *vs.* A; † $p < 0.05$ *vs.* D1; ‡ $p < 0.05$ *vs.* D2. A = arrival; D1 = Day 1; D2 = Day 2; D3 = Day 3; FRAP = ferric reducing antioxidant power; GPx = glutathione peroxidase; Norm = normoxia; SOD = superoxide dismutase.

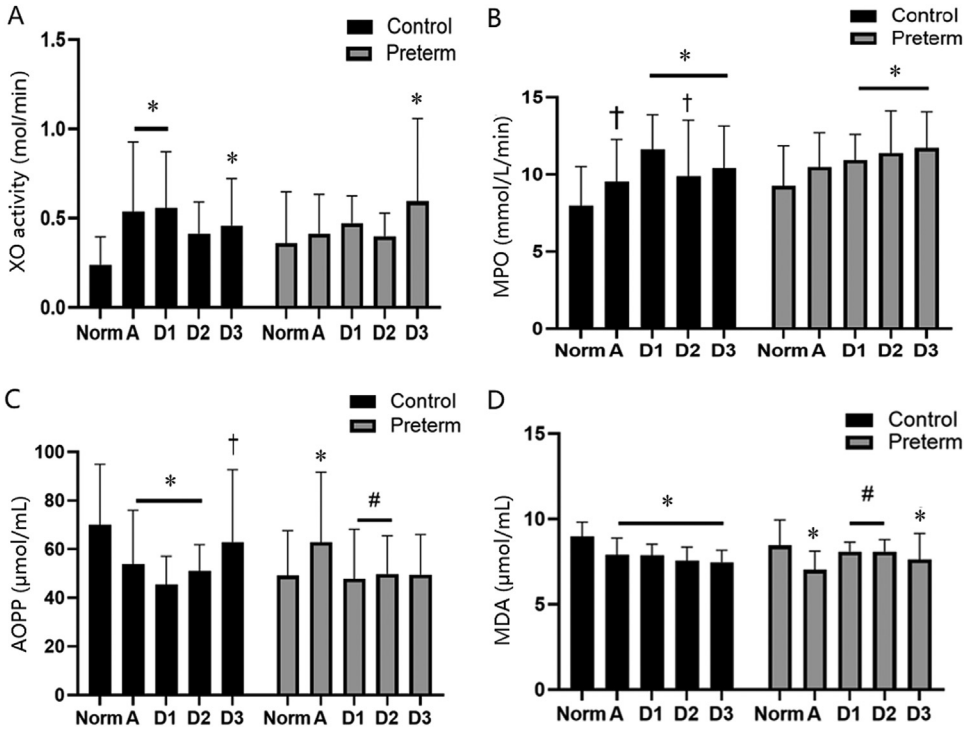

Fig. 2. Plasmatic activity of (A) XO; (B) MPO; (C) AOPP; (D) plasmatic concentration of MDA in the preterm group (light gray) and in the control group (dark) at the different time points. * $p < 0.05$ *vs.* Norm; $^{\#}$ $p < 0.05$ *vs.* A; † $P < 0.05$ *vs.* D1. A, arrival; AOPP = advanced oxidation protein product; D1 = Day 1; D2 = Day 2; D3 = Day 3; MDA = malondialdehyde; MPO = myeloperoxidase; Norm = normoxia; XO = xanthine oxidase.

at A (+28%, $p = 0.0444$) (Fig. 2C). MDA decreased in the control group compared to normoxia from A to D3 (−17%, $p = 0.0005$). In contrast, MDA decreased at A and D3 in the preterm group compared to normoxia.

3.3. NO metabolites

Nitrites increased at D3 (+42%, $p = 0.0166$) compared to D1 in the control group, and there is also a decrease at D1 (−41%, $p = 0.0091$) in the preterm group (Fig. 3). Nitrotyrosine decreased only at D3 (−25%, $p = 0.0247$) in the control group, as compared to A and D1, whereas a decrease at D2 (−21%, $p = 0.0013$) was observed in the preterm group when compared to Norm and D1.

The morning pulse saturation at sea level (normoxia) and at high altitude at D1, D2, and D3 are presented in Table 2. Compared to the control group, the saturation of the preterm group was lower at D1 ($p < 0.05$).

Table 2
Morning pulse oxygen saturation (%) data at sea level (normoxia) and at high altitude at Day 1, Day 2, and Day 3 (mean ± SD).

Group	Morning pulse oxygen saturation (%)			
	Sea level Normoxia	High altitude		
		Day 1	Day 2	Day 3
Control	97.07 ± 1.10	86.90 ± 1.73	87.71 ± 2.43	88.56 ± 2.19
Preterm	97.00 ± 1.03	84.70 ± 2.75*	85.89 ± 1.76	86.78 ± 1.86

* $p < 0.05$ indicates significant difference between Preterm Day 1 and Control Day 1.

4. Discussion

The aim of the present study was to investigate the effects of prolonged high-altitude exposure on oxidative stress markers, pro-/anti-oxidant enzyme activity, and NO metabolite kinetics in healthy adults born preterm and age- and exercise capacity-matched controls. This is the first study to investigate these responses throughout several days of hypobaric hypoxic exposure in these populations.

Adults born preterm expressed unique oxidative stress dynamics compared to the control participants. Indeed, acute increases in antioxidant enzyme activity (SOD and catalase) and the protein oxidation marker AOPP were observed in the preterm group, although these returned to baseline levels at D1 or D2. There was no significant change in the control group for these markers. Conversely, the pro-oxidant enzyme XO activity increased only at the end of the prolonged hypoxia exposure (D3) for the preterm, while it only increased acutely (i.e., arrival and D1) in the control group. These differences in oxidative stress kinetics in response to prolonged hypoxia suggest specific redox balance modulation in prematurely-born adults.

In the preterm group, plasmatic SOD total activity (including SOD1 + SOD2) and catalase increased immediately upon arrival at high-altitude, whereas there was no change in the control group. This suggests that preterm individuals likely have a higher oxidative stress response to hypoxia than the control group, possibly due to increased radical production (superoxide and H_2O_2). Regarding SOD, we can note that at high altitude, SOD2 is generally more stimulated than

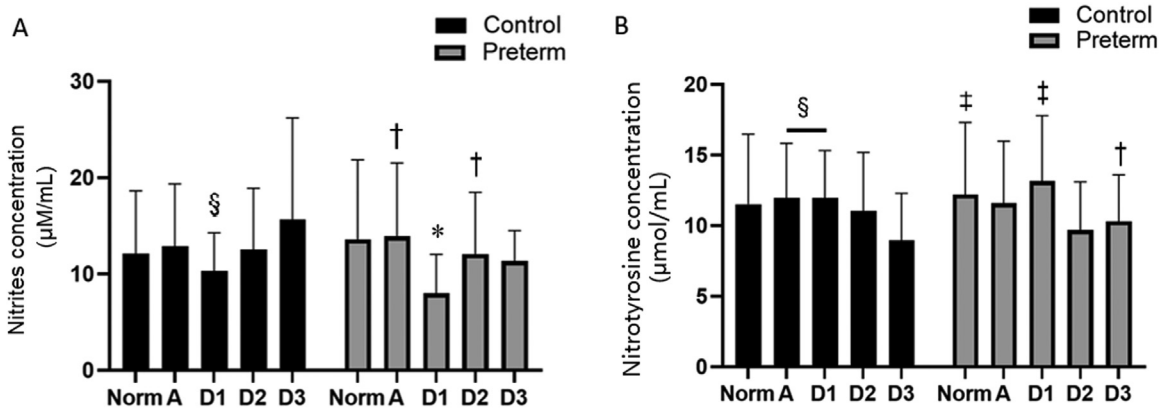

Fig. 3. Plasmatic concentration of (A) nitrites; (B) nitrotyrosine in the preterm group (light gray) and in the control group (dark) at the different time points. * $p < 0.05$ *vs.* Norm; $^{\#}$ $p < 0.05$ *vs.* A; † $p < 0.05$ *vs.* D1; ‡ $p < 0.05$ *vs.* D2; § $p < 0.05$ *vs.* D3, tendency $p = 0.0543$. A = arrival; D1 = Day 1; D2 = Day 2; D3 = Day 3; Norm = normoxia.

SOD1.[33] Indeed, SOD2, located directly in the mitochondria, plays a critical role in scavenging ROS generated during the increased metabolic activity that accompanies hypoxic conditions at high altitudes. The transient increase observed here could therefore be mainly due to an increase in SOD2 activity rather than SOD1, which is contained in the cytosol.[33] Over the following days, the AOPP levels decreased likely due to an augmentation of antioxidant enzyme activity. Interestingly, these transient increases in antioxidant enzyme activity, SOD, and catalase are concomitant to a higher oxygen desaturation, which is only significant the first day of hypoxic exposure (Table 2). These results confirm previous studies showing a relationship between oxygen desaturation and the magnitude of oxidative stress,[34] and they suggest that the highest oxygen desaturation of preterm participants during the first hour of hypoxic exposure may generate more ROS and thus better stimulate antioxidant enzyme activity. On the contrary to SOD and catalase, our results show that the increase of the pro-oxidant enzyme XO's activity is delayed in the preterm compared to controls. It appears, therefore, that the hypoxic stimulus duration and/or intensity was insufficient for prematurely-born participants to stimulate XO. On the other hand, this could also suggest that the XO pathway may be less sensitive to hypoxic stress in the preterm participants. This AOPP increase only at arrival in preterm subjects was, however, not explained by XO activity (which increased only on D3) or MPO activity (which was similar in both groups).

Other redox mechanisms generating superoxide, such as NADPH oxidase activation or nitric oxide synthase (NOS) uncoupling, could be involved in this specific response to hypoxic exposure.[35] The higher radical production could also originate in the mitochondria (i.e., Kumari et al.[36] reported that mitochondrial respiration in the peripheral blood mononuclear cells of prematurely-born individuals was greater than in a full-term group). This should consequently reduce ROS production in full-term individuals.[37,38] Further experiments seem warranted to confirm these hypotheses. An increase in GPx activity from A to D3 was seen regardless of birth status in this study, contradicting the results of Martin et al.[16] The discrepancy between the GPx and catalase kinetics, given that both reduce H_2O_2, may be due to a higher affinity and lower scavenging efficiency of GPx to H_2O_2. Under conditions of high H_2O_2 production, as observed in hypoxia in preterm individuals, the hypoxia-induced pro-oxidant stimulus could be sufficient to overwhelm GPx and, thus, stimulate catalase.[39,40]

In hypoxia, MPO catalyzes the formation of hypochlorous acid. This acid can cross cell membranes and cause protein fragmentation and subsequent oxidative stress.[41] The increase from D1 to D3 in MPO activity in both groups suggests that premature birth might not influence mechanisms in hypoxia with no specific adaptation, as conversely seen in SOD, catalase, and XO. For the lipid oxidation marker MDA, the same kinetics were also observed in both groups. The decrease in MDA in preterm and full-term individuals may be the result of increased GPx activity. However, these results contrast with those of AOPP, although this could be explained by their different production pathways. MDA is the end-product of PUFA peroxidation. Plasma MDA is also a reflection of whole-body rich-PUFA oxidation, and it has low sensitivity to pro-oxidant stimuli.[42] Plasma AOPP is primarily the result of advanced protein oxidation by ROS formed via myeloperoxidase reaction during oxidative/chlorine stress.[28] Regarding NO metabolites, no specific differences were observed during prolonged altitude exposure between the groups for nitrites. This is in contrast to previous studies, which have reported that people born preterm could present attenuated NO metabolism in acute hypoxia.[16] The nitrites decrease on D1 in both groups is certainly due to radical overproduction limiting NO bioavailability.[43,44]

The pro-oxidant activity of XO appears to be less sensitive to a hypoxia stimulus, whereas the antioxidant activity of SOD and catalase seems transiently upregulated in preterm individuals as an acute burst of ROS production upon initial exposure to hypoxia. We speculate that this pro-oxidant/anti-oxidant-specific response among preterm-born adults may be a hormetic adaptation.[35] Specifically, while neonatal oxidative stress may lead to initial detrimental effects, the chronic oxidative stress seen in

preterm adults[6] may promote adaptation across the lifespan that may even exceed normal function.[45] The understanding of these underlying specific modulations of oxidative stress require further investigations. In this context, the oxidative stress experienced by preterm individuals during pregnancy and neonatal development has been shown to cause DNA methylation.[46] Indeed, we recently reported that antioxidant and gene polymorphisms in prematurely born individuals was associated with hypoxia-related oxidative stress.[47] This suggests that DNA methylation polymorphism could be one of the potential mechanisms explaining the difference in oxidative stress responses to prolonged high-altitude that we observed in this study between term and preterm individuals.

5. Limitations

Despite its novelty, the present study has some limitations that we would like to acknowledge. First, MDA is criticized as a marker of lipid peroxidation as it is rapidly metabolized by the liver.[48] Markers more sensitive and more specific to lipid peroxidation exist, such as isoprostanes, whose assays are based on chromatography techniques associated with mass spectroscopy.[49] However, although less sensitive, the hypoxic stimulus is considered strong enough to observe changes in MDA concentrations.

There are also limitations related to the field testing at high altitude. While the exercise testing performed during the sojourns might have modulated the observed oxidative stress differences,[20] it is important to emphasize that these tests were standardized and conducted at the same time points for all participants. Additionally, this study only focused on male participants, so we cannot directly extend the results to female participants. In particular, hormonal changes throughout the menstrual cycle are known to influence oxidative stress,[50] exercise,[51] and hypoxic responses.[52]

6. Conclusion

This is the first study to examine the effects of prolonged hypoxic exposure on the kinetics of oxidative stress, antioxidant markers, and NO metabolism in a sample of prematurely born but otherwise healthy adults. It appears that preterm individuals exhibit different oxidative stress and antioxidant responses to prolonged hypoxia compared to their term-born counterparts. Further research seems warranted to establish the underlying mechanisms through which preterm birth (and associated treatments) may induce the results observed in this study.

Authors' contributions

GPM conceived the research, obtained the financial support, contributed to the experimental design, and analyzed and interpreted the data; TD conceived the research and obtained the financial support, and contributed to the experimental design; GM contributed to the experimental design, collected the data and analyzed and interpreted the data; BJN contributed to the experimental design and collected the data; GG contributed to the experimental design; MCD and VP analyzed the plasma samples for oxidative stress markers, antioxidant enzymes, and nitric oxide metabolites; analyzed and interpreted the data; and drafted the manuscript. All authors critically revised the draft. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and stating the results of the present study does not constitute an endorsement by American College of Sports Medicine. The authors thank all the volunteers who enthusiastically participated in this research project. Also, the authors would like to acknowledge Armando Chanoine and his team at the Torino Hut, where the high-altitude data collection was carried out, and Skyway Mont Blanc, who supported the transport of the equipment and participants to the Torino Hut. This work was funded by the Swiss National Science Foundation (SNSF Grant No. 320030L_192073 to GM) and the Slovenian Research Agency (ARRS Grant No. N5-0152 to TD).

Supplementary materials

Supplementary materials associated with this article can be found in the online version at doi:10.1016/j.jshs.2025.101034.

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