



## Effect of pre-term birth on oxidative stress responses to normoxic and hypoxic exercise

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### ABSTRACT

Pre-term birth is a major health concern that occurs in approximately 10% of births worldwide. Despite high incidence rate, long-term consequences of pre-term birth remain unclear. Recent evidence suggests that elevated oxidative stress observed in pre-term born infants could persist into adulthood. Given that oxidative stress is known to play an important role in response to physical activity and hypoxia, we investigated whether oxidative stress responses to acute exercise in normoxia and hypoxia may be differently modulated in pre-term vs. full-term born adults. Twenty-two pre-term born and fifteen age-matched full-term born controls performed maximal incremental cycling tests in both normoxia (FiO<sub>2</sub>: 0.21) and normobaric hypoxia (FiO<sub>2</sub>: 0.13; simulated altitude of 3800 m) in blinded and randomized manner. Plasma levels of oxidative stress (advanced oxidation protein products [AOPP] and malondialdehyde), antioxidant (ferric reducing antioxidant power, glutathione peroxidase, catalase [CAT] and superoxide dismutase [SOD]) and nitrosative stress markers (nitrotyrosine, nitrite and total nitrite and nitrate [NO<sub>x</sub>]) were measured before and immediately after each test. AOPP (+24%,  $P < 0.001$ ), CAT (+38%,  $P < 0.001$ ) and SOD (+12%,  $P = 0.018$ ) and NO<sub>x</sub> (+17%,  $P = 0.024$ ) significantly increased in response to exercise independently of condition and birth status. No difference in response to acute exercise in normoxia was noted between pre-term and full-term born adults in any of measured markers. Hypoxic exposure during exercise resulted in significant increase in AOPP (+45%,  $P = 0.008$ ), CAT (+55%,  $P = 0.019$ ) and a trend for an increase in nitrite/nitrate content (+35%,  $P = 0.107$ ) only in full-term and not pre-term born individuals. These results suggest that prematurely born adult individuals exhibit higher resistance to oxidative stress response to exercise in hypoxia.

### 1. Introduction

Pre-term birth (PTB), defined as birth occurring prior to the 37th

week of gestation, represents approximately 10% of the births worldwide [1] and is among the leading causes of death for the under-5 year olds [2]. However, continuous progress in neonatology during the last

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**Abbreviations list**

<b>AMS</b>	acute mountain sickness
<b>AOPP</b>	advanced oxidation protein products
<b>CAT</b>	catalase
<b>DAN</b>	2,3-diaminonaphthalene
<b>dSpO<sub>2</sub></b>	arterial blood desaturation
<b>dVE</b>	ventilation variation
<b>FRAP</b>	ferric reducing antioxidant power
<b>GPx</b>	glutathione peroxidase
<b>GSH</b>	reduced glutathione
<b>HVR</b>	hypoxic ventilatory response
<b>H<sub>2</sub>O<sub>2</sub></b>	hydrogen peroxide

<b>MDA</b>	malondialdehyde
<b>NADPH</b>	nicotinamide adenine dinucleotide phosphate hydrogen
<b>NOx</b>	total nitrite and nitrate
<b>NTB</b>	nitrobluetetraazolium
<b>PTB</b>	pre-term birth
<b>O<sub>2</sub><sup>·-</sup></b>	superoxide anion
<b>ROS</b>	reactive oxygen species
<b>SOD</b>	superoxide dismutase
<b>SpO<sub>2</sub></b>	capillary oxygen saturation
<b>TBA</b>	2-thiobarbituric
<b>VE</b>	ventilation
<b>VO<sub>2</sub></b>	oxygen uptake

50 years resulted in significantly greater survival rates [3], consequently leading to increased pre-term born population. A growing body of literature describes the long-term consequences of PTBs [4,5]. In particular, many of the physiological mechanisms linked to the detrimental consequences of PTB could be associated with elevated oxidative stress levels [6]. However, surprisingly only one study to date investigated the oxidative stress levels in adolescents (13–15 years) born very pre-term [7] and no data exists on the oxidative stress modulation in adults pre-term born [8].

Oxidative stress is defined as an imbalance between the generation of oxidant species, also termed reactive oxygen species (ROS), and antioxidants in favor of the former [9]. It is well established that many external factors can modulate oxidative stress level including physical exercise [10,11] and hypoxia [11,12]. In addition, the combination of both hypoxia and acute exercise is also known to have cumulative augmentative effect on oxidative stress [11,13–16]. However, the deleterious outcomes related to hypoxia-induced increased oxidative stress remain controversial and might be limited to extreme altitude [16] or to pathological conditions [17]. Nevertheless, the oxidative stress responses to both, exercise [10] and hypoxia [18] seem to importantly modulate the physiological adaptation of humans to these two stimuli.

Several studies reported reduced physical capacity in normoxia in adults born pre-term compared to those born full-term [19–23]. Additionally, it has been shown that at rest, hypoxic ventilatory response (HVR), defined as the ability to change ventilation in function of blood oxygen saturation, was lower in adults born pre-term than in adults born full-term [24]. However, to our knowledge, only one study investigated physical performance under hypoxia in pre-term and reported no difference with adults born full-term [23]. Moreover, because of the previously reported higher oxidative stress levels in adults born pre-term [7] and the above mentioned importance of oxidative stress adaptation to chronic exercise and hypoxia, the interaction between these two factors warrants scrutiny.

Accordingly, we aimed to investigate the oxidative stress responses to acute exercise in normoxia and normobaric hypoxia in individuals born pre-term as compared to age and aerobic capacity matched controls born at full-term.

## 2. Material and methods

### 2.1. Participants

Collectively, thirty-seven healthy men volunteered and gave written informed consent to participate in this study. All participants were free of cardiorespiratory and haematological diseases and were not exposed to altitudes above 1500 m during the one-month period prior to the study. The baseline characteristics of the participants are outlined in Table 1. Twenty-two participants were born pre-term and 15 were born full-term. The experimental protocol was approved by the National

Medical Ethics Committee of Slovenia and performed in accordance with the principles of the Declaration of Helsinki. The study was also pre-registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/NCT02780908?term=NCT02780908&rank=1) (NCT02780908) (<https://clinicaltrials.gov/ct2/show/NCT02780908?term=NCT02780908&rank=1>).

### 2.2. Incremental exercise tests

The participants performed two graded exercise test on an electromagnetically braked cycle-ergometer (Ergo Bike Premium, Daum electronics, Fürth, Germany) under normoxic ( $F_{iO_2}=0.21$ ;  $P_{iO_2}=147\text{mmHg}$ ) and normobaric hypoxic ( $F_{iO_2}=0.13$ ;  $P_{iO_2}=91\text{mmHg}$ ) conditions in a randomized manner. They were blinded in regards to  $F_{iO_2}$  of the gas mixture they were inspiring on both occasions. Both tests were performed at the same time of the day for each individual. The test protocol started at 60W with an increase of 40W every 2 min until volitional exhaustion. The normoxic and hypoxic tests were separated by exactly 7 days.

Throughout the tests the participants breathed through a facemask (Vmask, 7500 series, Hans Rudolph Inc., Shawnee, USA) and oxygen uptake ( $VO_2$ ) and ventilation (VE) were measured using a metabolic cart (Quark CPET, Cosmed, Rome, Italy). Capillary oxygen saturation ( $SpO_2$ ) was measured using a transcutaneous finger pulse oximetry device (Nellcor, BCI 3301, Boulder, USA).

### 2.3. Physiological response measurement

The methodological details of the cardiorespiratory measurements during both, normoxic and hypoxic incremental exercise tests are detailed elsewhere [25]. Arterial blood desaturation ( $dSpO_2$ ) was calculated as the difference between  $SpO_2$  measured at volitional exhaustion during the incremental test and the  $SpO_2$  at rest before beginning the test. The ventilation variation (dVE) was defined as the difference between the VE measured at volitional exhaustion during the incremental test and the VE at rest before beginning the test. HVR was computed as  $\frac{dVEh}{dSpO_2h}$  where  $dVEh$  represents the difference between ventilation at

**Table 1**  
Baseline characteristics of the participants.

Variable	Full-term ( $n = 15$ )		Pre-term ( $n = 22$ )	
	Mean	SD	Mean	SD
Age (years)	22	2	21	2
Body mass (kg)	73	6	69	7
Height (cm)	180	5	175***	7
BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	23	2	23	3
Gestational age (weeks)	39	2	29***	3
$\dot{V}O_{2\text{max}}$ ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	52	5	48	6

SD, standard deviation; BMI, body mass index;  $\dot{V}O_{2\text{max}}$ , maximal oxygen consumption. \*\*\* $P < 0.0001$  vs term.

exhaustion in hypoxia and ventilation at rest in normoxia and  $dSpO_2h$  represents the difference between  $SpO_2$  at exhaustion in hypoxia and  $SpO_2$  at rest in normoxia [26,27].

#### 2.4. Blood sampling

Six mL of venous blood were obtained from the antecubital vein of the seated participants before and within 1 min following the incremental exercise. Blood samples were drawn into ethylenediaminetetraacetic acid blood collection tubes, centrifuged (10min at 3500rpm, 4 °C) and the plasma was aliquoted into three 1.5 mL cryotubes, which were immediately frozen to -20 °C and to -80 °C.

#### 2.5. Biochemical analysis

All spectrophotometry and fluorometry measurements were performed with TECAN Infinite 2000 plate reader (Männedorf, Switzerland) as previously reported [28].

#### 2.6. Oxidative stress markers

Advanced oxidation protein products (AOPP) levels were measured via spectrophotometry by reading at 340 nm 40  $\mu$ L of plasma diluted in 200  $\mu$ L of PBS 1X and with 20  $\mu$ L of acetic acid (99–100%) in 96 well microtest plates. AOPP level was computed using chloramine-T standard solution, which absorb at 340 nm in presence of potassium iodide.

Malondialdehyde (MDA) level was determined by adding NaOH, 2-thiobarbituric acid (TBA) and HCl solutions to 50  $\mu$ L of plasma. After 1 h at 100 °C, MDA form a complex with TBA that can be measured by spectrophotometry in 96 well microtest plates at 532 nm. MDA level was computed using 1,1,3,3-tetraethoxypropane as standard.

#### 2.7. Antioxidant enzymes

Catalase activity was determined by measuring the kinetics of formaldehyde apparition formed by the reaction between methanol and hydrogen peroxide ( $H_2O_2$ ), which is catalyzed by catalase. 30  $\mu$ L of methanol (100%) and 20  $\mu$ L of  $H_2O_2$  solution (0.14%) were added to 20  $\mu$ L of plasma diluted in 100  $\mu$ L of PBS 1X in 96 well microtest plates. Twenty minutes later the reaction was stopped by adding 30  $\mu$ L of potassium hydroxide solution ( $10.69\text{mol}\cdot\text{L}^{-1}$ ). Formaldehyde was revealed by adding 30  $\mu$ L of purpald solution ( $0.20\text{mol}\cdot\text{L}^{-1}$ ) and its concentration was measured 5 min later by spectrophotometry at 540 nm and computed using formaldehyde standards.

Glutathione peroxidase (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 presence of glutathione reductase and reduced glutathione. Glutathione reductase, NADPH ( $10\text{mmol}\cdot\text{L}^{-1}$ ) and reduced glutathione solutions were added to 20  $\mu$ L of plasma diluted in 200  $\mu$ L of PBS 1X in 96 well microtest plates. 30  $\mu$ L of  $H_2O_2$  solution was then added and NADPH oxidation into  $NAD^+$  was measured during 5 min by spectrophotometry

at 340 nm.

Superoxide dismutase (SOD) activity measurement was based on the higher affinity of SOD to react with superoxide anion ( $O_2^{\cdot-}$ ) than nitrobluetrazolium (NTB), which produce detectable blue formazan. 250  $\mu$ L of a solution containing NTB, trimahydrochloride, diethylenetriaminepentaacetic acid and hypoxanthine is added to 20  $\mu$ L of plasma in 96 well microtest plates. Then, 20  $\mu$ L of xanthine oxidase ( $1.02\text{U}\cdot\text{mL}^{-1}$ ) were added and react with hypoxanthine to produce  $O_2^{\cdot-}$ . Appearance of blue formazan is measured by spectrophotometry at 560 nm during 5 min. SOD activity is computed by subtracting the rate of blue formazan appearance with deproteinized plasma (blank) to those with plasma sample.

#### 2.8. Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was determined by measuring the ability of the plasma to reduce ferric into ferrous iron. 17  $\mu$ L of ferric chloride solution ( $20\text{mmol}\cdot\text{L}^{-1}$ ), 17  $\mu$ L of tripyridyl-triazine solution ( $8\text{mmol}\cdot\text{L}^{-1}$ ) and 167  $\mu$ L of acetate solution ( $300\text{mmol}\cdot\text{L}^{-1}$ ) were added to 20  $\mu$ L of plasma diluted in 40  $\mu$ L of  $H_2O$  in 96 well microtest plates. Ferrous iron forms a complex with tripyridyl-triazine at low pH that can be measured by spectrophotometry at 593 nm and computed with ferrous iron solution ( $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ ) standards.

#### 2.9. Metabolites of NO

Nitrite levels were detected by using 2,3-diaminonaphtalene (DAN) that fixes nitrite and emits at 450 nm after an excitation at 365nm. 18  $\mu$ L of DAN solution containing DAN and HCl was added to 10  $\mu$ L of plasma diluted in 90  $\mu$ L of  $H_2O$  in 96 well microtest plates. 10 min later, the reaction was stopped with 18  $\mu$ L of NaOH solution. Nitrite level was measured by fluorometry (excitation at 365 nm and emission at 450 nm) and computed with  $\text{NO}_2$  standards.

To measure total nitrite and nitrate ( $\text{NO}_x$ ) level, nitrate was reduced into nitrite and nitrite was then measured as described above. 40  $\mu$ L of nitrate reductase solution was added to 10  $\mu$ L of plasma in 96 well microplates and 15 min later 50  $\mu$ L of  $H_2O$  was added. Then nitrite level is then determined as described above.

#### 2.10. Statistics

R (version 3.3.2) with *nlme* [29] and *multcomp* [30] packages was used to perform linear mixed effects analyses with condition (normoxia vs. hypoxia), time (pre vs. post exercise) and birth (full-term vs. pre-term) and their interactions as fixed effects and a random effect for participants after checking for normality and homoscedasticity. Significance level was set *a priori* at  $P < 0.05$  and a tendency was set at  $P < 0.1$ .

**Table 2**

Physiological response to graded exercise in normoxia and hypoxia in adults born full-term or pre-term.

Birth	Full-term (n = 15)		Pre-term (n = 22)		P values		
	Normoxia	Hypoxia	Normoxia	Hypoxia	Hypoxia	Birth	Birth×hypoxia
$\dot{V}O_{2\text{peak}}$ ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	52 (5)	45 (4)	48 (6)	42 (7)	< 0.0001	0.08	NS
PPO (W)	322 (33)	273 (28)	272 (39)	235 (36)	< 0.0001	0.008	0.01
$dSpO_2$ (%)	5.64 (4.14)	23.71 (4.75)	2.29 (2.69)	21 (5.18)	< 0.0001	0.006	NS
dVE ( $\text{L}\cdot\text{min}^{-1}$ )	110.1 (25.1)	124.9 (26.2)	99.5 (28.4)	107.3 (31.2)	0.049	0.09	NS
HVR ( $\text{L}\cdot\text{min}^{-1}\cdot\%^{-1}$ )	5.44 (1.80)		5.55 (1.98)			NS	

Values are presented as means (SD). Abbreviations:  $\dot{V}O_{2\text{peak}}$ , peak oxygen consumption; PPO, peak power output  $dSpO_2$ , arterial blood oxygen desaturation; dVE, ventilation variation; HVR, hypoxic ventilatory response; NS, not significant. These results are detailed elsewhere [25].

### 3. Results

#### 3.1. Exercise cardio-respiratory responses

The general cardio-respiratory responses to normoxic and hypoxic exercise are detailed elsewhere [25] but are summarized in Table 2 for the convenience of the reader. Expectedly, dSpO<sub>2</sub> was significantly higher during acute exercise in hypoxia than in normoxia independently of birth (3.5% during normoxic exercise vs. 21.6% during hypoxic exercise; pooled data;  $P < 0.0001$ ) but dSpO<sub>2</sub> was lower in participants born pre-term than full-term (-22%,  $P < 0.01$ ) independently of the condition. Furthermore, dVE during an acute exercise was greater in hypoxia than in normoxia independently of birth (+11%,  $P < 0.05$ ) and tended to be lower in participants born pre-term than full-term in normoxia and hypoxia ( $P = 0.09$ ). However, there was no difference in exercise HVR between the full-term and pre-term born participants.

#### 3.2. Oxidative stress responses to exercise

The responses of the measured oxidative stress and antioxidant markers to exercise are detailed in Table 3 and Fig. 1. While plasma AOPP significantly increased in response to exercise independently of the condition and/or birth status (+24%,  $P < 0.001$ ), the levels of MDA remained constant ( $P = 0.182$ ). Similarly, the catalase and SOD activity significantly increased in response to exercise regardless of birth and condition (+38%,  $P < 0.001$  and +12%,  $P = 0.018$  respectively for catalase and SOD) while GPx activity significantly decreased following exercise (-22%,  $P < 0.001$ ). FRAP tended to decrease following exercise independently of the condition and birth status ( $P = 0.087$ ). Acute exercise also induced significantly increased NOx levels in both groups (+17%,  $P = 0.024$ ) but did not change the levels of nitrite.

#### 3.3. Oxidative stress responses to hypoxia

The responses of antioxidant and oxidative stress markers to hypoxic exercise are detailed in Table 3 and Fig. 1. Plasma AOPP levels increased significantly in response to hypoxia only in the full-term born individuals (+45% in full-term vs +5% in pre-term,  $P = 0.008$ ) independently to exercise condition (i.e. exercise or rest) (Fig. 1A). However, no significant differences between MDA and FRAP responses to normoxic and hypoxic exercise were noted between full-term and pre-term born individuals. While the activity of SOD increased significantly in hypoxia compared to normoxia independently of birth and exercise condition (+14%,  $P = 0.007$ ), the catalase activity was only increased in hypoxia in the full-term born participants (+55% in full-term vs +3% in pre-term,  $P = 0.019$ ) independently to exercise condition (i.e. exercise or rest) (Fig. 1C). The GPx levels tended to decrease in response to hypoxia in both groups ( $P = 0.091$ ). Plasma nitrite levels

were significantly lower in hypoxia than in normoxia (-16%,  $P = 0.015$ ) independently of birth and exercise condition (i.e. exercise or rest) whereas hypoxia-induced increase in NOx levels trend to be only noted in the full-term born individuals (+35% in full-term vs +5% in pre-term,  $P = 0.107$ ) (Fig. 1H).

### 4. Discussion

The present study aimed to investigate the potential differential oxidative and nitrosative stress responses to acute exercise in normoxia and hypoxia in individuals born pre-term as compared to matched controls born at full-term. Our data show a similar exercise-induced increase in oxidative stress and antioxidant activity in pre-term and full-term. In addition, and in contrast to full-term born adults, the plasma AOPP, catalase and NOx responses to hypoxia does not increase compared to normoxic conditions in adults born prematurely and suggest higher resistance to oxidative stress in this population.

#### 4.1. Oxidative stress responses to exercise

As already demonstrated in adults born full-term [10,11], acute exercise resulted in increased oxidative stress markers and antioxidant enzymes activity in the pre-term born individuals. This is most likely due to exercise-induced ROS overproduction [10]. Independently of birth, we found that acute exercise augmented the plasma levels of AOPP, while no significant change was noted in MDA. The changes in MDA levels in response to acute graded exercise remains controversial [31]. Indeed, some studies reported increased level of plasma MDA in similar condition [13,32–35] while others did not find any significant changes [14,36–38]. The observed non-significant increase in MDA (+9%,  $P = 0.182$ ) could be explained by the reported moderate sensitivity and specificity of the employed MDA assay [39]. The catalase and SOD activity increased in response to exercise independently of birth, confirming the previously documented exercise-related upregulation of antioxidant enzymes activity [40]. However, in contrast to some studies [40,41], we observed a decreased GPx activity in response to exercise regardless of the birth status. Nevertheless, this could be explained by the fact that reduced glutathione (GSH) is a limiting substrate for GPx activity and that lower GSH content in erythrocytes in response to acute exercise has been shown [42]. Since no effect of prematurity has been observed in response to acute exercise regarding oxidative stress markers, further investigations are clearly warranted to determine whether adults born pre-term may also have the same oxidative stress responses than adults born full-term after prolonged, chronic or intermittent exercise training.

#### 4.2. Oxidative stress responses to hypoxia

The additive effects of hypoxia during exercise are clearly reflected in the increased AOPP levels observed in the full-term born individuals.

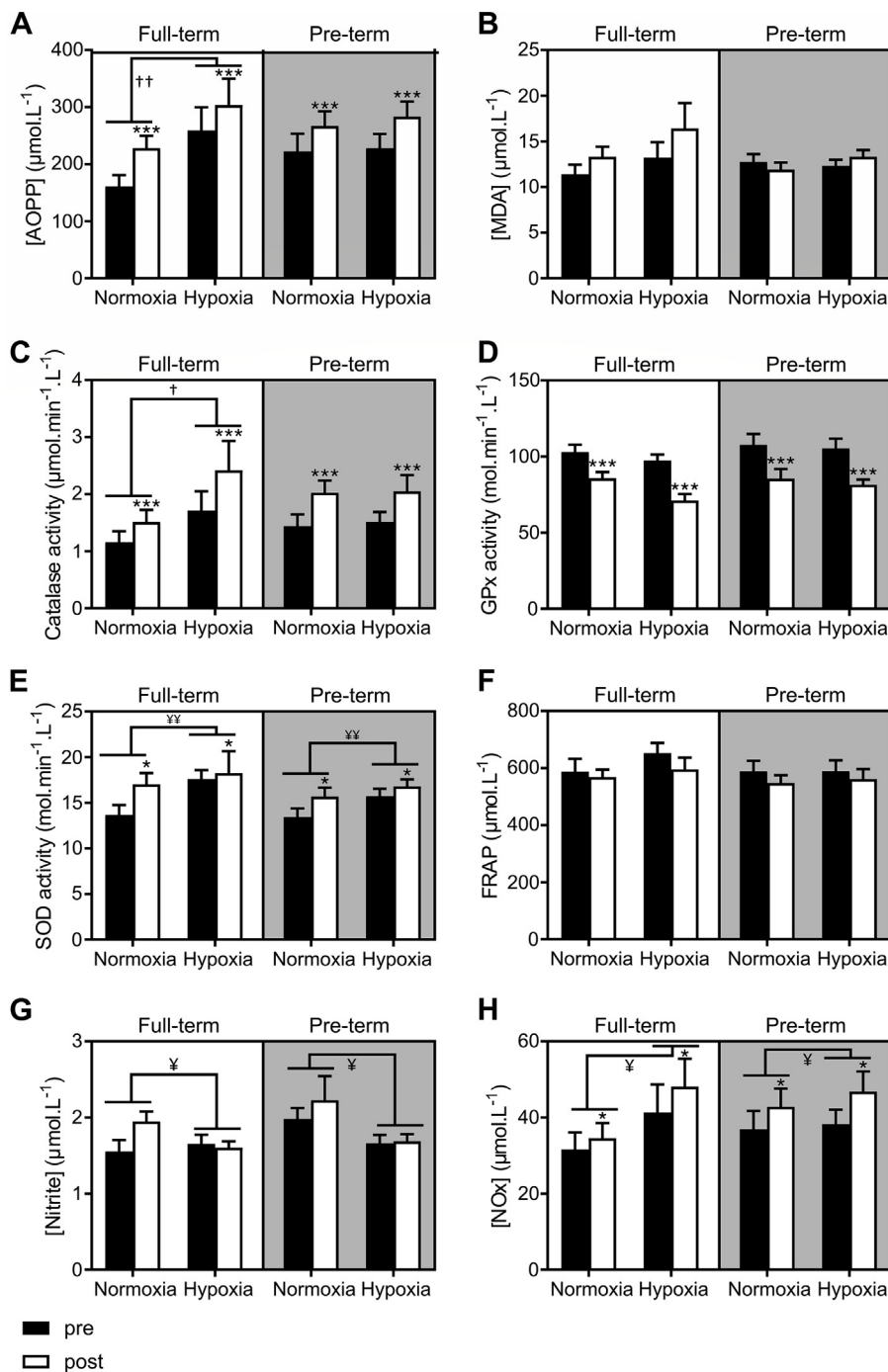
**Table 3**

P values for the main effects of condition (normoxia or hypoxia), birth (full-term or pre-term) and exercise (pre or post), and their interactions for oxidative or nitrosative stress plasma level.

Effect	Oxidative stress markers		Antioxidant enzymes				NO metabolism markers	
	AOPP	MDA	CAT	GPx	SOD	FRAP	Nitrite	NOx
Condition	<b>0.003<sup>a</sup></b>	0.115	<b>0.024<sup>a</sup></b>	0.091	<b>0.007<sup>a</sup></b>	0.278	<b>0.015<sup>a</sup></b>	<b>0.023<sup>a</sup></b>
Birth	0.735	0.319	0.854	0.278	0.237	0.449	0.119	0.687
Exercise	<b>&lt; 0.001<sup>a</sup></b>	0.182	<b>&lt; 0.001<sup>a</sup></b>	<b>&lt; 0.001<sup>a</sup></b>	<b>0.018<sup>a</sup></b>	0.087	0.221	<b>0.024<sup>a</sup></b>
Condition×birth	<b>0.008<sup>a</sup></b>	0.234	<b>0.019<sup>a</sup></b>	0.346	0.564	0.367	0.218	0.107
Condition×exercise	0.919	0.318	0.679	0.519	0.230	0.861	0.208	0.556
Birth×exercise	0.832	0.140	0.912	0.858	0.823	0.937	0.898	0.663
Condition×birth×exercise	0.551	0.872	0.483	0.590	0.602	0.551	0.653	0.937

AOPP, advanced oxidation protein products; CAT, catalase activity; FRAP, ferric reducing antioxidant power; GPx, glutathione peroxidase activity; NOx, total nitrite and nitrate; SOD, superoxide dismutase activity.

<sup>a</sup> Significant effects or interactions.



**Fig. 1.** AOPP (A), MDA (B), catalase activity (C), GPx (D), SOD activity (E), FRAP (F), nitrite (G) and NOx (H) levels before (black columns) and immediately following exercise (white columns) under normoxic and hypoxic conditions in the full-term ( $n = 15$ ) and pre-term born adults ( $n = 22$ ). Data are expressed as means  $\pm$  SEM. AOPP, advanced oxidation protein products; FRAP, ferric reducing antioxidant power; GPx, glutathione peroxidase; NOx, total nitrite and nitrate; pre, pre-exercise; post, post-exercise; SOD, superoxide dismutase. Differences pre vs. post exercise independently of birth and hypoxic/normoxic conditions: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ . Differences hypoxia vs. normoxia independently of exercise (pre/post): †† $P < 0.01$ , † $P < 0.05$ . Differences hypoxia vs. normoxia independently of exercise (pre/post) and birth: ¥  $P < 0.05$ , ¥¥  $P < 0.01$ .

These findings also support a growing body of literature indicating that acute hypoxic exposure augments oxidative stress [13,14,28]. Interestingly, we failed to observe a concomitant significant increase in MDA (+10%,  $P = 0.115$ ) although differences between lipid and protein oxidation in response to hypoxia has been previously observed [14]. The observed higher SOD, catalase activities and NOx levels in hypoxia also lend further support to the hypothesis that both ROS production and antioxidant activity are increased under hypoxic conditions [11].

However, in contrast to full-term born individuals, the pre-term born group did not display the above noted AOPP increase (see Fig. 1). This suggests a beneficial adaptation to hypoxia at least on the redox balance level. This is further confirmed by the lack of catalase activity increase in response to hypoxia in adults born pre-term, even though an increase was noted in the full-term born group. In contrast to SOD and

catalase, GPx activity was decreased regardless of the birth status. The response of GPx to hypoxic exercise could potentially be explained by a lower GSH content also reported in erythrocytes in response to altitude exposure [42]. Interestingly, we found that hypoxia-related NOx levels increase trend to occur only in full-term born individuals (+35% vs. +7% in participants born pre-term). This observation suggests that, like oxidative stress, the NO metabolism response to hypoxia may also be blunted in the pre-term born adult individuals.

The observed blunted oxidative stress and NO responses to hypoxia in the pre-term born individuals are quite surprising. Indeed, it has been shown that, the resting levels of 8-isoprostane are higher in exhaled breath condensate of adolescents (13–15 years) born very pre-term compared to those born at full-term [7] suggesting that a higher oxidative stress level could persist at adulthood in people born pre-term

[8]. However, no significant differences were observed between the two groups in baseline oxidative stress markers. Nevertheless, and although we hypothesized that an exacerbated oxidative stress in response to both, hypoxia and graded exercise would be noted in the pre-term born individuals, it seems that prematurely born adults developed adaptive mechanisms to face up to high oxygen variations early in life, known to generate high amount of ROS, that may limit oxidative stress and NO metabolism to hypoxia. Whether this greater resistance to oxidative stress response to hypoxia could be beneficial or detrimental adaptive mechanism in the pre-term born adults needs to be determined.

#### 4.3. Exercise cardio-respiratory responses

While Bates et al. observed a reduced HVR at rest in adults born pre-term [24], we confirmed their findings at rest [25] but not during exercise. This could mean that the stimulus of an incremental exercise on ventilation can outmatch/override the potential effect of hypoxia in the participants born pre-term. In any case, the blunted HVR at rest in participants born pre-term could be linked with the lower oxidative stress in response to hypoxia in participants born pre-term since a positive correlation between that changes in oxidative stress and HVR has previously been reported [43,44]. However, ventilatory response to hypoxia has also been related to the risk of altitude illness but with very limited sensitivity and specificity [27]. Interestingly, there seems to be no epidemiologic data on the incidence of acute mountain sickness (AMS) in the population born pre-term. Given the established influence and contribution of oxidative stress to AMS [45], our data provide strong impetus for future investigations on the AMS modulation in adults born pre-term since lower hypoxia-induced oxidative stress response was noted in this population. We should however be cautious regarding the direct translation of the present results to real high-altitude scenarios (hypobaric hypoxia) since we previously demonstrated that normobaric hypoxic exposure (i.e. simulated altitude) results in a lower oxidative stress increase than exposure to hypobaric hypoxia [28,46]. Nevertheless, since both groups were exposed to the same, normobaric hypoxic condition the observed difference between the two groups in oxidative stress responses provide important new insight into the consequence of prematurity.

## 5. Conclusions

In conclusion, similarly to the full-term born adults, the pre-term individuals displayed an increased oxidative stress, antioxidant activity and NO metabolism in response to acute exercise. However, and in contrast to the full-term born individuals, we report for the first time that hypoxia did not increase plasma AOPP, catalase and NOx levels in the pre-term born cohort suggesting that they developed adaptive processes that lead to greater resistance to oxidative stress in response to acute hypoxic exposure. Although we did not find significant difference in the exercise HRV in adults born pre-term, mechanisms regarding ventilatory and oxidative stress modulation in the pre-term born individuals warrants further scrutiny to expand our understanding.

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## Author contribution statement

GM, DO, TD and VP participated in design the study; AM, MM, TD and VN performed the experiments; MM, DO and TD included the patients; AM, GM, CF, TD and VP analyzed and interpreted the results; AM, TD and VP wrote the manuscript; AM, EG, TD and VP revised the

manuscript; AM, EG, GM, DO, MM, CF, TD and VP reviewed manuscript drafts and edited the manuscript.

## Declaration of competing 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2020.101497>.

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