ORIGINAL RESEARCH

Duration of neonatal oxygen supplementation, erythropoiesis and blood pressure in young adults born preterm

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ABSTRACT

Background Although erythropoiesis is impaired and anaemia frequent in neonates born preterm, haematopoiesis in adults born preterm has not been previously studied.

Objective We, thus, aimed to evaluate haemoglobin and erythropoietin levels in young adults born preterm, to identify neonatal events associated with erythropoiesis in adulthood and to examine the relationships of haemoglobin levels with respiratory function and blood pressure.

Methods We assessed a cohort of 101 young adults (ages 18–29) born preterm (≤29 weeks of gestation), in comparison to 105 full-term controls. We measured haemoglobin, erythropoietin levels and blood pressure. We also assessed respiratory function using spirometry.

Results Compared with controls, tobacco use and sex-adjusted haemoglobin levels were 5.3 (95% CI 2.9 to 7.7) g/L higher in preterm-born individuals, but erythropoietin levels were similar. Duration of oxygen supplementation in the neonatal period was independently associated with higher haemoglobin levels in the preterm group. In young adults born preterm with bronchopulmonary dysplasia, airflow limitation was associated with higher haemoglobin levels. Both systolic (SBP) and diastolic (DBP) blood pressure were increased in individuals born preterm (p=0.042 and p=0.0008, respectively). Higher haemoglobin levels were associated with higher SBP and DBP, independently of term or preterm status. Mediation analysis suggests that haemoglobin increase contributes to 37% and 32% of the effect of preterm birth on SBP and DBP, respectively.

Conclusions Haemoglobin levels are higher in young adults born preterm, while erythropoietin levels are similar, especially in case of bronchopulmonary dysplasia and airflow limitation, and haemoglobin increase is associated with elevated blood pressure in this population.

Key messages

What is the key question?
► Erythropoiesis is disturbed in preterm neonates, but whether these alterations persist later in life in unknown.

What is the bottom line?
► Haemoglobin levels are higher in young adults born preterm, and this increase is associated with bronchopulmonary dysplasia.

Why read on?
► This study is the first to demonstrate that young adults born preterm show higher haemoglobin values, which are associated with higher blood pressure, and suggests long-term disruption of erythropoiesis.

INTRODUCTION

Improvements in neonatal care have resulted in an increase in the survival rate of preterm (gestational age (GA) <37 weeks) neonates.[1] Worldwide, preterm birth accounts for 10% of births, including 1.5% of very preterm births.[2] Very preterm birth is associated with early occurrence of high blood pressure (BP),[3 4] and increased risk of ischaemic cardiovascular disease,[5] but pathophysiological mechanisms leading to this increase in BP remain to be determined. Very preterm birth is also associated with a higher risk of airflow obstruction, which is further increased in subjects with a history of bronchopulmonary dysplasia (BPD).[6]

During the neonatal period, infants born preterm are exposed to prolonged oxygen supplementation. In addition, they frequently present with anaemia, secondary to phlebotomy blood loss, iron deficiency and diminished erythropoietin (EPO) production,[7 8] resulting in red blood cell transfusions. We hypothesise that these neonatal events durably affect erythropoiesis regulation following preterm birth, leading to a difference in haemoglobin levels in young adults born preterm compared with those born term. As increased haemoglobin and haematocrit levels have been associated with an elevation of BP in healthy adults,[9–11] we further postulate that this difference could have an effect on BP.

In the present study, we, thus, aimed to assess EPO and haemoglobin levels in young adults born preterm, to evaluate the impact of neonatal comorbidities on erythropoiesis in adulthood and to determine if differential erythropoiesis in adults born preterm could participate to the increase in BP in this population.

Concise methods

Detailed methods are available in the online supplementary methods.

Study population

We obtained data from the Health of Adults born Preterm Investigation (HAPI) project that evaluated the health of young adults (18–29 years) born at ≤29 weeks of GA and compared them to individuals born full-term (≥37 weeks GA). Participants with severe neurocognitive impairment and pregnancy were excluded.

Clinical outcomes

On the day of the visit, each participant underwent clinical assessment. Systolic BP (SBP) and diastolic BP (DBP) were measured after seated rest for 5 min, in duplicate using an automated oscillometric device. Spirometry, including measurement of forced expiratory volume in 1 s (FEV_1), was performed according to the American Thoracic Society/European Respiratory Society guidelines.[12] FEV_1 Z-scores were calculated using the Global Lung Initiative 2012 reference values.[13] Mild BPD was defined as oxygen requirement for ≥28 days and breathing room air at 36 weeks postmenstrual age (PMA); moderate to severe BPD was defined as oxygen requirement at 36 weeks PMA, per National Institute of Child Health and Human Development (NICHD) criteria' .[14]

Biology and ultrasonography measurements

Blood was collected on the morning of the assessment after overnight fasting. Total blood count was measured at the Sainte-Justine University Hospital clinical biochemistry laboratory. EPO, soluble vascular endothelial growth factor receptor 1 (VEGF-R1 or sFlt-1), human soluble endoglin (CD105) and VEGF were measured in the serum. Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine values using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 formula.

Statistical analysis

For continuous variables, data were presented as mean±SD or median (IQR, 25%–75%) and between-group comparisons were performed using Student's t or Mann-Whitney U tests for normally distributed and non-normally distributed variables, respectively. Categorical variables were presented as n (%) with between-group comparisons done with the Fisher's exact test. P values<0.05 were considered statistically significant. Correlations between continuous variables were assessed using Spearman's r coefficient and test. Due to the low number of missing data, we performed primary analysis on complete cases with exclusion of missing data. We performed sensitivity analyses for missing data using multiple imputations as described in the online supplementary methods.

RESULTS

Population characteristics

The study cohort consisted of 105 term-born and 101 preterm-born participants. Characteristics of the study cohort are shown in table 1, clinical and biochemical measurements are provided in table 2. Participants born preterm had a higher SBP and DBP, and decreased respiratory function, compared with those born term. Comparisons of these clinical characteristics between individuals born preterm with and without BPD are shown in online supplementary table 1. Among those born preterm, a history of BPD was associated with a significantly lower GA and a higher number of red blood cell transfusions in the neonatal period. FEV_1 Z-score adjusted for tobacco use and sex was significantly lower in the preterm group, both in those with BPD (by 1.05 SD, 95% CI 0.73 to 1.37) and with no BPD (by 0.57 SD, 95% CI 0.15 to 0.99).

Haematological characteristics

Haematological characteristics of the study population are presented in table 3, and the proportion of participants with values outside laboratory range is provided in online supplementary table 2. Haemoglobin and haematocrit levels were higher in young adults born preterm, while mean globular volume and mean corpuscular haemoglobin concentration did not differ. Both leucocyte and platelet counts were similar in both groups. The significant increase in haemoglobin levels in the preterm group remained after adjustment for sex and protein levels ($p<0.001$), suggesting the increase in haemoglobin levels was not explained by haemoconcentration. EPO levels were similar between the two groups, and haemoglobin increase was associated with a decrease in EPO levels ($r=-0.174$, $p=0.016$) which was similar in both groups (correlation coefficients $r=-0.202$ and $r=-0.201$ for the term and the preterm groups, respectively). In comparison to term controls, tobacco use and sex-adjusted haemoglobin levels were 6.2 g/L (95% CI 3.5 to 9.0) higher in preterm adults with BPD and 3.7 g/L (95% CI 0.3 to 7.1) higher in preterm adults without BPD. Duration of oxygen supplementation and red blood cell transfusions in the neonatal period were associated with increased haemoglobin levels within the preterm group. None of the other studied neonatal factors were associated with haemoglobin levels after adjustment for sex and tobacco use (table 4). EPO levels were not associated with neonatal oxygen use, or with any other neonatal factor, even after adjustment for sex, tobacco use and haemoglobin level. We did not observe any significant between-group differences in the levels of angiogenesis biomarkers.

Relationships between haemoglobin, EPO and current clinical characteristics

[15 16 15 16] Male sex and preterm birth were strong predictors of higher haemoglobin levels. Current tobacco use was also associated with increased haemoglobin levels, and this association remained after adjustment for sex. We did not find an association between the eGFR and haemoglobin levels. In females, the number of days since last periods was not associated with a difference in haemoglobin or in EPO levels. Age and body mass index were not related to haemoglobin levels in our cohort, but age range in our study sample was narrow. There was no significant association between EPO and preterm birth in the univariate analysis. After adjustment for sex and haemoglobin levels in a multivariate analysis, preterm birth was significantly associated with increased EPO levels (table 5).

Erythropoiesis and adult respiratory function

FEV_1 Z-score was significantly lower in individuals born preterm. We aimed to determine if an association between airflow limitation and haemoglobin existed in our cohort. For this analysis, we excluded subjects receiving chronic medication for asthma (n=6 (6%) and 13 (13%) for the term and preterm groups, respectively), in whom measured spirometry parameters may not reflect baseline respiratory function. For each one-unit decrease in FEV_1 Z-score, sex-adjusted and tobacco use-adjusted haemoglobin levels increased by 2.60 g/L ($p=0.029$) in individuals born preterm with BPD, while no significant change was observed in those born preterm without BPD or in those born term (figure 1). The association between FEV_1 Z-score and haemoglobin levels was similar in those born preterm with mild

Table 1 Study population

Characteristic	Term (n=105) Missing n (%)	Term (n=105) Mean (SD) or n (%)	Preterm (n=101) Missing n (%)	Preterm (n=101) Mean (SD) or n (%)	P value
Age, years	0 (0)	23.2±2.5	0 (0)	23.2±2.2	0.99
Male sex, n (%)	0 (0)	43 (41)	0 (0)	45 (45)	0.67
Height, cm	0 (0)	171±9	0 (0)	165±9	<0.0001
Weight, kg	0 (0)	69.2±14.9	0 (0)	62.1±12.6	0.0003
Body mass index, kg/m^2	0 (0)	23.7±4.3	0 (0)	22.6±3.8	0.066
Current tobacco use*, n (%)	0 (0)	20 (19)	0 (0)	23 (23)	0.61
Excessive weekly alcohol consumption†, n (%)	13 (12)	11 (12)	4 (4)	10 (10)	0.818
Recreational drug use, n (%)	0 (0)	23 (22)	0 (0)	20 (20)	0.86
Physical activity sessions, <4/month	6 (6)	29 (29)	0 (0)	34 (35)	0.44
Parental history of hypertension, n (%)	0 (0)	39 (37)	0 (0)	45 (45)	0.32
Caucasian origin, n (%)	0 (0)	95 (90)	0 (0)	91 (90)	1.00
No of days since last periods‡	24 (39)	18.7±14.9	16 (29)	15.9±11.3	0.34
Education higher than high school, n (%)	7 (7)	70 (71)	8 (8)	60 (65)	0.35
Maternal characteristics					
Maternal age at delivery, n (%)	32 (30)	28.9±4.9	22 (22)	31.0±5.1	0.011
Maternal history of pre-eclampsia, n (%)	0 (0)	7 (7)	0 (0)	22 (22)	0.0023
Neonatal characteristics					
Gestational age, weeks	1 (1)	39.6±1.1	0 (0)	27.1±1.4	–
Birth weight, g	0 (0)	3421±387	0 (0)	965±230	–
Birth weight percentile§	0 (0)	49.3±24.4	0 (0)	35.3±17.1	<0.0001
Small for gestational age§, n (%)	1 (1)	7 (7)	0 (0)	6 (6)	1.00
Antenatal corticosteroids, n (%)	0 (0)	0 (0)	2 (2)	41 (41)	–
Use of surfactant, n (%)	0 (0)	0 (0)	5 (5)	46 (48)	–
Bronchopulmonary dysplasia	NA		2 (2)		
None		105 (100)		33 (33)	–
Mild		0 (0)		29 (29)	
Moderate to severe		0 (0)		37 (37)	
No of red blood cell transfusions during the neonatal period	0 (0)	0 (0)	9 (9)	6.8±6.3	–
At least one major neonatal complication¶, n (%)	0 (0)	0 (0)	0 (0)	54 (53)	–
Estimated daily dietary intake					
Iron, mg	13 (12)	5.96±7.7	4 (4)	5.79±5.77	0.86
B_6 vitamin, mg	13 (12)	1.83±0.86	4 (4)	1.80±1.25	0.83
B_{12} vitamin, µg	13 (12)	13.7±7.3	4 (4)	13.9±10.7	0.86

Results shown as mean±SD or n (%) unless stated otherwise. Comparisons were performed using Student's t-test or the Fisher's exact test.
*Current tobacco use: regular or occasional cigarette smoking, excluding former smokers.
†Excessive alcohol consumption: ≥14 drinks per week in males and ≥7 drinks per week in females.
‡For females.
§Birth weight percentiles (small for gestational age <10th percentile) according to Hadlock *et al*[15] (preterm group) or Kramer *et al*[16] (term group).
¶Major neonatal complications include: moderate to severe bronchopulmonary dysplasia, grade 3–4 intraventricular haemorrhage or cystic periventricular leukomalacia, retinopathy of prematurity ≥stage 3, patent ductus arteriosus ligation, necrotizing enterocolitis and postnatal sepsis.
NA, not applicable.

and moderate to severe BPD (online supplementary figure 1). However, a higher haemoglobin level was also observed in adults born preterm with normal respiratory function (online supplementary table 3).

Erythropoiesis and angiogenesis biomarkers in association with BP

We aimed to determine if haemoglobin increase could participate to the increase in BP observed in participants born preterm, using a mediation analysis. Tobacco use was not retained for adjustment as its effect was not significant in this model (B=−1.36, p=0.52 and B=−1.64, p=0.25 for SBP and DBP, respectively). We observed a significant association between haemoglobin levels and both SBP and DBP. We found no significant interaction with sex for associations of haemoglobin with SBP (p for interaction=0.55) and DBP (p for interaction=0.31). Association between haemoglobin levels and BP remained significant after adjustment for sex and term/preterm status (online

Table 2 Clinical characteristics

Characteristic	Term (n=105) Missing n (%)	Term (n=105) Mean (SD) or n (%)	Preterm (n=101) Missing n (%)	Preterm (n=101) Mean (SD) or n (%)	P value
Spirometry					
FEV_1, L	9 (9)	3.89±0.79	8 (8)	3.25±0.74	<0.0001
FEV_1, percent predicted	9 (9)	100±10.8	8 (8)	89.1±12.7	<0.0001
FEV_1, Z-score	9 (9)	0.012±0.926	8 (8)	−0.915±1.071	<0.0001
FVC, L	9 (9)	4.7±1	8 (8)	4.2±1	0.0010
FVC, percent predicted	9 (9)	103.4±10.9	8 (8)	98.6±11.4	0.0030
FVC, Z-score	9 (9)	0.28±0.899	8 (8)	−0.12±0.956	0.0034
FEV_1/FVC	9 (9)	0.831±0.066	8 (8)	0.78±0.09	<0.0001
FEV_1/FVC, Z-score	9 (9)	−0.44±0.9	8 (8)	−1.15±1.07	<0.0001
Venous blood gas					
Venous pH	24 (23)	7.38±0.03	26 (26)	7.38±0.03	0.16
Venous pCO_2, mm Hg	23 (22)	43.7±4.6	25 (25)	43.0±4.3	0.73
Venous bicarbonate, mmol/L	23 (22)	24.2±1.4	26 (26)	24.3±1.8	0.63
Pulse oximetry (SpO_2) ≥95%	30 (29)	75 (100)	27 (27)	74 (100)	1.00
Chronic use of asthma medication, n (%)	0 (0)	6 (6)	0 (0)	13 (13)	0.093
Systolic blood pressure, mm Hg	1 (1)	116±13	4 (4)	120±14	0.042
Diastolic blood pressure, mm Hg	1 (1)	67.9±7.6	4 (4)	71.8±8.4	0.0008
Metabolic and biochemistry measurements					
eGFR, mL/min/1.73 m^2	2 (2)	116±12	5 (5)	118±12	0.25
Fasting glucose, mmol/L	2 (2)	4.76±0.47	5 (5)	4.78±0.42	0.71
LDL cholesterol, mmol/L	2 (2)	2.35±0.65	5 (5)	2.28±0.61	0.43
HDL cholesterol, mmol/L	2 (2)	1.27±0.26	5 (5)	1.28±0.25	0.69
Triglycerides, mmol/L	2 (2)	0.86±0.351	5 (5)	0.889±0.445	0.62
Total serum protein, g/L	2 (2)	65.4±3.6	6 (6)	66.5±4.5	0.056

eGFR, estimated glomerular filtration rate; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; pCO_2, partial pressure of carbon dioxide; SpO2, peripheral oxygen saturation.

supplementary table 4). After adjustment for haemoglobin levels, the association between BP (both SBP and DBP) and term/preterm status was weaker, while the association between BP and haemoglobin level remained similar. Sex-adjusted mediation analysis showed that haemoglobin was a mediator for the increase in BP in young adults born preterm (average causal mediation effect, 1.03 (0.05–2.27) for SBP and 1.14 (0.48–2.11) for DBP, p=0.040 and p=0.0008 respectively). Using this analysis, we estimated that 37% and 32% of the effect of preterm birth on SBP and DBP, respectively, was mediated by haemoglobin levels (figure 2). Similar results were obtained after stratification by sex, although some associations were not significant likely due to a loss of power (online supplementary figure 2). EPO levels were not associated with BP in this cohort. Among the measured biomarkers of angiogenesis, only soluble endoglin was associated with an increase in SBP. However, this association did not remain significant after adjustment for sex (online supplementary table 4).

Sensitivity analyses

We performed sensitivity analyses of tables 4 and 5, online supplementary table 4 and figure 2 for missing data using multiple imputations and obtained similar results. Results of sensitivity analyses are shown in the sensitivity analyses section of the online supplementary file.

DISCUSSION

In this research, we demonstrate that erythropoiesis is increased in young adults born preterm, that this increase is associated with history of BPD and poorer lung function and that the resulting elevation in haemoglobin levels may participate to the increase in BP observed in this population.

Children born preterm have lower haemoglobin levels in the neonatal period. Phlebotomy and iron deficiency have an important role in anaemia of prematurity, but cannot be held fully responsible for low haemoglobin levels. In infants born preterm, compared with term-born infants, EPO production in response to anaemia is diminished.[7] Erythroid progenitor cells of infants born preterm with anaemia of prematurity are, however, highly responsive to EPO,[17] suggesting that inadequate EPO production, but not bone marrow responsiveness to EPO, has a causal role in anaemia of prematurity. One of the proposed mechanisms for EPO deficiency in infants born preterm is that their primary production site of EPO is the liver, with a progressive increase in EPO production from the kidney after 30 weeks of gestation.[18] Hypoxia and anaemia are major stimulants of EPO production; however, liver EPO production is less responsive to hypoxia and anaemia than kidney EPO production, which could explain the lower response to anaemia observed in neonates born preterm.[19] It has been estimated that up to 80% of very low birth weight infants (VLBW, <1.5 kg) and 95% of extremely low birth weight

Table 3 Haematological analyses according to sex and preterm status

	Male			Female		
	Term	Preterm	P value	Term	Preterm	P value
Complete blood count n, missing, n (%)	n=42 Missing: 1 (2)	n=45 Missing: 0 (0)		n=60 Missing: 2 (3)	n=52 Missing: 4 (7)	
Haemoglobin, g/L	142.2±10.1	149.2±8.9	0.0009	124.3±7.9	128.4±8.1	0.0086
Haematocrit, %	41.4±2.9	43.4±2.5	0.0013	36.9±2.4	38.2±2.2	0.0033
Mean globular volume, µ	88.2±3.5	88.3±3.5	0.86	89.2±4	88.7±3.4	0.53
Mean corpuscular haemoglobin concentration	343.2±9.8	344±9.6	0.71	337.3±8.5	336.3±7.5	0.52
Red blood cells, per mm^3	4.71±0.4	4.92±0.37	0.012	4.14±0.27	4.31±0.3	0.0026
Leucocytes, per mm^3	5.99±1.78	6.19±1.48	0.56	6.41±1.58	6.47±1.88	0.86
Neutrophils, per mm^3	3.27±1.43	3.45±1.24	0.52	3.57±1.35	3.55±1.23	0.94
Lymphocytes, per mm^3	1.92±0.66	1.94±0.47	0.87	2.16±0.8	2.19±1.25	0.88
Eosinophils, per mm^3	0.195±0.146	0.193±0.127	0.95	0.155±0.108	0.138±0.099	0.40
Basophils, per mm^3	0.007±0.026	0.013±0.034	0.34	0.013±0.047	0.023±0.088	0.48
Monocytes, per mm^3	0.57±0.22	0.58±0.19	0.89	0.50±0.13	0.55±0.25	0.14
Platelets (/mm^3)	221±50	208±40	0.22	234±47	237±49	0.69
Biomarkers						
EPO, U/L	6.94±2.98	7.45±3.08	0.44	7.56±3.74	8.7±3.58	0.11
Soluble endoglin, ng/mL	1.82 (1.54 to 2.17)	1.68 (1.34 to 1.93)	0.12	1.38 (1.11 to 1.53)	1.21 (1.02 to 1.43)	0.085
VEGF, ng/mL	0.080 (0.040 to 0.096)	0.077 (0.045 to 0.094)	0.93	0.061 (0.039 to 0.093)	0.065 (0.046 to 0.107)	0.28
sFlt-1, ng/mL	0.216 (0.172 to 0.239)	0.192 (0.164 to 0.242)	0.53	0.187 (0.169 to 0.225)	0.179 (0.151 to 0.208)	0.24

Results shown as mean±SD or median (25%–75%) and comparisons were performed using Student's t-test or Mann-Whitney U test, when appropriate. Missing values for complete blood count are provided in the table. EPO levels were missing in 3 (3%) term and 9 (9%) preterm participants, soluble endoglin levels were missing in 22 (21%) term and 17 (17%) preterm participants, VEGF levels were missing in 20 (19%) term and 13 (13%) preterm participants, and sFlt-1 levels were missing in 48 (46%) term and 60 (41%) preterm participants.
EPO, erythropoietin; VEGF, vascular endothelial growth factor.

(ELBW, <1 kg) infants receive blood transfusions in the neonatal period.[20]

Although haemoglobin levels spontaneously increase after a few weeks of age in neonates born preterm, erythropoiesis had not previously been studied in adults born preterm. Only scarce and conflicting data had previously been reported regarding haemoglobin levels in preterm born adults. Vrijlandt *et al* found no difference in haemoglobin levels (but did not provide the values) between adults born term and preterm, in a study including 42 adults born preterm with a mean GA of 30 weeks.[21] In contrast, and in line with our results, Vollsæter *et al* observed higher haemoglobin levels in young adults born extremely preterm (46 adults born preterm with a mean GA of 27 weeks), although the difference was significant only in males, possibly due to a lack of power in the female group.[22] Differences in GA and BPD prevalence between the studies may explain these conflicting results. Neither study further explored haematopoiesis in adults born preterm.

In our study, and in contrast with the neonatal period, young adults born preterm, compared with controls born full term, had higher haemoglobin levels, both in males and in females. Haemoglobin increase was associated with duration of neonatal oxygen supplementation and a higher number of red blood cell transfusions in the neonatal period. As expected, EPO levels decreased when haemoglobin levels increased in both groups. However, despite higher haemoglobin levels in the preterm group, EPO levels were similar in both groups, and EPO levels were higher in the preterm group after adjustment for haemoglobin level, suggesting that EPO production was responsible for increased haemoglobin levels in individuals born preterm. Preterm birth is associated with an abrupt and early transition to ex utero environment, with blood oxygen saturation levels increasing rapidly from physiological fetal levels (45%–55%) to adult values.[23] Early exposure to higher oxygen concentrations, neonatal oxygen supplementation and repeated adult red blood cell transfusions that result in a transition from fetal to adult haemoglobin predominance (with a different O_2 affinity) in early life[24] may durably alter oxygen-sensing pathways in preterm neonates that could result in a long-term upregulation of erythropoiesis in individuals born preterm. Since red blood cell transfusions in the neonatal period and duration of neonatal oxygen supplementation were associated with each other, the observational nature of our study does not allow to distinguish the effects of these factors separately.

Children and young adults born preterm are at increased risk of airflow obstruction that is only partially reversible after administration of bronchodilators,[25] similarly to what is observed in chronic obstructive pulmonary disease (COPD). COPD is associated with chronic hypoxaemia; increased levels of EPO have been observed in patients with COPD, even in those with polycythaemia.[26] Long-term oxygen treatment results in normalisation of haemoglobin levels in COPD patients with high basal haemoglobin levels.[27] Similarly, exposure of healthy subjects to high-altitude hypoxic conditions results in increased EPO production and haemoglobin concentrations, which allow to maintain normal arterial oxygen contents.[28] In our study,

Table 4 Influence of neonatal comorbidities on haemoglobin and EPO levels

	Haemoglobin, g/L		EPO, U/L		
	B (unadjusted)	B (model 1)	B (unadjusted)	B (model 1)	B (model 2)
Term					
Birth weight percentile (per 1% increase)†	−0.02 (−0.12 to 0.09)	0.01 (−0.07 to 0.08)	0.02 (−0.01 to 0.04)	0.02 (−0.01 to 0.04)	0.02 (−0.01 to 0.04)
Pre-eclampsia	3.13 (−6.49 to 12.74)	2.97 (−3.83 to 9.78)	−0.88 (−3.53 to 1.77)	−0.86 (−3.53 to 1.81)	−0.56 (−3.17 to 2.05)
Preterm					
Gestational age	−0.17 (−2.15 to 1.80)	−0.06 (−1.29 to 1.17)	0.13 (−0.38 to 0.64)	0.14 (−0.36 to 0.64)	0.14 (−0.36 to 0.64)
Birth weight percentile (per 10% increase)†	0.16* (0.01 to 0.32)	0.01 (−0.10 to 0.11)	0.00 (−0.04 to 0.04)	0.01 (−0.03 to 0.05)	0.01 (−0.03 to 0.06)
Pre-eclampsia	0.82 (−5.83 to 7.47)	1.57 (−2.58 to 5.72)	−1.08 (−2.76 to 0.60)	−1.28 (−2.92 to 0.36)	−1.22 (−2.89 to 0.44)
Antenatal corticosteroids	−2.13 (−7.58 to 3.31)	0.35 (−3.11 to 3.82)	−0.52 (−1.95 to 0.92)	−0.82 (−2.24 to 0.59)	−0.82 (−2.26 to 0.61)
Neonatal oxygen supplementation (per 10 days)	0.81* (0.30 to 1.32)	0.45* (0.11 to 0.79)	−0.07 (−0.21 to 0.06)	−0.07 (−0.22 to 0.07)	−0.06 (−0.21 to 0.09)
Red blood cell transfusions (per five transfusions)	3.30* (1.23 to 5.36)	1.69* (0.29 to 3.08)	−0.32 (−0.90 to 0.26)	−0.21 (−0.79 to 0.38)	−0.13 (−0.73 to 0.48)

Estimated effects of antenatal factors on haemoglobin and erythropoietin levels, obtained using univariate and multivariate linear regression. Model 1: adjustment for sex and tobacco use. Model 2: adjustment for sex, tobacco use and haemoglobin level.
Results are shown as the unstandardised regression coefficient B (95% CI).
*P<0.05.
†Birth weight percentiles according to Hadlock *et al*[15] (preterm group) or Kramer *et al*[16] (term group).
EPO, erythropoietin.

respiratory function was significantly altered in the preterm group, especially in those with BPD. In preterm-born participants with a history of BPD, haemoglobin levels increased with FEV_1 decline, suggesting that haemoglobin levels in these participants could increase, in part, as a response to chronic respiratory dysfunction. However, all participants, including those with airflow limitation, had normal pulse oximetry at rest, and haemoglobin levels were increased even in preterm

Table 5 Association of haemoglobin and EPO to current clinical characteristics

	Haemoglobin, g/L		EPO, U/L		
	B (unadjusted)	B (model 1)	B (unadjusted)	B (model 1)	B (model 2)
Male sex	19.64* (17.09 to 22.19)	–	−0.87 (−1.84 to 0.10)	–	–
Preterm birth	6.39* (2.79 to 10.00)	5.39* (2.96 to 7.81)	0.81 (−0.16 to 1.77)	0.86 (−0.10 to 1.82)	1.29* (0.30 to 2.29)
Body mass index, kg/m^2	0.33 (−0.12 to 0.77)	0.21 (−0.09 to 0.51)	0.08 (−0.04 to 0.20)	0.08 (−0.03 to 0.20)	0.10 (−0.02 to 0.22)
Age, year	0.32 (−0.48 to 1.11)	0.04 (−0.50 to 0.59)	0.20 (0.00 to 0.40)	0.22* (0.01 to 0.42)	0.23* (0.02 to 0.43)
Current tobacco smoking	7.47* (3.04 to 11.90)	3.63* (0.53 to 6.74)	−0.73 (−1.91 to 0.46)	−0.56 (−1.75 to 0.64)	−0.33 (−1.54 to 0.88)
No of days since last periods†	−0.08 (−0.22 to 0.05)	–	0.04 (−0.03 to 0.10)	–	0.03 (−0.03 to 1.10)
eGFR, 10 mL/min/1.73 m^2	−0.24 (−1.82 to 1.34)	−0.70 (−1.77 to 0.38)	0.20 (−0.22 to 0.62)	0.23 (−0.19 to 0.65)	0.18 (−0.24 to 0.60)
Fasting glucose, mmol/L	4.96* (0.89 to 9.03)	−1.03 (−3.96 to 1.90)	−0.89 (−2.00 to 0.22)	−0.70 (−1.85 to 0.45)	−0.79 (−1.93 to 0.36)
LDL cholesterol, mmol/L	1.85 (−1.20 to 4.90)	0.67 (−1.42 to 2.76)	−0.53 (−1.30 to 0.25)	−0.49 (−1.26 to 0.28)	−0.44 (−1.23 to 0.35)

Estimated effects of current clinical characteristics on haemoglobin and EPO levels, obtained using univariate and multivariate linear regression. Model 1: Adjusted for sex. Model 2: Adjusted for sex and haemoglobin level. Results are shown as the unstandardised regression coefficient B (95% CI).
*P<0.05.
†Females only, no adjustment for sex.
eGFR, estimated glomerular filtration rate; EPO, erythropoietin; LDL, low-density lipoprotein.

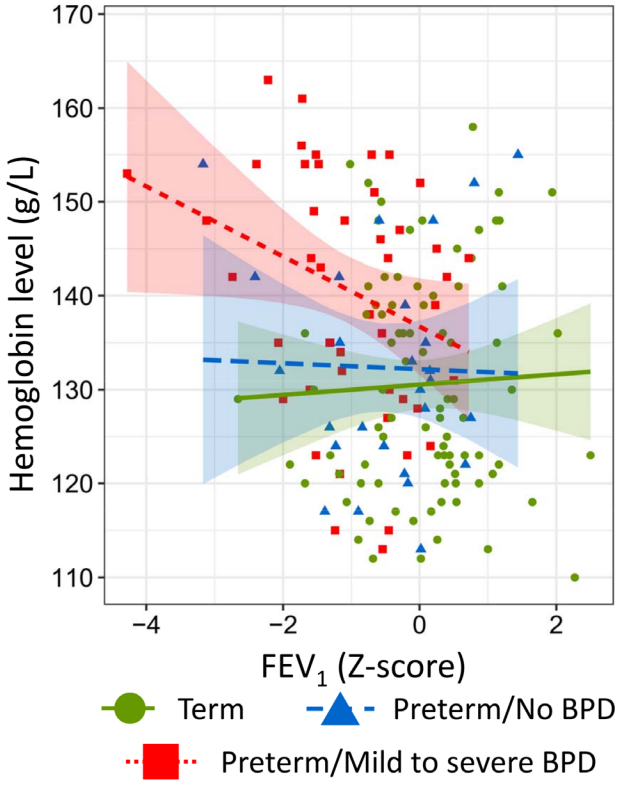

Figure 1 Association of haemoglobin and respiratory function, according to term/preterm and bronchopulmonary dysplasia (BPD) status. Participants using chronic asthma medication (inhaled corticosteroids including fluticasone propionate, ciclesonide, budesonide; long-acting β2 adrenergic receptor agonist including salmeterol xinafoate, formoterol fumarate and montelukast) excluded. FEV_1, forced expiratory volume in 1 s.

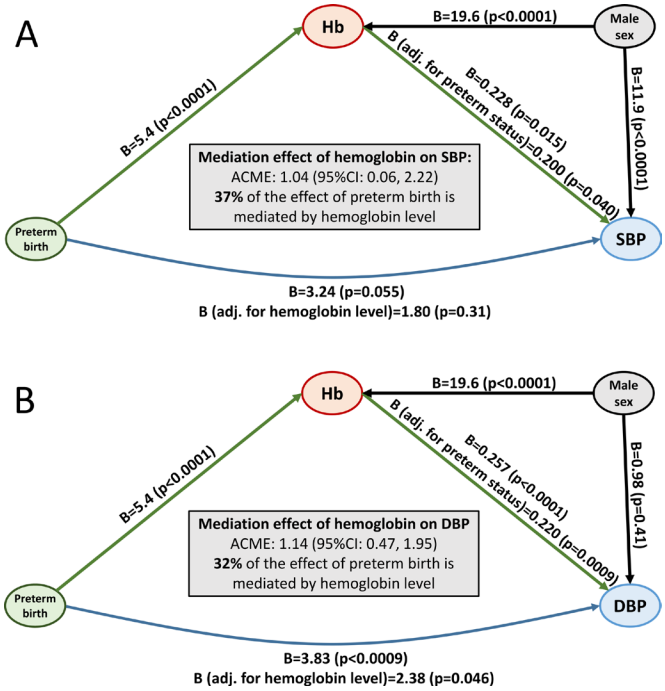

Figure 2 Mediation analysis to assess the participation of haemoglobin levels to the increase in (A) systolic blood pressure and (B) diastolic blood pressure observed in young adults born preterm. All estimations are adjusted for sex. ACME, average causal mediation effect; B, Unstandardised regression coefficient; DBP, diastolic blood pressure; Hb, haemoglobin; SBP, systolic blood pressure.

participants with normal spirometry values, suggesting the observed differences in haemoglobin levels between individuals born term and preterm are not solely the consequence of chronic respiratory dysfunction in adulthood. We found no evidence of intermittent hypoxia that could explain our findings. We previously reported the absence of ECG changes during exercise in a subset of term and preterm participants from the HAPI study who performed exercise testing and had continuous electrocardiographic and pulse oximetry monitoring.[29] Pulse oximetry was ≥95% at baseline in all participants. No participant, whether term or preterm, stopped exercise testing due to desaturation as defined by the American Heart Association[30] (absolute decrease in pulse oximetry ≥5%) in this study. In addition, although we did not assess sleep disordered breathing in this study, we previously reported no difference in BP sleep dipping between term and preterm individuals.[31] In line with these results, Björkqvist *et al* found no difference in sleep duration or sleep quality in young adults born preterm.[32]

Haemoglobin increase may have adverse consequences. In patients with polycythaemia vera, a myeloproliferative disorder, a high haematocrit was associated with an increased risk of hypertension and patients with hypertension had a higher risk of arterial thrombotic events.[33] In healthy individuals, haemoglobin levels have been shown to be positively correlated with SBP and DBP, both in males and in females.[9] Lowlanders exposed to high altitude exhibit, in addition to haemoglobin increase, an increase in systemic BP during the initial days[10] that persists even after 12 months in altitude.[11]

In this study, mediation analysis suggests that the increase in haemoglobin observed in the preterm population could participate to their increase in BP. The increase in BP observed in the preterm group, although modest, is consistent with what was previously reported in a meta-analysis by Hovi *et al*.[3] Mild increase in BP is nevertheless associated with an increase in the prevalence of hypertension in this population, as we[4] and others[34] previously reported. Even small increases in BP are associated with an increase in cardiovascular risk.[35] As the increased long-term cardiovascular,[5] cerebrovascular[36] and renal[37] risk associated with preterm birth have been well documented, the impact of haemoglobin on BP in those born preterm is likely to be of clinical relevance.

The pathophysiological mechanisms that lead to increased BP in this specific population had however not previously been elucidated. A recent meta-analysis of nine cohorts of young adults born preterm did not identify any neonatal risk factor for increased BP in this population.[3] We previously reported an inverse correlation between kidney size and BP in very preterm young adults, but the causality of this association was not established.[31] In this study, we did not identify any association between haemoglobin or EPO levels with kidney function that was in normal ranges for all participants. Lewandowski *et al*[38] observed higher levels of soluble endoglin levels and sFlt-1 in preterms, in association with high BP, which was not observed in our study. Differences between our's and Lewandowski *et al*'s studies include different mean GA (27.1 and 30.3 weeks, respectively) and body mass index (22.6 and 24.9 kg/m^2, respectively) that may explain these discrepancies.

Vascular alterations associated with preterm birth could contribute to hypertension in adults born preterm. However, a previous study has shown that arterial stiffness, known to be associated with cardiovascular outcomes,[39] measured by the Cardio-Ankle Vascular Index and brachial ultrasound was similar between adults born term and preterm.[40] Another study suggested that capillary rarefaction in adults born preterm could be associated with increased BP in this population.[38] It would thus be interesting, in a future study, to examine the association between capillary density, haemoglobin levels and BP in adults born preterm.

The renin–angiotensin system, which plays a key role in BP control, is involved in erythropoiesis regulation. Angiotensin II type I (AT1) receptor stimulation by angiotensin II has been shown to enhance EPO-stimulated erythroid proliferation in vitro,[41] and the use of ACE inhibitor enalapril corrects post-transplant erythrocytosis in kidney transplant recipients.[42] Treatment with ACE inhibitors decreases haemoglobin levels, BP and proteinuria in individuals with altitude polycythaemia.[43] High-oxygen neonatal exposure in rats is a recognised model of the preterm birth related deleterious cardiovascular conditions, with myocardial hypertrophy, fibrosis and activation of the renin-angiotensin system. We have previously shown that neonatal blockade of AT1 receptor using losartan prevents the occurrence of these oxygen-induced cardiac abnormalities in rats.[44] Blockade of the renin–angiotensin system may, therefore, represent the treatment of choice for high BP in preterm-born hypertensive patients. Whether treatment should be initiated in young preterm-born adults before the occurrence of hypertension remains to be investigated.

There are several limitations to our study. First, participants to our study were born between 1987 and 1997. Marked changes in neonatal practices have occurred during and since this period. Use of surfactant was only 48% in our cohort despite very low GA, due to the increasing use of surfactant starting 1990. Continuous positive airway pressure and more restrictive guidelines for blood transfusion have been introduced since. Results obtained in our study concerning higher BP and altered respiratory function are consistent with other studies conducted in young adults born preterm in the same period as our study participants,[3 45] but also with studies conducted in children born more recently.[46 47] Whether the observed associations between haemoglobin levels and preterm birth will remain in more contemporary cohorts will have to be assessed in the future. Second, the proportion of women born term with low haemoglobin levels was high in our study. Prevalence estimates of anaemia in young women vary widely. According to WHO, 10% of women of reproductive age have anaemia in Canada.[48] We are not aware of studies of the prevalence of anaemia conducted specifically in Québec, but the prevalence of anaemia in the same population group is 18% in France and 16% in Belgium. Prevalence estimates from our study (22%) are close to the ones obtained from France and Belgium. This is in line with the fact that the population of Québec (and of our study, which mostly recruited French-speaking participants born in Québec) mainly descends from French-speaking Europeans. Since haemoglobin electrophoresis was not performed, we cannot exclude the presence of haemoglobin variants that could explain the differences between groups. Thus, this single-centre, single-time point study will require confirmation in other cohorts and in time, in a longitudinal study.

This research shows, for the first time, that erythropoiesis is increased in young adults born very preterm. Haemoglobin increase is associated with poorer adult respiratory function, prolonged neonatal oxygen supplementation and blood transfusions in the neonatal period, suggesting these neonatal interventions could alter erythropoiesis response to anaemia and hypoxaemia. A higher haemoglobin level could further participate to the increase in BP observed in young adults born preterm.

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Contributors TML and AMN conceived the HAPI study and obtained the funding. AF, TML and AMN designed and performed the analysis, and wrote the paper. AF, CG-B, ROF and AC collected the data. YDP participated to the interpretation of the results. All authors revised the manuscript.

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Author note 140-character Tweet: Increased haemoglobin levels in adults born preterm linked to bronchopulmonary dysplasia and blood pressure.

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