



# Association between maternal haemoglobin concentrations and maternal and neonatal outcomes: the prospective, observational, multinational, INTERBIO-21st fetal study



Eric O Ohuma, Nusrat Jabin, Melissa F Young, Terrence Epie, Reynaldo Martorell, Juan Pablo Peña-Rosas, Maria Nieves Garcia-Casal, INTERBIO-21st Consortium, Aris T Papageorgiou, Stephen H Kennedy, Jose Villar

## Summary

**Background** Anaemia in pregnancy is a global health problem with associated maternal and neonatal morbidity and mortality. We aimed to investigate the association between maternal haemoglobin concentrations during pregnancy and the risk of adverse maternal and neonatal outcomes.

**Methods** In this prospective, observational, multinational, INTERBIO-21st fetal study conducted at maternity units in Brazil, Kenya, Pakistan, South Africa, and the UK, we enrolled pregnant women (aged  $\geq 18$  years, BMI  $< 35$  kg/m<sup>2</sup>, natural conception, and singleton pregnancy) who initiated antenatal care before 14 weeks' gestation. At each 5 $\pm$ 1 weekly visit until delivery, information was collected about the pregnancy, as well as the results of blood tests taken as part of routine antenatal care, including haemoglobin values. The outcome measures were maternal (gestational diabetes, pregnancy-induced hypertension, and preterm premature rupture of membranes) and neonatal outcomes (small for gestational age, preterm birth, and acute respiratory distress syndrome).

**Findings** Between Feb 8, 2012, and Nov 30, 2019, 2069 women (mean age 30.7 years [SD 5.0]) had at least one routinely haemoglobin concentration measured at 14–40 weeks' gestation, contributing 4690 haemoglobin measurements for the analysis. Compared with a haemoglobin cutoff of 110 g/L, the risk was increased more than two-fold for pregnancy-induced hypertension at haemoglobin concentrations of 170 g/L (risk ratio [RR] 2.29 [95% CI 1.19–4.39]) and higher, for preterm birth at haemoglobin concentrations of 70 g/L (RR 2.04 [95% CI 1.20–3.48]) and 165 g/L (RR 2.06 [95% CI 1.41–3.02]), and for acute respiratory distress syndrome at haemoglobin concentrations of 165 g/L (RR 2.84 [95% CI 1.51–5.35]). Trimester-specific results are also presented.

**Interpretation** Our data suggests that the current WHO haemoglobin cutoffs are associated with reduced risk of adverse maternal and neonatal outcomes. The current haemoglobin concentration cutoffs during pregnancy should not only consider thresholds for low haemoglobin concentrations that are associated with adverse outcomes but also define a threshold for high haemoglobin concentrations given the U-shaped relationship between haemoglobin concentration and adverse neonatal and maternal outcomes.

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## Introduction

Anaemia in pregnancy, due primarily to iron or folate deficiency, is associated with increased risk of maternal mortality,<sup>1</sup> caesarean section,<sup>2</sup> and adverse outcomes, including preterm birth, small for gestational age (SGA),<sup>3</sup> and perinatal and neonatal mortality.<sup>4,5</sup> The global prevalence of anaemia in pregnant women aged 15–49 years, estimated in 2019, was 36.5% (95% uncertainty interval 34.0–39.1), based on a haemoglobin threshold of less than 110 g/L, as defined by WHO.<sup>6</sup> WHO and the Centers for Disease Control and Prevention (CDC) have established trimester-specific cutoffs for maternal anaemia ( $< 110$  g/L, 105 g/L, and 110 g/L for the first, second, and third trimesters of pregnancy, respectively).<sup>7–9</sup>

However, there is little evidence that specific haemoglobin thresholds predict health risk or protection for the mother and her child.<sup>10</sup> The lowest rates of low

birthweight and preterm birth appear to occur when maternal haemoglobin concentrations are 95–105 g/L during the second trimester of pregnancy,<sup>11,12</sup> and 95–125 g/L at term.<sup>13,14</sup> Haemoglobin concentrations higher than 130 g/L at sea level have also been associated with negative pregnancy outcomes.<sup>11–15</sup> Large retrospective epidemiological studies,<sup>11,12,16</sup> and one prospective study in China,<sup>17</sup> have shown that both low and high haemoglobin concentrations during pregnancy are associated with increased risks for low birthweight and preterm birth. Additionally, there is no consensus on the timing of haemoglobin measurements or the cutoffs associated with various functional outcomes due to significant study heterogeneity.<sup>10,18</sup> One meta-analysis<sup>18</sup> has shown increased odds of intrauterine growth restriction in women with anaemia during pregnancy, whereas another study<sup>16</sup> showed no such association.

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Maternal, Adolescent, Reproductive, and Child Health (MARCH) Centre, Department of Infectious Disease Epidemiology and International Health, London School of Hygiene & Tropical Medicine, London, UK (E O Ohuma PhD); Hubert Department of Global Health, Emory University, Atlanta, GA, USA (M F Young PhD, Prof R Martorell PhD); Department of Nutrition and Food Safety, World Health Organization, Geneva, Switzerland (J P Peña-Rosas PhD, M N Garcia-Casal PhD); Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine (N Jabin MSc, T Epie MSc), Nuffield Department of Women's & Reproductive Health

(Prof A T Papageorgiou MD, Prof S H Kennedy MD, Prof J Villar MD), and Oxford Maternal & Perinatal Health Institute, Green Templeton College (Prof A T Papageorgiou, Prof S H Kennedy, Prof J Villar), University of Oxford, Oxford, UK

Correspondence to: Dr Eric O Ohuma, Maternal, Adolescent, Reproductive and Child Health (MARCH) Centre, Department of Infectious Disease Epidemiology and International Health, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK [eric.ohuma@lshtm.ac.uk](mailto:eric.ohuma@lshtm.ac.uk)

## Research in context

### Evidence before this study

Anaemia is a condition in which blood has reduced oxygen carrying capacity to the tissues due to a low number of red blood cells, or a reduction in the amount of haemoglobin. In pregnancy, both low and high haemoglobin concentrations have been associated with increased risk of adverse maternal and neonatal outcomes. However, little is known about the gestational age-specific haemoglobin thresholds that predict health risk or protection for mothers and their infants. The current haemoglobin cutoffs for defining anaemia in pregnancy only address values below the thresholds and were largely derived from expert technical consultations or non-prospective, country-specific studies mainly conducted in the USA. There is a paucity of data relating maternal and neonatal health outcomes to maternal haemoglobin concentrations during pregnancy to determine if revisions to the current haemoglobin cutoffs and definitions are required.

### Added value of this study

To our knowledge, our study is the first to use high-quality individual-level data from five multinational sites to quantify

the relative risk of adverse maternal and neonatal outcomes according to haemoglobin concentrations during pregnancy when compared with the current trimester-specific WHO haemoglobin cutoffs of 110 g/L and 105 g/L. These associations were also quantified by trimester of pregnancy.

### Implications for all the available evidence

Our study provides evidence to help inform the definition of optimal haemoglobin concentrations in pregnancy based on associations with common adverse maternal and neonatal outcomes. Using robust analyses, we show predicted relative risks by levels of haemoglobin, which strongly suggest that a common optimal range of haemoglobin concentrations by trimester, associated with reduced risks of adverse pregnancy and neonatal outcomes. An optimal haemoglobin concentrations range might be considered to reduce the risk of adverse outcomes.

In 2019, a meta-analysis reported that a haemoglobin concentration of less than 110 g/L is associated with pre-eclampsia, preterm birth, SGA, stillbirth, post-partum haemorrhage, and perinatal and neonatal mortality, whereas a haemoglobin concentration of higher than 130 g/L is associated with pre-eclampsia, SGA, and stillbirth.<sup>10</sup> However, the authors noted that the results differed by timing of haemoglobin measurement, haemoglobin cutoffs used, study design, and sample size. They also argued for a longitudinal study linking haemoglobin values during pregnancy to longer term maternal and child outcomes, given the paucity of currently available data.<sup>10</sup>

The prospective, observational, multinational, INTERBIO-21st fetal study is such a study.<sup>19</sup> The longitudinal haemoglobin values were obtained from a cohort of pregnant women recruited in maternity units in six countries who initiated antenatal care before 14 weeks' gestation and were seen every 5±1 weeks until delivery. Thereafter, the infants' health, growth, and development were monitored up to 2 years of age. Using these prospective multinational haemoglobin data during pregnancy and linked neonatal outcome data, we aimed to study how different maternal haemoglobin concentrations are associated with the risk of some adverse maternal and neonatal outcomes.<sup>19,20</sup>

## Methods

### Study design and participants

The prospective, observational, INTERBIO-21st fetal study was conducted between Feb 8, 2012, and Nov 30, 2019 at six cities: Pelotas (Brazil), Nairobi (Kenya), Karachi

(Pakistan), Soweto (South Africa), Mae Sot (Thailand), and Oxford (UK). Detailed information about each study site has already been published.<sup>19,20</sup>

We enrolled 3598 women who initiated antenatal care before 14 weeks' gestation, as determined by ultrasound dating,<sup>21</sup> irrespective of their pregnancy risk profile for adverse maternal or neonatal outcomes. 3446 (96%) women were monitored throughout pregnancy to delivery. The health, growth, and development of their children were then monitored up until 2 years of age. The only inclusion criteria were maternal age (of at least 18 years), BMI of less than 35 kg/m<sup>2</sup>, natural conception, and singleton pregnancy. After the dating ultrasound scan, the women were seen every 5±1 weeks until delivery.

The INTERBIO-21st fetal study and its ancillary studies were approved by the Oxfordshire Research Ethics Committee C (reference 08/H0606/139), the research ethics committees of the individual participating institutions, as well as the corresponding regional health authorities where the project was implemented. All participants provided written informed consent for the use of their clinical data.

### Procedures

Data collection relating to pregnancy in the INTERBIO-21st fetal study followed the same strategy as in the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project.<sup>22</sup> In brief, a comprehensive set of variables was obtained prospectively using data collection forms and an electronic data entry system specifically developed for the study. Baseline information

For more on the **INTERBIO-21st Study** see [www.interbio21.org.uk](http://www.interbio21.org.uk)

For more on the **INTERGROWTH-21st Project** see <https://www.intergrowth21.org.uk/>

included demographic and nutritional characteristics, medical, gynaecological, and obstetric history, and current pregnancy-related conditions. Pregnancy follow-up information included routine standard antenatal care variables, current health, use of nutritional supplements or medication, and referral to another level of care or hospital. Maternal morbidity was recorded, including diagnoses such as gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, and preterm labour.

The haemoglobin tests were taken as part of routine antenatal care and so only the results of those tests performed were available for analysis. Information on the reported use of preventive or therapeutic iron, calcium, and folic acid supplements was collected from the medical records. Although study sites were not asked to follow a specific protocol, we carefully documented the biochemical methods of haemoglobin determination used. All sites assessed haemoglobin from venous blood samples using various commercially available and reliable equipment,<sup>23</sup> commonly used in routine patient care (eg, automatised colorimetry, automatised turbidimetry, high-efficiency liquid chromatography autoanalyser [Sysmex Corporation, Kobe, Japan], automated flow fluorescent analyser, photometric method using automated cell counter, and cyanide-free sodium lauryl sulphate). No adjustment was made for the different methods in the analyses.

### Outcomes

Maternal outcomes consisted of pregnancy-induced hypertension, defined as blood pressure more than 140/90 mm Hg without proteinuria; gestational diabetes, defined as any degree of glucose intolerance with onset or first recognition during pregnancy; and preterm premature rupture of membranes (PPROM). Outcomes such as maternal admission to intensive care ( $n=42$ ), need for maternal blood transfusion ( $n=8$ ), and pre-eclampsia, defined as blood pressure more than 140/90 mm Hg with proteinuria ( $n=46$ ) could not be evaluated because there were scarce events reported.

Neonatal outcomes consisted of preterm birth, defined as live birth before 37 weeks' gestation; acute respiratory distress syndrome, defined as neonate demonstrating clinical features with abnormal chest x-ray that required oxygen at 6 h of life and continued need of respiratory support and surfactant therapy within first days of life; large for gestational age, defined as birthweight more than the 90th percentile of the INTERGROWTH-21st newborn size by gestational age and sex standard, and SGA, defined as birthweight less than the 10th percentile of the INTERGROWTH-21st newborn size by gestational age and sex standard.<sup>24</sup> Birthweight was ideally obtained within 12 h of birth (and no later than 24 h), using an electronic scale (Seca, Hamburg, Germany) at all sites (sensitivity of 10 g up to 20 kg). Neonatal death at discharge was not evaluated because the few events reported in the sites ( $n=20$ ).

### Statistical analysis

We did not perform a formal sample size calculation but our large sample size and longitudinal study design allowed us to explore several maternal and neonatal outcomes that are associated with maternal haemoglobin during pregnancy. Descriptive analyses were used to summarise maternal demographic characteristics, previous pregnancy outcomes, reproductive history, and characteristics of present pregnancy using frequencies and percentages for categorical data and mean (SD) or median (IQR) for continuous variables, as appropriate.

We adopted the quantitative approaches used in non-inferiority trials to determine whether the predicted risk associated with a particular haemoglobin value was not meaningfully higher than that associated with a reference haemoglobin cutoff (ie, haemoglobin value of 110 g/L or 105 g/L). We identified haemoglobin concentrations from the gestational maternal haemoglobin continuum where risks become meaningfully increased compared with the nadir of risk (ie, the maternal haemoglobin value at which risks of adverse outcomes are lowest). All analyses were adjusted for relevant maternal factors, such as maternal age, maternal BMI, nulliparity, previous caesarean section, malaria, HIV, history of hypertension, history of diabetes, history of miscarriages, and pregnancy termination. We implemented this approach in three steps using maternal haemoglobin values. First, we defined a reference maternal haemoglobin threshold to represent the nadir of risk using a maternal haemoglobin value of 110 g/L, which is the WHO and CDC threshold for defining anaemia in the first and third trimesters of pregnancy (and a 105 g/L threshold for defining anaemia in the second trimester in sensitivity analyses).<sup>8,9</sup> Second, we quantified the predicted risk of adverse maternal and neonatal outcomes as a function of continuous maternal haemoglobin-modelled maternal haemoglobin using a generalised linear model (binomial distribution) with a log-link to describe predicted risks at a given haemoglobin value; and finally, we used the regression equation from the aforementioned second step to compute relative risks and associated 95% CIs by comparing the predicted relative risk at the reference maternal haemoglobin threshold of 110 g/L to the predicted risk below and higher than the referent haemoglobin value of 110 g/L (a referent haemoglobin value of 105 g/L was also performed as part of sensitivity analyses). To ensure robustness of the results, we performed several sensitivity analyses: (1) analysis excluding each study site at a time to explore the effect of the study site on the results; (2) analysis for country-specific models to explore whether similar patterns were consistent across all countries and determine whether there were country-specific influences on the overall analysis; (3) analysis accounting for altitude of the study sites to investigate its impact on the association between haemoglobin concentration and adverse maternal and neonatal health outcomes—of the five study sites, three

were at low altitudes (Pelotas, Brazil [7m]; Karachi, Pakistan [10m]; Oxford, UK [61m]) whereas two were at high altitudes (Nairobi, Kenya [1795m]; Soweto, South Africa [1632m]); (4) analysis excluding maternal comorbidities: cancer, thalassaemia, and sickle cell disease, as well as HIV, to evaluate their impact on the overall results; (5) analysis to investigate the impact of multiple haemoglobin measures on the results (ie, by excluding multiple haemoglobin measures per woman); and (6) analysis adjusting for iron supplementation during pregnancy.

As the relation between maternal haemoglobin and pregnancy outcomes follows a non-linear shape in risk,<sup>10</sup> we used restricted cubic splines as they are a flexible, non-linear approach. We used five knots (default knot locations corresponding to the 5th, 27·5th, 50th, 72·5th and 95th centile positions) for each of the models. Separate models were fitted for maternal haemoglobin measurements taken in the second and third trimesters of pregnancy to explore the influence of timing of haemoglobin measurement on maternal and neonatal outcomes. We could not fit a separate model for the first trimester of pregnancy due to an insufficient number of haemoglobin measures ( $n < 30$ ).

All analyses were performed using R (version 4.3.0) and STATA (version 15.1).

### Role of the funding source

The INTERBIO-21st fetal study sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

### Results

At clinics in Mae Sot, Thailand, haematocrit was measured instead of haemoglobin concentrations. Therefore, all 578 women at this site were excluded, and the present analyses are thus based on 2069 women who had at least one haemoglobin concentration measured at 14–40 weeks' gestation (figure 1). The 2069 participants contributed 4690 haemoglobin measures with a median of three haemoglobin measures per woman. Each site's contribution to the current analyses ranged from 10·5% (Pelotas,  $n=217$ ) to 30·6% (Soweto,  $n=633$ ). The other sites contributed 22·8% (Oxford,  $n=472$ ), 15·0% (Karachi,  $n=311$ ), and 21·1% (Nairobi,  $n=436$ ), respectively (appendix p 8).

Of the 2069 women enrolled between 9 weeks' gestation and before 14 weeks' gestation from Feb 8, 2012, to Nov 30, 2019, 1071 (51·8%) had a BMI of 25 kg/m<sup>2</sup> or higher and were considered to have overweight (25·0–29·9 kg/m<sup>2</sup>) or obesity ( $\geq 30$  kg/m<sup>2</sup>); although an inclusion criterion was BMI  $< 35$  kg/m<sup>2</sup>, 944 (45·6%) had normal weight (18·5–24·9 kg/m<sup>2</sup>), and 54 (2·6%) had underweight ( $< 18$ ·5 kg/m<sup>2</sup>). In 2069 women, the mean age was 30·7 years (SD 5·0); 1605 (77·6%) were married or cohabiting, 76 (3·7%) had either primary education or no education, compared with 856 (41·4%) who

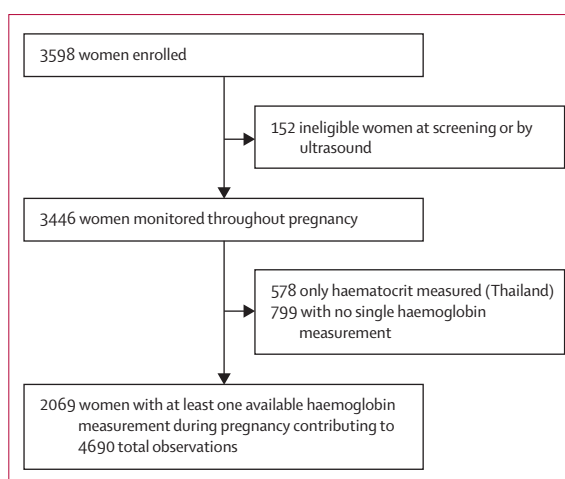


Figure 1: Flow chart of participants in the study

had at least a university education, and 565 (27·3%) were nulliparous. The median gestational age at delivery was 39·1 weeks (IQR 38·0–40·3). The median number of haemoglobin measurements per woman was three (IQR 2–5) and the median haemoglobin value was 120 g/L (IQR 111–129; table 1). The distribution and summary of maternal haemoglobin values according to gestational age and study site are shown in the appendix (pp 8–9).

The total prevalence of caesarean section was 44·5% ( $n=921$ ) and was highest in Pelotas (75·6%), Karachi (59·2%), and Soweto (53·4%), and lowest in Oxford (17·8%), and Nairobi (34·6%). The total prevalence of preterm labour was 3·1% ( $n=64$ ), pregnancy-induced hypertension 5·8% ( $n=120$ ), SGA 11·6% ( $n=239$ ), preterm birth 13·4% ( $n=277$ ), PPRM 3·6% ( $n=74$ ), and large for gestational age 11·7% ( $n=243$ ). 89 (4·3%) newborns had acute respiratory distress syndrome and 224 (10·8%) were admitted to intensive care (table 2). The women's medical conditions and obstetric history are summarised in the appendix (p 5).

The distribution of maternal haemoglobin for those women who reported use of iron (1254 [60·6%]; median haemoglobin concentration 118 g/L [IQR 107–129]), calcium (693 [33·5%]; median haemoglobin concentration 118 g/L [107–128]), folic acid (1543 [74·6%]; median haemoglobin concentration 119 g/L [108–129]); both iron and folic acid (1081; [52·3%]; median haemoglobin concentration 118 g/L [108–128]); iron, folic acid and calcium (500 [24·2%]; median haemoglobin concentration 118 g/L [107–128]); iron or calcium or folic acid (1753 [84·7%]; median haemoglobin concentration 119 g/L [108–128]), or any supplementation (1964 [94·9%]; median haemoglobin concentration 119 g/L [109–128]) obtained from the medical records are shown in the appendix (pp 6, 10). Over 90% of study participants in Soweto, Pelotas, and Karachi reported use of iron or folic acid supplements compared with 75% in Nairobi and 62% in Oxford. Less than half of all participants from

See Online for appendix

|  | Pelotas, Brazil<br>(n=217) | Nairobi, Kenya<br>(n=436) | Karachi,<br>Pakistan<br>(n=311) | Soweto, South<br>Africa (n=633) | Oxford, UK<br>(n=472) | Total (n=2069) |
|--|----------------------------|---------------------------|---------------------------------|---------------------------------|-----------------------|----------------|
| Maternal age, years                          | 28.9 (5.4)                 | 30.7 (4.1)                | 30.3 (4.5)                      | 31.0 (5.8)                      | 31.2 (4.7)            | 30.7 (5.0)     |
| Marital status                               |                            |                           |                                 |                                 |                       |                |
| Married or cohabiting                        | 197 (90.8%)                | 392 (89.9%)               | 310 (99.7%)                     | 255 (40.3%)                     | 451 (95.6%)           | 1605 (77.6%)   |
| Separated or divorced                        | 2 (0.9%)                   | 0 (0%)                    | 1 (0.3%)                        | 2 (0.3%)                        | 1 (0.2%)              | 6 (0.3%)       |
| Single                                       | 18 (8.3%)                  | 44 (10.1%)                | 0 (0%)                          | 376 (59.4%)                     | 20 (4.2%)             | 458 (22.1%)    |
| Educational level                            |                            |                           |                                 |                                 |                       |                |
| No school attended                           | 0 (0%)                     | 0 (0%)                    | 4 (1.3%)                        | 1 (0.2%)                        | 0 (0%)                | 5 (0.2%)       |
| Primary                                      | 39 (18.0%)                 | 2 (0.5%)                  | 11 (3.5%)                       | 19 (3.0%)                       | 0 (0%)                | 71 (3.4%)      |
| Professional or technical training           | 12 (5.5%)                  | 153 (35.1%)               | 12 (3.9%)                       | 103 (16.3%)                     | 88 (18.6%)            | 368 (17.8%)    |
| Secondary                                    | 87 (40.1%)                 | 5 (1.1%)                  | 51 (16.4%)                      | 464 (73.3%)                     | 162 (34.3%)           | 769 (37.2%)    |
| University                                   | 79 (36.4%)                 | 276 (63.3%)               | 233 (74.9%)                     | 46 (7.3%)                       | 222 (47.0%)           | 856 (41.4%)    |
| Occupational status                          |                            |                           |                                 |                                 |                       |                |
| Clerical support, service, or Sales          | 60 (27.6%)                 | 47 (10.8%)                | 0 (0%)                          | 88 (13.9%)                      | 138 (29.2%)           | 333 (16.1%)    |
| Managerial, professional, or technical       | 45 (20.7%)                 | 335 (76.8%)               | 72 (23.2%)                      | 18 (2.8%)                       | 233 (49.4%)           | 703 (34.0%)    |
| Skilled manual work                          | 10 (4.6%)                  | 8 (1.8%)                  | 29 (9.3%)                       | 52 (8.2%)                       | 4 (0.8%)              | 103 (5.0%)     |
| Unskilled manual work                        | 2 (0.9%)                   | 0 (0%)                    | 2 (0.6%)                        | 67 (10.6%)                      | 14 (3.0%)             | 85 (4.1%)      |
| Housework                                    | 63 (29.0%)                 | 15 (3.4%)                 | 201 (64.6%)                     | 69 (10.9%)                      | 73 (15.5%)            | 421 (20.3%)    |
| Student                                      | 10 (4.6%)                  | 23 (5.3%)                 | 4 (1.3%)                        | 23 (3.6%)                       | 10 (2.1%)             | 70 (3.4%)      |
| Other  | 27 (12.4%)                 | 8 (1.8%)                  | 3 (1.0%)                        | 316 (49.9%)                     | 0 (0%)                | 354 (17.1%)    |
| Number of previous pregnancies               |                            |                           |                                 |                                 |                       |                |
| 0  | 124 (57.1%)                | 179 (41.1%)               | 76 (24.4%)                      | 38 (6.0%)                       | 148 (31.4%)           | 565 (27.3%)    |
| 1  | 65 (30.0%)                 | 144 (33.0%)               | 88 (28.3%)                      | 172 (27.2%)                     | 176 (37.3%)           | 645 (31.2%)    |
| 2  | 17 (7.8%)                  | 75 (17.2%)                | 58 (18.6%)                      | 186 (29.4%)                     | 74 (15.7%)            | 410 (19.8%)    |
| ≥3   | 11 (5.1%)                  | 32 (7.3%)                 | 83 (26.7%)                      | 214 (33.8%)                     | 72 (15.3%)            | 412 (19.9%)    |
| Missing                                      | 0 (0%)                     | 6 (1.4%)                  | 6 (1.9%)                        | 23 (3.6%)                       | 2 (0.4%)              | 37 (1.8%)      |
| Diastolic blood pressure, mm Hg              | 116 (11.7)                 | 110 (10.4)                | 108 (10.5)                      | 114 (13.2)                      | 116 (12.3)            | 113 (12.3)     |
| Systolic blood pressure, mm Hg               | 73.0 (8.44)                | 70.0 (7.93)               | 69.8 (7.71)                     | 72.2 (10.6)                     | 71.3 (9.14)           | 71.2 (9.19)    |
| BMI  |                            |                           |                                 |                                 |                       |                |
| Normal weight (18.5–24.9 kg/m <sup>2</sup> ) | 107 (49.3%)                | 197 (45.2%)               | 143 (46.0%)                     | 221 (34.9%)                     | 276 (58.5%)           | 944 (45.6%)    |
| Underweight (<18.5 kg/m <sup>2</sup> )       | 4 (1.8%)                   | 9 (2.1%)                  | 15 (4.8%)                       | 14 (2.2%)                       | 12 (2.5%)             | 54 (2.6%)      |
| Overweight (25.0–29.9 kg/m <sup>2</sup> )    | 77 (35.5%)                 | 159 (36.5%)               | 102 (32.8%)                     | 239 (37.8%)                     | 133 (28.2%)           | 710 (34.3%)    |
| Obese (≥30.0 kg/m <sup>2</sup> )             | 29 (13.4%)                 | 71 (16.3%)                | 51 (16.4%)                      | 159 (25.1%)                     | 51 (10.8%)            | 361 (17.4%)    |
| Haemoglobin concentration, g/L               | 121 (114–128)              | 125 (116–133)             | 110 (102–119)                   | 123 (114–135)                   | 119 (112–126)         | 120 (111–129)  |
| Data are mean (SD), n (%), or median (IQR).  |                            |                           |                                 |                                 |                       |                |

**Table 1:** Baseline sociodemographic characteristics of the women (n=2069) in the INTERBIO-21st fetal study who contributed data to the present analyses

Oxford (34%) and Nairobi (44%) reported use of iron during their pregnancy (appendix p 6).

The risk of preterm birth (RR 1.54 [95% CI 1.15–2.07]; 277 participants; five sites) and acute respiratory distress syndrome (1.68 [1.01–2.80]; 89 participants; five sites) increased by more than 50% for haemoglobin concentrations of 85 g/L and the risk was approximately two-fold higher for haemoglobin concentrations of 70 g/L for preterm birth (2.04 [1.20–3.48]; 277 participants; five sites) when compared to the reference haemoglobin of 110 g/L (figure 2; table 3). Similarly, haemoglobin concentrations of 145 g/L for preterm birth (RR 1.31 [95% CI 1.05–1.62]; 277 participants; five sites) and acute respiratory distress syndrome (1.63 [1.15–2.32]; 89 participants; five sites) were also associated with

increased risk and there was more than a two-fold increased risk for haemoglobin concentrations of 165 g/L for preterm birth (2.06 [1.41–3.02]; 277 participants; five sites) and acute respiratory distress syndrome (2.84 [1.51–5.35]; 89 participants; five sites) when compared with the reference haemoglobin of 110 g/L. Risks of preterm birth and acute respiratory distress syndrome followed a U-shape continuously across gestational ages and higher risks were observed higher or lower than the reference haemoglobin value of 110 g/L. For SGA, we did not observe any significant association with haemoglobin concentrations.

In the second trimester, compared with the haemoglobin cutoff of 110 g/L, haemoglobin concentrations below 105 g/L were not associated with an



|   | Pelotas, Brazil<br>(n=217) | Nairobi,<br>Kenya<br>(n=436) | Karachi,<br>Pakistan<br>(n=311) | Soweto,<br>South Africa<br>(n=633) | Oxford, UK<br>(n=472) | Total<br>(n=2069) |
|---|----------------------------|------------------------------|---------------------------------|------------------------------------|-----------------------|-------------------|
| <b>Neonatal outcomes</b>                          |                            |                              |                                 |                                    |                       |                   |
| Birthweight, g                                    | 3120 (474)                 | 3280 (462)                   | 2870 (493)                      | 2910 (627)                         | 3350 (521)            | 3110 (570)        |
| Birthweight <2500 g (low birthweight)             | 22 (10.1%)                 | 15 (3.4%)                    | 49 (15.8%)                      | 115 (18.2%)                        | 25 (5.3%)             | 226 (10.9%)       |
| Birthweight >4000 g                               | 3 (1.4%)                   | 23 (5.3%)                    | 0 (0%)                          | 16 (2.5%)                          | 41 (8.7%)             | 83 (4.0%)         |
| Gestational age                                   | 38.5 (2.07)                | 39.8 (1.79)                  | 37.5 (3.04)                     | 37.5 (4.43)                        | 39.7 (1.66)           | 38.6 (3.19)       |
| Preterm birth (gestational age <37 weeks)         | 27 (12.4%)                 | 19 (4.4%)                    | 74 (23.8%)                      | 133 (21.0%)                        | 24 (5.1%)             | 277 (13.4%)       |
| Newborn admission to intensive care               | 22 (10.1%)                 | 30 (6.9%)                    | 23 (7.4%)                       | 110 (17.4%)                        | 39 (8.3%)             | 224 (10.8%)       |
| Acute respiratory distress syndrome               | 5 (2.3%)                   | 18 (4.1%)                    | 9 (2.9%)                        | 42 (6.6%)                          | 15 (3.2%)             | 89 (4.3%)         |
| Small for gestational age                         | 18 (8.3%)                  | 44 (10.1%)                   | 38 (12.2%)                      | 107 (16.9%)                        | 32 (6.8%)             | 239 (11.6%)       |
| Large for gestational age                         | 14 (6.5%)                  | 43 (9.9%)                    | 20 (6.4%)                       | 106 (16.8%)                        | 60 (12.7%)            | 243 (11.7%)       |
| <b>Maternal outcomes</b>                          |                            |                              |                                 |                                    |                       |                   |
| Pregnancy-induced hypertension                    | 16 (7.4%)                  | 7 (1.6%)                     | 23 (7.4%)                       | 38 (6.0%)                          | 36 (7.6%)             | 120 (5.8%)        |
| Gestational diabetes                              | 17 (7.8%)                  | 11 (2.5%)                    | 77 (24.8%)                      | 8 (1.3%)                           | 13 (2.8%)             | 126 (6.1%)        |
| Pre-eclampsia                                     | 0 (0%)                     | 5 (1.1%)                     | 11 (3.5%)                       | 8 (1.3%)                           | 7 (1.5%)              | 31 (1.5%)         |
| Preterm premature rupture of membranes            | 16 (7.4%)                  | 22 (5.0%)                    | 3 (1.0%)                        | 13 (2.1%)                          | 20 (4.2%)             | 74 (3.6%)         |
| Preterm labour (labour <37 weeks gestational age) | 32 (14.7%)                 | 4 (0.9%)                     | 12 (3.9%)                       | 5 (0.8%)                           | 11 (2.3%)             | 64 (3.1%)         |
| <b>Mode of delivery</b>                           |                            |                              |                                 |                                    |                       |                   |
| Vaginal spontaneous                               | 50 (23.0%)                 | 269 (61.7%)                  | 111 (35.7%)                     | 270 (42.7%)                        | 301 (63.8%)           | 1001 (48.3%)      |
| Caesarean   | 164 (75.6%)                | 151 (34.6%)                  | 184 (59.2%)                     | 338 (53.4%)                        | 84 (17.8%)            | 921 (44.5%)       |
| Vaginal assisted                                  | 2 (0.9%)                   | 10 (2.3%)                    | 7 (2.3%)                        | 2 (0.3%)                           | 84 (17.8%)            | 105 (5.1%)        |
| Assisted breech or breech extraction              | 0 (0%)                     | 0 (0%)                       | 0 (0%)                          | 0 (0%)                             | 1 (0.2%)              | 1 (0.1%)          |

Data are mean (SD) or n (%).

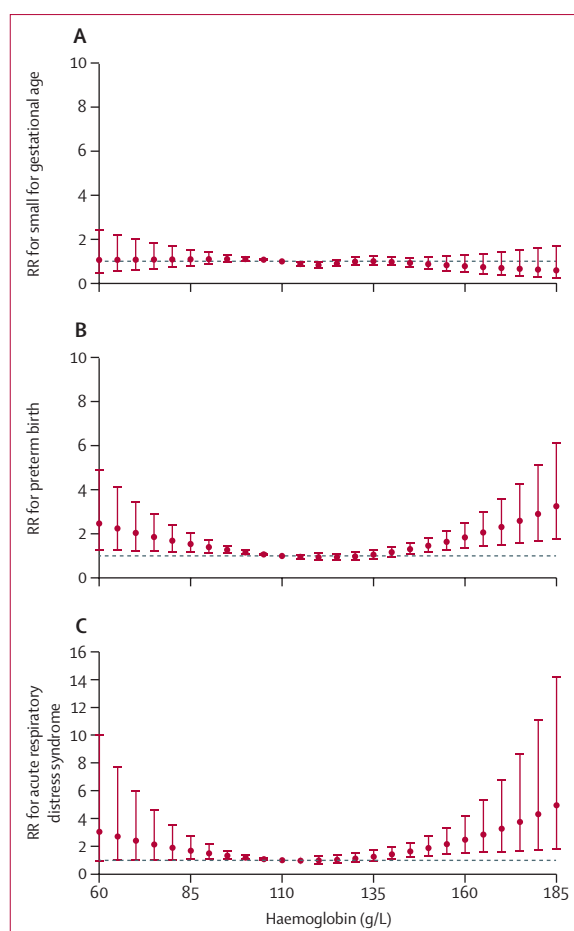
**Table 2: Maternal and neonatal outcomes of the women (n=2069) in the INTERBIO-21st fetal study who contributed data to the present analyses**

increased risk of SGA or acute respiratory distress syndrome and a haemoglobin concentration less than 95 g/L was not significantly associated with an increased risk of preterm birth. However, the risk of preterm birth increased by more than 70% for haemoglobin concentrations of 165 g/L (RR 1.71 [95% CI 1.02–2.85]; 225 participants; five sites; figure 3, table 3). In the third trimester, the risk of preterm birth (RR 1.68 [95% CI 1.15–2.44]; 51 participants; five sites) and acute respiratory distress syndrome (1.83 [1.02–3.29]; 22 participants; five sites) increased by more than 65% for haemoglobin concentrations of 85 g/L, and there was a more than two-fold increased risk for preterm birth (2.21 [1.23–3.96]; 51 participants; five sites) and acute respiratory distress syndrome (2.67 [1.06–6.74]; 22 participants; five sites) when haemoglobin concentrations were 75 g/L (figure 3, table 3) when compared with the reference haemoglobin of 110 g/L. This increase in risk is also observed for lower haemoglobin concentrations.

Overall, there was more than a 50% higher risk of pregnancy-induced hypertension for haemoglobin concentrations higher than 160 g/L (RR 1.67 [95% CI 1.04–2.67]; 120 participants; five sites) and the risk was more than two-fold for haemoglobin concentrations higher than 170 g/L (2.29 [1.19–4.39]; 120 participants; five sites). There was a reduced risk of pregnancy-induced hypertension at a haemoglobin concentration of

105 g/L (RR 0.88 [95% CI 0.80–0.96]; 120 participants; five sites), and in the third trimester (0.76 [0.63–0.93]; 38 participants; five sites). There was an overall reduction of the risk of gestational diabetes at a haemoglobin concentration of 120 g/L (RR 0.66 [95% CI 0.49–0.90]; 126 participants; five sites) and in the third trimester (0.65 [0.47–0.89]; 48 participants; five sites) and 125 g/L in the second trimester (0.56 [0.32–0.98]; 78 participants; five sites). There was no overall statistically significant association for PPRM, but in the second trimester the risk of PPRM increased by more than two-fold at a haemoglobin concentration of 160 g/L (2.83 [1.02–7.81]; 45 participants; five sites).

The association based on study site altitude is presented in the appendix (p 12). Overall, we observed a similar association pattern between maternal haemoglobin levels and adverse health outcomes in the entire sample and across high-altitude and low-altitude study sites. However, we found that in low-altitude sites, there was a higher risk ratio for adverse health outcomes, such as pregnancy-induced hypertension (haemoglobin above 125 g/L), preterm birth (haemoglobin above 140 g/L), and acute respiratory distress syndrome (haemoglobin above 140 g/L), with increased haemoglobin levels compared with the overall sample and high-altitude study sites. Furthermore, the site-specific sensitivity analysis showed similar trends in maternal and neonatal outcomes (appendix pp 11, 16).



**Figure 2: Association between overall haemoglobin concentration in pregnancy and neonatal outcomes**  
Risk ratios (RRs) with 95% CIs for neonatal outcomes: (A) small for gestational age, (B) preterm births, and (C) acute respiratory distress syndrome by overall recorded haemoglobin concentrations in pregnancy.

We also conducted an analysis excluding comorbidities such as cancer, thalassaemia, sickle cell disease, and HIV, and found minimal changes to the overall results (appendix pp 13–14). Additionally, adjusting for iron supplementation did not alter the results (appendix p 17). To address the potential bias from not considering multiple measures, we performed a sensitivity analysis. In this analysis, we randomly selected one haemoglobin measure per woman and used this cross-sectional dataset of haemoglobin measures to conduct key analyses. We compared risk ratio trends of pregnancy-induced hypertension, preterm birth, and acute respiratory distress syndrome, based on one haemoglobin measure per woman and multiple haemoglobin measurements, and found similar trends (appendix p 15).

## Discussion

Our analytical approach compared predicted risks of a given haemoglobin concentration with a reference haemoglobin value of 110 g/L<sup>25</sup>—the threshold for defining

anaemia in the first and third trimesters of pregnancy according to the WHO and CDC guidelines.<sup>8,9</sup> We used the recommendations set by WHO as they are in current use in most countries. WHO is in the process of reviewing the existing haemoglobin thresholds for different groups and populations,<sup>4</sup> as part of this process, we firmly believe that the findings from this work will make a valuable contribution to these ongoing discussions, particularly in determining whether the current WHO haemoglobin thresholds for pregnant women require revision.

Our findings suggest an association between maternal haemoglobin and both maternal and neonatal adverse outcomes. We found an increased risk for preterm birth and acute respiratory distress syndrome associated with maternal haemoglobin values and doubling of risk for haemoglobin values less than 70 g/L when compared to a reference value of 110 g/L. We have further showed a U-shaped relationship between maternal haemoglobin and preterm birth and acute respiratory distress syndrome: the risks increase for women with haemoglobin values less than 100 g/L or more than 145 g/L. For SGA, we did not observe any significant association with haemoglobin values. The increase in risk for preterm birth and acute respiratory distress syndrome differed by trimester: this finding for preterm birth was only in the second trimester, and for acute respiratory distress syndrome and preterm birth it was in the third trimester of pregnancy. For maternal outcomes, only pregnancy-induced hypertension was shown to be associated with maternal haemoglobin and the risk was more than two-fold for a haemoglobin value more than 170 g/L and in the third trimester. However, the risk of gestational diabetes was reduced for women with haemoglobin values less than 125 g/L, which was also observed in second and third trimesters. We observed more than a two-fold increased risk for PPROM only in the second trimester for a Hb value more than 160 g/L.

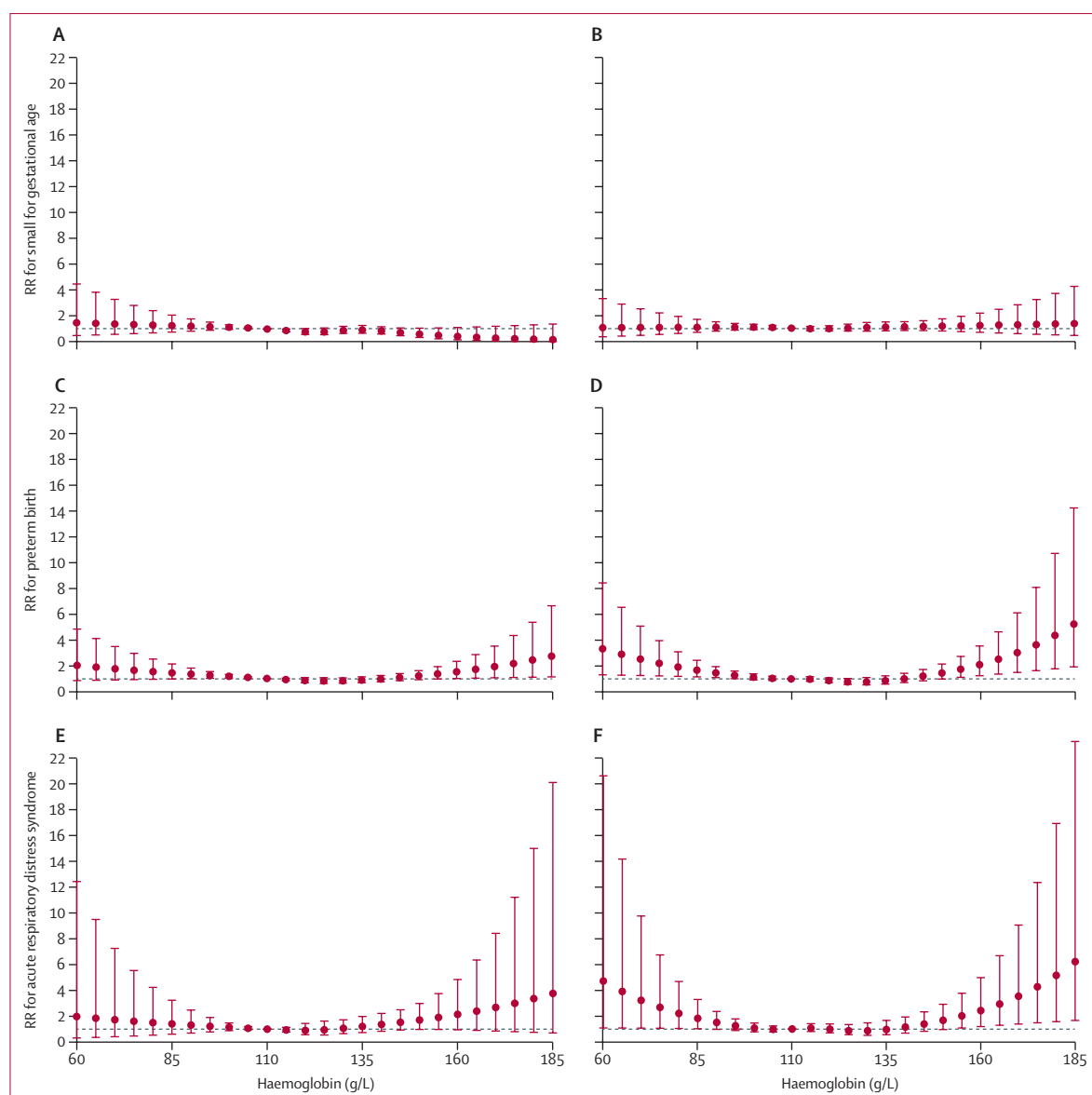
Our results are similar to those of Melissa F Young and colleagues<sup>10</sup> who showed in a meta-analysis that anaemia in pregnancy (haemoglobin <110 g/L) was associated with SGA (increased odds of 1.13-fold for haemoglobin ≤100 g/L and 1.29 for haemoglobin ≤70 g/L). In our study, the increased risk ratios for SGA were 1.11 for haemoglobin concentration of 100 g/L and 1.07 for haemoglobin concentration of 70 g/L. For preterm birth, Young and colleagues found increased odds of 1.47-fold for haemoglobin less than 100 g/L and 3.72 for haemoglobin less than or equal to 70 g/L, compared with 1.16 at haemoglobin of 100 g/L and 2.04 at haemoglobin of 70 g/L increased risk in our study. We did not find a significant association between SGA and haemoglobin values less than 70 g/L, perhaps because of a smaller sample size for haemoglobin values less than 70 g/L. Another study by Kathryn G Dewey and colleagues<sup>26</sup> confirmed a U-shaped curve for the risk of adverse birth outcomes with maternal haemoglobin values. Pre-conceptual and

|                     | Overall                    |   |   | Second trimester           |   |   | Third trimester           |  |   |
|---------------------|----------------------------|---|---|----------------------------|---|---|---------------------------|--|---|
|                     | SGA (n=239)<br>RR (95% CI) | Preterm birth<br>(n=277) RR<br>(95% CI) | Acute respiratory distress<br>syndrome<br>(n=89) RR<br>(95% CI) | SGA (n=177)<br>RR (95% CI) | Preterm birth<br>(n=225) RR<br>(95% CI) | Acute respiratory distress<br>syndrome<br>(n=67) RR<br>(95% CI) | SGA (n=62)<br>RR (95% CI) | Preterm birth<br>(n=51) RR<br>(95% CI) | Acute respiratory distress<br>syndrome<br>(n=22) RR<br>(95% CI) |
| Haemoglobin 60 g/L  | 1.06<br>(0.46–2.45)        | 2.47<br>(1.23–4.94)                     | 3.04<br>(0.92–10.04)  | 1.49<br>(0.49–4.49)        | 2.01<br>(0.84–4.83)                     | 1.98<br>(0.32–12.43)  | 1.04<br>(0.33–3.29)       | 3.33<br>(1.31–8.44)                    | 4.72<br>(1.08–20.64)  |
| Haemoglobin 65 g/L  | 1.07<br>(0.51–2.24)        | 2.25<br>(1.22–4.14)                     | 2.70<br>(0.94–7.77)   | 1.44<br>(0.54–3.85)        | 1.88<br>(0.86–4.09)                     | 1.85<br>(0.36–9.50)   | 1.04<br>(0.38–2.86)       | 2.90<br>(1.29–6.55)                    | 3.90<br>(1.07–14.18)  |
| Haemoglobin 70 g/L  | 1.07<br>(0.56–2.04)        | 2.04<br>(1.20–3.48)                     | 2.40<br>(0.96–6.01)   | 1.39<br>(0.59–3.30)        | 1.75<br>(0.88–3.47)                     | 1.73<br>(0.41–7.25)   | 1.05<br>(0.44–2.50)       | 2.53<br>(1.26–5.09)                    | 3.23<br>(1.07–9.76)   |
| Haemoglobin 75 g/L  | 1.08<br>(0.63–1.87)        | 1.86<br>(1.18–2.92)                     | 2.13<br>(0.98–4.65)   | 1.34<br>(0.64–2.83)        | 1.64<br>(0.91–2.95)                     | 1.61<br>(0.47–5.54)   | 1.05<br>(0.51–2.18)       | 2.21<br>(1.23–3.96)                    | 2.67<br>(1.06–6.74)   |
| Haemoglobin 80 g/L  | 1.09<br>(0.69–1.71)        | 1.69<br>(1.16–2.46)                     | 1.90<br>(1.00–3.60)   | 1.30<br>(0.70–2.42)        | 1.53<br>(0.93–2.50)                     | 1.50<br>(0.53–4.24)   | 1.06<br>(0.59–1.91)       | 1.92<br>(1.19–3.10)                    | 2.21<br>(1.04–4.68)   |
| Haemoglobin 85 g/L  | 1.09<br>(0.76–1.56)        | 1.54<br>(1.15–2.07)                     | 1.68<br>(1.01–2.80)   | 1.26<br>(0.76–2.08)        | 1.43<br>(0.96–2.12)                     | 1.40<br>(0.61–3.24)   | 1.06<br>(0.67–1.68)       | 1.68<br>(1.15–2.44)                    | 1.83<br>(1.02–3.29)   |
| Haemoglobin 90 g/L  | 1.10<br>(0.84–1.43)        | 1.40<br>(1.12–1.74)                     | 1.50<br>(1.03–2.18)   | 1.22<br>(0.83–1.78)        | 1.33<br>(0.98–1.80)                     | 1.31<br>(0.69–2.48)   | 1.07<br>(0.76–1.49)       | 1.46<br>(1.10–1.95)                    | 1.51<br>(0.97–2.36)   |
| Haemoglobin 95 g/L  | 1.11<br>(0.93–1.32)        | 1.27<br>(1.10–1.48)                     | 1.33<br>(1.03–1.71)   | 1.18<br>(0.90–1.53)        | 1.24<br>(1.01–1.53)                     | 1.22<br>(0.79–1.90)   | 1.07<br>(0.84–1.36)       | 1.28<br>(1.01–1.61)                    | 1.25<br>(0.88–1.78)   |
| Haemoglobin 100 g/L | 1.11<br>(0.99–1.23)        | 1.16<br>(1.05–1.28)                     | 1.18<br>(1.01–1.39)   | 1.14<br>(0.97–1.33)        | 1.16<br>(1.03–1.31)                     | 1.14<br>(0.88–1.48)   | 1.07<br>(0.88–1.30)       | 1.13<br>(0.92–1.39)                    | 1.07<br>(0.78–1.46)   |
| Haemoglobin 105 g/L | 1.08<br>(1.01–1.15)        | 1.07<br>(1.00–1.14)                     | 1.07<br>(0.97–1.19)   | 1.08<br>(1.01–1.16)        | 1.08<br>(1.02–1.14)                     | 1.07<br>(0.95–1.20)   | 1.05<br>(0.91–1.21)       | 1.03<br>(0.89–1.21)                    | 0.98<br>(0.77–1.24)   |
| Haemoglobin 110 g/L | 1.00 (ref)                 | 1.00 (ref)                              | 1.00 (ref)  | 1.00 (ref)                 | 1.00 (ref)                              | 1.00 (ref)  | 1.00 (ref)                | 1.00 (ref)                             | 1.00 (ref)  |
| Haemoglobin 115 g/L | 0.88<br>(0.78–1.00)        | 0.96<br>(0.85–1.09)                     | 0.97<br>(0.79–1.19)   | 0.88<br>(0.78–1.00)        | 0.92<br>(0.83–1.01)                     | 0.94<br>(0.78–1.14)   | 0.95<br>(0.80–1.13)       | 0.98<br>(0.82–1.17)                    | 1.07<br>(0.80–1.41)   |
| Haemoglobin 120 g/L | 0.83<br>(0.68–1.02)        | 0.94<br>(0.77–1.15)                     | 0.98<br>(0.70–1.37)   | 0.77<br>(0.58–1.03)        | 0.84<br>(0.66–1.07)                     | 0.9<br>(0.57–1.44)  | 0.96<br>(0.78–1.18)       | 0.87<br>(0.70–1.08)                    | 0.99<br>(0.70–1.39)   |
| Haemoglobin 125 g/L | 0.9<br>(0.74–1.09)         | 0.94<br>(0.78–1.14)                     | 1.03<br>(0.75–1.41)   | 0.78<br>(0.56–1.08)        | 0.80<br>(0.61–1.06)                     | 0.94<br>(0.54–1.63)   | 1.00<br>(0.77–1.31)       | 0.77<br>(0.57–1.03)                    | 0.87<br>(0.56–1.35)   |
| Haemoglobin 130 g/L | 0.99<br>(0.80–1.22)        | 0.98<br>(0.79–1.20)                     | 1.12<br>(0.80–1.57)   | 0.89<br>(0.65–1.20)        | 0.82<br>(0.64–1.04)                     | 1.06<br>(0.65–1.72)   | 1.05<br>(0.76–1.44)       | 0.77<br>(0.53–1.10)                    | 0.86<br>(0.51–1.48)   |
| Haemoglobin 135 g/L | 1.01<br>(0.81–1.26)        | 1.05<br>(0.85–1.32)                     | 1.25<br>(0.87–1.78)   | 0.92<br>(0.67–1.27)        | 0.87<br>(0.67–1.12)                     | 1.21<br>(0.74–1.98)   | 1.08<br>(0.79–1.48)       | 0.85<br>(0.59–1.23)                    | 0.96<br>(0.56–1.67)   |
| Haemoglobin 140 g/L | 0.98<br>(0.78–1.22)        | 1.17<br>(0.94–1.45)                     | 1.42<br>(1.01–2.01)   | 0.84<br>(0.61–1.17)        | 0.96<br>(0.74–1.23)                     | 1.36<br>(0.83–2.22)   | 1.10<br>(0.81–1.50)       | 1.01<br>(0.71–1.43)                    | 1.15<br>(0.68–1.93)   |
| Haemoglobin 145 g/L | 0.93<br>(0.72–1.20)        | 1.31<br>(1.05–1.62)                     | 1.63<br>(1.15–2.32)   | 0.72<br>(0.48–1.08)        | 1.07<br>(0.83–1.38)                     | 1.53<br>(0.93–2.50)   | 1.13<br>(0.81–1.57)       | 1.21<br>(0.85–1.73)                    | 1.38<br>(0.82–2.33)   |
| Haemoglobin 150 g/L | 0.88<br>(0.63–1.22)        | 1.47<br>(1.16–1.85)                     | 1.87<br>(1.27–2.77)   | 0.60<br>(0.34–1.07)        | 1.20<br>(0.90–1.60)                     | 1.71<br>(0.98–2.99)   | 1.15<br>(0.78–1.72)       | 1.46<br>(0.99–2.15)                    | 1.67<br>(0.96–2.92)   |
| Haemoglobin 155 g/L | 0.83<br>(0.54–1.26)        | 1.64<br>(1.25–2.16)                     | 2.15<br>(1.37–3.40)   | 0.51<br>(0.24–1.08)        | 1.35<br>(0.95–1.92)                     | 1.91<br>(0.97–3.75)   | 1.18<br>(0.73–1.91)       | 1.75<br>(1.12–2.74)                    | 2.01<br>(1.08–3.77)   |
| Haemoglobin 160 g/L | 0.78<br>(0.47–1.32)        | 1.84<br>(1.33–2.54)                     | 2.47<br>(1.44–4.24)   | 0.43<br>(0.16–1.12)        | 1.52<br>(0.99–2.33)                     | 2.14<br>(0.95–4.84)   | 1.21<br>(0.67–2.16)       | 2.10<br>(1.24–3.55)                    | 2.43<br>(1.19–4.98)   |
| Haemoglobin 165 g/L | 0.74<br>(0.40–1.38)        | 2.06<br>(1.41–3.02)                     | 2.84<br>(1.51–5.35)   | 0.36<br>(0.11–1.16)        | 1.71<br>(1.02–2.85)                     | 2.40<br>(0.90–6.36)   | 1.23<br>(0.62–2.46)       | 2.52<br>(1.37–4.65)                    | 2.93<br>(1.29–6.69)   |
| Haemoglobin 170 g/L | 0.70<br>(0.34–1.46)        | 2.31<br>(1.49–3.60)                     | 3.27<br>(1.57–6.80)   | 0.30<br>(0.07–1.21)        | 1.92<br>(1.05–3.51)                     | 2.68<br>(0.85–8.42)   | 1.26<br>(0.57–2.81)       | 3.03<br>(1.50–6.12)                    | 3.54<br>(1.38–9.06)   |
| Haemoglobin 175 g/L | 0.66<br>(0.28–1.54)        | 2.59<br>(1.56–4.30)                     | 3.75<br>(1.62–8.67)   | 0.25<br>(0.05–1.26)        | 2.16<br>(1.07–4.33)                     | 3.00<br>(0.80–11.21)  | 1.29<br>(0.52–3.21)       | 3.64<br>(1.63–8.09)                    | 4.27<br>(1.48–12.36)  |
| Haemoglobin 180 g/L | 0.63<br>(0.24–1.62)        | 2.90<br>(1.64–5.14)                     | 4.31<br>(1.67–11.09)  | 0.21<br>(0.03–1.32)        | 2.42<br>(1.10–5.35)                     | 3.36<br>(0.75–15.00)  | 1.32<br>(0.47–3.69)       | 4.37<br>(1.78–10.72)                   | 5.16<br>(1.57–16.94)  |

Data are RR (95% CI). SGA model was adjusted for: maternal age, maternal BMI, nulliparity, previous caesarean section, malaria, HIV, history of hypertension, history of diabetes, history of miscarriages, and termination. Preterm birth model was adjusted for: maternal age, maternal BMI, nulliparity, previous caesarean section, malaria, HIV, history of hypertension, history of diabetes, history of miscarriages, and termination. Acute respiratory distress syndrome model was adjusted for: maternal age, maternal BMI, history of hypertension, and history of diabetes. SGA=small for gestational age. RR=risk ratio.

**Table 3: Neonatal outcomes**





**Figure 3: Association between haemoglobin concentration in second and third trimester of pregnancy and neonatal outcomes**

Risk ratios (RRs) with 95% CIs for small for gestational age, preterm births, and respiratory distress syndrome by recorded haemoglobin concentrations during second trimester (A, C, and E on left) and third trimesters of pregnancy (B, D, and F on right).

first-trimester haemoglobin values are important clinically because they affect the timing and delivery of antenatal care and interventions in pregnancy. The second trimester is of particular importance in reviewing the haemoglobin cutoffs for anaemia, given changes in blood volume and variation in recommendations for haemoglobin cutoffs during this period. Further research is needed to confirm these findings and understand the mechanisms that might underlie differential associations based on the timing of haemoglobin measurement.

The study by Young and colleagues revealed a paucity of data linking anaemia to maternal and long-term child

health outcomes. They therefore suggested that a longitudinal study needed to be performed with haemoglobin values during pregnancy linked to outcomes to fill this gap.<sup>10</sup> The key strength of our study is that we have met that need, confirming and quantifying the association between maternal haemoglobin concentrations and maternal and neonatal health outcomes. We have showed a U-shaped relationship between maternal haemoglobin in pregnancy and neonatal outcomes (ie, both low and high haemoglobin concentrations are associated with adverse outcomes in agreement with previous studies).<sup>26</sup> The lowest rates of low birthweight and premature birth appear to occur when maternal

haemoglobin concentrations are at 95–105 g/L during the second trimester of gestation,<sup>11,12</sup> and at 95–125 g/L at term.<sup>13,14</sup> Haemoglobin concentrations higher than 130 g/L at sea level have also been associated with negative pregnancy outcomes.<sup>11–15</sup> Large epidemiological retrospective studies,<sup>11,12,16</sup> and one prospective study in China,<sup>17</sup> have shown that both low and high haemoglobin concentrations during pregnancy are associated with increased risks for low birthweight and preterm birth. We have also conducted robust and rigorous statistical analyses using continuous values of haemoglobin that enabled assessment of the shape of the relationships to determine possible points of inflection (eg, data-driven cutoffs) and separate analyses by timing of haemoglobin measurement.

Our results are limited to women contributing to these analyses and were from study sites in five geographically diverse countries ranging in altitudes (from 10 m to <1800 m above sea level). We found that in low-altitude sites, with an increased haemoglobin level the risk ratios of adverse health outcomes, such as pregnancy-induced hypertension, preterm birth, and respiratory distress syndrome, were higher compared with the overall sample and high-altitude study sites. However, we were limited by small sample sizes to explore other important outcomes, such as stillbirth, perinatal and neonatal mortality, post-partum haemorrhage, pre-eclampsia, and maternal mortality. We did not have information on other outcomes, such as postnatal depression, nor did we capture symptoms of anaemia, such as fatigue and dyspnoea, which affect a woman's quality of life. Hence, we were unable to establish respective associations between maternal haemoglobin and these outcomes. Another limitation is that it was not possible to analyse the data based on supplementation doses and duration. A Cochrane review on the use of daily iron supplements during pregnancy<sup>27</sup> found that women who received iron were on average likely to have higher haemoglobin levels at term (mean difference 8.88 g/L [95% CI 6.96–10.80]; reported in 19 studies involving 3704 women) and women who received iron were at higher risk of high haemoglobin concentrations at term (defined as haemoglobin >130 g/L at 37 weeks' gestation or more) at term (average RR 3.07 [95% CI 1.18–8.02]; reported in eight studies involving 2156 women).<sup>27</sup> The oxygen-carrying capacity of anaemia during pregnancy can affect the placenta. Hypoxia of the placenta is thought to be associated to complications of pregnancy, including pre-eclampsia, intrauterine growth restriction, and SGA.<sup>28</sup> It has been hypothesised that both anaemia and elevated haemoglobin levels during pregnancy might lead to suboptimal oxygen and nutrients supply to the placenta and fetus, and subsequently to maternal, placental, and fetal complications, and it is through this mechanism that haemoglobin concentrations during pregnancy can affect pregnancy outcomes.<sup>29</sup> Finally, the association between maternal haemoglobin and adverse maternal outcomes

should be interpreted with caution as we did not collect any data on plasma volume expansion to determine whether haemoconcentration due to pre-eclampsia was the cause of high haemoglobin levels or biomarkers to determine the cause of anaemia, such as iron-deficiency, infection, or inflammation.

Maternal anaemia during pregnancy might affect neurocognitive development in children in the long term, resulting in poor school attendance followed by suboptimal economic productivity in adulthood.<sup>30,31</sup> Annual economic loss because of iron deficiency anaemia in ten developing countries was estimated at \$16.78 per capita (in 1994 US\$), or 4% of gross domestic product.<sup>32</sup> Additionally, long-term effects on both the auditory and sensory systems have been reported.<sup>33</sup> Future studies should aim to quantify the associated increased risks of maternal haemoglobin and neurocognitive developmental outcomes and determine if separate cutoffs are required for certain high-risk populations, such as young pregnant adolescents and twin pregnancies.

In summary, from a multinational longitudinal study, we have described association patterns between maternal haemoglobin concentrations in pregnancy and adverse maternal and neonatal health outcomes. The finding of risks relative to haemoglobin concentrations suggests that clinical and public health benefit might arise from using an optimal range of haemoglobin concentrations throughout pregnancy that are associated with lower risk of maternal and neonatal outcomes.

#### Contributors

JV and SHK were responsible for conceiving the INTERGROWTH-21st Project. The INTERBIO-21st fetal study is an extension to the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) Project. JPPR and MNGC conceived the protocol for this analysis in collaboration with EOO and JV. EOO performed the statistical analyses on the maternal haemoglobin data in collaboration with NJ. EOO and JV had full access and verified the data. EOO wrote the paper in collaboration with JV, SHK, TE, MY, and RM, with input from all co-authors. All co-authors read the report, made suggestions about its content, and agreed with submission of the manuscript.

#### Declaration of interests

We declare no competing interests.

#### Data sharing

Upon a reasonable request for academic purposes and in adherence to the limitations set by informed consent, anonymised data will be provided. The corresponding author should be contacted for such requests. The INTERBIO-21st Consortium Executive Committee will review each request, and on approval, the researcher will be required to sign a data access agreement with the INTERBIO-21st Consortium.

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