# Fetal plasma erythropoietin concentration in severe growth retardation

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**OBJECTIVE:** The aim of this study was to determine whether hypoxemia induces an increase in plasma erythropoietin concentration in human fetal life and, if so, whether this response stimulates fetal erythropoiesis.

**STUDY DESIGN:** The plasma erythropoietin concentration in blood samples from 33 small-for-gestational-age fetuses at 26 to 38 weeks' gestation was measured. Measurements were compared with the reference range for gestation, and associations with $Po_2$, pH, and erythroblast and erythrocyte counts were examined.

**RESULTS:** The mean plasma erythropoietin concentration in the small-for-gestational-age fetuses was significantly increased, and the degree of increase was significantly associated both with fetal acidemia and, more strongly, with fetal erythroblastosis.

**CONCLUSION:** Erythropoietin production in response to tissue hypoxia occurs from at least 26 weeks' gestation with measurable physiologic effects on erythropoiesis. Furthermore, more accurate assessment of tissue oxygenation may be obtained by measuring the erythroblast count rather than the blood pH.
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**Key words:** Cordocentesis, intrauterine growth retardation, erythroblast count, erythropoietin, fetal hypoxia

Antenatal studies involving fetal blood samples obtained by cordocentesis and Doppler investigations have demonstrated that in some small-for-gestational-age (SGA) fetuses at least two factors contribute to tissue hypoxia. First, there is hypoxemic hypoxia (low blood oxygen content) caused by reduced uteroplacental perfusion and oxygen transport to the fetus.[1, 2] Second, ischemic hypoxia occurs in the splanchnic, renal, pulmonary, and musculoskeletal tissues as a result of redistribution in fetal blood flow with preferential shunting to the brain, heart, and adrenals.[2, 3]

In adult life tissue hypoxia is known to increase renal production of erythropoietin.[4] The resulting increase in erythropoietin concentration induces a rise in red blood cell mass by stimulating proliferation, differentiation, and maturation of erythroid precursors.[4] Animal studies have demonstrated that during fetal life plasma erythropoietin concentrations increase in response to hypoxia.[4, 5] Furthermore, studies examining umbilical cord blood from human pregnancies at delivery have established that plasma erythropoietin levels are increased in patients where intrauterine hypoxia is suspected.[6, 7]

In most mammals fetal erythropoietin production occurs in the liver with a gradual switch to the kidney around the time of birth.[4, 5, 8] However, hematopoietic maturation and the switch from liver to kidney as the predominant site of erythropoietin production varies in different species. Therefore findings from animal studies may not reflect human physiologic conditions. Similarly, because response to tissue hypoxia or effects on erythropoiesis may be altered around the time of birth or by the process of delivery itself, findings from postnatal human studies may not reflect antenatal physiologic responses.

The aim of this study was to determine whether hypoxemia induces an increase in plasma erythropoietin concentration in human fetal life and, if so, whether this response stimulates fetal erythropoiesis.

## Patients and methods

Plasma erythropoietin concentration was measured in 33 SGA fetuses referred to our unit for further assessment at 26 to 38 (mean 31) weeks' gestation (Fig. 1). In

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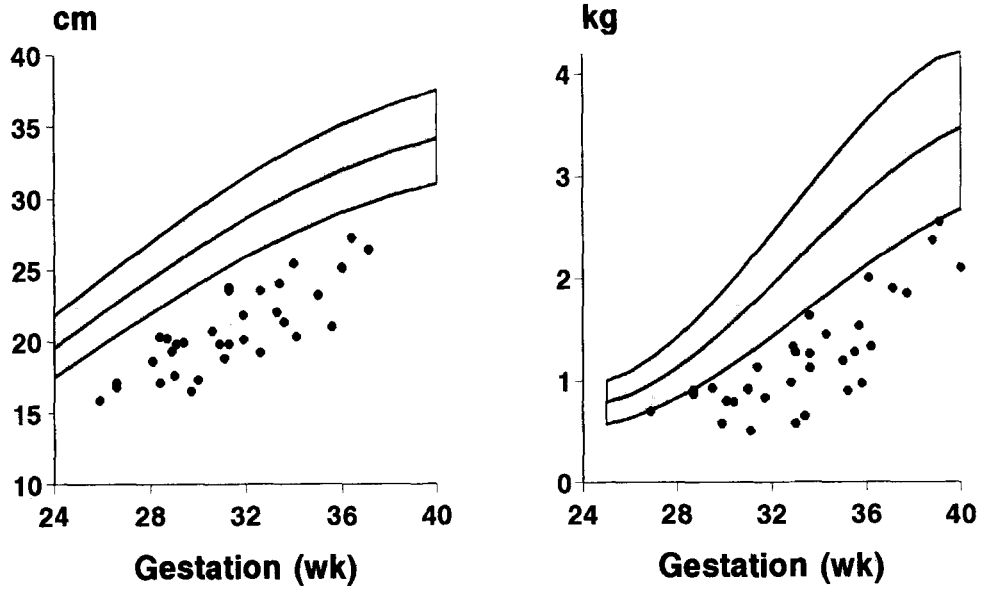


**Fig. 1.** Abdominal circumference *(left)* and birth weight *(right)* of 33 SGA fetuses plotted on appropriate reference range (mean, 5th and 95th percentiles) for gestation.

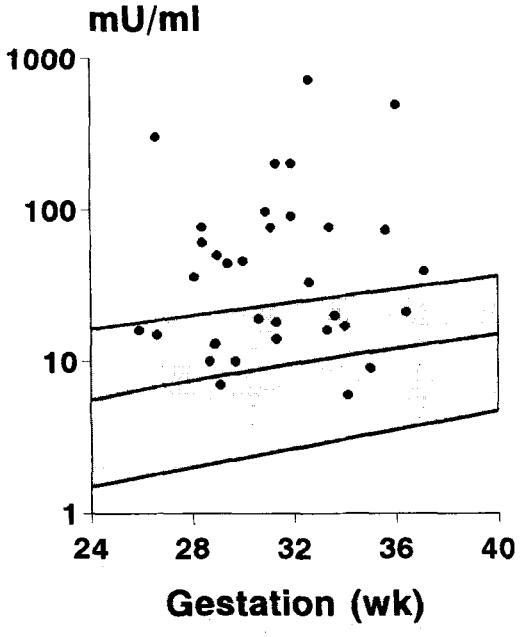


**Fig. 2.** Umbilical venous plasma erythropoietin concentration in 33 SGA fetuses plotted on reference range (mean, 5th and 95th percentiles) for gestation.

all cases ultrasonographic examination had demonstrated that the fetal abdominal circumference was below the 5th percentile for gestation, and Doppler ultrasonographic investigation of the uterine and umbilical arteries was suggestive of placental insufficiency. In seven patients there was oligohydramnios, and in an additional 17 cases the amniotic fluid volume was subjectively assessed by ultrasonography to be reduced. Subsequently, all fetuses were demonstrated to be chromosomally and anatomically normal. At delivery all infants had a birth weight below the 5th percentile for gestation (Fig. 1).[9] There were two intrauterine and three neonatal deaths; the remaining 27 babies are alive.

Continuous-wave Doppler studies (Doptek, Chichester, England) of the uterine and umbilical arteries were performed immediately before cordocentesis, and in all cases there was an early diastolic notch in the waveform from at least one of the uterine arteries or absent end-diastolic frequencies in the waveform from an umbilical artery.[10, 11]

Umbilical venous blood samples were obtained by cordocentesis, which was performed without maternal sedation or fetal paralysis.[1] Fetal blood $Po_2$ and pH were measured in samples (250 μl) collected into heparinized syringes (Radiometer ABL 330, Copenhagen). Fetal blood (180 μl) was also collected into 20 μl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mmol/L sodium chloride), and contamination with maternal blood was excluded by the acid-elution (Kleihauer-Betke) method. The erythrocyte and total nucleated cell counts were determined with a Coulter Stacker Automated Cytometer (Coulter Electronics, Luton, England). Blood films were stained by the May-Grünwald-Giemsa method, and the number of erythroblasts was counted.

The samples for erythropoietin were stored at −20° C and assayed in one batch with an erythropoi-

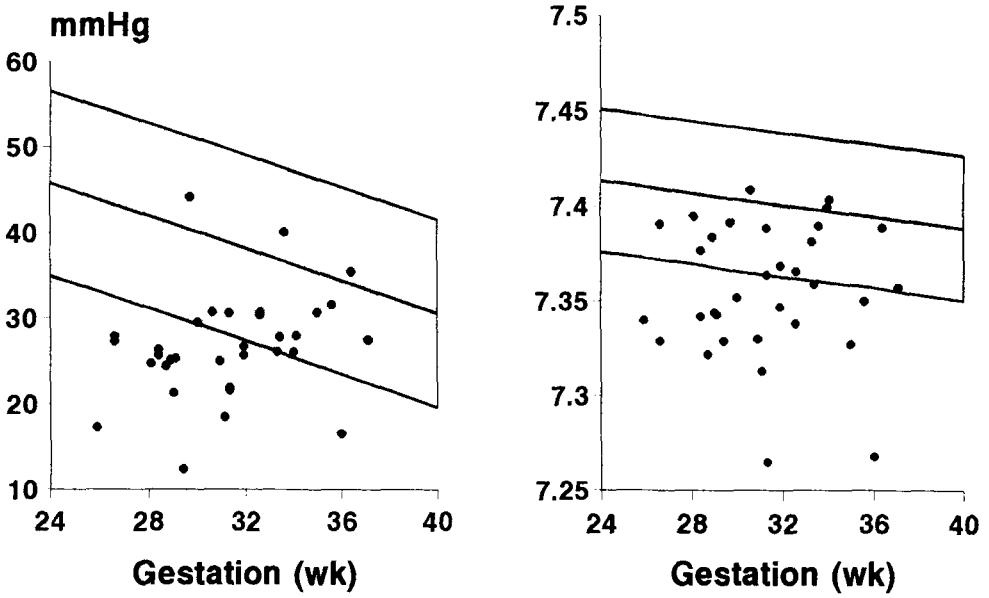


**Fig. 3.** Umbilical venous blood oxygen tension *(left)* and pH *(right)* in 33 SGA fetuses plotted on appropriate reference range (mean, 5th and 95th percentiles) for gestation.

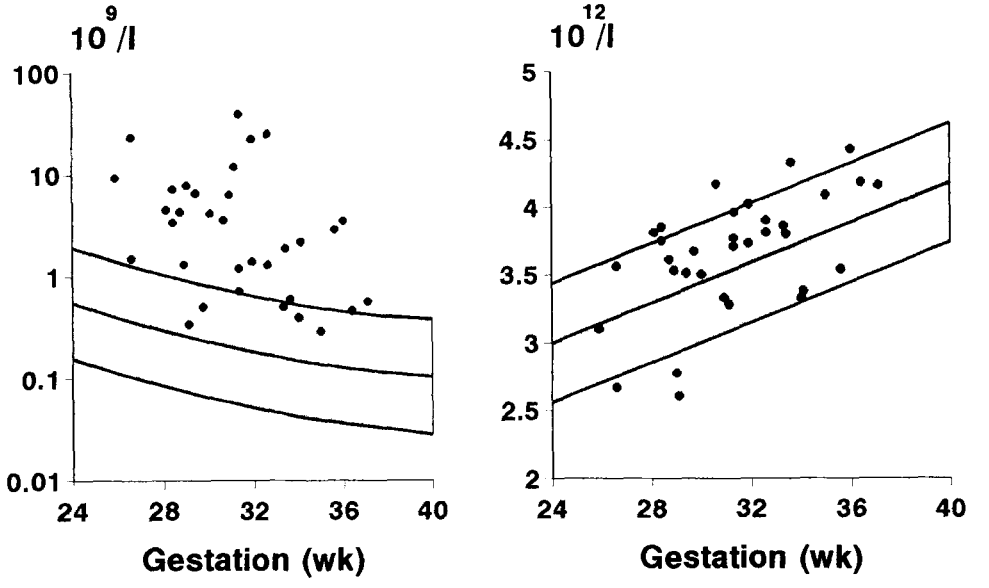


**Fig. 4.** Umbilical venous blood erythroblast *(left)* and erythrocyte *(right)* counts in 33 SGA fetuses plotted on appropriate reference range (mean, 5th and 95th percentiles) for gestation.

etin enzyme immunoassay kit (Clinigen, Amgen Diagnostics, Thousand Oaks, Calif.). The reference curve was standardized against the World Health Organization second international reference preparation of human urinary erythropoietin. The limit of sensitivity of the assay was determined to be 2 mU/ml (limit at 3 SD from the zero erythropoietin standard).

**Statistical analysis.** Because all parameters measured change with gestation, individual values were expressed as the number of standard deviations by which the measurements differed from the appropriate normal mean for gestation (Δ-values).[1, 2, 12]

The Wilcoxon test was used to examine whether measurements in the SGA group differed significantly from the appropriate normal mean for gestation. Regression analysis was then applied to examine whether

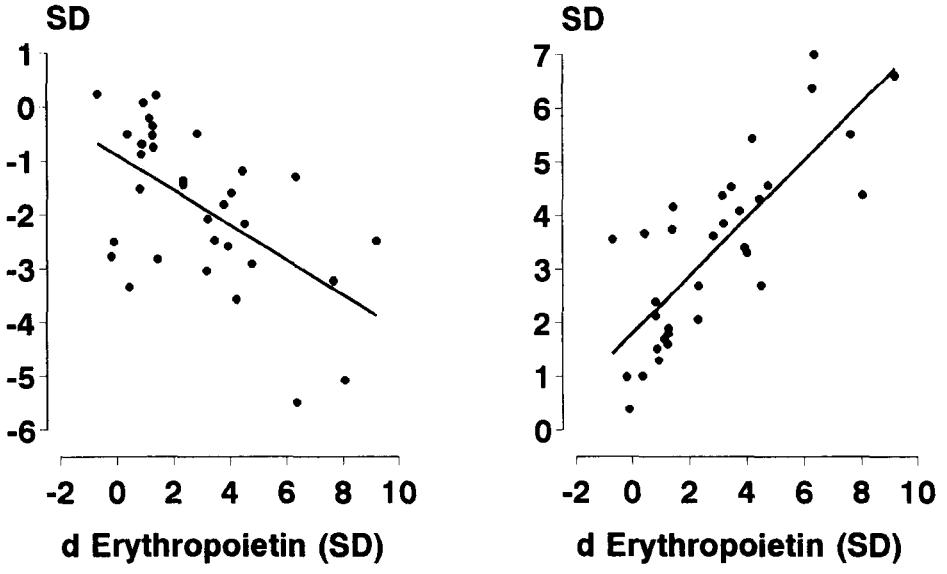


**Fig. 5.** Degree of acidemia *(left)* and erythroblastosis *(right)* in association with extent to which plasma erythropoietin was increased.

Δ-$Po_2$, Δ-pH, and Δ–erythroblast or erythrocyte counts were significantly related to Δ erythropoietin concentration.

Multiple regression was applied to examine whether blood gases and plasma erythropoietin contributed independently in the prediction of changes in erythropoiesis.

## Results

In the 33 SGA fetuses the mean plasma erythropoietin concentration was significantly higher than the appropriate normal mean for gestation (Fig. 2; erythropoietin mean difference 2.31 SD, $p < 0.0001$), and the mean umbilical venous blood $Po_2$ and pH were significantly reduced (Fig. 3; $Po_2$ mean difference $-1.93$ SD, $p < 0.0001$; pH mean difference $-1.59$ SD, $p < 0.0001$). The mean erythroblast count was significantly higher, but the mean erythrocyte count was not significantly different from the appropriate normal means for gestation (Fig. 4; erythroblast count mean difference 3.56 SD, $p < 0.0001$; erythrocyte count mean difference 0.77 SD).

The Δ–fetal erythropoietin was significantly associated with Δ-pH and Δ–erythroblast count (Fig. 5; $r = -0.57$, residual SD > 1.18, $p < 0.001$ and $r = 0.80$, residual SD > 1.04, $p < 0.001$) but not with gestation ($r = -0.002$), Δ-$Po_2$ (Fig. 6, $r = -0.23$), or Δ–erythrocyte count (Fig. 6, $r = 0.25$). The correlation coefficient was higher and the residual SD was lower for the association between Δ-erythropoietin and Δ–erythroblast count than between Δ–erythropoietin and Δ-pH (Fig. 5). Furthermore, a multiple regression model with Δ-erythropoietin and Δ-pH did not provide a significantly better prediction of Δ–erythroblast count than that obtained from Δ-erythropoietin alone ($F$ to remove pH = 0.99, $t = 1.0$, $p = 0.33$).

## Comment

This study has demonstrated that in pregnancies complicated by severe intrauterine growth retardation fetal plasma erythropoietin concentration is increased and the degree of increase is significantly associated with the degree of fetal acidemia. These findings are in keeping with data from postnatal, adult, and animal studies and establish that in human fetal life erythropoietin production in response to tissue hypoxia occurs from at least 26 weeks' gestation.[4-8]

There was no significant association between Δ-erythropoietin and Δ-$Po_2$. This implies that the deficit in umbilical venous blood oxygen tension does not provide an accurate prediction of the degree of tissue hypoxia, which is produced by a combination of decreased tissue perfusion and blood oxygen content. Umbilical venous acidemia causes a right shift in the hemoglobin-oxygen saturation curve, which for a given $Po_2$ results in reduced blood oxygen content.[13]

In the SGA fetuses increased erythropoietin production was assoicated with erythroblastosis but not with an increase in the number of erythrocytes. In normal human fetal life the number of circulating erythroblasts decreases exponentially with gestation, reaching a plateau at 24 to 26 weeks' gestation.[14] This decrease coincides with the switch from hepatic to medullary erythropoiesis. With liver erythropoiesis erythroblasts enter the peripheral circulation freely, whereas with marrow erythropoiesis the nucleated erythroid precursors are confined to the parenchyma in which hematopoiesis takes place.[15] Erythroblastosis in SGA fetuses

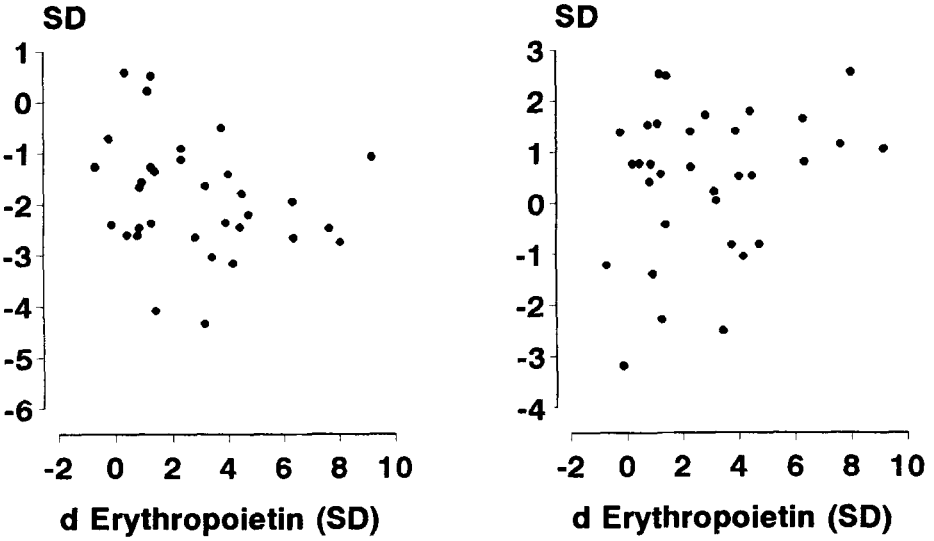


**Fig. 6.** Δ — Umbilical venous $Po_2$ and Δ–erythrocyte count in association with Δ-erythropoietin concentration. Δ-Values represent number of standard deviations by which measurements differed from appropriate normal mean for gestation.

may be caused by a delay in the switch from hepatic to marrow erythropoiesis. However, such a developmental delay would not explain the correlation between increased plasma erythropoietin and increased numbers of circulating erythroblasts.

More plausible explanations for the erythroblastosis of SGA fetuses include erythropoietin-mediated premature release of red blood cells from the bone marrow or recruitment of hepatic erythropoiesis; the data of the current study do not allow distinction between these hypotheses. However, evidence for hypoxia-induced extramedullary erythropoiesis has been provided by Naeye,[16] who demonstrated persistent hepatic erythropoiesis in infants with chronic neonatal hypoxia.

Blood pH is widely accepted as an index of fetal oxygenation. The finding that the degree of increase in plasma erythropoietin was more strongly associated with the degree of erythroblastosis than with acidemia suggests that the erythroblast count may provide a better measure of tissue oxygenation. Supportive evidence is provided by the finding that neonatal asphyxia is more accurately predicted by the presence of erythroblastosis than by low pH in cord blood at delivery.[17]

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