*British Journal of Nutrition* (2008), **100**, 408–417
doi:10.1017/S000711450889438X


# Nutritional anaemia dysregulates endocrine control of fetal growth

S. Mahajan[1]*†, R. Aalinkeel[1], P. Shah[2], S. Singh[3] and N. Kochupillai[1]

[1]*Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*
[2]*Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas, MD Anderson Cancer Center, Box 301402, Houston, TX 77230, USA*
[3]*Department of Obstetrics and Gynaecology, Joan C Edwards School of Medicine, 1600 Medical Center Drive, P.O. Box 4500, Huntington, VA 25701, USA*

*(Received 21 May 2007 – Revised 16 November 2007 – Accepted 19 November 2007 – First published online 13 February 2008)*

Severe anaemia is associated with increased low birth weight and Barker's hypothesis of 'fetal origins' proposes that a nutritional insult during critical periods of development results in adaptations that predispose individuals to adult onset diseases. We hypothesize that endocrine alterations may occur in the maternal–fetal milieu as a consequence of nutritional anaemia during pregnancy. We examined the quantitative variations in hormonal profiles in paired maternal and cord blood samples obtained from mothers and their neonates who were classified based on maternal anaemia status. Our results show that: (1) 74·6 % of the mothers enrolled in the study were anaemic, of which 85·2 % had moderate anaemia and 14·7 % had severe anaemia; (2) anthropometric parameters measured in the mothers indicate that severely anaemic mothers had a significantly low pre- and post-pregnancy weight, a significantly decreased maternal fundal height and abdominal circumference; (3) anthropometric measures in the neonates born to severely anaemic mothers show a significant reduction in ponderal index, birth weight and placental weight; (4) significant increase in both maternal, fetal insulin-like growth factor 1, ferritin levels and increased maternal erythropoietin levels were observed with an increase in severity of anaemia; (5) decreased $T_3$ and increased prolactin levels were observed in the maternal blood of severely anaemic mothers as compared with the control group. An insight into the endocrine modulation to overcome a growth disadvantage due to nutritional anaemia in pregnancy may lead to a better understanding of fetal adaptations invoked when the maternal-placental nutrient supply fails to meet the fetal nutrient demand.

**Maternal malnutrition: Nutritional anaemia: Hormones: Anthropometrics: Fetal growth**

Anaemia in the developing world is most commonly caused by Fe deficiency, which not only impairs the production of red cells in the blood, but also affects general cell growth and proliferation[1–4]. Fe deficiency is considered to be one of the most prevalent forms of malnutrition, yet there has been a lack of consensus about the nature and magnitude of the health consequences of Fe deficiency in populations. Nutritional anaemia is defined as a condition in which the Hb content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency[1–6].

In developing countries, the incidence of nutritional anaemia in women and, particularly, among expectant mothers is alarmingly high. In India, nutritional anaemia is a serious public health problem, particularly during pregnancy, since Fe-deficiency anaemia (IDA) is observed in approximately 70 % of pregnant women; 15–30 % of all maternal deaths are anaemia related[7]. Fe deficiency during pregnancy promotes reductions in Fe supplies, causing anaemia to develop in the neonate[1–6]. IDA is associated with a >2-fold increase in the risk of preterm delivery[8–10]. Maternal anaemia when diagnosed before mid pregnancy is also associated with an increased risk of preterm birth. IDA is relatively common in the third trimester of pregnancy, but causal associations with low birth weight and compromised neonatal Fe status are difficult to establish. Supplementation with Fe is generally recommended during pregnancy to meet the Fe needs of both mother and fetus.

Current knowledge indicates that IDA in pregnancy is a risk factor for preterm delivery and subsequent low birth weight. Fe supplements improve Fe status during pregnancy, even for women who enter pregnancy with reasonable Fe stores, and provide some protection against Fe deficiency in subsequent pregnancies[11]. Inadequate intake of Fe from the diet during pregnancy can lead to compromised haematological status of the neonate without indications of growth retardation or impaired neurological function at birth[12]. While Fe supplementation increases maternal Fe status and stores, it has also recently been linked to maternal complications such as gestational diabetes and increased oxidative stress during pregnancy[13–15].

**Abbreviations:** EGF, epidermal growth factor; GH, growth hormone; hPL, placental lactogen; IDA, Fe-deficiency anaemia; IGF, insulin-like growth factor; PRL, prolactin; TSH, thyroid-stimulating hormone.

* **Corresponding author:** Dr Supriya D. Mahajan, fax +1 716 859 2999, email smahajan@buffalo.edu
† **Present address:** Department of Medicine, Div of Allergy, Immunology, and Rheumatology, 310 Multi Research Bldg, Buffalo General Hospital, 100 High Street, Buffalo, NY 14 203, USA.

The amount of Fe accumulated by the fetus during intra-uterine life is proportional to the amount of weight it gains[13–16]. Gestational conditions that result in lower newborn Fe stores include severe maternal Fe deficiency, maternal hypertension with intra-uterine growth retardation and maternal diabetes mellitus[17–19]. Very low birth weight premature infants are also at risk for early postnatal Fe deficiencies because they accrete less Fe during gestation, grow more rapidly postnatally during catch-up growth. Maternal and perinatal outcome is normal in situations of mild anaemia, while severe anaemia is associated with increased low birth weight babies.

Several hormones and growth factors are known to play important roles in the tissue accretion and differentiation in the fetus[20,21]. The perinatal period presents a highly synchronized sequence of metabolic and endocrine events, which are very important for normal growth and development of the fetus[20–22]. Maternal nutrients are essential for growth and development of the fetus and transport of these nutrients occurs via the placental blood supply. The placenta is important in the production and transport of growth-promoting hormones. Several studies including our own have shown that low birth weight and prenatal stress results in the modulation of the hypothalamic–pituitary–adrenal axis. Changes in the concentrations of hormones occur in response to nutritional restriction during gestation. In general, nutritional restriction reduces fetal nutrient availability, resulting in a decrease in the anabolic hormone concentrations such as insulin, insulin-like growth factor (IGF)-I, thyroxine ($T_4$) and an increase in catabolic hormone concentrations such as cortisol, catecholamines and growth hormone (GH)[20–25]. The precise pattern of endocrine changes may depend on the duration and magnitude of the nutritional insult.

Limited information is available in literature with regard to the endocrine alterations in the maternal–fetal milieu as a consequence of nutritional anaemia during pregnancy. In order to investigate the endocrine adaptation mechanisms by the fetus due to maternal anaemia, we examined the quantitative variations in hormonal and growth factor profiles in paired maternal and cord blood samples obtained from mothers and their neonates who were classified based on the severity of maternal anaemia during pregnancy.

## Experimental methods

Pregnant mothers (*n* 300), who were registered and being followed up in the antenatal clinic in a prominent national government hospital in New Delhi, India, and their newborns were the source of the clinical material for the present study. The study design and the consent forms were approved by the appropriate institutional review board and the study was conducted based strictly on their recommended guidelines. Subjects were enrolled in the present study during the first trimester between 6 and 12 weeks during their first visit to the antenatal clinic after their pregnancy was confirmed, after obtaining an informed consent. A detailed clinical obstetric history of the patient was documented and early pregnancy weight was noted. All patients enrolled in the study were regularly followed up at monthly intervals in the antenatal clinic and were monitored for gestational diabetes, hypertension and any other infections. If any abnormalities were observed, those patients were excluded from the study. Gestational age was assessed from the date of the last menstrual period and only term pregnancies (38–42 weeks) were included in the study. Maternal blood (10 ml) was drawn at term prior to delivery of the neonates, before the onset of labour. At birth, cord blood (20 ml) was collected from the placental end. Blood samples were centrifuged to separate the plasma and aliquoted and stored at −20°C until further use. Endocrine parameters were measured in these aliquoted paired maternal and cord blood samples. The endocrine parameters measured represent random levels since none of the samples taken was in the fasting state. Weight (g) and length (cm) of the neonate were recorded as soon as the baby was dried and cleaned after delivery. Anthropometric data were obtained from the mothers and the neonates. Maternal weight gain (kg) during pregnancy and maternal early-pregnancy weight (kg) were also measured, which gave an indication of the short- and long-term nutritional status of the mothers. Maternal nutritional status and fetal growth status was assessed using a combination of anthropometric and biochemical parameters, such as BMI calculated from the height and weight of the mother, mid-arm circumference (cm), abdominal girth or abdominal circumference (cm) and fundal height (cm). Ponderal index (birth weight/birth length$^{(3)}$) was also calculated from the neonates' weight and length at birth. Standard internationally accepted methodologies were used to measure all the anthropometric parameters in both the mother and the neonate[26–32]. All these measures were recorded at every wellness visit to the antenatal clinic. The haematological status of the mothers and neonates was measured using total Hb measurement. Additionally, ferritin levels indicative of Fe reserve were measured in both the mothers and their neonates. The biochemical parameters measured to assess nutritional status were maternal and fetal total protein and serum albumin levels. The methodology used to measure total serum proteins was the standard Biuret reaction in our routine clinical laboratory using an automated sampler.

### *Study design*

According to WHO, 43 % of all non-pregnant women aged 15–49 years in developing countries develop anaemia during pregnancy[31–33]. Anaemia is recognized as a risk factor for maternal mortality when associated with antepartum and post partum haemorrhage and low birth weight. WHO defines anaemia as a Hb level below 12 g/dl in non-pregnant women and Hb level below 11 g/dl in pregnant women. These numbers are based on average levels around the world, for all women regardless of their age[31–33]. There is much controversy as to what the cut-off point for anaemia should be, particularly in the developing world, since the WHO criteria are proposed based on statistical computations that incorporate data of the Hb levels of a healthy population worldwide. However, some researchers believe that cut-off points should be based on country-specific reference groups and should reflect differences in Hb levels of that specific race and population. In the present study, we used a cut-off point of Hb $< 11$ as indicative of anaemia, since it best reflected our patient population. We classified the mothers into three study groups based on the WHO classification of severity of anaemia: Group I (normal control – no anaemia, Hb $> 11{\cdot}0$ g/dl); Group II (moderate anaemia,

Hb > 7–10·9 g/dl); Group III (severe anaemia, Hb < 6·9 g/dl). Exclusion criteria of the study included both pre- and post-term neonates and their mothers and any other clinical disorders known to predispose the mothers to intra-uterine growth retarded births other than malnutrition and/or anaemia, such as pre-eclampsia, renovascular disease, chronic hypertension, vasculopathy from diabetes, drug abuse, genetic or congenital abnormalities, inborn errors of metabolism, twin births and toxic exposure. Additionally, patients with glucose-6-phosphate dehydrogenase deficiency, thalassaemia and sickle cell anaemia have been excluded from this study.

To examine the endocrine profile of mothers and their neonates in the three study groups, an array of hormones and growth factors relevant to fetal growth and development, such as GH, prolactin (PRL), insulin, placental lactogen (hPL), thyroid hormones ($T_4$, $T_3$, $rT_3$, thyroid-stimulating hormone (TSH)), IGF-1 and epidermal growth factor (EGF) were assayed in both cord blood and maternal blood using a combination of in-house RIA and commercially available kits. The standards, iodination material for preparation of the radiolabelled antigen (Amersham Biosciences Inc, Piscataway, NJ) and the specific antisera were obtained from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD and the assays were performed using a precise protocol provided by this program. Peptide hormones, GH, PRL, hPL (www.humc.edu/hormones) were assayed using the reagents from National Institutes of Health – National Institute of Diabetes and Digestive and Kidney Diseases – National Hormone and Pituitary Program. Insulin, TSH, $T_4$ and $T_3$ (Catalogue no. DSL-10-1600, Catalogue no. DSL-5300, Catalogue no. DSL-3200, and Catalogue no. DSL-3100 respectively; Diagnostic System Laboratories Inc (DSL) Webster, TX, USA) and r-T3, IGF-1 and EGF (Catalogue no. 29-BC-1115, Catalogue no. 22-IGF-R20 and Catalogue no. 45-EGFH-0061 respectively; Alpco Diagnostics, Salem, NH, USA) were assayed using commercially available kits from these vendors. All the assays were highly specific and sensitive with an intra- and inter-assay CV less than 10 %. The minimum detectability of the GH, PRL, hPL, insulin, IGF-1 and EGF assays were 0·23 ng/ml, 0·4 ng/ml, 0·015 mU/l, 0·4 μU/ml, 0·078 ng/ml and 0·06 ng/ml respectively. The minimum detectability of the $T_3$, $T_4$, $rT_3$ and TSH assays were 2·5 pg/ml, 5·0 pg/ml, 1 pg/ml and 0·03 μU/ml respectively.

### *Statistical analysis*

Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were performed using a Bonferroni adjustment. The tables contain the *P* values for these pair wise group comparisons. All data analysis was done using the normal control group as a referent group. Additionally, multivariate regression analysis was done to investigate relationships between maternal Hb and the endocrine parameters that are important markers of nutritional anaemia. The statistical software used was Analyse-it (version 1.73; Analyse-it for Microsoft® Excel®, Leeds, UK).

## Results

A total of 300 cases were enrolled in the present study. Only term pregnancies with an uneventful perinatal period were included in the study. The patient population was representative of the majority of the urban and rural, low and middle socioeconomic class of the society. The mean gestational age of the mothers was 39·1 (SD 1·3) weeks. Gestational age was assessed from the date of the last menstrual period. The mean age of the mothers in the study was 21·3 (SD 0·6) years. The mean gestational age was not significantly different between the three study groups and values were 39·4 (SD 0·42) and 39·7 (SD 0·63) weeks in severe and moderately anaemic mothers respectively as compared with normal mothers (40·1 (SD 0·57) weeks). Further, the mean maternal age was not significantly different in the three study groups and mean age of severe and moderately anaemic mothers was 19·4 (SD 1·2) and 20·9 (SD 1·4) years respectively as compared with that of the normal mothers (21·8 (SD 0·75) years).

### *Anthropometric, biochemical and haematological measures of maternal–fetal growth and nutritional status*

Nutritional status of the mothers enrolled in the present study was assessed using both anthropometric and biochemical measurements. The anthropometric parameters measured in mothers included the mean pre-pregnancy maternal weight, maternal weight at delivery, maternal height, maternal mid-arm circumference, maternal fundal height and maternal abdominal circumference. Fetal growth status was assessed by measuring neonatal birth weight, neonatal length, placental weight, Rohrer's ponderal index. The biochemical parameters measured in both maternal blood and paired cord blood samples were total protein and serum albumin. Additionally, Hb levels were assessed in both maternal blood and paired cord blood samples, which were indicative of the haematological status of the mothers and their neonates. Of the mothers enrolled in the present study, 74·6 % were found to be anaemic, of which 85·2 % had moderate anaemia, while 14·7 % had severe anaemia.

Table 1 outlines the anthropometric indices measured in the mothers and their neonates. Statistical comparisons were made with regard to the normal control group. The current results show that maternal pre-pregnancy weight (kg) was significantly lower in (Group III) mothers who have severe anaemia as compared with the normal control group of mothers. A similar trend was observed with regard to maternal weight at delivery (kg), which was significantly lower in severely anaemic mothers as compared with the normal control group of mothers. No significant differences in maternal height and maternal mid-arm circumference were observed between the three study groups. However, maternal abdominal circumference (cm) was significantly lower in severely anaemic mothers as compared with the normal control group of mothers. A similar trend was observed with regard to maternal fundal height (cm), which was significantly lower in the severely anaemic group as compared with the normal control group. We observed a significant decrease in birth weight (g) of neonates born to mothers with moderate to severe anaemia as compared with the non-anaemic mothers. No significant difference was observed with regard to neonatal length (cm); however, ponderal index (ratio) and placental weight (g) were significantly decreased in the neonates born to severely anaemic mothers, as compared with the ponderal index and placental weight values in the neonates

British Journal of Nutrition

**Table 1.** Anthropometric indices of maternal and fetal nutritional status measured in the three study groups‡
(Mean values and standard deviations)

| | | Group I (*n* 76) | | Group II (*n* 191) | | Group III (*n* 33) | |
|---|---|---|---|---|---|---|---|
| Anthropometric parameters | Unit | Mean | SD | Mean | SD | Mean | SD |
| Maternal nutritional status assessment | | | | | | | |
| Maternal early-pregnancy weight | kg | 48·04 | 5·47 | 47·00 (*P*=0·0001)† | 5·02 | 45·58 (*P*=0·006)* | 4·25 |
| Maternal weight at delivery | kg | 54·71 | 6·88 | 54·06 (*P*=0·0001)† | 6·38 | 51·48 (*P*=0·009)* | 6·23 |
| Maternal height | cm | 155·53 | 4·78 | 154·59 | 4·51 | 154·25 | 5·83 |
| Maternal mid-arm circumference | cm | 21·6 | 3·2 | 21·61 | 2·92 | 20·75 | 4·03 |
| Maternal BMI | index | 20·1 | 1·4 | 19·6 | 0·8 | 18·9 | 1·1 |
| Maternal fundal height | cm | 30·8 | 3·37 | 30·6 | 2·95 | 29·64 (*P*=0·027)* | 2·92 |
| Maternal abdominal circumference | cm | 87·27 | 7·41 | 87·09 (*P*=0·004)† | 6·35 | 83·6 (*P*=0·005)* | 6·32 |
| Fetal nutritional status assessment | | | | | | | |
| Birth weight | g | 2802·3 | 408·18 | 2701·48 (*P*=0·03)* (*P*=0·001)† | 410·11 | 2442·42 (*P*=0·0003)* | 504·75 |
| Neonatal length | cm | 49·667 | 1·86 | 49·43 | 1·80 | 49·11 | 2·92 |
| Ponderal index | ratio | 2·29 | 0·29 | 2·24 (*P*=0·003)† | 0·34 | 2·05 (*P*=0·0001)* | 0·31 |
| Weight placenta | g | 434·67 | 111·28 | 417·47 | 111·62 | 380·03 (*P*=0·02)* | 130·09 |

Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were also performed using a Bonferroni adjustment.
* (*P*-value) indicates a statistically significant difference for pair-wise group comparisons between severe or moderate anaemia *v.* non-anaemic controls.
† (*P*-value) indicates a statistically significant difference for pair-wise group comparisons between moderate anaemia *v.* severe anaemia.
‡ For details of subjects and procedures, see Experimental methods.

born to normal mothers. These results indicate that substantial nutritional anaemia is associated with an increased incidence of low birth weight.

Comparison of the anthropometric parameters between the moderate (Group II) and severe (Group III) anaemia groups indicated that the maternal early-pregnancy weight, maternal weight at delivery and maternal abdominal circumference were significantly lower in the severely anaemic mothers as compared with the moderately anaemic mothers. Neonates born to Group III mothers had significantly lower birth weight and ponderal index as compared with those born to Group II mothers.

Table 2 outlines the biochemical and haematological indices measured in the mothers and their neonates. We measured the serum albumin and total protein levels as biochemical markers of nutritional status in the maternal blood and cord blood in the three study groups. The present data indicate that there was no significant difference in the fetal biochemical status as indicated by no statistically significant differences in serum albumin and total protein in the three study groups. However, significantly lower total protein (mg/dl) levels were observed in severely anaemic mothers compared with the normal mothers. Mean Hb (g/l) levels measured in moderately anaemic and in severely anaemic mothers were significantly lower compared with levels in normal mothers. However, the cord blood Hb levels were significantly lower only in the neonates born to severely anaemic mothers compared with the control group. No statistically significant difference was observed in the maternal ferritin levels between the moderately anaemic and the severely anaemic groups compared with the normal control group. However, a significant increase in the fetal Fe stores as reflected by the serum ferritin (g/l) levels in the cord blood were observed in the neonates born to moderately anaemic mothers and neonates born to severely anaemic mothers compared with the normal control group. Interestingly, the levels of cord blood erythropoietin (g/l) were unchanged in the three study groups; however, maternal erythropoietin levels were significantly higher in the moderately anaemic mothers as well as the severely anaemic mothers compared with the normal control mothers.

Comparison of the biochemical and haematological indices between the moderate (Group II) and severe (Group III) anaemia groups indicated that maternal total protein was significantly lower in the severely anaemic mothers compared with the moderately anaemic mothers. Maternal erythropoietin levels were significantly higher in the severely anaemic mothers compared with the moderately anaemic mothers.

### *Endocrine parameters in cord blood in the study groups*

We measured the levels of peptide hormones (GH, PRL, insulin, hPL), growth factors (IGF-1 and EGF) and thyroid hormones ($T_3$, $T_4$, $rT_3$ and TSH) in the cord blood and maternal blood in the three study groups. The levels of these hormones in cord blood of neonates born to mothers who moderately anaemic (Group II) and severely anaemic (Group III) compared with neonates born to mothers normal mothers (Group I) are shown in Table 3. The data show no statistically significant differences in levels of GH (ng/ml), PRL (ng/ml), insulin (mU/ml), hPL (mU/l), EGF (ng/ml) or thyroid hormones ($T_3$ (ng/dl), $rT_3$ (ng/dl) and TSH (μU/ml)) between the three groups. However, a significant increase in IGF-1 (ng/ml) levels was observed in the cord blood of neonates born to severely anaemic mothers compared with the control group. Additionally, the thyroid hormone cord blood $T_4$ levels (μg/dl) were significantly lower in both the neonates born to moderately anaemic and severely anaemic mothers compared with the neonates born to normal mothers.

Comparison of the endocrine parameters in the cord blood of neonates born to moderately anaemic (Group II) mothers and severely anaemic (Group III) mothers indicated that the levels of PRL, insulin, IGF-1 and hPL were significantly higher in the neonates born to severely anaemic mothers compared with those born to moderately anaemic mothers. Cord blood EGF levels were significantly higher in neonates born to moderately anaemic mothers compared with neonates born to severely anaemic mothers.

British Journal of Nutrition

British Journal of Nutrition

**Table 2.** Biochemical and haematological indices of nutritional status measured in paired maternal and cord blood samples in the three study groups‡
(Mean values and standard deviations)

| | | Group I (*n* 76) | | Group II (*n* 191) | | Group III (*n* 33) | |
|---|---|---|---|---|---|---|---|
| Biochemical and haematological parameters | Unit | Mean | SD | Mean | SD | Mean | SD |
| Maternal biochemical status | | | | | | | |
| Total protein | mg/dl | 7·17 | 0·88 | 7·15 ($P$=0·002)† | 1·02 | 6·47 ($P$=0·02)* | 1·79 |
| Serum albumin | mg/dl | 3·80 | 0·66 | 3·70 | 0·72 | 3·56 | 1·13 |
| Fetal biochemical status | | | | | | | |
| Total protein | mg/dl | 7·27 | 1·07 | 7·14 | 1·02 | 7·03 | 1·29 |
| Serum albumin | mg/dl | 4·03 | 0·68 | 4·04 | 0·79 | 4·11 | 0·62 |
| Maternal haematological status | | | | | | | |
| Hb | g/l | 11·81 | 0·55 | 9·76 ($P$=5·8 × $10^{-40}$)* ($P$=0·0001)† | 0·83 | 6·30 ($P$=3·6 × $10^{-61}$) | 0·62 |
| Ferritin | g/l | 37·18 | 24·82 | 54·27 ($P$=0·06)* | 42·2 | 46·06 | 44·91 |
| Erythropoietin | g/l | 32·3 | 23·34 | 558·16 ($P$=0·001)* ($P$=0·009)† | 46·92 | 83·0 ($P$=0·0001)* | 63·8 |
| Fetal haematological status | | | | | | | |
| Hb | g/l | 12·99 | 1·15 | 12·87 ($P$=0·001)† | 1·33 | 11·42 ($P$=0·045)* | 3·00 |
| Ferritin (g/l) | g/l | 97·53 | 50·6 | 104·59 ($P$=0·01)* | 53·32 | 117·24 ($P$=0·007)* | 54·4 |
| Erythropoietin | g/l | 43·9 | 26·45 | 43·25 | 39·08 | 44·7 | 36·2 |

Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were also performed using a Bonferroni adjustment.
*($P$-value) indicates a statistically significant difference for pair-wise group comparisons between severe or moderate anaemia *v.* non-anaemic controls.
†($P$-value) indicates a statistically significant difference for pair-wise group comparisons between moderate anaemia *v.* severe anaemia.
‡For details of subjects and procedures, see Experimental methods.

*Endocrine parameters in maternal blood in the study groups*

Data shown in Table 4 indicate no statistically significant differences in maternal GH, insulin and EGF levels in the maternal blood of moderately and severely anaemic mothers compared with the normal control (Group I) mothers. Significant differences were observed in maternal PRL levels (ng/ml), which were significantly higher in the moderately anaemic and severely anaemic mothers compared with the normal mothers. The hPL levels (mU/l) were significantly lower in the severely anaemic mothers compared with the normal control mothers. A significant increase in maternal IGF-1 (ng/ml) levels was observed in both the moderately and severely anaemic mothers compared with the control group.

Maternal thyroid hormone $T_4$ (μg/dl) and rT3 (ng/dl) levels were not significantly different between the moderately and severely anaemic mothers compared with the Group I mothers. However, maternal blood T3 (ng/dl) levels were significantly lower in severely anaemic mothers compared with the Group I mothers, indicating development of a hypothyroid state with the progression of severity of anaemia.

In the maternal compartment, only the EGF (ng/ml) levels were significantly higher in Group II mothers compared with Group III mothers.

*Regression analysis*

We did regression analysis to evaluate associations between maternal Hb values and the various endocrine parameters. The correlation coefficient ($r$) and the corresponding $P$ values indicate the strength and direction of a linear relationship between two random variables. We observed significant associations between maternal Hb and cord ferritin, maternal erythropoietin and cord and maternal IGF-1 levels, indicating that these endocrine parameters are significantly modulated in nutritional anaemia (Fig. 1A–D).

## Discussion

Fe deficiency is the most common cause of nutritional anaemia. During pregnancy there is a high risk of developing it due to the increase of Fe requirements for fetal and maternal tissue growth. Globally, 50 % of anaemia cases are assumed to be attributable to Fe deficiency and the prevalence of this micronutrient deficiency remains a significant public health problem[1–4]. Anaemia has been recognized as being associated with protein–energy malnutrition since the earliest classified descriptions, but the pathogenesis of anaemia is complex and involves several variables[34–40]. The aetiology of anaemia remains unexplained, despite careful investigations, primarily due to the difficulties of assessment of micronutrient sufficiency or deficiency in pregnancy as well as the compounded problem of the interaction between other common infections and micronutrient deficiency states during pregnancy.

In the present study, we believe that there is a co-existence of anaemia and malnutrition in our patient cohorts since the majority of the subjects belonged to low socioeconomic strata of society and were likely to have a certain degree of nutritional restriction. This assumption is substantiated by the results of all the anthropometric parameters measured in

**Table 3.** Endocrine parameters measured in the cord blood samples of neonates belonging to the three study groups‡ (Mean values and standard deviations)

| | | Group I (*n* 76) | | Group II (*n* 191) | | Group III (*n* 33) | |
|---|---|---|---|---|---|---|---|
| Cord blood | Unit | Mean | SD | Mean | SD | Mean | SD |
| Peptide hormones | | | | | | | |
| GH | ng/ml | 33·50 | 17·2 | 37·12 | 23·55 | 32·23 | 26·50 |
| PRL | ng/ml | 313·97 | 30·10 | 264·96 (*P*=0·0001)† | 27·17 | 340·26 | 32·9 |
| Insulin | mU/ml | 20·43 | 13·26 | 21·25 (*P*=0·01)† | 17·17 | 30·33 | 26·98 |
| hPL | mU/l | 76·62 | 16·6 | 54·67 (*P*=0·0001)† | 7·95 | 103·10 | 21·8 |
| Growth factors | | | | | | | |
| IGF-1 | ng/ml | 204·04 | 103·51 | 232·15 (*P*=0·0001)† | 115·34 | 324·63 (*P*=0·01)* | 116·34 |
| EGF | ng/ml | 3·94 | 2·79 | 4·32 (*P*=0·027)† | 3·25 | 3·03 | 1·62 |
| Thyroid hormones and thyroid-stimulating hormone | | | | | | | |
| T4 | μg/dl | 8·85 | 3·06 | 8·14 (*P*=0·05)* | 3·15 | 8·07 | 3·34 |
| T3 | ng/dl | 47·76 | 16·91 | 51·10 | 26·61 | 46·29 | 23·14 |
| r-T3 | ng/dl | 217·13 | 88·81 | 215·27 | 96·42 | 249·67 | 134·62 |
| TSH | μU/ml | 5·10 | 3·67 | 5·07 | 4·05 | 5·27 | 3·12 |

GH, growth hormone; PRL, prolactin; hPL, placental lactogen; IGF, insulin-like growth factor; EGF, epidermal growth factor; TSH, thyroid-stimulating hormone.
Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were also performed using a Bonferroni adjustment.
* (*P*-value) indicates a statistically significant difference for pair wise group comparisons between severe or moderate anaemia *v.* non-anaemic controls.
† (*P*-value) indicates a statistically significant difference for pair wise group comparisons between moderate anaemia *v.* severe anaemia.
‡ For details of subjects and procedures, see Experimental methods.

the mothers, namely, the pre-pregnancy weight, maternal weight at delivery, fundal height and abdominal girth with the exception of maternal height and maternal mid-arm circumference, which were significantly lower in the group constituting the severely anaemic mothers and were indicative of the fact that these mothers were nutritionally restricted both prior to and during pregnancy. A significant decrease in neonatal birth weight was observed in neonates born to both moderately and severely anaemic mothers. However, in the neonates born to severely anaemic mothers, other anthropometric measures of fetal growth such as ponderal index and placental weight were also significantly lower compared with the normal control group, indicating significant growth restriction due to nutritional anaemia in the mothers. Substantial IDA (usually considered to be $<8{\cdot}0$ g/dl) has been reported to be associated with an increased incidence of low birth weight[41–44].

We observed a significant reduction in serum total protein levels in the maternal blood of severely anaemic mothers. This suggests the co-existence of a significant degree of energy deprivation in addition to $B_{12}$/folic acid deficiency and/or vitamin A deficiencies in this group of mothers[45]. Anaemia in pregnant mothers may be associated with malnutrition and lack of Fe/folic acid supplementation during pregnancy may contribute to an increased nutritional insult to the fetus, by further contributing to fetal growth retardation.

A significant decrease in Hb concentrations is observed in the three study groups, as expected, based on the study

**Table 4.** Endocrine parameters measured in the maternal blood samples of mothers belonging to the three study groups‡ (Mean values and standard deviations)

| | | Group I (*n* 76) | | Group II (*n* 191) | | Group III (*n* 33) | |
|---|---|---|---|---|---|---|---|
| Maternal blood | Unit | Mean | SD | Mean | SD | Mean | SD |
| Peptide hormones | | | | | | | |
| GH | ng/ml | 13·54 | 11·38 | 14·59 | 12·67 | 11·17 | 7·14 |
| PRL | ng/ml | 346·45 | 231·7 | 429·80 (*P*=0·02)* | 303·06 | 493·21 (*P*=0·02)* | 324·6 |
| Insulin | mU/ml | 111·83 | 16·9 | 100·87 | 12·44 | 97·31 | 15·04 |
| hPL | mU/l | 3220·0 | 1409·53 | 3007·03 | 1604·4 | 2590·79 (*P*=0·01)* | 1180·44 |
| Growth factors | | | | | | | |
| IGF-1 | ng/ml | 114·38 | 16·78 | 144·67 (*P*=0·01)* | 52·59 | 151·7 (*P*=0·05)* | 65·03 |
| EGF | ng/ml | 8·09 | 2·47 | 8·26 (*P*=0·0001)† | 3·08 | 6·01 | 3·39 |
| Thyroid hormones and thyroid-stimulating hormone | | | | | | | |
| T4 | μg/dl | 9·67 | 2·94 | 9·93 | 3·24 | 9·74 | 3·17 |
| T3 | ng/dl | 124·75 | 4·06 | 122·82 | 42·17 | 109·47 (*P*=0·04)* | 40·01 |
| r-T3 | ng/dl | 30·40 | 14·2 | 28·30 | 11·07 | 32·89 | 16·66 |
| TSH | μU/ml | 1·71 | 1·18 | 2·01 | 1·54 | 1·68 | 0·80 |

GH, growth hormone; PRL, prolactin; hPL, placental lactogen; IGF, insulin-like growth factor; EGF, epidermal growth factor; TSH, thyroid-stimulating hormone.
Statistical analysis was done using ANOVA. Multiple comparisons between the study groups were also performed using a Bonferroni adjustment.
* (*P*-value) indicates a statistically significant difference for pair-wise group comparisons between severe or moderate anaemia *v.* non-anaemic controls.
† (*P*-value) indicates a statistically significant difference for pair-wise group comparisons between moderate anaemia *v.* severe anaemia.
‡ For details of subjects and procedures, see Experimental methods.

British Journal of Nutrition

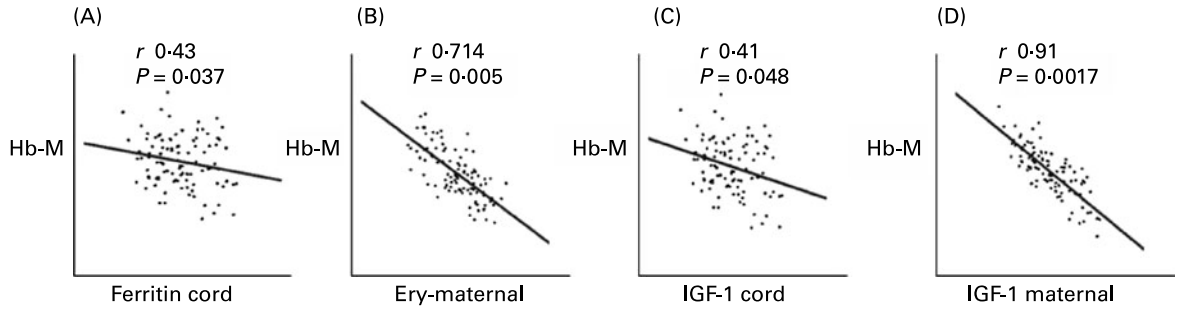


**Fig. 1.** Graphical representation of regression analysis between maternal Hb and the endocrine parameters. (A) Correlation between maternal Hb and cord ferritin levels; (B) correlation between maternal Hb and maternal erythropoietin levels (Ery-maternal); (C) correlation between maternal Hb and cord insulin-like growth factor (IGF)-1 levels; (D) correlation between maternal Hb and maternal IGF-1 levels; showing significant associations between maternal Hb and cord ferritin, and cord and maternal IGF-1 levels indicating that these endocrine parameters are significantly modulated in nutritional anaemia (*r* correlation coefficient; $P<0{\cdot}05$ indicates statistical significance). For details of subjects and procedures, see Experimental methods.

design. However there is only a small difference in Hb levels in the cord blood of neonates born to moderately and severely anaemic mothers when compared with the normal levels in the control group neonates. Dewey *et al.* [46] have shown that neonates have a high concentration of circulating Hb and that Fe availability in the neonates is via destruction of erythrocytes and that this Fe is also used for the synthesis of new Hb, thus reducing the need for exogenous Fe[47–51]. The significantly increased levels of serum ferritin in the cord blood of neonates born to moderately and/or severely anaemic mothers are indicative of a sizeable Fe reserve in the liver of these neonates. Ferritin and transferring/transferrin receptor are all Fe regulatory proteins that are involved in the modulation of the cellular defence mechanisms against stress and inflammation, and ferritin levels are known to be regulated by cytokines, oxidants and growth factors. Ferritin changes in hypoxia are mediated by altered regulation of the Fe responsive proteins[52,53]. The increase in both the maternal and fetal serum ferritin levels with the degree of severity of anaemia observed in the present study may be an adaptive mechanism in pregnancy to ensure prolonged survival under a growth-restricted environment. Thus, these changes in ferritin levels are a result of the hypoxic insult that causes an increase in pro-inflammatory cytokines that modulate the hypothalamic–pituitary–adrenal axis. Pro-inflammatory cytokines can modulate the production of the Fe-regulatory hormone hepcidin, thereby altering Fe metabolism resulting in a decrease in serum Fe and total Fe binding capacity and an increase serum ferritin level that reflects augmented ferritin synthesis[54]. We believe that chronic nutritional anaemia results in hypoxic stress *in utero*, which induces both functional and structural adaptations within the hypothalamo–pituitary–adrenocortical axis, resulting in alterations in endocrine responsiveness in the maternal–feto–placental milieu.

It is evident from the comparisons between the moderately anaemic and the severely anaemic group of mothers and their neonates that with the increase in the severity of anaemia there is a significant decrease in maternal early-pregnancy weight, maternal weight at delivery and maternal abdominal circumference, birth weight, ponderal index, total protein, while an inverse relationship was observed with both cord and maternal erythropoietin levels. The decrease in these afore-mentioned anthropometric and biochemical measures of nutritional status of the mother and the neonate confirms the co-existence of anaemia and maternal malnutrition. Further, there is a quantitative decrease in these parameters with the progression of the severity of nutritional anaemia.

Since hypoxia is known to be the main stimulus for increased erythropoietin gene expression and erythropoietin production[53,55,56], it is not surprising that an inverse correlation is observed between both cord and maternal erythropoietin levels and severity of anaemia. Erythropoietin is an erythroid-specific glycoprotein hormone, which is an important factor involved in the regulation of erythrocyte production. The increase in maternal erythrocyte mass and the growth of the fetus during pregnancy require increased amounts of Fe, leading to a rapid decline in maternal Fe stores[47–56]. IDA in pregnancy acts as a stimulus for erythropoietin induction. An increase in plasma volume is observed during gestation, and maternal Hb concentrations continue to decrease despite the erythropoiesis process[49–56]. Inadequate nutritional supplementation and or energy restriction during this stage will result in anaemia that can impair the liberation of oxygen to the fetus, thus interfering with normal intrauterine growth. Fe deficiency may also affect important enzymes, such as cytochromes, causing oxidative damage to erythrocytes and the fetoplacental unit[47–56]. Our regression analysis shows a positive correlation between the cord blood erythropoietin levels and severity of anaemia.

The hormones and growth factors evaluated in the present study are known to play important roles in the tissue accretion and differentiation in the fetus in several mammalian species[56–58]. The perinatal period presents a highly synchronized sequence of metabolic and endocrine events, which are very important for normal growth and development of the fetus[56–60]. Significantly higher levels of IGF-1 were observed in the cord blood of neonates born to severely anaemic mothers. The fetus is known to control the placental utilization of substrates via its blood concentrations of oxygen and glucose via IGF-I; additionally, the levels of hPL, can alter the stability and concentrations of IGF-binding proteins and the levels of circulating IGF. IGF-1 may have both anabolic and anti-catabolic effects in the fetus. hPL, in addition to helping to mobilize nutrients from maternal sources, has also been shown to stimulate fetal IGF-1 production by interacting with GH receptors[61]. hPL has been shown to stimulate ornithine decarboxylase activity in fetal liver and promote amino acid transport in fetal muscle and enhance DNA

synthesis and IGF-1 production in mouse fibroblasts. IGF-1 has been shown to have metabolic, mitogeneic and differentiation functions in fetal and neonatal tissues. Several hormones may promote growth and differentiation of fetal tissue through the common pathway of IGF generation[62–64]. We have previously reported that neonates born to malnourished and anaemic mothers also show a significant increase in IGF-1 levels[59]. Erythropoietin therapy has been reported to enhance erythropoiesis and modulates the serum concentration of IGF-I[53,64,65]. The enhanced erythropoiesis that is reflected by the increased serum erythropoietin levels in the anaemic mothers may be responsible for the increased IGF-1 concentration observed in that group. Regression analysis of our data showed significant associations between maternal Hb and cord ferritin, maternal erythropoietin and cord and maternal IGF-1 levels, indicating that all of these parameters play a key role in endocrine regulation in nutritional anaemia. Cord blood levels of insulin and IGF-1 were significantly higher in the neonates born to severely anaemic mothers compared with those born to moderately anaemic mothers. Although when statistical comparisons between the severely anaemic group and normal control group for these parameters were done, no statistical significance was attained.

The cord blood PRL and hPL values of neonates born to moderately anaemic mothers were lower than those of the normal controls, as well as the neonates born to severely anaemic mothers. Although a significant *P* value was obtained on comparison between the moderately anaemic and the severely anaemic groups, no plausible explanation can be provided for this discrepancy other than the fact that this discordance is most likely due to the large variance in the number of subjects within each patient cohort. Cord and maternal EGF levels were significantly higher in neonates born to moderately anaemic mothers compared with neonates born to severely anaemic mothers. This can be explained by the fact that EGF can selectively impair erythropoiesis and induce a decline in Hb levels[65–67].

We observed a decrease in $T_4$ levels in the cord blood of neonates born to moderately and severely anaemic mothers. Also, the levels of maternal $T_3$ were significantly lower in the severely anaemic mothers. Thyroid hormones are critical to the growth and development of the human fetus. We and other investigators have documented that malnutrition during pregnancy induces a hypoxic suppression of thyroid function, or a hypothyroid state with a consequent decrease in metabolic rate and oxygen consumption, which would be a beneficial adaptation of the fetus to the stimulus of maternal nutrient restriction[60]. The significant decrease in cord blood $T_4$ and maternal $T_3$ levels may be indicative of a disrupted thyroid hormone balance that may eventually result in a hypothyroid state should the severity of the anaemic insult continue.

A previous study reports that hPL is a potent haemopoietic hormone[67]. However, we can attribute decreased levels of hPL in the severely anaemic mothers in the present study to a significantly smaller placental size in that group. The changes in the maternal–fetal endocrine milieu in the severely anaemic study group described earlier suggest that nutritional anaemia imposes a growth restriction *in utero*. Although there are several studies highlighting the effects of anaemia on fetal growth and development, the literature is not conclusive on the influence of anaemia in pregnancy. More frequent preterm births and low birth weights have been reported in the majority of studies that have considered mild to moderate maternal anaemia; however, there are very few reports on the endocrine changes in both the maternal and fetal compartments as a consequence of anaemia and correlations with its severity. Many studies, however, have indicated that routine Fe supplementation during pregnancy may have beneficial effects on pregnancy outcome. Fe deficiency no doubt has deleterious consequences on maternal and fetal health[68].

The present study has shown that anaemia in pregnancy most definitely influences fetal outcome. Additionally, the more the magnitude of the anaemic insult, the greater the effect on fetal growth due to a significant endocrine modulation in the maternal fetoplacental unit. The endocrine profile in such a dataset is a unique set of information that may lead to a better understanding of maternal–fetal physiology and about how environmental factors such as maternal anaemia can adversely affect fetal development and long-term neonatal outcome. The present data are extremely important, particularly in the light of Barker's hypothesis, which suggests that alterations in the maternal endocrine, nutritional and metabolic environment disrupt the developmental trajectory of the fetus and can lead to adult diseases. Additional research studies such as ours need to be done to elucidate the mechanisms underlying the prevalence of nutritional anaemia and the complex relationship between maternal nutrition and endocrine modulation that result in a beneficial adaptation of the fetus under situations of maternal nutritional deprivation, which are common in developing countries.

## Acknowledgements

The authors would like to thank Dr Meharban Singh MD, Head of Department of Pediatrics and Neonatology, All India Institute of Medical Sciences, New Delhi, India and Dr Krishna Singh MD, Head of Department of Obstetrics and Gynecology, Safdarjung Hospital, New Delhi for their support towards the successful completion of this study. The authors gratefully acknowledge the NIH-NIADDK-NHPP hormone assay program for supply of assay reagents. The authors also gratefully acknowledge the funding support from the Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. There is no conflict of interest between the authors.

## References

1. Woodruff AW (1968) Anaemia associated with protein-calorie malnutrition. In *Calorie Deficiencies and Protein Deficiencies. Proceedings of a Colloquium held in Cambridge*, pp. 165–173 [RA McCance and EM Widdowson, editors]. London, UK: J & A Churchill.
2. DeMaeyer E & Adiels-Tegman M (1985) The prevalence of anaemia in the world. *World Health Stat Q Rep* **38**, 302–316.
3. World Health Organization Working Group (1982) Hereditary anaemia: genetic basis, clinical features, diagnosis and treatment. *WHO Bulletin* **60**, 643–660.
4. Dempster WS, Sive AA, Rosseau S, Malau H & Heese HV (1995) Misplaced iron in Kwashiorkor. *Eur J Clin Nutr* **49**, 208–210.
5. Scholl TO (2005) Iron status during pregnancy: setting the stage for mother and infant. *Am J Clin Nutr* **81**, 1218S–1222S.

British Journal of Nutrition

6. Rahman M, Roy SK, Ali M, Mitra AK, Alam AN & Akbar MS (1993) Maternal nutritional status as a determinant of child health. *J Trop Pediatr* **39**, 86–88.
7. Thangaleela T & Vijayalakshmi P (1994) Impact of anaemia in pregnancy. *Indian J Nutr Diet* **31**, 251–256.
8. Dallman PR (1993) Iron deficiency anemia: a synthesis of current scientific knowledge and US recommendations for prevention and treatment. In *Iron Deficiency Anemia* [R Earl and CE Woteki, editors]. Washington, DC: Committee on the Prevention, Detection, and Management of Iron Deficiency Anemia among US Children and Women of Childbearing Age, Food and Nutrition Board, Institute of Medicine; National Academy Press.
9. Duncan B, Schifman RB, Corrigan JJ Jr & Schaefer C (1985) Iron and the exclusively breast-fed infant from birth to six months. *J Pediatr Gastroenterol Nutr* **4**, 421–425.
10. Earle R & Woteki CE (1993) *Iron Deficiency Anemia: Recommended Guidelines for the Prevention, Detection, and Management among U.S. Children and Women of Childbearing Age*. Food and Nutrition Board, Institute of Medicine, pp. 1–35. Washington, DC: National Academy Press.
11. Fomon SJ (1991) Requirements and recommended dietary intakes of protein during infancy. *Pediatr Res* **30**, 391–395.
12. Golub MS, Hogrefe CE, Tarantal AF, Germann SL, Beard JL, Georgieff MK, Calatroni A & Lozoff B (2006) Diet-induced iron deficiency anemia and pregnancy outcome in rhesus monkeys. *Am J Clin Nutr* **83**, 647–656.
13. Hill JM (1989) Comments on: Brain iron: a lesson from animal models. *Am J Clin Nutr* **50**, 618–629.
14. Idiradinata P & Pollitt E (1993) Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* **341**, 1–4.
15. Jelliffe DB (1968), *Child Nutrition in Developing Countries: A Handbook for Fieldworkers*, Public Health Service Pub No 1822, pp. 75. Washington, DC: US Department of Health, Education and Welfare.
16. Lozoff B, Jimenez E & Wolf AW (1991) Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* **325**, 687–694.
17. Pollitt E (1993) Iron deficiency and cognitive function. In *Annual Review of Nutrition*, vol. 13, pp. 521–537 [RE Olson, DM Bier and DB McCormick, editors]. Palo Alto, CA: Annual Reviews.
18. Smith Q (1990) Regulation of metal uptake and distribution within brain. In *Nutrition and the Brain*, pp. 25–74 [RJ Wurtman and JJ Wurtman, editors]. New York: Raven Press.
19. Youdim MBH, Ben-Shachar D & Yehuda S (1989) Putative biological mechanisms of the effect of iron deficiency on brain biochemistry and behavior. *Am J Clin Nutr* **50**, 607–617.
20. Gluckman PD & Pinal CS (2003) Regulation of fetal growth by the somatotrophic axis. *J Nutr* **133**, 1741S–1746S.
21. Nieto-Diaz A, Matorras R, Villar J & Serra M (1996) Intrauterine growth retardation at term: association between anthropometric and endocrine parameters. *Acta Obstet Gynecol Scand* **75**, 127–131.
22. Godfrey KM & Barker DJ (1995) Maternal nutrition in relation to fetal and placental growth. *Eur J Obstet Gynecol Reprod Biol* **61**, 15–22.
23. Fowden AL, Li J & Forhead AJ (1998) Glucocorticoids and the preparation for life after birth: are there long term consequences of the life insurance? *Proc Nutr Soc* **57**, 113–122.
24. Fowden AL & Forhead AJ (2001) The role of hormones in intrauterine development. In *Lung Biology in Health and Disease*, Vol. **151**, pp. 199–228 [DJP Barker, editor]. New York: Marcel Dekker.
25. Fowden AL & Forhead AJ (2004) Endocrine mechanisms of intrauterine programming. *Reproduction* **127**, 515–526.
26. UNICEF/WHO (2004) Low birth weight: country, regional and global estimates, New York. In *Monitoring Low Birth Weight: An Evaluation of International Estimates and an Updated Estimation Procedure*, WHO Bulletin [A Blanc and T Wardlaw, editors]. Geneva: WHO.
27. World Health Organization (1995) Maternal anthropometry and pregnancy outcomes. A WHO collaborative study. *WHO Bulletin* **73**, Suppl. 1–98.
28. De Onis M & Habicht JP (1996) Anthropometric reference data for international use. Recommendations from the World Health Organisation Expert Committee. *Am J Clin Nutr* **64**, 650–658.
29. Mohan M, Prasad SR, Chellani HK & Kapani V (1990) Intrauterine growth curves in north Indian babies: weight, length, head circumference and ponderal index. *Indian Pediatr* **27**, 43–51.
30. World Health Organization (1992) *Low Birth Weight*. A tabulation of available information, WHO/MCH/92.2. Geneva: WHO.
31. World Health Organization (1997) *National Reports on the Third Evaluation of the Implementation of 'Health for All' Strategies*. New Delhi: WHO Global Database.
32. ACC/SCN (2000) *Fourth Report on the World Nutrition Situation*. Geneva: ACC/SCN in collaboration with IFPRI.
33. World Health Organization Scientific Group (1968) *Nutritional Anaemias*. Report of a WHO Scientific Group Meeting, Geneva, 13–17 March 1967. Geneva: WHO.
34. Nieto-Diaz A, Matorras R, Villar J & Serra M (1996) Intrauterine growth retardation at term: association between anthropometric and endocrine parameters. *Acta Obstet Gynecol Scand* **75**, 127–131.
35. Godfrey KM & Barker DJP (1995) Maternal nutrition in relation to fetal and placental growth. *Eur J Obstet Gynecol Reprod Biol* **61**, 15–22.
36. Barker DJP, Eriksson JG, Forsen T & Osmond C (2002) Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* **31**, 1235–1239.
37. Bateson P, Barker DJP, Clutton-Brock T, *et al.* (2004) Developmental plasticity and human health. *Nature* **430**, 419–421.
38. Bhargava M, Iyer PU, Kumar R, Ramji S, Kapani V & Bhargava SK (1991) Relationship of maternal serum ferritin with foetal serum ferritin, birth weight and gestation. *J Trop Pediatr* **37**, 149–152.
39. Hirve SS & Ganatra BR (1994) Determinants of low birth weight: a community based prospective cohort study. *Indian Pediatr* **31**, 1221–1225.
40. Onadeko MO, Avokey F & Lawoyin TO (1996) Observations of stillbirths, birthweight and maternal haemoglobin in teenage pregnancy in Ibadan, Nigeria. *Afr J Med Med Sci* **25**, 81–86.
41. Scholl TO, Hediger ML, Fischer RL & Shearer JW (1992) Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. *Am J Clin Nutr* **55**, 985–988.
42. Scholl TO & Hediger ML (1994) Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *Am J Clin Nutr* **59**, Suppl. 492S–500S.
43. Singla PN, Tyagi M, Kumar A, Dash D & Shankar R (1997) Fetal growth in maternal anaemia. *J Trop Pediatr* **43**, 89–92.
44. Spinillo A, Capuzzo E, Piazzi G, Nicola S, Colonna L & Iasci A (1994) Maternal high-risk factors and severity of growth deficit in small for gestational age infants. *Early Hum Dev* **38**, 35–43.
45. Swain S, Singh S, Bhatia BD, Pandey S & Krishna M (1994) Maternal hemoglobin and serum albumin and fetal growth. *Indian Pediatr* **31**, 777–782.
46. Dewey KG, Beaton G, Fjeld C, Lonnerdal B & Reeds P (1996) Protein requirements of infants and children. *Eur J Clin Nutr* **150**, S119–S147.
47. Bentley DP (1985) Iron metabolism and anaemia in pregnancy. *Clin Haematol* **14**, 613–628.
48. Bothwell TH (2000) Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr* **72**, 257S–264S.


British Journal of Nutrition

49. Fenton V, Cavill I & Fisher J (1977) Iron stores in pregnancy. *Br J Haematol* **37**, 145–149.
50. Huch R & Huch A (1993) Maternal and fetal erythropoietin: physiological aspects and clinical significance. *Ann Med* **25**, 289–293.
51. Rikonen S, Saijonmaa O, Järvenpää AL & Fyhrquist F (1994) Serum concentrations of erythropoietin in healthy and anaemic pregnant women. *Scand J Lab Invest* **54**, 653–657.
52. Torti FM & Torti SV (2002) Regulation of ferritin genes and protein. *Blood* **99**, 3505–3516.
53. Jelkmann W (2003) Erythropoietin. *J Endocrinol Invest* **26**, 832–837.
54. Ganz T (2006) Molecular pathogenesis of anemia of chronic disease. *Pediatr Blood Cancer* **46**, 554–557.
55. Watt F, Taylor JED & Nestadt A (1962) Erythroid hypoplasia in kwashiorkor. *Br Med J* **1**, 73–75.
56. Howells MR, Jones SE, Napier JAF, Saunders K & Cavill I (1986) Erythropoiesis in pregnancy. *Br J Haematol* **64**, 595–599.
57. Fowden AL, Giussani DA & Forhead AJ (2006) Intrauterine programming of physiological systems: causes and consequences. *Physiology (Bethesda)* **21**, 29–37.
58. Clark PM (1998) Programming of the hypothalamo–pituitary–adrenal axis and the fetal origins of adult disease hypothesis. *Eur J Pediatr* **157**, Suppl. 1, S7–S10.
59. Mahajan SD, Singh S, Shah P, Gupta N & Kochupillai N (2004) Effect of maternal malnutrition and anemia on the endocrine regulation of fetal growth. *Endocr Res* **30**, 189–203.
60. Mahajan SD, Aalinkeel R, Singh S, Shah P, Gupta N & Kochupillai N (2005) Thyroid hormone dysregulation in intrauterine growth retardation associated with maternal malnutrition and/or anemia. *Horm Metab Res* **37**, 633–640.
61. Alsat E, Marcotty C, Gabriel R, Igout A, Frankenne F, Hennen G & Evain-Brion D (1995) Molecular approach to intrauterine growth retardation: an overview of recent data. *Reprod Fertil Dev* **7**, 1457–1464.
62. Bassett NS, Oliver MH, Breier BH & Gluckman PD (1990) The effect of maternal starvation on plasma insulin-like growth factor I concentrations in the late gestation ovine fetus. *Pediatr Res* **27**, 401–404.
63. Shambaugh G 3rd, Glick R, Radosevich J & Unterman T (1993) Insulin-like growth factor-I and binding protein-1 can modulate fetal brain cell growth during maternal starvation. *Ann N Y Acad Sci* **692**, 270–272.
64. Sheashaa HA, Khalil A, Aarman MM, El-Shahat FB, Selim A & El-Gawad SS (2005) Correction of hemodialysis anemia is associated with significant increase in serum concentration of IGF-I in patients treated with erythropoietin: a randomized controlled study. *Int Urol Nephrol* **37**, 153–158.
65. Vannasaeng S, Fucharoen S, Pootrakul P, Ploybutr S & Yansukon P (1991) Pituitary function in thalassemic patients and the effect of chelation therapy. *Acta Endocrinol (Copenh)* **124**, 23–30.
66. Juhl CO, Vinter-Jensen L, Myhre-Jensen O, Jensen LS & Dajani EZ (1995) Epidermal growth factor can selectively impaired erythropoiesis and induce a decline in hemoglobin levels. *Clin Sci (Lond)* **89**, 453–458.
67. Berczi I & Nagy E (1991) Placental lactogen is a haemopoietic hormone. *Br J Haematol* **79**, 355–358.
68. van den Broek N (2003) Anaemia and micronutrient deficiencies. *Br Med Bull* **67**, 149–160.


British Journal of Nutrition