Third trimester fetal growth and umbilical venous blood concentrations of IGF-1, IGFBP-1, and growth hormone at term

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Abstract
Insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-1 (IGFBP-1) and growth hormone (GH) concentrations were measured in umbilical venous blood after delivery of 78 term newborn infants. Three groups of pregnancies were prospectively identified during the third trimester, according to fetal size and subsequent fetal growth, assessed by repeated ultrasound scans. Fetal size was considered either appropriate for gestational age (AGA) or small for gestational age (SGA), according to whether the first ultrasound measurement of abdominal circumference was equal to or above, or below the tenth centile for gestational age, respectively. Subsequent fetal growth was quantified by the change in the standard deviation score of abdominal circumference measurements between the first and last scans before delivery. Fetal growth retardation (FGR) was defined as a (negative) change in SD score of greater than −1·5. Eighteen SGA fetuses with evidence of FGR had significantly lower IGF-1 (median 0·05 (range 0·0–0·24) U/ml) at delivery than 35 SGA fetuses with normal growth (median 0·13 (range 0·0–0·94) U/ml; P<0·05) and 25 AGA fetuses with normal growth (median 0·31 (range 0·0–0·84) U/ml; P<0·05). The median concentration in the SGA group with normal growth was also significantly lower than that of the AGA group with normal growth. There were no significant differences in IGFBP-1 or GH concentrations between the three groups.

These observations indicate that umbilical blood concentrations at birth of IGF-1, but not IGFBP-1 or GH, relate to both fetal size and fetal growth during the third trimester of pregnancies reaching term.
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Keywords: umbilical blood, insulin-like growth factor-1, insulin-like growth factor binding protein-1, growth hormone.

Insulin-like growth factors (IGFs) are mitogenic peptides produced by mesenchymal cells. They are structurally similar to proinsulin. Insulin-like growth factor-1 (IGF-1) may act in a paracrine or autocrine manner[1] by stimulating cellular proliferation and differentiation in target cells.[2] Several studies have shown a positive association between birthweight and IGF-1 concentrations in umbilical venous blood at delivery,[3–8] although one found an inverse correlation.[9] In the circulation IGF-1 is bound to specific proteins and six IGF binding proteins (IGFBPs) have been described.[10 11] Fetal concentrations of IGF-1 are low but studies suggest its half-life may be prolonged by being bound to IGFBP-1[12] and/or IGFBP-3.[11] Reports of the association between birthweight and IGFBP-1 concentrations have generally shown a negative correlation,[6 9 12] although one study did not find any association.[7] Growth hormone (GH) does not appear to be related to birthweight.[13]

Studies of circulating concentration of IGF-1 and IGFBP-1 in the fetus have all used birthweight as a measure of fetal growth. However, low birthweight is a poor measure of fetal growth retardation[14] which is better defined as reduced change in fetal size over time.[15 16]

We designed a prospective study to relate umbilical vein concentrations of IGF-1, IGFBP-1, and GH at birth to fetal size and growth, assessed using repeated ultrasound measures of abdominal circumference during the third trimester of pregnancy.

Methods
Seventy eight pregnancies, thought clinically to be small during the third trimester, were recruited into the study following referral to the radiology department. In 53 cases the fetus was found to be SGA with an abdominal circumference of <10th centile.[16] A further 25 cases were AGA with an abdominal circumference between the 10th and 90th centiles. All women had been scanned between 18 and 21 weeks' gestation at which time measurements of biparietal diameter and femur length were consistent with being within seven days of certain menstrual dates. All pregnancies continued until 37 weeks or more. The study was approved by the hospital ethics committee and all women gave informed consent.

Fetal abdominal circumference was measured by ultrasound scans at intervals of one or two weeks on at least three occasions before delivery. At each scan the abdominal circumference value was expressed as a standard deviation (SD) score using our own, published, reference standards.[16] The SD score was calculated as the measured abdominal circumference value minus the mean value for the gestation, divided by the SD of the mean for that gestation. Fetal growth was quantified by determining the change in SD score between

Table 1 Description of groups according to fetal size and growth

	Normal size	Small size (abdominal circumference of <10th centile)	
	Normal growth (n=25)	Normal growth (n=35)	Growth retardation (n=18)
Gestation (days)	274 (254–289)	269 (253–290)	272 (257–292)
Birthweight (g)	3570 (3200–4200)	2659 (1701–3120)*	2277 (1360–2900)*†
Ponderal index	2·60 (2·38–2·98)	2·45 (2·13–2·42)*	2·28 (1·90–2·93)*†
MAC:HC ratio	0·31 (0·28–0·35)	0·28 (0·22–0·33)*	0·26 (0·23–0·30)*†
Skinfold thickness (mm)			
Subscapular	4·6 (2·9–6·1)	3·1 (2·2–4·4)*	2·7 (2·0–4·3)*†
Triceps	4·7 (3·1–6·2)	3·1 (2·2–4·4)*	2·7 (1·8–4·3)*†

Values are median (range). MAC=mid arm circumference. HC=head circumference.
Statistical differences: *0·05 compared with normal size, normal growth group. †0·05 compared with small size, normal growth group.

the first ultrasound scan after recruitment and the last scan before delivery. A change in score of greater than −1·5 was used to define fetal growth retardation as this was the cutoff, determined using ROC curves, which best predicted neonatal morphometry indicative of fetal growth retardation[17] as well as adverse perinatal outcome related to fetal growth retardation.[18]

Immediately after delivery, and before expulsion of the placenta, the umbilical cord was clamped in two places. Three millilitres of umbilical venous blood were collected into a plain tube and centrifuged within five minutes of collection at 3500 rpm for 10 minutes. Aliquots of sera were stored at −30°C until further analysis. Serum IGF-1 was measured, after acid-ethanol extraction of its binding proteins, by radioimmunoassay (RIA) using a polyclonal rabbit antiserum (R557A) raised against purified human IGF-1.[19] The sensitivity of this assay was 0·07 U/ml. Intra-assay coefficient of variation (CV) values were 11·3% at 0·23 U/ml and 6·5% at 1·23 U/ml. Interassay CV values were 10·5% at 0·38 U/ml and 12·1% at 0·99 U/ml. Serum concentrations of IGFBP-1 were measured using a specific RIA using purified antigen obtained from Dr S Drop (Rotterdam, Holland). Tracer was prepared by iodination of antigen using the chloamine-T method followed by separation on a short Sephadex G75 column. Antiserum was used at a final dilution of 1 in 10 000 which bound about 60% of iodinated tracer. Bound and free antigen were separated using a solid phase second antibody which was cellulose coated with donkey antirabbit antibody (Sac-Cel, Wellcome, Beckenham, Kent, England). The intra-assay CV was 4·4% at 252 ng/ml and the interassay CV was 6·5% at 290 ng/ml. GH concentrations were measured using a solid phase immunoradiometric assay with a sensitivity of 0·2 mU/l. Intra-assay CV values were 5·1, 2·4, and 2·6% at 0·8, 4·5, and
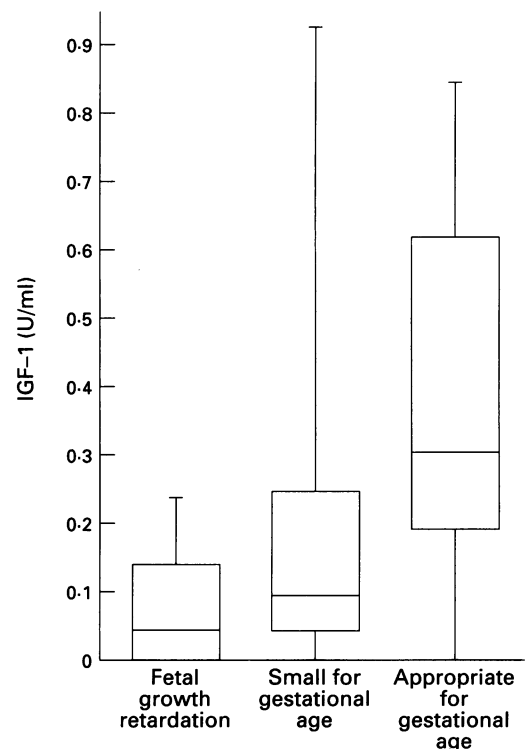

Figure 1 Box and whisker plot (median, range, and interquartile values) of umbilical vein serum concentrations of insulin-like growth hormone-1 (IGF-1) at birth according to fetal size and growth: size small for gestational age with fetal growth retardation; size small for gestational age and normal growth; size appropriate for gestational age and normal growth.

86·5 mU/l, respectively. Interassay CV values were 3·3, 5·2, and 5·5% at 7·7, 21·7, and 45·8 mU/l, respectively.

On the second day of life, standard neonatal morphometric indices (ponderal index (PI), mid-arm circumference (MAC):head circumference (HC) ratio, and subscapular and triceps skinfold thicknesses) were determined.[20] Crown-heel length was measured to the nearest mm using an 'infantometer' with the head against the head plate and the knees fully extended. The foot plate was brought into contact with the foot in its entire length. Skinfold thickness was measured using Holtain calipers with small surface areas which exert a pressure of 10 g/mm² over the whole range of measurements. Mean values for each group of babies were compared.

Fetuses fell into one of three groups according to size and subsequent growth: AGA with normal growth; SGA with normal growth; and SGA with fetal growth retardation. The relation between the different endocrine factors and the change in SD scores were assessed by linear regression analysis. Significance of differences in neonatal morphometric measurements and umbilical vein blood concentrations of growth factors between groups was assessed by the Mann-Whitney U test and accepted if P<0·05.

Table 2 Umbilical venous blood data according to fetal size and growth

	Normal size	Small size (abdominal circumference of <10th centile)	
	Normal growth (n=25)	Normal growth (n=35)	Growth retardation (n=18)
IGF-1 (U/ml)	0·31 (0·00–0·84)	0·13 (0·00–0·94)*	0·05 (0·00–0·24)*†
IGFBP-1 (ng/ml)	186 (52–1060)	220 (36–528)	232 (76–760)
Growth hormone (mU/l)	72·8 (24·8–1001·0)	69·3 (19·4–1235·0)	100·4 (12·4–1003·0)

Values are median (range). IGF=insulin-like growth factor; IGFBP=IGF binding protein.
Statistical differences: *0·05 compared with normal size, normal growth group. †0·05 compared with small size, normal growth group.

Results

Of the 53 SGA fetuses, 18 showed ultrasound evidence of fetal growth retardation (small size, growth-retarded group) and 35 showed normal growth (small size, normal growth group). All

Figure 2 Box and whisker plot (median, range, and interquartile values) of umbilical vein serum concentrations of insulin-like growth hormone-1 binding protein (IGFBP-1) at birth, according to fetal size and growth: size small for gestational age with fetal growth retardation; size small for gestational age and normal growth; size appropriate for gestational age and normal growth.
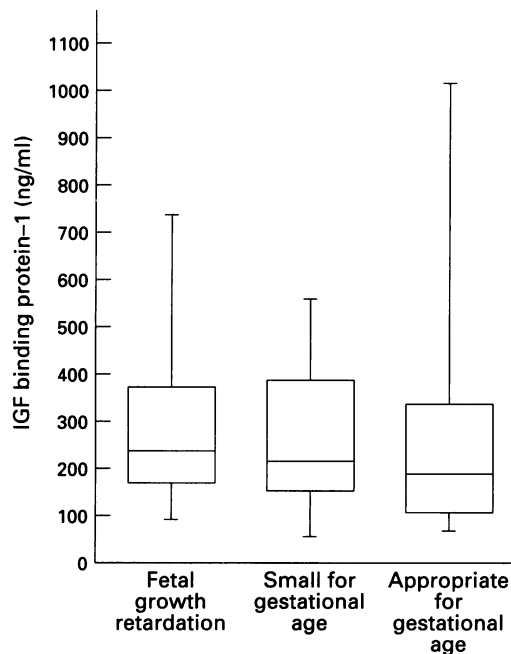
25 of the AGA fetuses had normal antenatal growth (normal size, normal growth group). Median values of birthweight, neonatal PI, MAC:HC ratio, and subscapular and triceps skinfold thickness were significantly different in the three groups, being lowest in the small size, growth-retarded group and highest in the normal size, normal growth group (table 1).

Median umbilical vein concentrations of IGF-1, IGFBP-1, and GH are shown in table 2 and figures 1–3. Serum IGF-1 was significantly lower in the small size, growth-retarded group compared with both other groups, and was significantly lower in the small size, normal growth group compared with the normal size, normal growth group (fig 1). There were no significant differences between the groups for IGFBP-1 (fig 2) or GH concentrations (fig 3). There was a significant correlation between umbilical vein IGF-1 concentrations and the change in SD score (IGF-1 = $0 \cdot 21 + 0 \cdot 04$
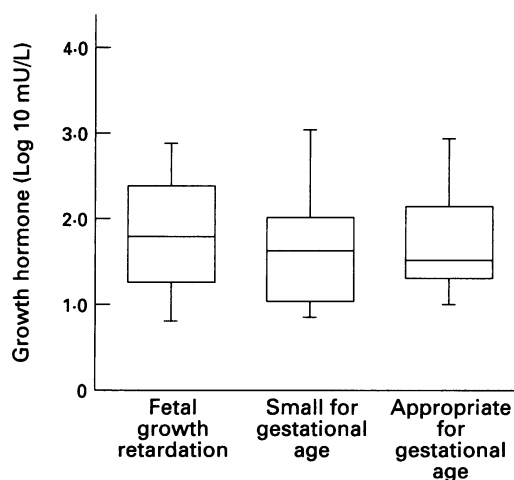

Figure 3 Box and whisker plot (median, range, and interquartile values) of umbilical vein serum concentrations of growth hormone at birth according to fetal size and growth: size small for gestational age with fetal growth retardation; size small for gestational age and normal growth; size appropriate for gestational age and normal growth.

change in SD score, SD = $0 \cdot 015$, $R^2 = 8 \cdot 9\%$, P = $0 \cdot 004$) as illustrated in fig 4. There was no significant correlation between change in SD score and IGFBP-1 or GH concentrations.

Discussion

Previous cross-sectional studies have related circulating concentrations of IGF-1 in umbilical cord blood to birthweight at a variety of gestational ages. Rather than use birthweight as a measure of fetal growth we used serial ultrasound measures of fetal size obtained prospectively during the third trimester of pregnancy. We found a significant correlation between serum IGF-1 concentrations in umbilical venous blood at birth and both fetal size (at recruitment into the study) and growth (change between first and last SD score of abdominal circumference measurement), as assessed by ultrasound during the third trimester of pregnancy. IGFBP-1 and GH concentrations showed no such association with fetal size or growth.

Our method of assessing fetal growth produced three distinct groups in terms of indices of neonatal body proportion as well as birthweight. We have already reported that this methodology identifies abnormal neonatal morphometry indicative of fetal growth retardation in SGA babies.[17] A change in SD scores of −1·5 was determined by ROC curves to be the cutoff point below which fetal growth retardation was best predicted. We have also shown that SGA babies identified as growth retarded by this method are more likely to be delivered by caesarean section, be acidaemic at birth, and be admitted to the neonatal intensive care unit.[18] The neonatal indices of body proportion clearly show that this methodology is successful in differentiating low birthweight babies into groups with and without evidence of fetal growth retardation.

The relation between fetal growth and circulating concentrations of IGF-1 in umbilical venous blood is in line with the findings of most previous studies which found a positive correlation between birthweight and IGF-1.[3–8] One study reported that IGF-1 concentrations were not reduced in SGA babies.[21] However, in a large study of more than 500 cord samples, IGF-1 concentrations were 40% lower in SGA babies and 28% higher in large-for-dates
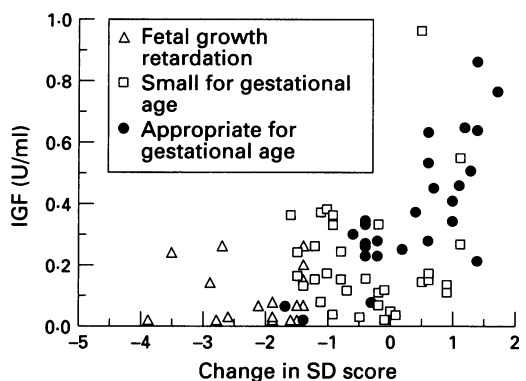

Figure 4 Umbilical vein serum concentrations of IGF-1 plotted according to change in SD score. The three groups of fetal size and growth are shown by different symbols.

babies compared with AGA babies.[6] A study of growth restricted fetal sheep also showed a positive relation between IGF-1 and fetal body weight, fetal liver weight, PO_2 and glucose at all gestations.[22] Their model of placental restriction resulted in fetal growth retardation associated with significantly reduced concentrations of IGF-1. This adds weight to our finding of significantly lower concentrations of IGF-1 in SGA babies with fetal growth retardation, and suggests that a real interruption of fetal growth in utero, as opposed to small size (and low birthweight), is related to a reduction in normal placental function.

The bioavailability of IGFs is thought to be determined by the relative proportions of specific IGF binding proteins. Circulating concentrations of IGFs in the fetus are very low compared with adult values but increase throughout gestation.[5 8 11] However, measured concentrations may not reflect bioactivity. IGF-1 and IGFBP-3 concentrations are strongly correlated[7] and concentrations of both increase substantially with gestation.[11] The formation of complexes may be one way by which bioavailability of IGF-1 is maintained throughout pregnancy. Three groups of workers have reported an inverse correlation between birthweight and IGFBP-1[6 12 21] and a study of growth restricted fetal rats also showed higher levels.[23] A recent Finnish study also reported an inverse relation between IGFBP-1 in amniotic fluid at 16 weeks' gestation and subsequent birthweight.[24] However, we did not find a significant relation between IGFBP-1 concentrations and fetal size or growth rate determined by ultrasound despite the range of birthweights in this study. This suggests that the control mechanisms responsible for IGF-1 and IGFBP-1 concentrations are influenced in different ways by the process responsible for fetal growth retardation.

Our methodology did not show any association between concentrations of GH and fetal size or growth. There is a reported lack of correlation between fetal growth and GH,[13 25] and one study reported an absence of GH receptors in the fetus except in the liver.[26] However, other studies[27 28] have confirmed the presence of circulating GH and widespread tissue receptors (nervous system, mesenchymal, and growth plate). The role of GH and its association with IGF-1 during fetal life has yet to be fully determined.

The results from our study confirm that IGF-1 concentrations are lower in small than normal sized fetuses. The observation that IGF-1 concentrations were lower still in growth retarded small fetuses suggests that IGF-1 may be important in determining fetal growth rate as well as size. Whether these lower concentrations of IGF-1 reflect the mechanism of endocrine control or an overspill from mechanisms at the cellular level remains to be determined.

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