Markers of collagen metabolism and insulin-like growth factor binding protein-1 in term infants

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Abstract

Aim—To study the relation between fetal growth and markers of collagen metabolism and insulin-like growth factor binding protein-1 (IGFBP-1) in term infants.

Methods—Cord vein plasma was obtained from 67 term infants of gestational age 37.1–41.7 weeks (39 appropriate for gestational age (AGA), 11 large for gestational age (LGA; relative birth weight ≥ 2.0 SD), and 17 small for gestational age (SGA; relative birth weight ≤ -2.0 SD)) for analysis of markers of metabolism of collagen type I (PICP and ICTP) and III (PIIINP) and of IGFBP-1.

Results—Negative correlations existed between gestational age and PICP ($r = -0.294$, $p = 0.0158$), ICTP ($r = -0.338$, $p = 0.0052$), and PIIINP ($r = -0.432$, $p = 0.0003$). These correlations were also found in SGA infants (all $p < 0.05$). IGFBP-1 showed negative correlations with birth weight and relative birth weight ($r = -0.644$, $p = 0.0001$, and $r = -0.693$, $p = 0.0001$ respectively) but not with gestational age ($p>0.05$).

Conclusions—In the term fetus, collagen metabolism is primarily dependent on maturity and not on intrauterine growth status, whereas IGFBP-1 reflects intrauterine growth independently of maturity.

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Propeptides of collagen type I C-terminal propeptide (PICP) and collagen type III N-terminal propeptide (PIIINP) can be measured in plasma as markers of collagen formation.[1 2] Similarly, when collagen type I is degraded, its C-terminal cross linked telopeptide, ICTP, is formed.[3] In adults PICP and ICTP correlate with histomorphometric indices of bone formation and resorption.[4] In children, serum concentration of PIIINP correlates with growth velocity.[5 6] The concentration of PIIINP in cord blood has been reported to mirror the shape of the fetal somatic growth velocity curve (g/kg/day) during the second half of pregnancy.[7] At birth, small for gestational age (SGA) infants have lower concentrations of PIIINP than infants appropriate for gestational age (AGA).[8] However, the opposite has also been found.[9] Different results have been reported on the relation between fetal growth status and circulating concentrations of collagen propeptides at birth. Infants of diabetic mothers have been shown to have higher concentrations of ICTP, but not of PICP, than normal controls.[10] On the other hand, in term infants a negative correlation has been found between the concentration of ICTP in cord blood and birth weight.[11]

The action of insulin-like growth factors (IGFs) is modulated by IGF binding proteins (IGFBPs).[12–14] In the fetus the major source of IGFBP-1 is the liver, and the main regulator of IGFBP-1 in the circulation is insulin.[15] In the fetal circulation, the concentration of IGFBP-1 decreases with increasing gestational age; close to term this association seems to disappear.[16–18] In most studies, but not all, a negative correlation has been found between the concentration of IGFBP-1 in cord blood and birth weight.[16–18] An interrelation may also exist between IGFBP-1 and collagen metabolism. IGF-I stimulates collagen synthesis and accumulation of collagen mRNA in vivo and in vitro,[19 20] whereas IGFBP-1 inhibits the effects of IGF-I in a number of cell types and tissues.[21 22]

On the basis of the studies described above, it may be hypothesised that, in the term human infant at birth, collagen metabolism is dependent on somatic growth and maturity, and that IGFBP-1 reduces the turnover of collagen. Therefore the aims of this study were to clarify whether markers of the metabolism of collagen types I and III and IGFBP-1 reflect intrauterine growth and maturity in term infants, and whether any association exists between these markers and IGFBP-1.

Methods

The study population consisted of 67 healthy term newborns (36 boys and 31 girls). Table 1 presents the patient data. The study was approved by the local ethics committee. Relative birth weight was determined by reference to a Finnish newborn population of 74 766 singeltons born 1978–1982. Using infant birth weight, gestational age, and sex, the relative birth weight of each newborn infant was expressed in standard deviation units (SD units).[23] Of the infants studied, 39 were AGA (birth weight range 2640–4520 g; −1.9 to +1.9 SD), 11 were large for gestational age (LGA;

Table 1   Demographic data for the neonates studied

	Mean (SD)	Range
Gestational age (weeks)	39.1 (1.1)	37.1–41.7
Weight (g)	3436 (856)	1870–5545
Length (cm)	49.1 (2.9)	42–54
Relative birth weight (SD)	−0.16 (2.0)	−3.9–5.0
BMI (kg/m^2)	13.9 (2.4)	9.0–19.4
Placental weight (g)	641 (173)	340–1100
Head circumference (cm)	34.5 (2.0)	30–39
Apgar score at 5 minutes	9 (1)	4–10

BMI, body mass index.

Table 2 Demographic data for neonates with normal (AGA) and deviant growth (LGA and SGA).

	AGA	LGA	SGA
M/F	20/19	6/5	10/7
Gestational age (weeks)	39.4 (1.0)	38.7 (1.3)	38.8 (1.2)
Weight (g)	3557 (514)	4637 (422)★	2382 (273)★
Length (cm)	49.8 (1.9)	52.0 (1.8)★	45.7 (2.0)★
Relative birth weight (SD)	0.0 (1.1)	2.9 (1.0)★	−2.6 (0.6)★
BMI (kg/m^2)	14.1 (1.8)	17.2 (1.5)★	11.4 (1.0)★
Placental weight (g)	648 (135)	825 (120)★	444 (68)★
Head circumference (cm)	35.5 (1.5)	37 (1.0)★	32.4 (1.0)★
Apgar scores at 5 minutes	9 (1)	9 (1)	9 (1)

Data presented as numbers or mean (SD).

★$p < 0.05$ *v* AGA.

AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age; BMI, body mass index.

birth weight range 4125–5545 g; 2.0–5.0 SD), and 17 were SGA (range 1870–2725 g; −3.9 to −2.0 SD) (table 2). Twenty one of the infants were delivered by caesarean section; of these, eight were LGA and three SGA. Four of the mothers had insulin dependent diabetes mellitus and one pre-eclampsia. Patients with major malformations were excluded. In a subset of 36 infants, samples were available for measurement of IGFBP-1. Birth weight, relative birth weight, and gestational age in this group did not differ significantly from those of the whole study population.

Blood samples from the umbilical vein were drawn at birth into tubes containing EDTA. The tubes were centrifuged at 1000 *g* and plasma was stored at −20°C until analysis. PICP, ICTP, and PIIINP were determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland).[1–3] IGFBP-1 was analysed by immunoenzymometric assay as described previously.[24] The detection limit of the assay is 0.5 µg/l. The intra-assay and interassay coefficients of variation were 3.4% and 7.4% respectively. Body mass index (BMI) was calculated as weight (kg)/height (m)^2.

Patient data are given as mean (SD) (table 1). The results are given as median and quartiles. Correlations of PICP, ICTP, PIIINP, and IGFBP-1 concentrations were calculated with simple and multiple regression after logarithmic transformation. The effect of gestational age on correlations between variables was eliminated by calculation of partial correlations. Polynomial regression was used to study non-linearity. Differences between groups (AGA, SGA, LGA) were analysed using analysis of variance and Fisher's post hoc test after logarithmic transformation of the data. In analysis of variance, $p < 0.05$ was considered significant.

Results

A negative correlation existed between cord blood PIIINP and gestational age ($r = −0.432$, $p = 0.0003$; fig 1A). Also negative, but somewhat weaker, correlations were found between both PICP and ICTP and gestational age ($r = −0.294$, $p = 0.0158$, and $r = −0.338$, $p = 0.0052$ respectively; fig 1B,C). Table 3 shows concentrations of collagen markers and partial correlations between individual variables with gestational age partialled out. A weak positive correlation existed between PIIINP and relative birth weight ($r = 0.245$,
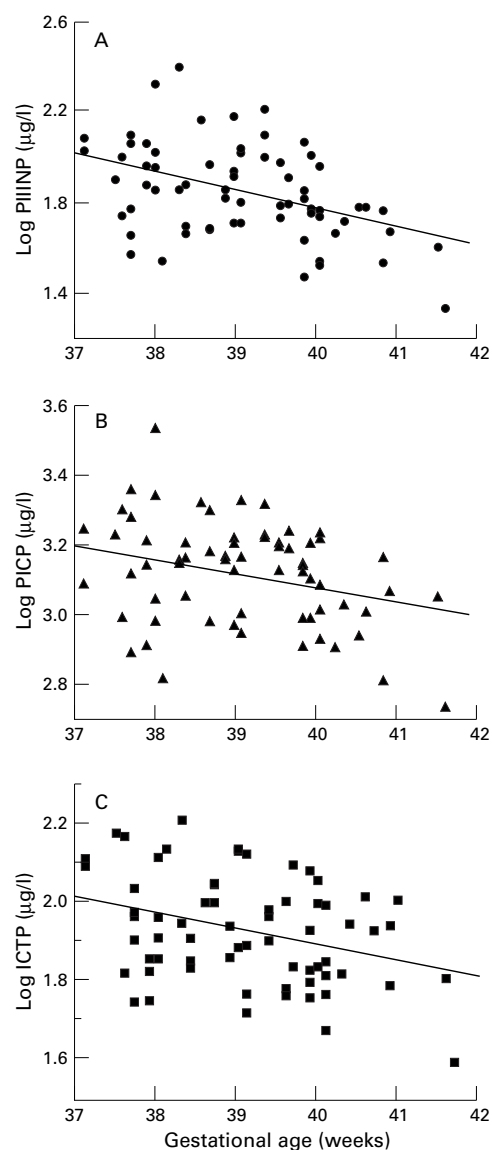

Figure 1 Correlation between gestational age and (A) collagen type III N-terminal propeptide (PIIINP; $r = −0.432$, $p = 0.0003$), (B) collagen type I C-terminal propeptide (PICP; $r = −0.294$, $p = 0.0158$), and (C) C-terminal cross linked telopeptide (ICTP; $r = −0.338$, $p = 0.0052$).

$p = 0.045$), but not with other patient data. PICP, ICTP, and PICP/ICTP showed no correlation with relative birth weight, demographic data, or biochemical variables. There was no evidence of significant non-linearity between patient data and markers of collagen metabolism.

IGFBP-1 was measured in 36 infants (table 4). Negative correlations were found between IGFBP-1 and birth weight ($r = −0.644$,

Table 3 Correlations between collagen concentrations with gestational age eliminated

Variable	PIIINP	PICP	ICTP	PICP/ICTP
PIIINP	1			
PICP	0.650	1		
ICTP	0.282	0.346	1	
PICP/ICTP	0.350	0.634	−0.479	1

PIIINP, collagen type III N-terminal propeptide; PICP, collagen type I C-terminal propeptide; ICTP, C-terminal cross linked telopeptide.

Table 4   Plasma concentrations of PICP, ICTP, PIIINP and IGFBP-1 for the whole study group and in the AGA, LGA, and SGA subgroups (median and quartiles)

Variable	All infants (n=67)	AGA (n=39)	LGA (n=11)	SGA (n=17)
PICP (µg/l) (median)	1396.0	1398.0	1411.0	1231.0
quartiles	995.3; 1651.5	981.0; 1648.5	1178.5; 1818.8	991.8; 1659.3
ICTP (µg/l) (median)	84.6	93.9	65.9*	71.2*
quartiles	66.7; 103.4	77.0; 109.3	60.6; 90.4	58.6; 91.0
PIIINP (µg/l) (median)	75.4	76.0	106.1*	55.5
quartiles	56.1; 104.7	59.7; 94.6	72.5; 123.3	44.3; 102.2
PICP/ICTP (median)	15.9	12.7	23.7*	15.9
quartiles	12.1; 21.2	11.6; 17.8	16.5; 25.2	13.7; 21.5
	n=36	n=15	n=10*	n=12
IGFBP-1 (µg/l) (median)	44.0	40.0	19.0*	110.0*
quartiles	23.0; 44.0	29.3; 71.5	9.9; 32.0	73.0; 172.0

*p < 0.05 *v* AGA.
PIIINP, collagen type III N-terminal propeptide; PICP, collagen type I C-terminal propeptide; ICTP, C-terminal cross linked telopeptide; IGFBP-1, insulin-like growth factor binding protein-1; AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age.
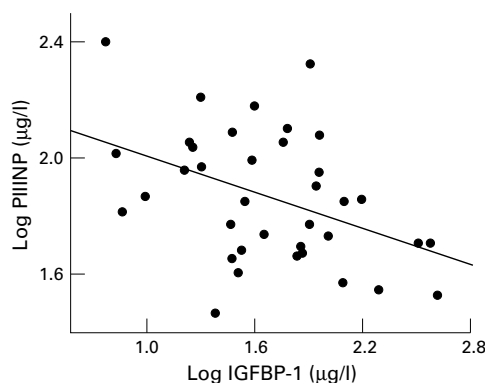

Figure 2   Correlation between insulin-like growth factor binding protein-1 (IGFBP-1) and collagen type III N-terminal propeptide (PIIINP; r = −0.424, p = 0.0089).

p = 0.0001), relative birth weight ($r = -0.693$, p = 0.0001), weight of the placenta ($r = -0.495$, p = 0.0022), BMI ($r = -0.688$, p = 0.0001), birth length ($r = -0.456$, p = 0.0045), and head circumference ($r = -0.497$, p = 0.0018). A negative correlation existed also between IGFBP-1 and PIIINP ($r = -0.424$, p = 0.0089; fig 2). There was no correlation between IGFBP-1 and gestational age ($r = 0.071$, p = 0.68). No significant differences were found in concentrations of collagen markers or IGFBP-1 with respect to sex or route of delivery (data not shown).

On the basis of their intrauterine growth, the patients were divided into three groups: AGA, LGA, and SGA (table 2). A higher concentration of PIIINP was found in LGA than in AGA or SGA infants, whereas the concentration of ICTP was lower in LGA and SGA than in AGA infants (table 4). However, no significant difference in ICTP was found between LGA and SGA. The ratio of PICP/ICTP was higher in LGA than in AGA. In LGA the concentration of IGFBP-1 was lower and in SGA higher than in AGA (all p < 0.05; table 4). In SGA infants, significant negative correlations existed between gestational age and PIIINP ($r = -0.757$, p = 0.0004), PICP ($r = -0.560$, p = 0.019), and ICTP ($r = -0.669$, p = 0.0033).

Discussion

In term fetuses we found a negative correlation between the metabolism of type I and III collagen and gestational age. This dependence was observed in collagen formation markers, PIIINP and PICP, as well as in the marker of degradation of type I collagen, ICTP. Moreover, the dependence was also found in the presence of fetal growth retardation—that is, in SGA infants.

A negative correlation between PIIINP and gestational age has previously been found in infants of 23–41 weeks gestation.[7] The present results show that this phenomenon is seen in term infants of 37–42 weeks gestation as well. The findings are also in accordance with previous data showing lower concentrations of PIIINP in SGA infants than in AGA infants.[8] Moreover, we found significantly higher PIIINP in LGA than in AGA infants, and particularly in SGA infants. This may reflect differences in somatic growth, and is supported by the correlation between birth weight and lean mass described previously.[25] These results are in contrast with a report by Yunoki *et al*,[9] in which no significant difference was found in this variable between LGA and SGA infants. This discrepancy may be due to different selection of subjects, as the previous report included neonates of 20–41 weeks of gestation.[9]

The negative correlation between gestational age and collagen metabolism was also seen in turnover markers of collagen type I. The inter-relation between maturity and markers of formation and degradation of collagen type I has not previously been studied at birth. At 4 weeks of age, preterm infants have been reported to have higher ICTP than term infants, whereas at this age no significant difference exists in PICP.[26] In children, PICP, the marker of collagen type I formation, and ICTP, the marker of collagen type I degradation, both correlate with growth velocity during puberty and treatment with growth hormone.[5 27] In the infants in this study, a correlation existed between PICP and gestational age, but no statistically significant difference in PICP was found between the groups. One factor that may contribute to this is the large biological variation observed in PICP in LGA infants. Therefore, in addition to maturity, PICP may also be associated with somatic growth during the late fetal period.

In contrast with PICP, ICTP concentrations were significantly lower in LGA and SGA than AGA infants. Interestingly, no significant difference was found in this variable between the LGA and SGA groups. The low ICTP in SGA infants is in accordance with the concept of ICTP reflecting growth velocity, as previously shown in children.[5] On the other hand, the finding of lower ICTP in LGA than AGA infants is also supported by a previous report.[2] Thus, in these two extremes of fetal growth, low ICTP may reflect two different metabolic states: in SGA infants it may be due to low collagen turnover, whereas in LGA infants it may indicate decreased degradation of collagen type I in a situation of considerable anabolic growth.

The PICP/ICTP ratio has been used as an indicator of the balance between collagen synthesis and degradation—for example, systemic glucocorticoid treatment causes a decrease in this ratio.[28] Accordingly, the higher PICP/ICTP

ratio in LGA infants may reflect increased collagen synthesis. However, as shown here, both PICP and ICTP correlate more closely with maturity than with fetal growth status. Therefore, this phenomenon may explain the somewhat inconsistent lack of difference in PICP/ICTP between AGA and SGA infants as well as between SGA and LGA infants.

Nutritional status and supply of dietary energy and protein are critical regulators of the circulating levels of IGFBPs.[12–14] We found that IGFBP-1 correlated inversely with the growth status of the fetus. This finding is in accordance with previous data.[16 17] In contrast with the data on markers of collagen I and III, there was no interdependence between gestational age and IGFBP-1 concentration. Therefore, in term infants of 37–42 weeks gestation, IGFBP-1 seems to be dependent on fetal growth status, and not on gestational age. In contrast with a previous study, we observed no effect on mode of birth on IGFBP-1 levels.[29] Animal studies have suggested that IGFBP-1, either directly or by blocking the action of IGF-I, may act as an inhibitor of collagen synthesis.[19 20 30–32] In this study, a significant negative correlation was found between PIIINP and IGFBP-1, a result that may support this phenomenon. However, no such correlation was found between IGFBP-1 and markers of collagen type I. Therefore, in the term fetus, the impact of maturity on collagen metabolism may predominate over other regulatory mechanisms.

In conclusion, in the human fetus at term— that is, at gestational age 37–42 weeks, a negative correlation exists between markers of collagen I and III and maturity. In contrast, IGFBP-1 shows dependence on fetal growth, but not on gestational age.

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