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A Longitudinal Study of Intrauterine Growth and the Placental Growth Hormone (GH)-Insulin-Like Growth Factor I Axis in Maternal Circulation: Association between Placental GH and Fetal Growth

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The aim of the study was 1) to evaluate the association of maternal serum levels of placental GH and IGF-I with fetal growth, and 2) to establish reference data for placental GH, IGF-I, and IGF-binding protein-3 (IGFBP-3) in normal pregnancies based on longitudinal measurements. A prospective longitudinal study of 89 normal pregnant women was conducted. The women had, on the average, seven blood samples taken and three ultrasound examinations performed. All had normal umbilical artery pulsatility indexes during pregnancy and gave birth to singletons between 37 and 42 wk gestation with birth weights above −2 SD. Placental GH levels were detectable in all samples from as early as 5 wk gestation and increased significantly throughout pregnancy to approximately 37 wk when peak levels of 22 ng/ml (range, 4.64–69.22 ng/ml) were reached. Subsequently, placental GH levels decreased until birth. The change in placental GH during 24.5–37.5 wk gestation was positively associated with fetal growth rate ($P = 0.027$) and birth weight ($P = 0.027$). Gestational age at peak placental GH values ($P = 0.007$) was associated with pregnancy length. A positive association between the change in placental GH and the change in IGF-I levels throughout gestation was found in a multivariate analysis ($r^2 = 0.42$; $P < 0.001$). There was no association between placental GH and IGFBP-3 levels. The change in IGF-I throughout gestation ($P = 0.039$), but not placental GH, was significantly positively associated with placental weight at birth. We found a significant association between placental GH and fetal growth. In addition, we found a highly significant association between the increase in placental GH and the increase in IGF-I. The gestational age at peak placental GH levels was associated with pregnancy length. (*J Clin Endocrinol Metab* 89: 384–391, 2004)

RECENTLY, CONSIDERABLE FOCUS has been directed toward the importance of fetal growth, as intrauterine growth restriction (IUGR) is associated with increased perinatal morbidity and mortality. Furthermore, low birth weight adjusted for gestational age may have negative long-term consequences particularly for cardiopulmonary and endocrinological disorders in adult life (1–3). However, the regulation of normal fetal growth still remains unclear.

Placental GH, a product of the GH-V gene, predominantly expressed in the syncytiotrophoblast cells (4) of the placenta, is thought to be involved in the regulation of fetal growth (5). Maternal serum levels of placental GH increase throughout pregnancy from 7 wk gestation (6) until term and gradually replace the pulsatile pituitary GH secretion (7, 8). With the onset of labor and the removal of the placenta after childbirth, there is a rapid fall in serum placental GH levels (placental GH half-life, 15 min) (6). In contrast to pituitary GH, placental GH is secreted continuously (8), which permits evaluation of placental GH levels from a single measurement. Several cross-sectional studies have shown that in both normal and pathological pregnancies the rise in placental GH is associated with a rise in maternal IGF-I levels (9, 10) and that reduced levels of both placental GH and maternal IGF-I are seen in women with IUGR pregnancies (5). Placental GH is not detectable in the fetal circulation, but it is believed to influence fetal growth indirectly by regulating the maternal substrate supply to the fetus. The physiological role, however, may also include a direct influence on placental development through an autocrine or paracrine mechanism, as suggested by the presence of GH receptors in the placenta (11).

To our knowledge no longitudinal data exist on the progressive rise in placental GH, IGF-I, and IGFBP-3 in relation to ultrasound assessment of intrauterine fetal growth in women with normal pregnancies. The aim of this study was 1) to evaluate the relationship of placental GH and maternal IGF-I with fetal growth, and b) to establish reference data for placental GH, IGF-I, and IGFBP-3 throughout normal pregnancies based on longitudinal measurements.

Subjects and Methods

Study participants and design

During the period of May 1999 to October 2001, 103 pregnant women were recruited consecutively from the first routine contact (between gestational wk 6–12) at a University Hospital in Copenhagen. After informed consent, all mothers were scheduled to have blood samples

Abbreviations: BMI, Body mass index; IGFBP, IGF-binding protein; IUGR, intrauterine growth restriction.

obtained approximately every 4–6 wk throughout pregnancy. All pregnant women were scheduled to have 3 ultrasound examinations. Eighty-nine women had normal singleton pregnancies and gave birth between 37–42 wk gestation to newborns with birth weights above −2 SD (12). Thus, 14 women were excluded due to preterm birth or small for gestational age newborn (n = 4), postmaturity (n = 2), miscarriage (n = 1), or personal reasons (n = 7). A total of 650 blood samples were obtained (with an average of 7 samples/woman; range, 3–9). All women had normal placental function as assessed by umbilical artery pulsatility index. Information regarding mother's diseases, smoking, parity, parturition, and childbirth history were obtained from medical records during the course of pregnancy and after birth.

Ultrasound scan

One examiner performed all ultrasound examinations using an ultrasound color Doppler equipment (2102 Scanner, B-K Medical, Copenhagen, Denmark) with a convex abdominal array 2.5–5 MHz MFI (multifrequency imaging) probe. The test-retest coefficient (same observer same fetus) was 4.6% (13). All women had a dating scan at approximately 17 wk gestation (range, 15–22). Fetal weight estimations were calculated at approximately 28 (range, 27–33) and 33 (range, 32–35) wk gestation from biparietal diameter and abdominal circumference using Warsof's equation (14).

Ethical aspects

The study was performed according to the Helsinki II Declaration and was approved by the local ethics committee and the Danish Registry Agency.

Laboratory methods

Nonfasting peripheral venous blood samples were taken from an antecubital vein between midmorning and early afternoon. Samples were separated by centrifugation and stored at −20 C until analysis.

Assays

Placental GH was measured in a solid phase ^{125}I-labeled immunoradiometric assay (Biocode, Liege, Belgium) using two specific monoclonal antibodies as previously described (4). In our laboratory the intraassay coefficients of variation were less than 8% (at 24.6 ng/ml; n = 20) and 6.3% (at 5.0 ng/ml; n = 20), and the interassay coefficients of variation were 6.1% (at 4.1 ng/ml; n = 20), 9.7% (at 22.2 ng/ml; n = 20), and 8.1% (at 35.6 ng/ml; n = 20). The limit of detection was less than 0.1 ng/ml, defined as zero standard +3 SD.

IGF-I was measured in all subjects with a RIA originally described by Bang *et al.* (15) with some modifications. Serum was extracted by acid/ethanol and cryoprecipitated before analysis to remove interfering binding proteins using monoiodinated Tyr^{31}-[^{125}I]des-(1–3) IGF-I as radioligand (16). Inter- and intraassay variations were both below 11% in the range measured. The limit of detection was 21 ng/ml. IGFBP-3 was determined by RIA, as described by Blum *et al.* (17). IGFBP-3 was measured on unprocessed serum using a polyclonal rabbit antiserum and a purified human IGFBP-3 fragment as standard and radioligand. Reagents for the analysis were obtained from Mediagnost GmbH (Tubingen, Germany). Inter- and intraassay variations were 7.3% and 3.5%, respectively. The limit of detection was 300 ng/ml.

Statistical analysis

The statistical analyses were carried out using the statistical package SPSS (version 11, SPSS, Inc., Chicago, IL) and SAS (version 6.12, SAS Institute, Cary, NC).

Descriptive statistics are given as median values and ranges, and the study population is grouped according to gender of the fetus. Square root and log transformation were applied to all hormones values to improve the approximation of normal distribution and linearize relationships.

The reference charts were constructed by use of random effects models to account for correlation within each subject. We assumed that the individual developments were approximately piecewise linear with individual variation of slopes and intercepts. The fitted random effects models were validated, and data were transformed to obtain residuals that were approximately normal. Based on the effects model with estimates of residual variation as well as variation within subjects, we constructed the model-based reference charts.

A linear regression model, fitted on every subject's data over time was applied to yield a slope value representing the rate of change in hormone levels per unit of time (gestational age in days) with corresponding intercepts (baseline values). Slope values and intercepts were calculated on individual hormonal changes throughout gestation and on the change between 24.5–37.5 wk gestation, as this time window represents a period when fetal weight velocity is at its maximum (18). The maximal hormone levels as well as the difference between maximal hormone level and hormone concentration in the last blood sample for placental GH and IGF-I were calculated. To determine factors of influence on placental GH, IGF-I, and IGFBP-3, the slopes were used as dependent variables in linear regression analyses describing the increase in individual hormone levels throughout gestation. All models included smoking habits, maternal age, social-economic status, parity, prepregnancy body mass index (BMI; weight/height^2), placental weight, and hormone levels (de-

TABLE 1. Description of study population

	n	Female	n	Male	n	Total
Gestational age at birth (d)	39	278 (267; 292)	50	280 (266; 296)	89	279 (266; 296)
Birth weight (g)	39	3640 (2955; 4580)	50	3600 (2950; 5000)	89	3610 (2950; 5000)
% Birth weight deviation	39	2.6 (−13.4; 28.8)	50	2.1 (−19.4; 40.7)	89	2.1 (−19.4; 40.7)
Mother's age (yr)	39	31 (22; 39)	50	32 (20; 45)	89	31 (20; 45)
Mother's height (m)	39	1.68 (1.6; 1.83)	50	1.69 (1.55; 1.83)	89	1.69 (1.55; 1.83)
Prepregnancy weight (kg)	39	67.0 (48; 100)	49	65 (51; 98)	88	65.5 (48; 100)
BMI (kg/m^2)	39	23 (17; 32)	49	23 (19; 36)	88	23 (17; 36)
Placental weight (g)	38	649 (400; 1200)	49	670 (420; 980)	87	670 (400; 1200)
Birth length (cm)	34	52 (48; 57)	49	52 (45; 59)	83	52 (45; 59)
Head circumference (cm)	28	35 (31; 37)	43	36 (30; 38)	71	35 (30; 38)
Delivery						
Cesarean	3		7		10	
Normal vaginal	30		37		67	
Vacuum extraction	6		6		12	
Parity						
0	14		23		37	
1	19		18		37	
≥2	6		9		15	
Smokers	15		15		30	
Nonsmokers	21		34		55	

Data are given as medians and ranges.

TABLE 2. Descriptive fetal ultrasound data

	n	Female	n	Male	N	Total
Gestational age at 1st exam (d)	39	123 (101; 154)	50	125 (105; 154)	89	123 (101; 154)
Gestational age at 2nd exam (d)	39	198 (188; 234)	50	199 (189; 212)	89	199 (188; 234)
Gestational age at 3rd exam (d)	37	233 (216; 247)	50	232 (224; 244)	87	232 (216; 247)
Fetal weight at 2nd exam (g)	39	1340 (995; 1807)	50	1361 (1014; 2043)	89	1350 (995; 2043)
Fetal weight at 3rd exam (g)	37	2314 (1525; 3106)	50	2300 (1550; 3232)	87	2314 (1525; 3232)
% Weight deviation from normal at 2nd exam	39	1.42 (−22.1; 23.7)	50	0.43 (−23.4; 27.1)	89	0.89 (−23.4; 27.1)
% Weight deviation from normal at 3rd exam	37	3.65 (−22.9; 36.1)	50	2.9 (−24.4; 32.6)	87	3.2 (−22.9; 36.1)
Pulsatility index 1st exam	25	1.2 (0; 2.1)	40	1.2 (0.7; 2.7)	65	1.2 (0; 2.7)
Pulsatility index 2nd exam	36	1.0 (0.7; 1.3)	49	1.0 (0.7; 1.4)	85	1.0 (0.7; 1.4)
Pulsatility index 3rd exam	38	0.9 (0.6; 1.3)	50	0.9 (0.4; 1.4)	88	0.9 (0.4; 1.4)

Data are given as medians with ranges.

pending on model) as explanatory variables. Backward stepwise regression models were adopted to determine factors predicting changes in placental GH, IGF-I, and IGFBP-3. To describe the fetal growth rate, individual slopes were calculated from assessment of birth weight and two fetal weight estimations at approximately 28 and 33 wk of gestation. Fetal growth rate, birth weight, and placental weight were used as univariate dependent variables in linear regression models with the slope of placental GH, IGF-I, and IGFBP-3 together with prepregnancy BMI, maternal age, smoking, parity, and fetal sex as explanatory variables. The intercepts of the biochemical markers were excluded because they were nonsignificant in these models. To determine whether placental GH or IGF-I had an influence on the time of parturition, maximal hormone values, the difference between maximal values and the last taken blood sample (Δ value), and gestational age at maximal concentrations were used as explanatory variables in a regression model, with gestational age at birth as the dependent variable. Residual plots were used to validate all regression models.

Results

The basic clinical characteristics of the study population are shown in Tables 1 and 2. We found no statistical significant differences between maternal and fetal characteristics of the study population with regard to the gender of the fetus.

Fetal growth

Median fetal weights during gestation in the three birth weight tertiles are shown in Fig. 1A. Fetal growth was linear in the period studied (approximate gestational wk 28–40) and estimated to a mean of 174 g/wk (range, 150–227 g/wk; Fig. 2A).

Longitudinal levels of placental GH

Placental GH levels were detectable in all available samples from as early as 5 wk gestation and increased significantly throughout pregnancy to a peak of 22 ng/ml (range, 4.64–69.22 ng/ml) at approximately 37 wk gestation (Fig. 2B). Subsequently, placental GH levels decreased until the time of birth, with the lowest values seen in women giving birth to the lightest children (Fig. 1B). In late second trimester and third trimester, placental GH levels showed large interindividual variation (Fig. 2B). The total change in placental GH was significantly negatively associated with maternal BMI ($P = 0.031$) and positively with the change in maternal IGF-I ($P = 0.037$) when the model was adjusted for fetal sex ($P = 0.128$). The change in placental GH between wk 24.5 and 37.5 was significantly related to fetal sex ($P = 0.048$); women carrying female fetuses had a tendency toward steeper placental GH slopes (Table 3).

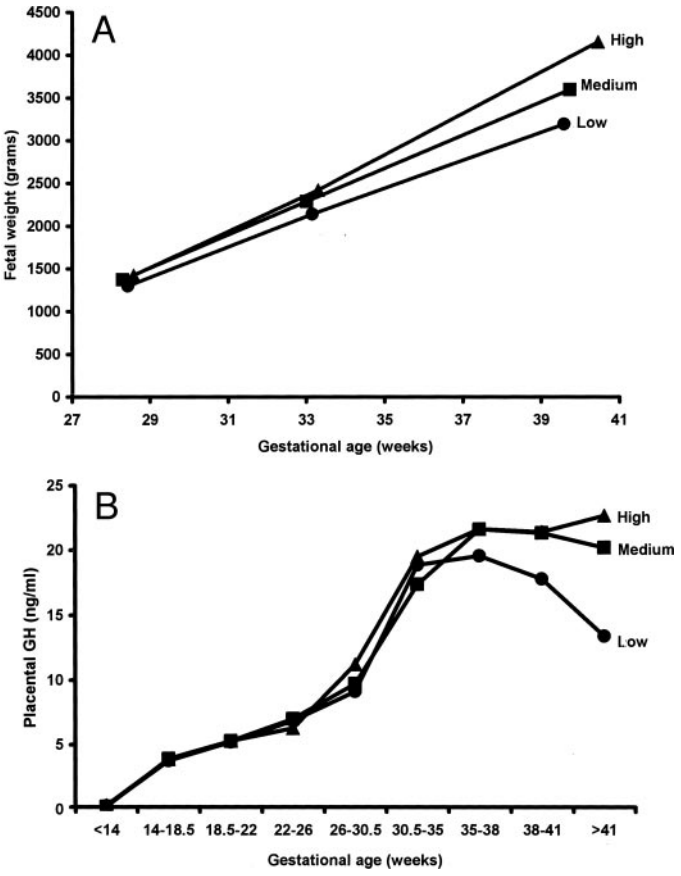

FIG. 1. A, Trend curves for estimated fetal weights and corresponding birth weights grouped by median birth weight tertiles in low (●), median (■), and high (▲) birth weights. B, Median placental GH levels in normal pregnant women grouped corresponding to the above-mentioned birth weight tertiles.

Longitudinal levels of IGF-I and IGFBP-3

Individual maternal IGF-I levels increased throughout pregnancy, reached a maximum of 436 ng/ml (range, 215–705) at approximately 37 wk gestation, and decreased before parturition (Fig. 2D). In late gestation IGF-I levels showed marked interindividual variation. The change in IGF-I was positively associated with the change in placental GH throughout gestation in a linear regression analysis ($r^2 = 0.1519$; $P < 0.001$; Fig. 3). In a multivariate analysis, the change in IGF-I was even more strongly associated with the

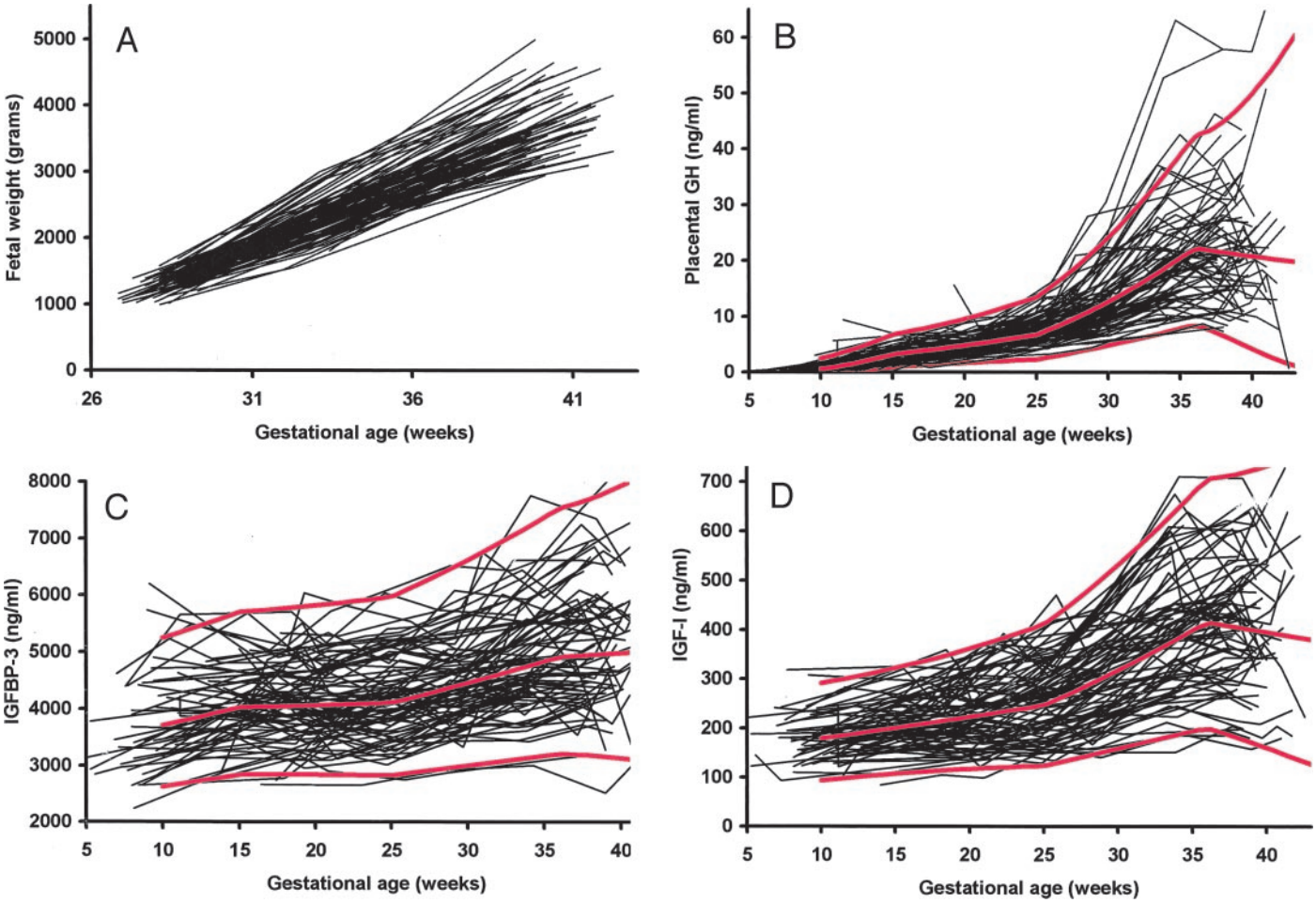

FIG. 2. Individual curves from a longitudinal study of 89 normal singleton pregnancies. All parameters are plotted against gestational age. A, Fetal weight estimates and birth weight; B, maternal plasma placental GH; C, maternal plasma IGFBP-3; D, maternal plasma IGF-I levels. The *thick red lines* represent the mean and 95% confidence interval.

change in placental GH ($P < 0.001$), the change in IGFBP-3 ($P < 0.001$), and maternal prepregnancy BMI ($P = 0.003$; Table 3). IGFBP-3 levels increased significantly throughout gestation ($P = 0.001$; Fig. 2C), and the peak of 5116 ng/ml (range, 3074–9924) was reached at approximately 37 wk gestation. The change in IGFBP-3 throughout gestation was positively associated with the change in IGF-I ($P < 0.001$; Table 3).

Relationship of placental GH and maternal IGF-I to fetal growth rate, birth weight, and placental weight

Fetal growth rate was significantly positively associated with the change in placental GH ($P = 0.027$) between gestational wk 24.5–37.5 in a model adjusted for confounders (Table 4). Using birth weight as the dependent variable, both the change in placental GH during 24.5–37.5 wk gestation ($P = 0.027$) and gestational age at birth ($P = 0.021$) were significantly positively associated with birth weight (Table 4). Inclusion of placental weight in the model eliminated the significant associations of placental GH and gestational age with birth weight, as placental weight is strongly associated with birth weight. Placental weight was significantly positively associated with gestational age at birth ($P = 0.049$) and the change in IGF-I throughout gestation ($P = 0.039$), but not with the change in placental GH (Table 4).

Relationship of maximal placental GH and IGF-I to age at time of birth

We found that the gestational age when maximum placental GH concentrations were reached was significantly positively associated with pregnancy length in days ($P = 0.007$). Neither the maximal concentration of placental GH, the difference between maximal levels and last taken blood sample, gestational age at maximal IGF-I levels, or maximum IGF-I level was significantly associated with pregnancy length.

Discussion

In a large cohort of normal pregnant women we found a significant positive association between maternal serum levels of placental GH and fetal growth. To our knowledge this association has not been demonstrated previously in a prospective longitudinal study. Placental GH levels increased during pregnancy, with large interindividual variation up to approximately 37 wk gestation and a decline thereafter. In-

TABLE 3. Univariate linear regression models with square root values of the change in placental GH, IGF-I, and IGFBP-3 used as dependent variables during total gestation or during 24.5–37.5 wk gestation

	Gestational period					
	24.5–37.5 wk gestation			Throughout gestation		
	Parameter estimate	95% CI	*P* value	Parameter estimate	95% CI	*P* value
Placental GH slope	**$r^2 = 0.098$**			**$r^2 = 0.253$**		
Prepregnancy BMI (kg/m^2)			0.163			**0.031**
17.00–20.00	5.7×10^{-3}	-2.4×10^{-3}; 1.4×10^{-2}	0.162	4.7×10^{-3}	8.3×10^{-4}; 8.56×10^{-3}	**0.018**
20.01–25.00	5.4×10^{-3}	-4.5×10^{-4}; 1.1×10^{-2}	0.070	3.1×10^{-3}	-3.5×10^{-4}; 5.8×10^{-3}	**0.028**
25.01–36.00	Ref		Ref	Ref		**Ref**
Fetal sex (male *vs.* female)	-5.16×10^{-3}	-1.0×10^{-2}; -3.6×10^{-5}	**0.048**	-1.8×10^{-3}	-4.2×10^{-3}; 5.3×10^{-4}	**0.128**
IGF-I slope	1.24×10^{-2}	-4.5×10^{-2}; 7.0×10^{-2}	0.670	0.17	0.10; 0.24	**<0.001**
IGF-I slope	**$r^2 = 0.158$**			**$r^2 = 0.42$**		
Prepregnancy BMI (kg/m^2)			0.515			**0.003**
17.00–20.00	-6.6×10^{-3}	-3.7×10^{-2}; 2.4×10^{-2}	0.666	-1.6×10^{-2}	-2.5×10^{-2}; -6.8×10^{-3}	**<0.001**
20.01–25.00	7.8×10^{-3}	-1.4×10^{-2}; 2.9×10^{-2}	0.483	-8.3×10^{-3}	-1.5×10^{-2}; -1.7×10^{-3}	**0.011**
25.01–36.00	Ref		Ref	Ref		**Ref**
Placental GH slope	0.351	−0.463; 1.166	0.393	1.0	0.5; 1.5	**<0.001**
IGFBP-3 slope	0.191	8.3×10^{-2}; 0.299	**<0.001**	0.2	0.1; 0.3	**<0.001**
IGFBP-3 slope	**$r^2 = 0.133$**			**$r^2 = 0.230$**		
IGF-I slope	0.708	0.309; 1.106	**<0.001**	0.937	0.572; 1.303	**<0.001**

The 95% confidence intervals (CI) are given. Significant *P* values are in *bold*.

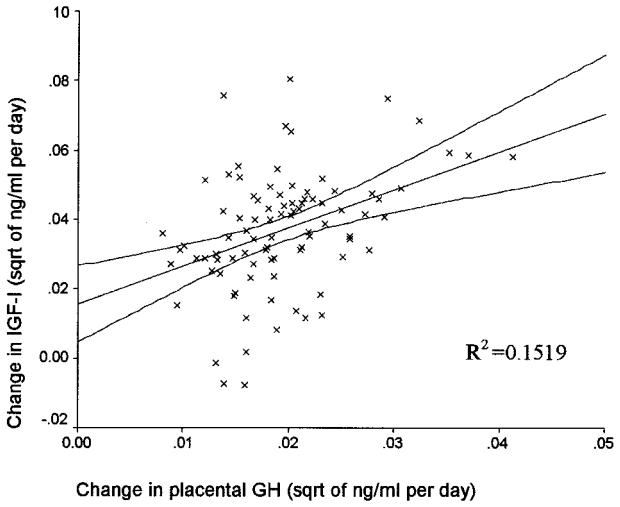

FIG. 3. Association between the change in maternal placental GH and the change in maternal IGF-I levels throughout gestation. Data shown here are square root transformed (sqrt).

terestingly, we found that the gestational age when peak concentrations of placental GH were reached was associated with the length of pregnancy, *i.e.* early maximal placental GH levels were associated with early onset of labor. In addition, we found a highly significant positive association between the increase in placental GH and the increase in IGF-I during pregnancy.

In the present study placental GH was positively associated with fetal growth during a period when fetal weight gain is large. The pregnancy-induced rise in placental GH explains less than 10% of the variation in fetal growth rate, which may reflect the limited number of subjects in our study. Nevertheless, our present findings, based on longitudinal assessments, are in accordance with data from a previous cross-sectional study of 455 pregnant women, in whom placental GH was positively associated with fetal weight at approximately 28 wk gestation (19). In addition, data from studies of pathological pregnancies suggested that placental GH levels were lower in women with IUGR compared with normal pregnancies (5, 9, 10). Placental GH mRNA expression per cell did not differ between normal and IUGR placentas (20), although controversy exists (21). Chowen *et al.* (20) found that the mean number of cells expressing placental GH mRNA per area was significantly greater in normal placentas. This suggests that decreased levels of placental GH in the maternal circulation in IUGR are not only due to a reduced placental size, but also to a reduced number of placental cells per area that are capable of producing this peptide.

Intriguingly, we found that the age at which placental GH peaks was positively associated with pregnancy length. Thus, the earlier the time of the placental GH peak during gestation, the earlier the child was born within 37–42 wk gestation. A preliminary study reported on significantly reduced placental GH levels at the onset of labor compared with levels obtained 2–4 d before birth (22). However, it remains to be seen whether placental GH may be implicated in the mechanisms of the onset of labor.

Placental GH, a product of the GH-V gene, is predominantly expressed in syncytiotrophoblast cells, and transcript levels increase with increasing formation of syncytiotrophoblasts (23, 24). Receptors for GH are present in the villous trophoblasts as well as the extravillous trophoblasts (25) and bind placental GH with similar affinity compared with pituitary GH. In addition, activation of the GH receptor by placental GH leads to same intracellular signaling compared with 22-kDa, pituitary-derived GH (26). Therefore, it has been speculated that placental GH acts as an auto- or paracrine trophic hormone on placental growth or, alternatively, affects placental function. We did not find any association between placental size at birth and the change in placental GH levels, which could suggest that placental GH may not be the primary trophic regulator of placental growth. However, this does not exclude placenta as the target organ for placental GH. Placental GH may still regulate the transport

TABLE 4. Fetal growth rate, birth weight, and placental weight, as dependent variables in linear regression models where confounders known to have effect on the above mentioned variables are included together with the slope of the square root of placental GH or IGF-I

	Gestational period					
	24.5–37.5 wk gestation			Throughout gestation		
	Parameter estimate	95% CI	*P* value	Parameter estimate	95% CI	*P* value
Fetal growth rate	**$r^2 = 0.079$**			**$r^2 = 0.078$**		
Placental GH slope	117.4	14.1; 220.7	**0.027**	85.7	−118.2; 289.6	0.40
Prepregnancy BMI (kg/m^2)			0.60			0.40
17.00–20.00	−1.0	−4.8; 2.7	0.58	-1.4×10^{-3}	−3.8; 3.8	0.99
20.01–25.00	−1.4	−4.1; 1.3	0.31	−0.51	−3.2; 2.1	0.70
25.01–36.00	Ref		Ref	Ref		Ref
Fetal sex (male *vs.* female fetuses[a])	0.4	−2.1; 2.9	0.75	−0.34	−2.6; 1.8	0.73
Smoking habits (smokers *vs.* nonsmokers[a])	−0.9	−3.5; 1.6	0.47	−0.54	−2.9; 1.8	0.65
Birth weight	**$r^2 = 0.09$**			**$r^2 = 0.057$**		
Prepregnancy BMI (kg/m^2)			0.345			0.564
17.00–20.00	−213.6	−528; 100.8	0.180	−163.5	−468.9; 141.9	0.290
20.01–25.00	−134.7	−360; 90.6	0.273	−71.5	−288.6; 145.5	0.514
25.01–36.00	Ref		Ref	Ref		Ref
Placental GH slope	9674	1131; 18218	**0.027**	10418	−4811; 25649	0.177
Gestational age at birth (d)	15.0	2.9; 27.6	**0.021**	15.8	3.2; 28.5	**0.014**
Fetal sex (male *vs.* female fetuses[a])	50.8	−146.3; 250.0	0.609	19.8	−208.2; 170.1	0.842
Placental weight	**$r^2 = 0.016$**			**$r^2 = 0.074$**		
IGF-I slope	103.9	−656.7; 864.6	0.786	1873	−93; 3653	**0.039**
Gestational age at birth (d)	4.4	-3.9×10^{-3}; 8.7	0.50	4.1	2.3×10^{-2}; 8.2	**0.049**

The slopes are calculated as a change during total gestation or between 24.5–37.5 weeks of gestation. The 95% confidence intervals (CI) are given. Significant *P* values are in *bold*.

of nutrients across the placenta to the fetus. Glucose transporters are localized in the placenta. The GLUT1 glucose transporter, present on both the microvillous and basal membranes of the syncytium, is the primary isoform involved in the transplacental movement of glucose. The GLUT3 protein is localized to the arterial component of the fetal vascular endothelium, where it may play a role in enhancing transplacental glucose transport (27). Transgenic mice overexpressing placental GH were severely insulin resistant, as manifested by fasting and postprandial hyperinsulinemia (28). Placental GH is therefore a likely candidate to mediate the insulin resistance of pregnancy. Whether placental GH stimulates placental expression of glucose transporters, thereby regulating fetal glucose uptake, remains speculative.

The correlations between the rise in placental GH and IGF-I levels validates previous observations based on small cross-sectional studies (5, 9, 10, 29). In our study this association was seen after adjusting for other factors, such as BMI and fetal sex, which have previously been shown to influence maternal placental GH levels (19, 30, 31). It has been shown that placental GH and maternal IGF-I levels increase in pregnancies of GH-deficient women (6, 32), supporting the hypothesis of placental GH, and not pituitary GH, being the main regulator of maternal IGF-I during pregnancy. We found that maternal IGF-I levels increased throughout gestation until 37 wk gestation, with a subsequent decrease until birth. In line with this finding, previous cross-sectional studies of normal and pathological pregnancies (diabetes or IUGR) (33–36) reported elevated IGF-I levels in late gestation compared with early gestation.

We found that the rate of increase in maternal IGF-I was not associated with fetal growth rate or birth weight. The possible mechanisms by which maternal IGF-I levels could influence fetal growth are uncertain. Decreased placental transport of certain amino acids has been described in IUGR pregnancies (37), and some animal studies suggest that maternal IGF-I may play a role in placental transfer of such nutrients. IGF-I treatment of pregnant sheep leads to an increase in glucose concentrations and amino acid uptake to the fetus (38). IGF-I infusions in pregnant mice and rats stimulate fetal weight with increasing litter size. thereby removing maternal constraint (39). Maternal IGF-I has been associated with intrauterine growth and birth weight in several species (40, 41). In humans, conflicting results exist on the relationship between maternal IGF-I levels in pregnancy and birth weight (36, 42–44). In our study of normal pregnant women without signs of placental dysfunction, there was no correlation of IGF-I to either fetal growth rate or birth weight. This is comparable to the finding of Holmes *et al.* (35), who reported an association between maternal IGF-I and birth weight in women with placental dysfunction, but not in healthy pregnant women. We found a positive association between the change in IGF-I throughout gestation and placental size, thereby suggesting a role for IGF-I in the regulation of placental growth. The increase in placental size may be associated with an increased transport of nutrients to the fetus due to an increase in the surface area for exchange.

Thus, we found that placental GH, but not IGF-I, was associated with fetal growth (but that they were interrelated). We believe that this could hypothetically suggest that placental GH influences fetal growth, whereas IGF-I may stimulate fetal growth indirectly by affecting placental size or function. Alternatively, maternal IGF-I may not be important for fetal growth at all. However, we cannot exclude that the differences may reflect the limited number of women in our study.

We found an increase in IGFBP-3 levels determined by RIA during pregnancy in accordance with the finding of Wang *et*

al. (45). In our study the increase in IGFBP-3 was significantly positively associated with the changes in IGF-I levels, but was not associated with the changes in placental GH. To our knowledge, no previous data exist on the relationship between IGFBP-3 and placental GH. However, we have not investigated IGFBP-3 protease activity, which is known to occur in human third trimester pregnancies (46). Therefore, we cannot take into account relative changes in affinity or binding kinetics of IGFBP-3 that occur during pregnancy due to proteolytic modification.

In conclusion, placental GH was positively associated with fetal growth rate between 24.5–37.5 wk gestation as well as with birth weight, whereas this association was not found for IGF-I. We found that the gestational age at which peak values of placental GH were reached was associated with the length of pregnancy. In addition, this paper presents longitudinal reference ranges for maternal serum levels of placental GH, IGF-I, and IGFBP-3 throughout normal pregnancies.

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