

Placental Growth Hormone (GH), GH-Binding Protein, and Insulin-Like Growth Factor Axis in Normal, Growth-Retarded, and Diabetic Pregnancies: Correlations with Fetal Growth*

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ABSTRACT

We previously described significant changes in GH-binding protein (GHBP) in pathological human pregnancy. There was a substantial elevation of GHBP in cases of noninsulin-dependent diabetes mellitus and a reduction in insulin-dependent diabetes mellitus. GHBP has the potential to modulate the proportion of free placental GH (PGH) and hence the impact on the maternal GH/insulin-like growth factor I (IGF-I) axis, fetal growth, and maternal glycemic status. The present study was undertaken to investigate the relationship among glycemia, GHBP, and PGH during pregnancy and to assess the impact of GHBP on the concentration of free PGH. We have extended the analysis of specimens to include measurements of GHBP, PGH, IGF-I, IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-2, and IGFBP-3 and have related these to maternal characteristics, fetal growth, and glycemia. The simultaneous measurement of GHBP and PGH has for the first time allowed calculation of the free component of PGH and correlation of the free component to indexes of fetal growth and other endocrine markers. PGH, free PGH, IGF-I, and IGF-II were substantially decreased in IUGR at 28–30 weeks gestation (K28) and 36–38 weeks gestation (K36). The mean concentration (\pm SEM) of total PGH increased significantly from K28 to K36 (30.0 ± 2.2 to 50.7 ± 6.2

ng/mL; $n = 40$), as did the concentration of free PGH (23.4 ± 2.3 to 43.7 ± 6.0 ng/mL; $n = 38$). The mean percentage of free PGH was significantly less in IUGR than in normal subjects (67% vs. 79%; $P < 0.01$). Macrosomia was associated with an increase in these parameters that did not reach statistical significance. Multiple regression analysis revealed that PGH/IGF-I and IGFBP-3 account for 40% of the variance in birth weight. IGFBP-3 showed a significant correlation with IGF-I, IGF-II, and free and total PGH at K28 and K36. Noninsulin-dependent diabetes mellitus patients had a lower mean percentage of free PGH (65%; $P < 0.01$), and insulin-dependent diabetics had a higher mean percentage of free PGH (87%; $P < 0.01$) than normal subjects. Mean postprandial glucose at K28 correlated positively with PGH and free PGH (consistent with the hyperglycemic action of GH). GHBP correlated negatively with both postprandial and fasting glucose. Although GHBP correlated negatively with PGH ($r = -0.52$; $P < .001$), free PGH and total PGH correlated very closely ($r = 0.98$). The results are consistent with an inhibitory function for GHBP *in vivo* and support a critical role for placental GH and IGF-I in driving normal fetal growth. (*J Clin Endocrinol Metab* 85: 1143–1150, 2000)

LATE PREGNANCY is a catabolic phase with redistribution of maternal nutrients to support fetal growth (1). Work by Gluckman (2) and others has shown fetal growth to be dependent on maternal substrate supply, and the hormonal mechanisms driving these changes are beginning to be elucidated. Placental GH (PGH) progressively replaces pituitary GH (hGH-N) in the maternal circulation from midgestation (3).

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Placental GH is a major regulator of maternal insulin-like growth factor I (IGF-I) (4, 5) and has a spectrum of metabolic activities comparable to that of hGH-N (6) and a similar somatogenic effect (7). The high levels of PGH measured from midgestation in human pregnancy (8) are likely to impact on placental metabolism and substrate supply to the fetus, either directly or mediated by IGF-I (2, 5, 9, 10). This is supported by observations that maternal concentrations of total PGH and IGF-I correlate strongly and are decreased in cases of intrauterine growth retardation (IUGR) (5, 8, 11) and that maternal IGF-I correlates with fetal femur length and birth weight (12). Furthermore, the administration of hGH to pregnant rats impacts on fetal weight (13, 14). It has been demonstrated that GH does not cross the placenta in pregnant rats (15), so it is likely that its effects on fetal growth are indirect and mediated by effects on maternal IGF-I concentrations and on substrate supply to the fetus.

The effects of PGH on maternal metabolism, on the placenta, and hence on fetal growth are potentially modulated by the high affinity GH-binding protein (GHBP) (16–18). In the maternal circulation GHBP concentrations are comparable or higher than the levels in the nonpregnant state, depending on gestational stage (19, 20). However, to date there have been no studies relating the concentration of free PGH to maternal biochemical parameters or to fetal growth. For better understanding of the GH axis during pregnancy it is necessary to have measurements of the gestational profile of free PGH in normal and pathological pregnancies. A comparison of the correlations between free PGH and growth parameters and between total PGH and growth parameters would also provide evidence relevant to the impact of maternal GHBP on fetal growth and, more generally, on the physiological role of GHBP in the control of GH action *in vivo*.

We have previously described significant changes in GH-binding protein (GHBP) in human pregnancy (19). That study demonstrated a reduction in GHBP with advancing gestation and a positive correlation between GHBP and maternal weight and body mass index (BMI). Another finding of that study was the substantial elevation of GHBP across all stages of gestation in cases of noninsulin-dependent diabetes mellitus (NIDDM), which contrasted with the reduction in GHBP in insulin-dependent diabetes mellitus (IDDM). The existence of divergent GHBP concentrations in these different forms of diabetes prompted the present study to investigate the relationship among changes in glycemia, PGH, and changes in GHBP during pregnancy.

Therefore in the present work we have extended the analysis of specimens from our previous study (19) to include measurements of PGH, free PGH, IGF-I, IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-2, and IGFBP-3, and we have related these to maternal characteristics, fetal growth, and glycemia. Finally, the present study was undertaken to determine whether any combination of these biochemical variables could predict birth weight.

Subjects and Methods

Consent forms and study protocols were approved by the Mater Mothers' Hospital and Queensland University of Technology ethics committees. Subjects gave formal written consent to participate in the study after receiving appropriate written and verbal information.

Patient characteristics have previously been described in detail (19). The current study used all available samples taken in the third trimester from patients with prepregnancy IDDM ($n = 13$), NIDDM ($n = 6$), and IUGR ($n = 16$) pregnancies. A subset of samples from normal pregnancies ($n = 23$) was analyzed for comparative purposes. Normal women underwent a 50-g nonfasting glucose challenge test at 28 weeks gestation to exclude gestational diabetes.

In the light of previous data regarding PGH secretion (4, 5, 8), samples taken at 28–30 weeks gestation (K28) or at 36–38 weeks gestation (K36) were analyzed. Ultrasonographic fetal assessments were performed at K28 and K36 to assess fetal growth.

Fetal outcome data collected at delivery included weeks of gestation, gender, birth weight, head circumference, and crown-heel length. The z (SD) scores for birth weight, corrected for gestational age and gender [$z = \text{birth weight} - \text{mean birth weight}/\text{SD}(\text{birth weight})$] were calculated for each baby to allow comparison of relative fetal growth across a range of gestational ages.

Categories of fetal growth were defined prospectively as follows: IUGR, birth weight less than 10th percentile; normal, birth weight more than 10th and less than 90th percentiles; and macrosomia, birth weight more than 90th percentile. Normative data were derived from a con-

temporary cohort of 21,221 singleton babies born at the Mater Mothers' Hospital.

Glycemic control records

In a subset of the patients with IDDM ($n = 9$) or NIDDM ($n = 6$), records of glycemic control were available from home monitoring and from clinic visits, all using the ACCUTREND reflectance glucose monitor (Roche Molecular Biochemicals, Mannheim, Germany). For each patient, the mean of self-monitored capillary glucose measurements taken while fasting and 2 h after breakfast was calculated in addition to a mean value for capillary glucose measurements taken at clinic visits. Mean capillary glucose values for each patient (fasting, postprandial, and at the clinic) were calculated using all available measurements between 20 and 30 weeks gestation. These mean values therefore represent overall glycemia around the time of the K28 blood sampling and have been used in the correlation analyses described below to explore the relationship between glycemia and parameters of GH metabolism.

Laboratory methods

All GHBP measurements were performed using the ligand immuno-functional assay for GHBP as reported previously (19, 21). PGH was assayed using the recombinant PGH standards and monoclonal antibodies E8 and 7C12 developed by Hennen's group (22–24) in an ^{125}I -labeled sandwich immunoassay assay. In our hands the mean intraassay coefficient of variation was 7.7%, and the interassay coefficient of variation was 9.3%. Free PGH was calculated according to the algorithm and computer program of Barsano and Baumann (25), as applied previously by Cramer *et al.* (26), using the paired values of GHBP and PGH measured in each serum sample and using the affinity for native human GHBP measured by Barnard *et al.* (18).

Serum IGF-I and IGF-II were assayed using modifications of published RIA protocols after extraction of serum with acetone and formic acid (27, 28). ^{125}I -Labeled IGF-I and IGF-II tracers were purchased from Amersham Pharmacia Biotech (Aylesbury, UK). Primary incubation with rabbit anti-IGF-I or IGF-II antiserum (GroPep Pty. Ltd., Adelaide, Australia) for 16 h was followed by immunoprecipitation with Sac-cel anti-rabbit antibody and counting. For the IGF-I, interassay CVs at the lower and upper ends of the sensitive range were 14.5% and 17.7%, respectively. For IGF-II, interassay CVs at the lower and upper ranges were 16.4% and 11.7%, respectively.

IGFBP-1, -2, and -3 were assayed using immunoradiometric assay kits supplied by Diagnostics Systems Laboratories, Inc. (Webster, TX). Reported intraassay CVs of these assays were 2.7–5.2% for IGFBP-1, 4.7–8.5% for IGFBP-2, and 1.8–3.9% for IGFBP-3. Interassay CVs for these assays were 3.5–6.0%, 4.5–7.4%, and 0.5–1.9% for IGFBP-1 to -3, respectively.

Statistical analysis

Differences between serum hormone concentrations at K28 and K36 and between groups of patients classified by growth (normal, IUGR, and macrosomic) and diagnosis (normal, IDDM, NIDDM, and IUGR) were analyzed using ANOVA and covariance. Significant differences were further examined using the least significant difference test. Linear correlation analysis was used to explore the relationships between continuous variables, and the r values quoted are Pearson's correlation coefficients.

Forward stepwise multiple regression analysis was used to develop a biochemical model for the prediction of birth weight using all GH-related variables and to examine the influence of glycemia on PGH and GHBP concentrations. In all cases, $F > 1.0$ was required for variables to enter the model. For all analyses, statistical significance was accepted at the 5% level on two-tailed testing. All statistical analyses were performed using Statistica for Windows (StatSoft, Tulsa, OK).

Results

Data from a total of 58 women with singleton pregnancies were included in this study. Maternal and fetal characteristics are summarized in Table 1. Women with NIDDM

TABLE 1. Maternal and fetal characteristics

Diagnostic group	n	Maternal age (yr)	Maternal wt(kg)	Maternal BMI (kg/m ²)	Birth wt (z-score)	Birth wt (g)	Head circumference (cm)	Crown-heel length (cm)
Normal	23	29.7 (1.0)	59.9 (2.0)	23.5 (0.8)	0.04 (0.16)	3324 (96)	34.5 (0.3)	50.5 (0.6)
IDDM	13	26.5 (1.5)	70.6 (4.0) ^a	26.4 (1.2)	0.76 (0.25) ^b	3346 (126)	34.3 (0.4)	51.4 (0.6)
NIDDM	6	27.7 (1.6)	95.0 (9.8) ^b	36.2 (2.7) ^c	0.53 (0.26)	3356 (131)	34.1 (0.4)	48.8 (1.5)
IUGR	16	27.4 (1.2)	56.6 (4.7) ^b	21.8 (1.9)	-1.8 (0.09) ^c	2326 (111) ^c	32.4 (0.4) ^c	45.8 (1.1) ^b

Characteristics of mothers recruited into the study and of their babies at birth, classified by diagnostic group. Birth weight SD (z-scores) are corrected for gestational age at delivery and for gender. Figures in parentheses are SEMs.

^a $P < 0.05$ vs. normals.

^b $P < 0.01$ vs. normals.

^c $P < 0.01$ vs. all other groups.

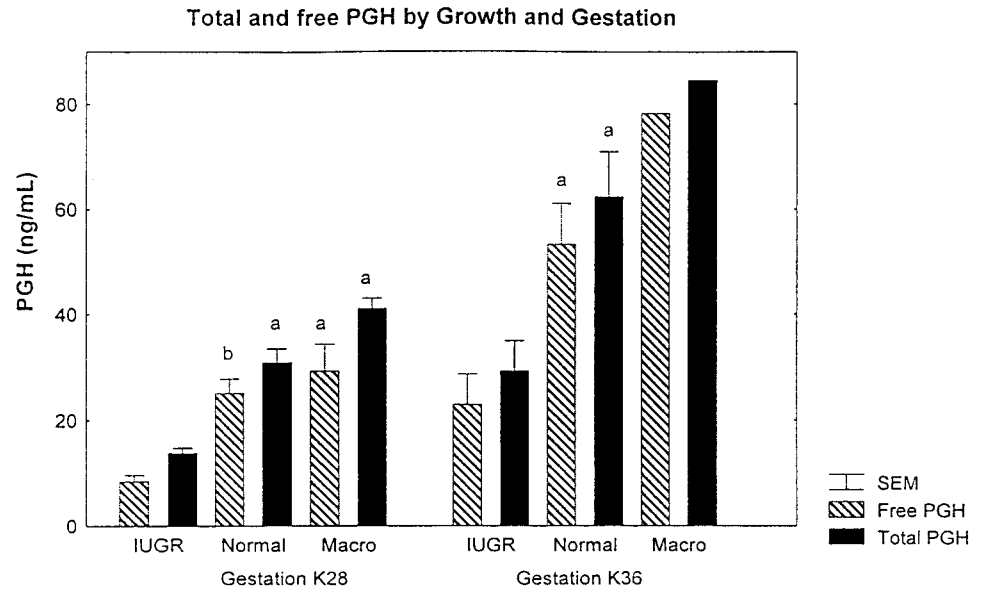


FIG. 1. Total and free PGH by growth category (IUGR, <10th percentile; Macro, >90th percentile) at K28 and K36. a, $P < 0.01$; b, $P < 0.05$ (vs. IUGR).

showed significantly greater body mass and BMI. Because of this and because our previous study (19) showed a positive correlation between BMI and GHBP levels, an analysis of covariance was performed using maternal weight and BMI as covariates when examining the outcome parameters. The results were congruent with those obtained without analysis of covariance. In the interests of simplicity the uncorrected means are reported in the tables and figures. As expected, birth weight z-scores were higher than normal in IDDM pregnancies ($P < 0.01$) and lower in IUGR ($P < 0.001$). The IUGR group also demonstrated significantly reduced head circumference and crown-heel length.

Effects of gestation

Mean maternal total PGH concentrations increased by 69% from K28 to K36 ($P < 0.01$). GHBP levels did not change significantly over this period, as noted in our previous study (19), but free PGH rose by 87% ($P < 0.01$). No significant differences were noted in levels of IGF-I, IGF-II, IGFBP-1, IGFBP-2, or IGFBP-3 at these two gestational ages.

Effects of diagnosis

At both K28 and K36, women recruited because of antenatally detected IUGR showed lower levels of PGH and free PGH than normal subjects [free PGH, 24.25 ± 2.62 ng/mL

(normal) vs. 8.46 ± 1.16 ng/mL (IUGR) at K28 ($P < 0.01$); 54.87 ± 8.31 ng/mL (normal) vs. 22.96 ± 5.67 ng/mL (IUGR) at K36 ($P < 0.01$); Fig. 1]. In addition, IUGR patients showed higher levels of GHBP at K28 and K36 (Figs. 2 and 3). Levels of IGF-I and IGF-II were reduced in IUGR (Figs. 4 and 5). Analysis of the IGFBPs showed no consistent pattern, although IGFBP-3 levels were somewhat lower in IUGR patients at K36 ($P < 0.05$).

The diabetic subjects showed some additional changes in GH-related parameters. Total PGH levels were similar to those in normal subjects, but reduction of GHBP in IDDM and elevation of GHBP in NIDDM compared to normal subjects (both $P = 0.02$) were noted at K28. As a consequence of changes in the GHBP concentration, at K28 the percentage of free PGH was lower in NIDDM subjects than in normal subjects (62% vs. 74%; $P < 0.05$) and higher in IDDM than in normal subjects (84% vs. 74%; $P < 0.05$). At K36 this trend was also significant, with a reduced percentage of free PGH in NIDDM (69% vs. 85%; $P < 0.05$) and an increased percentage in IDDM relative to that in normal subjects (91% cf. 85%; $P < 0.05$). IGFBP-2 levels were higher in IDDM and NIDDM subjects than in normal subjects at K28 ($P < 0.05$). Both NIDDM and IDDM patients demonstrated higher levels of PGH, free PGH, IGF-I, and IGF-II at K28 than the IUGR patients.

FIG. 2. PGH, GHBP, and free PGH at K28, classified by diagnostic group. a, $P < 0.01$ vs. normal subjects; b, $P < 0.05$ vs. normal subjects; c, $P < 0.01$ vs. IUGR; d, $P < 0.01$ vs. NIDDM; e, $P < 0.05$ vs. NIDDM

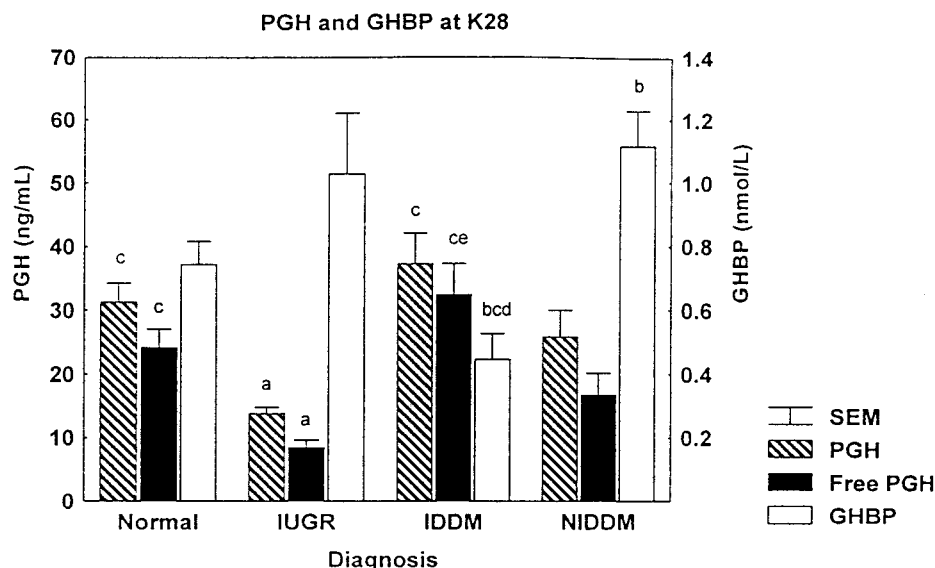
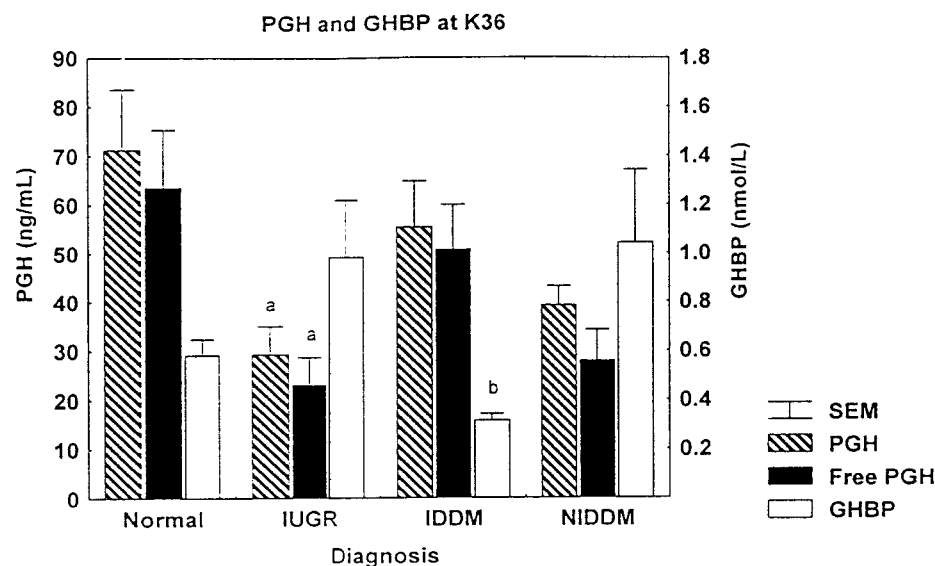


FIG. 3. PGH, GHBP, and free PGH at K36, classified by diagnostic group. a, $P < 0.01$; b, $P < 0.05$ (vs. normal subjects).



Effects of fetal size

The results were also analyzed using the predefined growth categories (IUGR, <10th percentile, $n = 16$; normal, $n = 36$; macrosomia, >90th percentile, $n = 6$), as outlined above. In this analysis, recruitment diagnosis was not considered.

The results found were congruent with those outlined above for the recruitment diagnosis, with reductions in PGH, free PGH, IGF-I, and IGF-II found in IUGR at both gestational ages ($P < 0.01$). Macrosomia was associated with slightly higher numerical values for most of these parameters, but the differences were not statistically significant. IGFBP-1 at K28 showed a significant negative correlation with birth weight ($r = -0.35$), but no correlation at K36.

Free vs. total GH

The calculated percentage of total placental GH present in the unbound state varied significantly with diagnosis (by

ANOVA, $P < 0.0001$), but not with gestation. In normal subjects $79 \pm 2\%$ ($n = 33$) of total PGH was unbound compared to $67 \pm 4\%$ in IUGR ($n = 19$; $P < 0.01$), $65 \pm 4\%$ in NIDDM ($n = 9$; $P < 0.01$), and $87 \pm 2\%$ in IDDM ($n = 17$; $P = 0.06$).

Correlation analysis

One of the primary aims of the study was to determine the value of estimations of maternal GH-related parameters as predictors of fetal growth. In the correlation analysis (see Tables 2 and 3), levels of hormonal parameters at K28 and K36 were correlated with birth weight z-scores, and the interrelationship of the various GH-related parameters was also explored.

Total PGH, free PGH, and IGF-II correlated positively with z-score at both K28 and K36. IGFBP-1 showed a negative correlation with z-score only at K28, whereas IGF-I correlated positively with birth weight at K36. As shown in Table

FIG. 4. Free PGH, IGF-I, and IGF-II and sum of IGF-I plus IGF-II by diagnostic category at K28. a, $P < 0.01$; b, $P < 0.05$ (vs. normal subjects).

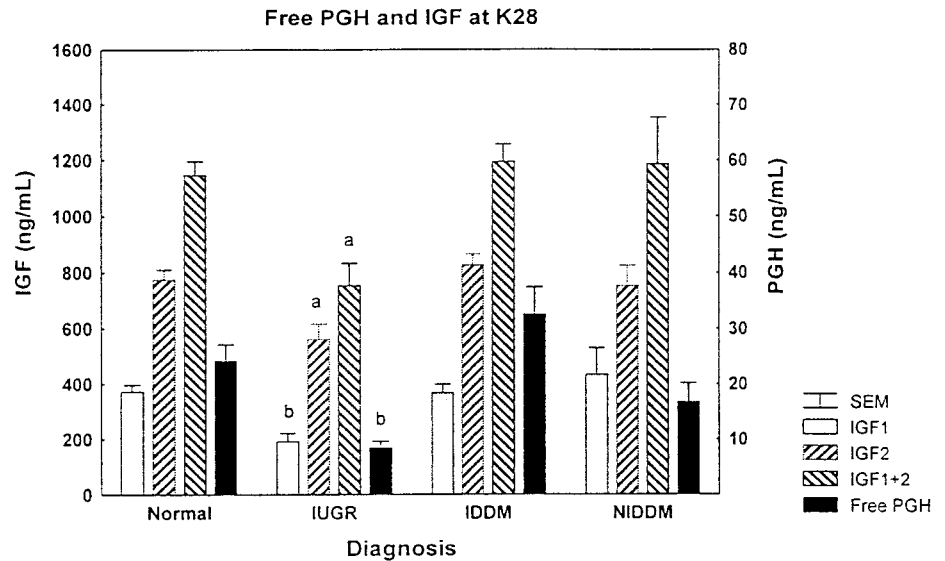
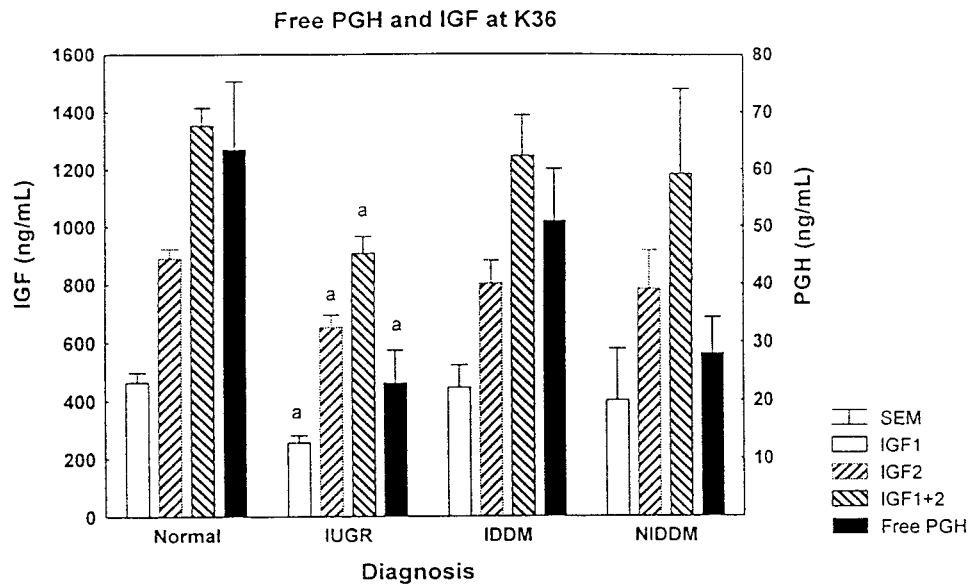


FIG. 5. Free PGH, IGF-I, and IGF-II and sum of IGF-I plus IGF-II by diagnostic category at K36. a, $P < 0.01$ vs. normal subjects



2, PGH, free PGH, and IGF-I measured at K36 correlated positively, whereas GHBP correlated negatively with head circumference and crown-heel length, suggesting an overall positive effect of PGH on fetal size. Interestingly, only PGH and free PGH correlated significantly with ponderal index (birth weight/crown-heel length³) which is a measure of relative fetal adiposity.

Forward stepwise multiple regression analysis was used to determine whether any combination of GH-related variables was able to predict birth weight z-score. At K28, an r of 0.64 ($r^2 = 0.41$; $P < 0.01$) was obtained, with IGF-II, IGFBP-1, PGH, GHBP, and IGFBP-3 retained in the model as significant variables. At K36 an r of 0.59 ($r^2 = 0.35$; $P < 0.01$) was noted, with IGF-I, free PGH, and IGFBP-3 retained in the model. These results suggest that these GH-related variables may account for up to 40% of the observed variance in birth weight.

PGH and free PGH correlated closely ($r = 0.98$ – 0.99). PGH correlated negatively with GHBP at both gestational ages. At

K28, PGH correlated positively with IGF-II, but not with IGF-I. At K36, significant positive correlations with both IGF-I and IGF-II were seen.

IGFBP-3 showed a significant positive correlation with IGF-I and IGF-II at both gestational ages. IGFBP-3 also correlated significantly with free and total PGH at K28 and K36.

Effects of glycemia

Linear correlation analysis of the effects of maternal glycemia on GH parameters showed significant effects related to postprandial glucose levels (Table 4). At K28, there was a positive correlation between mean postprandial glucose and PGH ($r = 0.57$; $P = 0.03$), free PGH ($r = 0.64$; $P = 0.01$), and IGFBP-3 ($r = 0.57$; $P = 0.03$). GHBP correlated negatively with both postprandial glucose ($r = -0.56$; $P = 0.03$) and fasting glucose ($r = -0.58$; $P = 0.03$) at K28. Insufficient glucose data were available at K36 for an adequate correla-

TABLE 2. GH parameters and growth parameters at birth

Variable	Gestation	Birth wt z-score	Head circumference	Crown-heel length	Ponderal index
PGH	28	0.39 ^a			
PGH	36	0.42 ^b	0.33 ^a	0.35 ^a	0.35 ^a
GHBP	28	-0.25			
GHBP	36	-0.25	-0.58 ^b	-0.60 ^b	
Free PGH	28	0.37 ^a			
Free PGH	36	0.43 ^b	0.34 ^a	0.43 ^b	0.38 ^a
IGF-I	28	0.27			
IGF-I	36	0.51 ^b	0.36 ^a	0.51 ^b	
IGF-II	28	0.45 ^b			
IGF-II	36	0.37 ^a		0.37 ^a	
IGFBP-1	28	-0.35 ^a			
IGFBP-1	36	0.04			
IGFBP-2	28	-0.003			
IGFBP-2	36	-0.06			
IGFBP-3	28	0.21			
IGFBP-3	36	0.18			

Pearson correlation coefficients between GH-related parameters measured in maternal serum at 28 and 36 weeks gestation and indexes of fetal growth at birth. All correlations are quoted for birth weight SD (z) score. For head circumference, crown-heel length, and ponderal index (weight/crown-heel length³), only statistically significant correlations are shown.

^a $P < 0.05$.

^b $P < 0.01$.

TABLE 3. Correlations: PGH and free PGH *vs.* other GH-related variables

Variable	PGH	Free PGH
a) Gestation K 28		
GHBP	-0.52 ^a	-0.66 ^a
IGF-I	0.19	0.12
IGF-II	0.55 ^a	0.55 ^a
IGFBP-1	0.13	0.17
IGFBP-2	0.30	0.43 ^a
IGFBP-3	0.43 ^a	0.43 ^a
b) Gestation K 36		
GHBP	-0.32 ^b	-0.37 ^b
IGF-I	0.52 ^a	0.52 ^a
IGF-II	0.32 ^b	0.33 ^b
IGFBP-1	-0.004	0.008
IGFBP-2	0.08	0.12
IGFBP-3	0.45 ^a	0.47 ^a

r values are shown.

^a $P < 0.01$.

^b $P < 0.05$.

tion analysis. Our previous study (19) showed a positive correlation between GHBP levels, and maternal prepregnancy weight and BMI. Because of the possibility that this effect may have been influencing our conclusions about the relationship between glycemia and PGH and GHBP levels, multiple regression analysis combining both measures of maternal size (weight, height, and BMI) and of maternal glycemia (fasting, postprandial, and clinic-measured glucose) as determinants of PGH and GHBP was also undertaken at K28. Data for all of these variables were available for 13 women.

Forward stepwise multiple regression with PGH as the dependent variable showed $r = 0.80$, $r^2 = 0.65$, and $P = 0.05$, with postprandial glucose, maternal BMI, fasting glucose, and maternal weight retained in the model. With GHBP as the dependent variable, the analysis showed $r = 0.89$, $r^2 =$

TABLE 4. Glycemia and GH parameters at gestation K 28

Variable	Mean clinic glucose	Mean fasting glucose	Mean postprandial glucose
PGH	0.49	0.24	0.57 ^a
GHBP	-0.39	-0.58 ^a	-0.56 ^a
Free PGH	0.48	0.38	0.64 ^b
IGF-I	-0.06	-0.23	-0.01
IGF-II	0.44	0.21	0.45
IGFBP-1	0.02	0.14	0.27
IGFBP-2	0.33	0.44	0.39
IGFBP-3	0.24	0.27	0.57 ^a

Pearson correlation coefficients between mean maternal glycemia (at clinic visits, fasting and 2 h postprandial) and GH-related parameters in patients with diabetes.

^a $P < 0.05$.

^b $P < 0.01$.

0.79, and $P < 0.001$, with maternal weight and postprandial glucose retained. Using free PGH as the dependant variable, we obtained $r = 0.83$, $r^2 = 0.69$, and $P < 0.02$, with postprandial glucose, BMI, and fasting glucose retained in the model.

These multiple regression results suggest that glycemia is related to PGH concentrations by mechanisms independent of maternal weight and BMI. Further, maternal weight and BMI influence both PGH and GHBP levels.

IGFBP-1 did not show significant correlation with indexes of glycemia at K28 or K36, but was significantly lower in NIDDM than in normal subjects at K28 ($P < 0.02$).

Discussion

There have been a number of reports supporting a critical role for maternal IGFs and maternal GH as determinants of fetal growth (11–14, 22). The present report is the first in which PGH, GHBP, IGFs, and IGFBPs in late pregnancy have all been measured in the same sample set. This has allowed calculation of the free component of placental GH and correlation of the free component with indexes of fetal growth and other endocrine markers.

Our previous study (19) of the same cohort and another study (29) suggested that there may be a link between the glycemic status of patients and the concentration of GHBP and, hence, the concentration of free GH. In the current work we have also attempted to address this hypothesis.

A highly significant correlation was found between PGH and birth weight and between free PGH with birth weight at both K28 and K36. This is consistent with the finding of Evain-Brion *et al.* for total PGH (11, 22). Correlations and significance levels were very similar for free and total PGH, reflecting the tight correlation between free and total PGH ($r = 0.98$ – 0.99). Note that IGF-I and IGF-II also correlated well with birth weight, consistent with previous studies. IGF-I and IGF-II correlated strongly with each other at both gestation stages, consistent with observations by Ferguson *et al.* (12). Interestingly, although PGH and free PGH concentrations doubled between K28 and K36, IGF concentrations did not change significantly, suggesting a decrease in IGF responsiveness to GH between these gestational ages. The elevation in GH along with its continuous, rather than pulsatile, secretion are congruent with the well known clinical

observation of increased glycemia and insulin resistance during later pregnancy.

IUGR patients showed higher levels of GHBP at K28 and K36 along with reduced PGH concentration. Fisker *et al.* (30) reported that GH replacement in GH-deficient adults resulted in a reduction in GHBP that was secondary to decreased adiposity. Another recent GH replacement study in GH-deficient adults (31) found a very strong three-way correlation ($r > 0.81$) among adiposity, serum leptin, and GHBP. Indeed, our earlier study (19) of normal and diabetic pregnancies found a significant correlation between BMI and GHBP. However, the elevated concentration of GHBP in IUGR pregnancy does not appear to result from increased adiposity, because the mean maternal BMI of the IUGR group was not significantly different from that in the normal group.

The present study was also undertaken to determine whether any combination of biochemical variables could predict birth weight. At K28 the combination of IGF-II, IGFBP-1, PGH, GHBP, and IGFBP-3 was the strongest predictor of birth weight. At K36 a combination IGF-I, free PGH, and IGFBP-3 was the best predictor. These results show that maternal GH-related parameters account for up to 40% of the observed variance in birth weight.

IGFBP-1 at K28 showed a significant negative correlation with birth weight ($r = -0.35$), but not at K36. This is consistent with the observations of Baldwin *et al.* (32). They reported a correlation coefficient at K20-K24 ($r = -0.368$) very similar to the one we measured at K28 ($r = 0.35$) and a weaker but significant negative correlation at K30-K34. In the nonpregnant state IGFBP-1 is thought to regulate the availability of IGF-I. In pregnancy its observed relationship to fetal size suggests a similar role that is independent of PGH status, as IGFBP-I showed no correlation with PGH, but showed a significant negative correlation with birth weight.

At both gestational ages IGFBP-3 correlated significantly with free and total PGH. IGFBP-3 is an index of functionality of the GH/IGF axis in the nonpregnant state (33) with the IGFBP-3 gene under direct control of a GH-responsive promoter (34). Our results suggest that this GH-responsive promoter is likely to have an active regulatory role during pregnancy.

There were significant correlations of PGH and free PGH with IGF-II at both gestational ages, but although the correlation coefficients for IGF-I were similar at K28 and K36, it was significant only at K36. Our finding is consistent with a number of studies (5, 8, 22) that showed decreased maternal serum IGF-I in IUGR, but differs from the results of Witznitzer *et al.* (35). The latter group did not find a correlation between maternal serum IGF-I and birth weight after week 37, but their study did not include cases of IUGR.

IGFBP-1 did not show a significant correlation with indexes of glycemia at K28 or K36. By contrast, Baldwin *et al.* (32) found a negative correlation in the third trimester between IGFBP-1 and glucose levels after a 50-g oral glucose tolerance test. Our different result probably reflects methodological differences, because our measurements were taken during fasting and 2 h after breakfast, rather than after an oral glucose load. Although IGFBP-1 did not show significant negative correlation with indexes of glycemia, it was

significantly lower in NIDDM patients than in normal subjects at K28 ($P < 0.02$). The latter result could be consistent with suppression of IGFBP-1 by elevated insulin, as observed in the nonpregnant state (36, 37).

PGH, free PGH, and IGFBP-3 were all strongly correlated with glycemia at K28. This is consistent with the well established antiinsulin and hyperglycemic action of GH (38, 39). Whereas GH correlated positively with glycemia, GHBP correlated negatively. This may be consistent with GHBP normally functioning to inhibit GH metabolic actions *in vivo* [as it does *in vitro* (40–43)]. Limited published data concerning PGH regulation *in vitro* and *in vivo* suggested inhibition of PGH secretion at high glucose concentrations and stimulation of PGH during hypoglycemia. Patel *et al.* (44) described a concentration-dependent inhibition of PGH secretion by glucose in human placental explants and in trophoblast cultures, whereas Bjorkland *et al.* (45) described a mean 27% increase in PGH during a hyperinsulinemic hypoglycemic clamp (glucose, 2.2 mmol/L) in pregnant IDDM subjects. However, this increase in PGH was detected at glucose levels well below the normal range. It appears to represent a counterregulatory response to hypoglycemia, as seen with pituitary GH in the nonpregnant state. Another group (46) described reduction in PGH concentrations during an oral glucose tolerance test in women with gestational diabetes. This also suggests an inhibitory effect of acute hyperglycemia on PGH secretion.

In light of these studies we might have expected to see a counterregulatory decrease in GH concentrations in response to hyperglycemia. However, we observed a positive correlation between PGH and glycemic status, suggesting that the physiological relationship between long term glycemia and PGH in pregnant patients with diabetes may be different. In our patients glucose was generally somewhat elevated chronically, and no patient demonstrated frank hypoglycemia. In the chronic situation we hypothesize that PGH levels are driving increased glycemia, rather than responding to glycemia in a counterregulatory mechanism.

In general, total PGH and free PGH correlate very closely. This would tend to keep the free or bioavailable GH within tightly set limits for a particular subject, particularly as secretion of PGH, unlike that of pituitary human GH, is not pulsatile (5, 47). Our observations in this and our previous study indicate that large excursions in GHBP concentration only appear to occur in chronic pathological states, such as NIDDM and IUGR, where the levels are elevated and cause a significant reduction in the free fraction of PGH.

In conclusion, maternal free PGH, total PGH, and IGF-I correlate significantly with fetal weight. Free and total PGH correlate strongly with postprandial glycemia at K28. Moreover, maternal GH-related parameters, taken together, account for up to 40% of the observed variance in birth weight. In rodents (48) maternal treatment with IGF-I has been associated with an increase in messenger ribonucleic acid for the placental glucose transporters GLUT1 and GLUT3. As GLUT1 is thought to be responsible for glucose transport from mother to placenta and GLUT3 to be responsible for glucose transport from placenta to fetus, this observation, if congruent in humans, would provide a causal link between maternal GH-related parameters and fetal size.

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