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Placental GH, IGF-I, IGF-Binding Protein-1, and Leptin during a Glucose Challenge Test in Pregnant Women: Relation with Maternal Body Weight, Glucose Tolerance, and Birth Weight

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Abstract: The prediction of birth weight may be improved by the measurement of hormones or growth factors in the mother. We measured body weight (BW) and plasma levels of placental GH (PGH), IGF-I, IGF-binding protein-1 (IGFBP-1), and leptin at the time of the glucose challenge test (GCT) in 289 women, who were pregnant with a single fetus, between 24 and 29 wk gestational age (GA). Delivery occurred 12 ± 2 (mean ± SD) wk later.

First, we examined which variables regulate these hormonal factors. Multiple regression showed that PGH concentrations were determined by GA at sampling and were negatively related to BW. IGF-I levels were mainly determined by PGH, and also by insulin, BW, and (negatively) age. IGFBP-1 concentrations were negatively determined by BW, insulin, and IGF-I. BW was also a powerful determinant of leptin levels, with insulin as a less robust determinant.

Second, we examined the relation to glucose levels. PGH, IGF-I, and IGFBP-1 concentrations were not correlated with post-GCT glucose levels and were comparable in women with a normal or disturbed GCT (glucose ≥7.8 mmol/liter; n = 72).

Finally, we examined the relation with birth weight and placental weight. Birth weight, corrected for GA and stratified into percentile groups, and the ponderal index at birth were strongly related to maternal BW, but not to maternal PGH, IGF-I, or IGFBP-1 levels. Neither was maternal leptin related to birth weight, but leptin concentrations were slightly higher in women who delivered obese babies. Placental weight was not related to any of the hormonal factors.

This prospective study indicates that the variation in circulating PGH, IGF-I, IGFBP-1, and leptin between 24 and 29 wk of pregnancy is strongly dependent on maternal BW, but is unrelated to glucose tolerance. In addition, the measurement of PGH, IGF-I, IGFBP-1, or leptin at the time of the GCT is not useful clinically to predict birth weight.

Abbreviations: BMI, Body mass index; BW, body weight; CV, coefficients) of variation; GA, gestational age; GCT, glucose challenge test; GDM, gestational diabetes mellitus; IGFBP-1, IGF binding protein 1; IUGR, *in utero* growth retardation; LSD, least significant difference; P, percentile; PGH, placental GH; PI, ponderal index; rh, recombinant human.

In utero growth retardation (IUGR) and overgrowth (macrosomia) are associated with increased perinatal complications, including fetal distress, neonatal hypoglycemia, and perinatal death. In addition, both IUGR and macrosomia have long-term effects on body size and composition and increase the risk of developing features of the metabolic syndrome (1). More specifically, thin and obese babies [*i.e.* babies with a low or high ponderal index (PI), respectively] appear to be the most at risk (1). The clinical and ultrasonic prediction of birth weight and PI may be improved by additional biochemical measurements. These potential biochemical markers include growth-related hormones and growth factors such as placental GH (PGH), IGF-I, IGF-binding protein-1 (IGFBP-1), and leptin, which could be measured in amniotic fluid or, preferably, in maternal serum at some point during pregnancy. These growth-related factors do not cross the placental barrier, but may affect fetal growth through their effects on the placenta (2).

PGH is secreted by the syncytiotrophoblast and is believed to be responsible for the gradual rise in serum IGF-I concentrations during the second half of pregnancy, because PGH and IGF-I levels are correlated (3, 4). Circulating IGFBP-1 increases from early pregnancy onward (5, 6) and is produced by both liver and decidua (7). There is also a surge in serum leptin during pregnancy, owing to the accumulation of adipose tissue and the

placental production of leptin (8, 9).

Several studies have demonstrated lower circulating levels of PGH and IGF-I, but higher levels of IGFBP-1, during the third trimester of pregnancies complicated by IUGR, in particular pregnancies with a deficient uteroplacental supply line (4, 10-14). Maternal serum IGFBP-1 was also found to correlate negatively with birth weight in normal (15,16) and diabetic (17) pregnancies. In addition, maternal leptin levels were inversely correlated with birth weight in adolescent pregnancies (18) and in pregnancies complicated by gestational diabetes mellitus (GDM) (19).

Methodological aspects limit the conclusions that can be drawn from these studies. In some studies, women were sampled throughout pregnancy (16). Other studies focused on pregnancies in which IUGR was already diagnosed (4, 11-14) and samples were taken shortly before delivery (4, 11-13); in these studies, lower PGH and IGF-I may reflect a lower placental mass. Some studies did not correct birth weight for gestational age (GA) (15,16). Finally, most studies did not correct for the effect of maternal body weight (BW), which has a powerful effect on maternal IGFBP-1 (16,20) and leptin levels (18) - and perhaps on PGH and IGF-I levels, and also on birth weight (21).

We therefore decided to study PGH, IGF-I, IGFBP-1, and leptin levels at the time of the glucose challenge test (GCT), because the number of blood samples performed on a routine basis during pregnancy is limited. A GCT is generally done between 24 and 28 wk as a screening test for GDM (22). Maternal BW at this time was included as a measurement.

The experimental setting offered the additional bonus to examine the relation of PGH and IGFBP-1 with glucose levels. Insulin resistance is well known to arise in the second half of pregnancy, accelerating the development of glucose intolerance (23). Although acromegaly and GH administration in nonpregnant individuals induce insulin resistance (24,25), the link between PGH and insulin resistance/glucose intolerance during pregnancy is unknown. Recently, PGH concentrations at 28 wk were found to be positively correlated with postprandial, but not fasting, glucose levels (14). On the other hand, the addition of glucose to villous tissue cultures resulted in a sharp reduction of PGH output *in vitro* (26). Increased IGFBP-1 levels during pregnancy may also promote glucose intolerance. Indeed, the acute infusion of IGFBP-1 blocked the hypoglycemic response to IGF-I and raised glucose levels in rats (27), and transgenic mice that overexpress the IGFBP-1 gene develop glucose intolerance (28).

Materials and Methods

Study population

The study protocol was approved by the Ethical Committee of the Katholieke Universiteit Leuven Faculty of Medicine. The study population consisted of women who had a GCT, ordered by their obstetrics/ gynecology physician, in the antenatal clinic between May and September 2000. Fasting or nonfasting women received 100 ml of a lemon-flavored 50% glucose solution (50 g of glucose) and received an extra 100 ml of water if they wanted it. GCTs were performed throughout the day, between 0900 and 1900 h. Patients were given a leaflet explaining the purpose of the study during the time they had to wait for the blood sampling. In the meantime, patients were requested not to eat, drink, smoke, or chew gum, and not to walk about. After 60 min, they were asked whether they consented to participate in the study, which consisted of donating an extra blood tube in addition to the tests ordered by the physician. This extra blood tube was kept at 4 C, centrifuged on a twice daily basis, aliquoted, and frozen at -80 C until assayed. Clinical data were retrieved from the computerized file notes after delivery; there was no interference with clinical management.

Three hundred thirty-nine samples were obtained; 50 samples were discarded, either because they were taken from women carrying twins (n = 5) or because they were taken at 30-wk GA or later (n = 39) or before 24-wk GA (n = 6); however, we included the 47 samples taken at 29-wk GA in the study. The recorded BW was determined at the time of the GCT; the recorded height was determined at the first antenatal visit, and the body mass index (BMI) was calculated. None of the mothers had known glucose intolerance before the GCT. In 14 patients (4.9%), glucose intolerance was clinically detected on the basis of the GCT result and a subsequent oral glucose tolerance test, and they were treated with a 1600-1800 kcal diabetes diet (n = 12) or with a diet combined with insulin (n = 2). Thirty-eight patients (13.7%) developed some form of hypertension during pregnancy, according to the classification of Davey and MacGillivray (29); 7 of them (2.5%) developed preeclampsia, 21 developed gestational hypertension, and 10 had chronic hypertension. Eleven patients dropped out from the antenatal clinic between the GCT and delivery, or they delivered elsewhere; thus, the delivery data

pertain to 278 women/newborns. Birth weight was compared with recently updated reference charts, derived from more than 429,000 births in Flanders, Belgium, and was stratified into percentile groups according to GA (30). The PI at birth was calculated as: birth weight in grams X 100/(length in centimeters) (3). Newborns were stratified into three categories: thin (PI <2.32), normal (PI between 2.32 and 2.84), and obese (PI ≥2.85) (31). There were no major congenital abnormalities in the cohort, except for one newborn who had a pulmonary artery abnormality that did not require neonatal surgery.

Assays

All assays were done in a single run, and all measurements were done in duplicate. Plasma glucose was measured by the glucose-oxidase method with a YSI 2300 Stat Plus glucometer (YSI, Inc., Yellow Springs, OH); we did not use the glucose values, measured for clinical management. Insulin was measured by RIA, with recombinant human (rh) insulin as the standard, and a polyclonal rabbit antiserum (32); the detection limit of this assay is 15 pmol/liter, and the between-assay coefficient of variation (CV) is 3.2-5.9%. PGH was measured by a two-site immunoradiometric assay, using rhPGH as the standard and two monoclonal antibodies. The detection limit is about 10 pmol/liter; within- and between-assay CV are 3.0-5.5% and 5.0-7.9%, respectively (33-35). IGF-I was measured by RIA after acid ethanol extraction, using rhIGF-I as the standard and a polyclonal antiserum raised in guinea pigs. The detection limit is about 5 nmol/liter, and within- and between-assay CV are 10.8 and 7.6%, respectively (35-37). IGFBP-1 was measured by RIA, using as standard IGFBP-1 purified from human amniotic fluid standardized to an enzyme-linked immunoassay from Medix Biochemica (Kauniainen, Finland) and an antiserum raised in rabbits, and was described in detail previously (37); the detection limit is about 10 pmol/liter, and within- and between-assay CV are 2.4-4.0% and 6.2-9.7%, respectively. Leptin was measured with a commercial RIA kit (Linco Research, Inc., St. Charles, MO); the detection limit is about 30 pmol/liter, and within- and between-assay CV are 3.4-8.3% and 3.0-6.2%, respectively.

Data analysis

Using a software program (NCSS, Kaysville, UT), analyses included: *t* tests and χ^2 tests, one-way ANOVA followed by Fisher's least significant difference (LSD) multiple comparison test, two-factor ANOVA, pair-wise Pearson correlation matrices, and multiple regression. Data are shown as means ± SD.

Results

General characteristics

Maternal age varied between 15 and 44 yr (Table 1). Using age distribution data, we calculated that the study cohort was older compared with the entire parturient population of Flanders, Belgium, in 1999 (38) (χ^2 test, $P <$ 0.0001). One hundred forty women (48%) were nulliparous, 99 (34%) were para 1, and 50 (17%) were multiparous. The large majority (n = 272, 94%) was of European (Caucasian) ancestry, whereas 17 were of non-European or uncertain ancestry. Mean GA at delivery was 38.9 wk (SD, 1.6; range, 26-42 wk); delivery occurred 12.1 wk after sampling (SD, 2.3; range, 4 d to 17 wk). Eighteen women (6.5%) delivered before 37 wk [in Flanders, the premature delivery rate for singleton pregnancies is 6.0% (38)]. The newborn population consisted of 141 girls (50.7%) and 137 boys. Mean birth weight was 3390 g (SD, 535; range, 520-4790), length was 50.5 cm (SD, 2.7; range, 28-56), head circumference was 34.6 cm (SD, 1.4; range, 29.5-38.5), and placental weight was 573 g (SD, 121; range, 100-1130). Regarding the birth weight percentile distribution, 8.3% were below or at the 10th percentile (P_{10}), 11.9% were between P_{11} and P_{25}, 22.3% were between P_{26} and P_{50}, 27.0% were between P_{51} and P_{75}, 15.5% were between P_{76} and P_{90}, and 15.1% were more than P_{90}.

There was a wide variation in most plasma parameters, particularly in insulin levels. Because the histogram of insulin levels showed an unequal distribution, we used $\log_{10}$-transformed values, which were compatible with a Gaussian distribution (data not shown).

TABLE 1. *Data of 289 pregnant women at the glucose challenge test*

	Mean	SD	Range
Age (yr)	29.7	4.6	15-44
Gestational age (wk)	26.8	1.6	24-29
Body weight (kg)	72.4	12.2	49.1-115
BMI (kg/m^2)	26.1	4.3	18.1-41.3
Plasma glucose (mmol/liter)	6.7	1.8	2.7-12.1
Plasma insulin (pmol/liter)	600	433	116-5112
Plasma PGH (pmol/liter)	492	289	53-2177
Plasma IGF-I (nmol/liter)	28.8	9.9	4.2-69.3
Plasma IGFBP-1 (pmol/liter)	1440	624	334-5222
Plasma leptin (pmol/liter)	800	435	91-2750

Correlation and multiple regression analysis of plasma PGH, IGF-I, IGFBP-1, and leptin levels

In Table 2, we show the correlation coefficients between maternal age, GA, BW, and BMI, and the various plasma parameters. On the basis of this analysis, independent variables were introduced for PGH, IGF-I, IGFBP-1, and leptin in a multiple regression analysis (Table 3); either BW or BMI was introduced, whichever correlated better. In the PGH model, only GA and BW were significant determinants of PGH levels. One-way ANOVA confirmed that PGH levels were related to GA; PGH levels were higher at 27-29 wk than 24-26 wk (Fig. 1, *left panel).* PGH concentrations gradually declined with increasing BW (Fig. 1, *right panel).* In the model for IGF-I, PGH was the most significant determinant, and then insulin; age (negatively) and BMI were less robust determinants. One-way ANOVA showed a trend *(*$P = 0.099$) for an effect of GA on IGF-I levels, and subsequent *post hoc* analysis showed that IGF-I levels were higher *(*$P < 0.05$) at 29 wk than between 24 and 27 wk (data not shown).

The BMI, as well as insulin and IGF-I levels, but not GA, were determinants of IGFBP-1 levels; none of these factors was predominant in the regression model, however. The simple R^2 *(i.e.* the R^2 value that would be obtained if IGFBP-1 were regressed only against one particular variable) was 9.9, 12.9, and 10.2% for BMI, insulin, and IGF-I, respectively.

The BMI was by far the most powerful determinant of plasma leptin levels, with insulin as a smaller determinant. The simple R^2 was 41.4 and 12.4% for BMI and insulin, respectively. Leptin levels were significantly different in each of the BMI quartiles (ANOVA, $P < 0.0001$) (data not shown).

Relation to plasma glucose levels

Seventy-two women (24.9%) had a plasma glucose level of at least 7.8 mmol/liter. Women with a positive GCT tended to have a higher BW *(*$P = 0.06$) and BMI *(*$P = 0.05$) than women with a normal GCT. Figure 2 shows that, in contrast to the difference in insulin levels, there was no difference in PGH, IGFBP-1, or leptin levels among women with a normal *vs.* abnormal GCT. Neither were plasma IGF-I levels different between the two groups *(*$P = 0.32$).

TABLE 2. *Pearson correlation coefficients between variables at the time of the glucose challenge test*

	Age	GA	BW/BMI	Glucose	Log_{10} insulin	PGH	IGF-I	IGFBP-1
BW/BMI	NS	NS						
Glucose	0.19^a	NS	0.16^a/0.17^a					
Log_{10} insulin	NS	NS	0.27^a/0.34^a	0.41^a				
PGH	NS	0.35^a	-0.29^a/-0.26^a	NS	-0.14^b			
IGF-I	-0.13^b	0.19^a	0.11^c/0.14^b	NS	0.27^a	0.25^a		
IGFBP-1	NS	-0.10^c	-0.27^a/-0.32^a	NS	-0.36^a	NS	-0.33^a	
Leptin	NS	NS	0.62^a/0.64^a	0.12^b	0.36^a	-0.22^a	0.14^b	-0.31^a

$^a P < 0.01$; $^b P < 0.05$; $^c P < 0.10$.

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TABLE 3. *Multiple regression of PGH, IGF-I, IGFBP-1, and leptin levels at the time of the glucose challenge test*

		T-value	*P*	R^2 (%)
PGH				
	GA	6.46	<0.0001	11.8
	BW	-5.20	<0.0001	7.6
	Leptin		NS	
	Intercept	-2.51	0.01	
	Total R^2 = 0.20 (*P* < 0.0001)			
IGF-I				
	Age	-2.15	0.03	1.4
	GA		NS	
	BMI	2.36	0.02	1.7
	Insulin	4.11	<0.0001	5.1
	PGH	5.76	<0.0001	10.0
	Leptin		NS	
	Intercept		NS	
	Total R^2 = 0.18 (*P* < 0.0001)			
IGFBP-1				
	GA		NS	
	BMI	-3.61	<0.001	3.7
	Insulin	-3.95	<0.001	4.4
	IGF-I	-4.17	<0.0001	5.0
	Leptin		NS	
	Intercept	12.92	<0.0001	
	Total R^2 = 0.22 (*P* < 0.0001)			
Leptin				
	BMI	12.24	<0.0001	31.1
	Glucose		NS	
	Insulin	3.13	0.001	2.0
	PGH		NS	
	IGF-I		NS	
	Intercept	-7.55	<0.0001	
	Total R^2 = 0.43 (*P* < 0.0001)			

R^2 (%), Incremental last R^2, i.e. R^2 that would be reduced if this variable were removed from the model.

FIG. 1. *Effect of increasing GA (in weeks, left panel) and body weight (in quartiles, right panel) on plasma placental GH concentrations. Data are shown as box plots; the number of data in each group is given in the box. Statistical procedure is one-way ANOVA, followed by Fisher's LSD multiple comparison test. Groups that are significantly different (P < 0.05) from one another are denoted by different letters.*

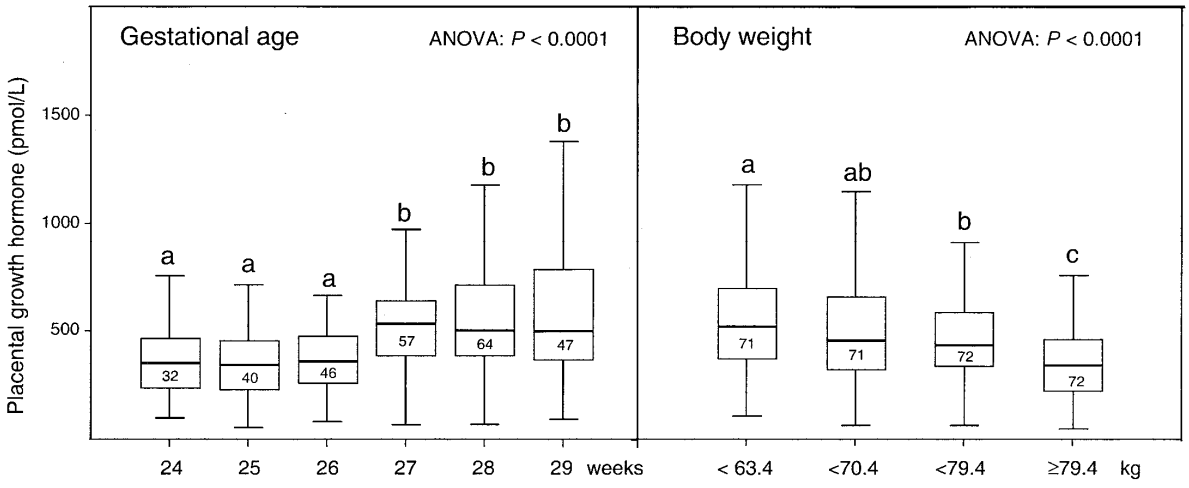
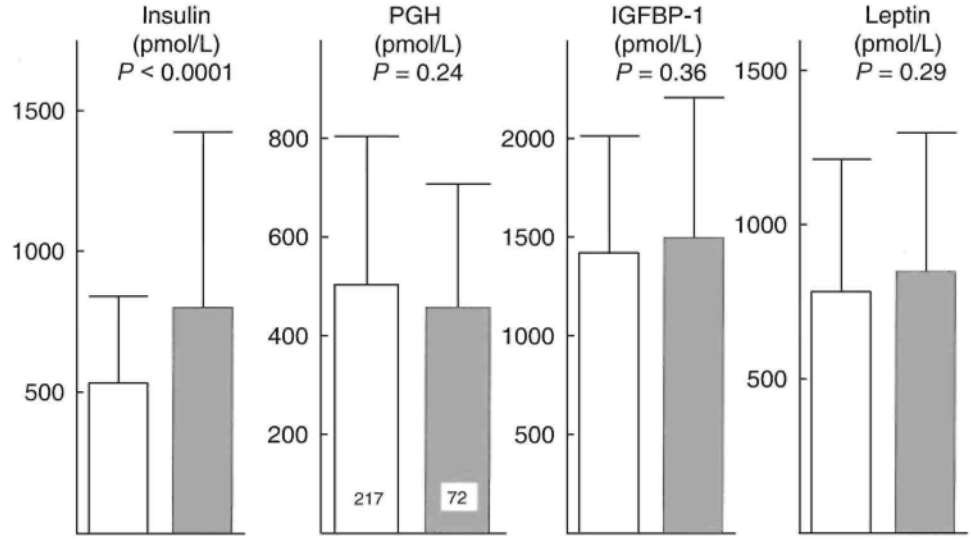


FIG. 2. *Comparison of insulin, PGH, IGFBP-1, and leptin concentrations between women with a normal GCT (white bars) and women with an impaired GCT (glucose level ≥ 7.8 mmol/ liter; hatched bars). Data are means ± SD; the number of data are given in the bars. Plasma glucose levels were 5.9 ± 1.1 mg/dl in the normal-GCT group, and 9.1 ± 1.0 mg/dl in the impaired-GCT group. Statistical procedure is unpaired t tests, taking the variance of the data into account.*



Parameters at birth

The birth weight percentile distribution was skewed to the right compared with Flemish reference charts (χ^2 test, $P = 0.003$) (38). As expected, birth weight, length, head circumference, and placental weight were highly correlated with one another and with GA at birth ($P < 0.001$). GA at birth was slightly higher in girls than in boys (39.1 ± 1.3 *vs.* 38.7 ± 1.9 wk; $P = 0.049$); none of the biometric parameters at birth were significantly different in girls *vs.* boys, although the PI tended to be higher in girls ($P = 0.07$).

Relation between maternal parameters at the GCT and birth and placental weights

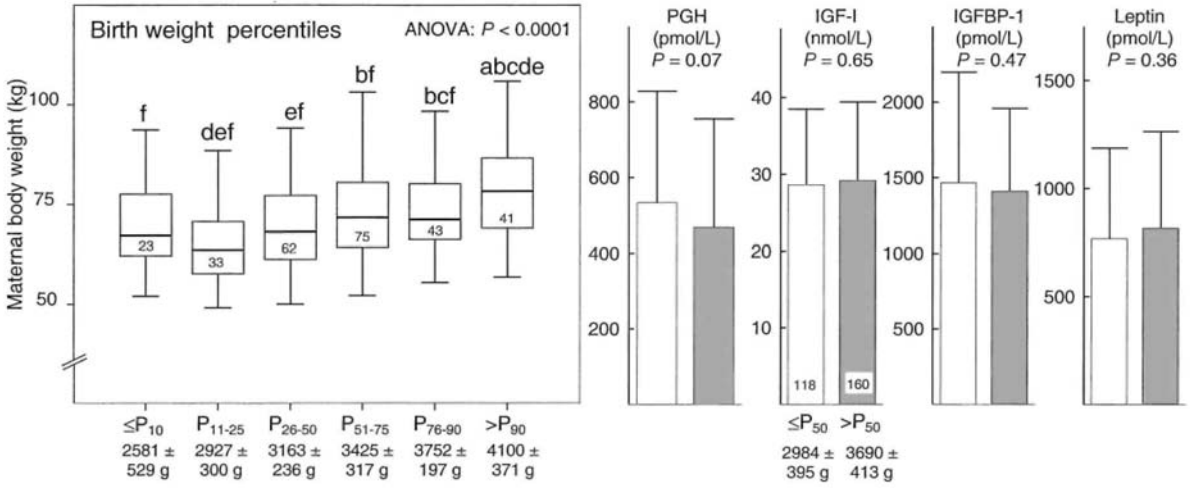
Maternal BW, height, and BMI were related to birth weight percentile distribution. The effect of BW is shown in Fig. 3 *(left panel);* the relation with height and BMI was equally strong (ANOVA, $P < 0.001$). In contrast, birth weight distribution was not significantly related to maternal PGH (ANOVA, $P = 0.30$), IGF-I ($P = 0.16$), IGFBP-1 ($P = 0.55$), or leptin levels ($P = 0.32$). The analyses remained unchanged when restricted to women who were not diagnosed with glucose intolerance, to women who remained normotensive throughout pregnancy, or to women of European ancestry (data not shown). Figure 3 *(right panel)* shows that there was no significant difference in PGH, IGF-I, IGFBP-1, and leptin levels between women who subsequently delivered babies no more than P_{50}, compared with women who delivered babies more than P_{50}.

The significant effect of GA at sampling on PGH levels might obscure a relation between PGH and birth weight. Hence, we performed two-factor ANOVA, with GA as the first factor and birth weight distribution ($\leq P_{50}$ *vs.* $> P_{50}$) as the second; GA was a significant ($P < 0.0001$) determinant of PGH levels, but not birth weight percentile ($P > 0.05$).

Placental weight was correlated with maternal BW at sampling ($R = 0.28$; $P < 0.0001$), but there was no correlation between placental weight and plasma PGH, IGF-I, IGFBP-1, and leptin.

We found no significant differences in BW/BMI or in any of the plasma parameters between mothers who were pregnant with either a female or a male fetus (data not shown).

FIG. 3. *Left, Maternal BW at the time of the GCT according to different birth weight P subgroups. Data are shown as box plots, and the number of data in each group is given in the box. Statistical procedure is one-way ANOVA, followed by Fisher's LSD multiple comparison test. Values that are significantly different ($P < 0.05$) from the P_{10} or less, P_{11-25}, P_{26-50}, P_{51-75}, P_{76-90}, and more than P_{90} groups are denoted by a, b, c, d, e, and f, respectively. Right, Comparison between plasma PGH, IGF-I, IGFBP-1, and leptin concentrations at the time of the GCT in women who delivered babies with a birth weight that was either no more than P_{50} (white bars) or more than P_{50} (hatched bars). Statistical procedure is unpaired t tests, taking the variance of the data into account.*



Relation between maternal parameters at the GCT and the PI at birth

At birth, 9% of the newborns were thin, 74% normal, and 17% obese. The PI at birth was related to maternal BW and BMI (ANOVA, $P = 0.02$ and $P = 0.008$, respectively). This effect was even more pronounced in the subgroup of women not diagnosed with glucose intolerance (95% of the cohort). Figure 4 *(left panel)* shows that BW was higher in women who later delivered obese babies, compared with thin or normal babies. Plasma glucose, insulin, PGH, IGF-I, and IGFBP-1 levels were not significantly different among women who delivered thin, normal, or obese babies (data not shown). However, leptin levels were higher in women who delivered obese babies compared with normal babies (Fig. 4, *right panel).*

Discussion

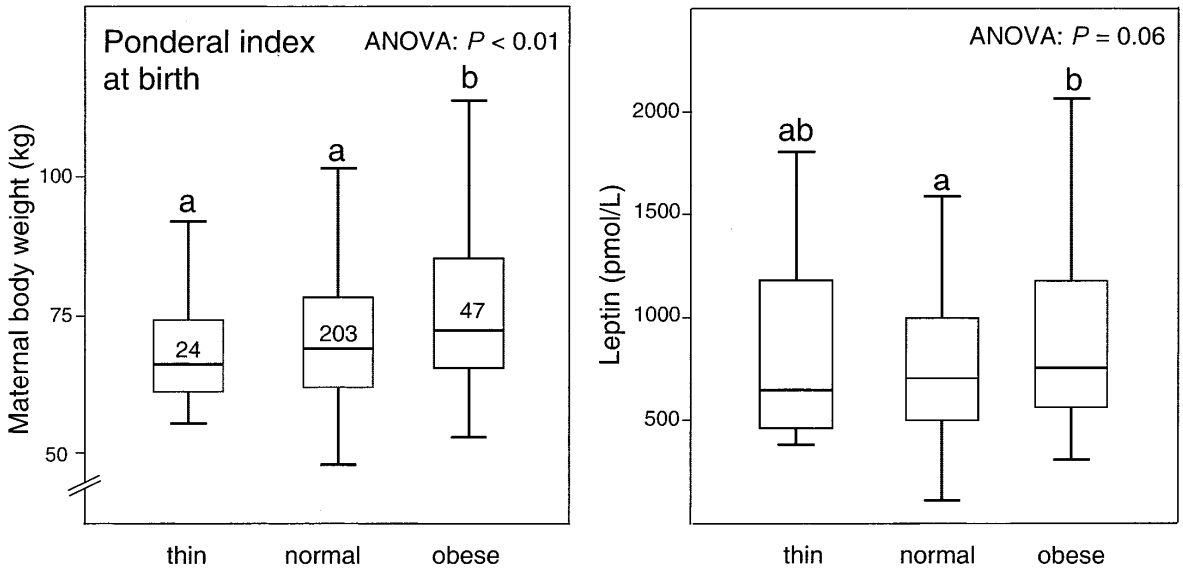
In this cohort of 289 mostly Caucasian women, 24.9% had a positive GCT (≥140 mg/dl), which is slightly higher than in a large cohort in Toronto, Canada (21%) (39). That the study cohort was at somewhat higher risk for GDM can be inferred from the finding that the cohort was older than the general Belgian parturient population. Indeed, some physicians followed the recent American Diabetes Association recommendation that Caucasian women younger than 25 yr of age without other risk factors need not be screened for GDM (22). We confirmed that maternal age was positively correlated with post-GCT glucose levels. In addition, in comparison with the Belgian newborn population, the birth weight distribution was slightly skewed to the right. However, in terms of parity and the incidence of preterm delivery, hypertension, *etc.,* the cohort is representative of the Belgian pregnant population.

We found that plasma PGH, IGF-I, IGFBP-1, and leptin levels at the GCT were all correlated with BW and BMI. Not surprisingly, the correlation was strongest by far for leptin. But we also demonstrated, for the first time, a relatively strong negative correlation between BW/BMI and PGH levels. Adiposity has been shown to negatively affect GH concentrations; in obese men, the number of GH secretory bursts is lower than in nonobese men, and GH clearance is accelerated (40). PGH has 93% DNA sequence identity with GH, but its secretion is nonpulsatile and depends primarily on the placental mass (34). It would be interesting to examine in a future study whether maternal BW affects PGH half-life. Differences in GH binding in the circulation do not explain the altered metabolic clearance rate of GH in obese individuals (40). Also, McIntyre *et al.* (14) found no meaningful differences in GH-binding protein levels among healthy women, diabetic women, and women with IUGR fetuses at 28-wk GA, and there was an excellent correlation between total and free PGH levels.

The concentrations of PGH, and to a lesser extent those of IGF-I, were positively correlated with GA at

sampling, confirming the well characterized rise in PGH and IGF-I levels during pregnancy (3, 4). Unlike BW/BMI, this effect can be observed within a 6-wk interval in the second half of pregnancy in a relatively large cohort of women. We did not confirm recently reported data that PGH levels were different according to the sex of the fetus (41). PGH was the major determinant of IGF-I concentrations, confirming the strong correlation between both variables in normal and IUGR pregnancies (3,4,10). We have reported that PGH and IGF-I concentrations were within the normal range and were highly correlated in a pregnant woman with absent GH secretion owing to *pit-1*-deficiency (35). The discrepant relation between BW and PGH (negative) *vs.* BW and IGF-I (positive) may be explained by the fact that insulin is a second important determinant of IGF-I levels. Finally, it is well known that IGF-I levels decline with age in men and nonpregnant women (36); the present study extends this data to pregnant women between 15 and 44 yr of age.

FIG. 4. *Maternal BW (left panel) and plasma leptin concentrations (right panel) at the time of the glucose challenge test, according to the PI of the newborn at birth: thin (PI < 2.32), normal (PI 2.32-2.84), or obese (PI ≥ 2.85). Data are shown as box plots, and the number of data are given in the box. Statistical procedure is one-way ANOVA, followed by Fisher's LSD multiple comparison test. Groups that are significantly different (P < 0.05) from one another are denoted by different letters.*



The IGFBP-1 levels in this study were three to four times higher than those we previously measured in nonpregnant women (37). The GCT stimulates insulin secretion, and the down-regulation of IGFBP-1 by insulin and IGF-I is well documented (7). However, Baldwin *et al.* found that IGFBP-1 levels 60 min after a 50-g glucose load at 20-24 wk were 95% of fasting levels (15). IGFBP-1 levels exhibit a diurnal variation with a nocturnal and early morning rise, but IGFBP-1 varied little between 0900 and 1900 h during the second half of pregnancy (42). We found that IGFBP-1 was negatively correlated with BMI and with insulin and IGF-I levels; the multiple regression was unable to give predominance to any of these factors. There is some evidence *in vitro* that pituitary GH suppresses the hepatic IGFBP-1 production (43), but we did not find a significant correlation between PGH and IGFBP-1 levels.

The effect of the GCT on leptin levels was probably small; in both healthy and GDM women, no difference was found between fasting and 30-min leptin levels during a 75-g glucose load at 28 wk (19). Again, plasma concentrations of leptin show a strong diurnal variation, with a nocturnal peak. During the day, a small, gradual rise was documented in nonpregnant women (44), which, if confirmed for pregnant women, would be a limitation of this study. In addition to its strong correlation with BMI, we found that leptin was also correlated with insulin levels. This confirms data in nonpregnant women and in men that insulin is related to leptin independently from its effect on adipose tissue (45-47).

At the outset of the study, we hypothesized that PGH and/or IGFBP-1 might be implicated in the development of insulin resistance and glucose intolerance during pregnancy. However, we found no evidence that supports this hypothesis; PGH and IGFBP-1 were not correlated with post-GCT glucose levels, and there was no difference in PGH and IGFBP-1 levels between women who had either a normal or an abnormal GCT. Hence, the diabetogenic effect of pregnancy is unlikely to be the result of PGH secretion or the increment in IGFBP-1

secretion.

We found that PGH, IGF-I, IGFBP-1, or leptin measurements, performed on average 12 wk before delivery, were not predictive of birth weight, the PI at birth, or placenta weight. There was only a trend for leptin levels to be higher in women who later delivered obese compared with normal babies. In contrast, maternal BW/BMI was a powerful determinant of birth weight percentile and the PI, confirming previous findings (21). Our finding appears to contradict previous reports of lower PGH and IGF-I, but higher IGFBP-1 and leptin, levels in women who delivered small-for-GA or IUGR babies (4, 10-14, 18). However, these studies focused on specific populations: pregnant teenagers, part of whom were still growing (18), or pregnancies in which IUGR had already been diagnosed by ultrasound, with or without a deficient uteroplacental supply line diagnosed by Doppler measurements (4, 10-14). Also, blood sampling was performed at delivery or shortly before delivery in some studies (11-13, 41). We conclude from our data that the measurement of PGH, IGF-I, IGFBP-1, or leptin in an unselected pregnant population undergoing a GCT is not predictive of birth weight.

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