

# Overexpression of Placental Leptin in Diabetic Pregnancy

## A Critical Role for Insulin

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**Leptin, a small peptide produced by adipocytes, is implicated in an increasing number of endocrine regulations, including adiposity, satiety, puberty, and fertility. Although the factors involved in controlling maternal and fetal weight gain during pregnancy have not been fully elucidated, leptin has recently emerged as such a potential factor. In our study, we report the presence of high amounts of leptin mRNA and immunoreactive protein in the human placenta, establishing the placental synthesis of this hormone. A large (three- to fivefold) augmentation in leptin mRNA and protein was found in placentas from insulin-treated diabetic women. This finding was associated with increased concentrations of leptin and insulin in venous cord blood without modification of maternal circulating leptin levels. These data provide evidence that the placenta is a site for regulated leptin production in utero. Insulin is likely to play a critical role in this regulation, thus emphasizing the importance of placental leptin signaling in diabetic pregnancy. *Diabetes* 47:847-850, 1998**

**T**he regulation of fetoplacental growth and development involves multiple metabolic and endocrine mechanisms, including progressive adaptations of maternal metabolism (1). Genetic and hormonal factors such as insulin, insulin-like growth factors, and cytokines play a crucial role in intrauterine development (2). In addition, an adequate transfer of maternal nutrients to the umbilical circulation is of major importance in increasing fetal size and weight (3). However, the factors controlling maternal and fetal weight gain during pregnancy are not known, and leptin, the protein encoded by the *ob* gene (4), has recently emerged as a potential factor (5,6). Increased circulating leptin levels are found with advancing gestation

(7), and leptin is also present in the amniotic fluid and the umbilical circulation (6-8).

Correlations have been found among plasma leptin level, BMI, and adipose tissue mass in both animals and humans (9,10). In addition, leptin might act as a sensor of energy balance in nonsteady situations (11). Consecutively, leptin expression is regulated by factors such as nutritional and hormonal challenges (12). Insulin might contribute to this regulation, because chronic insulin administration stimulates leptin production in rodents (13) and humans (14).

The present study investigates the regulation of placental leptin expression in utero. We report herein that leptin is overexpressed in placentas of diabetic women who require chronic insulin therapy. These data strongly suggest that insulin modulates expression of placental leptin, which may act as a circulating signal to control fetal homeostasis.

### RESEARCH DESIGN AND METHODS

**Patients.** Placentas were obtained at the end of term pregnancies of 25 (14 normal and 11 diabetic) women. Gestational age at delivery was calculated according to the last menstrual period and confirmed by 1st-trimester ultrasonography. Of the diabetic mothers, seven had IDDM, and four had gestational diabetes that required insulin therapy and that had been diagnosed using a standard 75-g oral glucose tolerance test according to World Health Organization criteria (15) (Table 1). Placental biopsies were performed immediately after delivery, and biopsy specimens were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Venous blood samples were collected from the mother and umbilical cord at the time of delivery. The blood was immediately centrifuged, and plasma was stored at  $-20^{\circ}\text{C}$  until analysis. All protocols were approved by the institutional review board of Cochin Faculty, University René Descartes, Paris, and all mothers gave informed consent. **RNA extraction and Northern blot analysis.** Total RNA was extracted from tissues and cultured cells according to the procedures of Chirgwin et al. (16) and Chomczynski and Sacchi (17), respectively. For Northern blot analysis, total RNA samples were electrophoresed on 1% agarose per 0.6 mol/l formaldehyde gels and transferred by capillarity to Hybond-N membranes (Amersham International, Les Ulis, France). Hybridizations were performed with an  $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ -labeled human leptin cDNA probe (18) using the Multiprime labeling system kit (Amersham). After autoradiography for 2-24 h, quantification of the signals was performed by scanning densitometry (GS-300 scanning densitometer; Hoefer Scientific, San Francisco, CA). After stripping of the leptin probe, the blots were rehybridized with a 24-mer antisense oligonucleotide specific for 18S rRNA to correct for the amount of RNA transferred to the filters.

**Biochemical assays.** Plasma leptin levels were measured by radioimmunoassay (Linco Research, St. Charles, MO). Placental leptin concentrations were determined on whole-tissue homogenates (10% vol/wt in 250 mmol/l sucrose and 25 mmol/l HEPES buffer [pH 7.4] containing 1 mmol/l phenylmethylsulfonyl fluoride [PMSF], 2 mmol/l aprotinin, and 2 mmol/l dithiothreitol [DTT]). Measurements were performed by radioimmunoassay (19), and linearity was established using increasing volumes (50-300  $\mu\text{l}$ ) of placental homogenates. Plasma insulin was measured by radioimmunoassay using reagents provided by CIS BIO International

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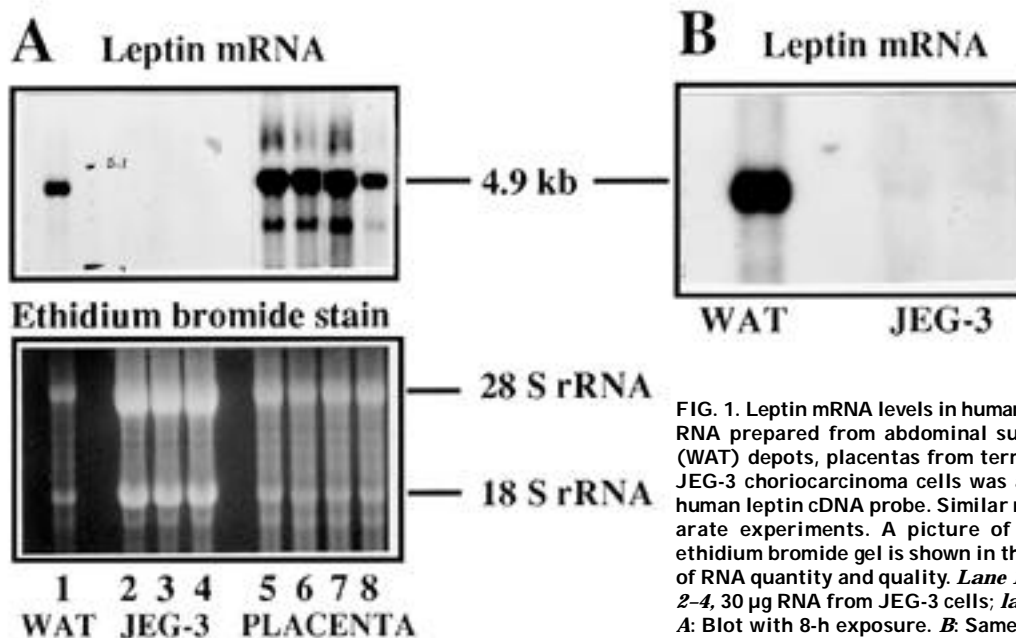


FIG. 1. Leptin mRNA levels in human placenta and adipose tissue. Total RNA prepared from abdominal subcutaneous white adipose tissue (WAT) depots, placentas from term pregnancies (39–40 weeks), and JEG-3 choriocarcinoma cells was analyzed by Northern blot with a human leptin cDNA probe. Similar results were obtained in three separate experiments. A picture of the ultraviolet transilluminated ethidium bromide gel is shown in the lower panel to allow assessment of RNA quantity and quality. Lane 1, 15 µg total RNA from WAT; lanes 2–4, 30 µg RNA from JEG-3 cells; lanes 5–8, 20 µg RNA from placenta. A: Blot with 8-h exposure. B: Same blot with 48-h exposure.

(Gif-sur-Yvette, France). Protein concentrations were determined with the standard Bio-Rad assay (Richmond, CA).

**Statistical analysis.** Results are expressed as means ± SE. Statistical analysis was performed by analysis of variance and Student's *t* test for unpaired data (Stat-work Software, Calabasas, CA).

**RESULTS**

**Placental leptin expression and synthesis.** Placental tissue samples from 14 women with uncomplicated term pregnancies and appropriate-for-gestational-age babies were examined for leptin mRNA by Northern blot analysis (Fig. 1). A major mRNA transcript of 4.9 kilobases (kb) was detected. In addition, lower amounts of a 2.4-kb transcript were present. Leptin mRNA levels expressed in densitometry units were similar in placenta (58 ± 12) and in maternal abdominal subcutaneous fat (60 ± 20). By contrast, low levels of leptin mRNA (2.0 ± 0.6) were found in JEG-3 cells, a human choriocarcinoma cell line (Fig. 1). Immunoreactive leptin was detected in all placenta samples analyzed (Table 1) with a mean level of 0.3 ng/mg protein.

**Regulation of placental leptin expression.** To investigate whether placental leptin mRNA expression can be regulated in utero, we obtained placentas from diabetic women requiring insulin therapy during pregnancy (Table 1). These women were hyperinsulinemic, and the mean increase in daily dose of exogenous insulin during pregnancy compared with prepregnancy dose was 0.38 ± 0.5 U/kg, indicating that they were insulin resistant. Plasma leptin concentrations were unchanged in diabetic mothers but increased 2.5-fold in the umbilical vein (Table 1). Placentas from women requiring insulin therapy had higher levels of leptin mRNA (threefold, *P* < 0.005) compared with nondiabetic women (Fig. 2). This increase in leptin mRNA was associated with a similar increase in placental leptin content detected as immunoreactive protein (Table 1).

**DISCUSSION**

In placentas from normal term pregnancies, leptin mRNA levels are similar to those detected in adipose tissue (Fig. 1), which has been the major source of leptin described so far.

Leptin mRNA is also present in JEG-3 choriocarcinoma cells, albeit at lower levels than in placenta, in agreement with findings in BeWo cells (7). The detection of immunoreactive protein in placental homogenates (Table 1) establishes that leptin mRNA is efficiently processed in the placenta, thus demonstrating an extended role of leptin in human reproduction. It also corroborates the recent findings of a direct synthesis of leptin by syncytiotrophoblast cells in human term placenta (7,20). Because pregnancy is associated with increased maternal fat stores that could favor maternal leptin production, the present study does not allow determination of the exact contribution of the placenta to the increased circulating leptin in pregnant women (6,7). How-

TABLE 1  
Clinical data of insulin-treated diabetic mothers and their newborns

Parameters	Subjects	
	Control	Diabetic
<i>n</i>	14	11
Gestational age (weeks)	39.6 ± 0.3	37.9 ± 0.2*
Maternal age (years)	31.6 ± 1.4	31.4 ± 1.1
Maternal weight at birth (kg)	72.9 ± 1.9	73.9 ± 1.9
Maternal weight gain (kg)	15.5 ± 0.7	12.5 ± 0.9†
Maternal BMI at birth (kg/m <sup>2</sup> )	27.1 ± 0.8	27.2 ± 1.1
Placental weight (g)	588 ± 41	555 ± 52
Birth weight (g)	3262 ± 8	3217 ± 107
Neonatal BMI (kg/m <sup>2</sup> )	13.4 ± 2.6	13.5 ± 0.5
Maternal plasma glucose (mg/dl)	86 ± 6	98 ± 7 (NS)
Umbilical plasma glucose (mg/dl)	64 ± 9	58 ± 6 (NS)
Maternal plasma insulin (µU/ml)	11.3 ± 1.6	18.6 ± 4.0†
Umbilical plasma insulin (µU/ml)	5.3 ± 1.1	27.3 ± 7.8†
Maternal plasma leptin (ng/ml)	17.8 ± 3.4	13.3 ± 2.1 (NS)
Umbilical plasma leptin (ng/ml)	7.9 ± 2.3	19.4 ± 4.5†
Placental leptin (ng/mg protein)	0.3 ± 0.07	1.5 ± 0.5*

Data are means ± SE. Statistically significant differences between control and insulin-treated diabetic groups: \**P* < 0.001; †*P* < 0.05.

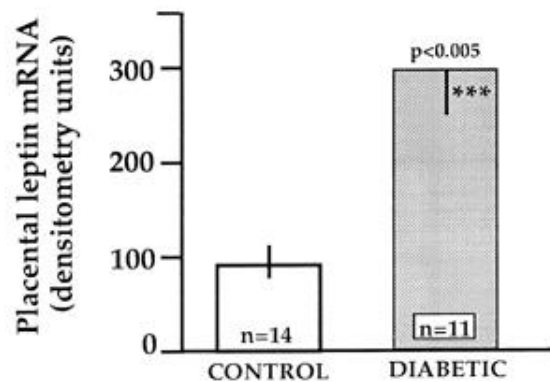


FIG. 2. Leptin mRNA expression in placentas of insulin-treated diabetic women. A total of 25 placentas were obtained from term pregnancies (38–41 weeks) and were subsequently divided into two groups: placentas from nondiabetic women (control subjects) and placentas from insulin-treated women (diabetic subjects). Total RNA was prepared and analyzed for leptin mRNA by Northern blot with 20  $\mu$ g run in each lane. Three separate Northern blots were analyzed by scanning densitometry. Results normalized for 18S rRNA signals are expressed as means  $\pm$  SE. \*\*\* $P$  < 0.005 for diabetic versus control group.

ever, the absence of correlation between maternal circulating and placental leptin concentrations in diabetic pregnancies (Table 1) does not favor the previous hypothesis that placental leptin is secreted in maternal circulation (7). In contrast, fetal circulating leptin is likely to reflect mostly the placental production, because umbilical leptin levels are increased in diabetic pregnancies (Table 1), but neonatal adiposity reflected by BMI is not modified. It is thus unlikely that fetal adipose mass contributed significantly to the umbilical hyperleptinemia.

The demonstration that placental leptin mRNA and protein contents are increased three- and fivefold, respectively, in women requiring chronic insulin therapy during pregnancy (Fig. 2 and Table 1) provides the first direct evidence that placental leptin production can be regulated in utero. Although several hormonal and metabolic changes that occur in insulin-resistant women could contribute to the regulation of placental leptin expression, it is tempting to speculate that maternal and/or fetal hyperinsulinemia is responsible for this regulation. Indeed, insulin regulation of leptin mRNA expression has been observed in adipose tissue of rodents (13,21) and humans (14,22). Our data further emphasize a role of insulin in regulating placental metabolic processes and fit with the idea that insulin is an important modulator of leptin gene expression, even though its mechanisms of action are yet to be elucidated (23).

Fetal hyperinsulinemia is commonly considered to be the cause of neonatal macrosomia. According to Pedersen (24), fetal hyperinsulinemia results from maternal hyperglycemia in poorly controlled diabetic pregnancies. In the present study, the normal weights of newborns of insulin-treated mothers are likely due to tight glycemic control during pregnancy, but surprisingly, these newborns are normoglycemic and hyperinsulinemic (Table 1). These findings could be explained by an accelerated maturation of fetal  $\beta$ -cells in response to chronic insulin treatment (25) or by a transplacental transfer of exogenous insulin (26). The hyperleptinemia developing during fetal life could compensate for

the adipogenic effect of insulin in fetuses of insulin-treated mothers. Indeed, it has been shown that leptin treatment induces a specific reduction of adipose tissue mass in rodents (27–29) and that leptin impairs metabolic action of insulin in isolated adipocytes (30), indicating that adipose tissue itself is a sensitive target for the action of its own product. These data support the hypothesis that placental leptin acts as an autocrine/paracrine modulator of placental functions as well as an endocrine signal for regulating fetal adipose stores.

In conclusion, we have shown that leptin gene expression and production are markedly elevated in placentas of diabetic women treated with insulin. The present findings provide direct evidence that leptin production can be regulated in utero and emphasize the role of placental leptin in human pregnancy.

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