Elevation of Plasma Leptin Levels During Pregnancy in Normal and Diabetic Women

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The impact of pregnancy and food intake on plasma leptin levels was investigated in insulin-dependent diabetes mellitus (IDDM) patients and healthy normal-weight women. Fourteen women with IDDM and 11 women with no diabetes or family history of diabetes were served a 707-kcal lunch in gestational weeks 34 to 38. Six breast-feeding women from each group were examined a second time within 1 month after delivery. Leptin levels were not different in the two groups either during pregnancy or postpartum. In addition to a positive correlation to body mass index (BMI), leptin levels tended to correlate with gestational weight gain. The leptin concentration during pregnancy was higher than the postpartum level, which was within the range of previously reported levels in non-obese nonpregnant women. Ingestion of the test meal did not affect leptin levels and there were no relationships between leptin and insulin or glucose, for either basal or postprandial (60-minute) levels. Only the insulin dose taken by the diabetic women correlated to leptin level. During pregnancy, there is an augmented energy expenditure and maternal metabolism is altered to increase fat stores. The present observation that leptin levels were elevated in pregnant women suggests an additional role for leptin in the accumulation of body fat.

RECENTLY, THE GENES responsible for obesity in *Lep/Lep* mice and humans were cloned.[1] The *Lep* gene and its protein product, leptin, are especially expressed in adipose tissue.[2-4] Leptin is considered to act centrally as a signaling factor that regulates body weight homeostasis through the control of appetite and energy expenditure.[1,5-7] Insulin appears to be a potent regulator of *Lep* gene expression and plasma leptin levels in the rat and mouse.[2,8,9] In humans, there is also a relationship between *LEP* gene expression and obesity, but a regulatory role for insulin is less clear. Both the *LEP* mRNA content of adipocytes and serum leptin levels are increased in obese subjects,[10,11] and body mass index (BMI) correlates to circulating leptin levels in both normal weight, obese, and non–insulin-dependent diabetes mellitus (NIDDM) patients.[12,13] Short-term (5-hour) hyperinsulinemia induced by insulin infusion has no effect on circulating leptin levels in these groups,[12,14] whereas prolonged (48- to 72-hour) hyperinsulinemia possibly causes an increase.[12] In addition, in vitro, long-term (96-hour) stimulation of adipocytes with insulin results in an increased release of leptin, as well as an increase in *LEP* mRNA levels.[12]

In rodents, glucocorticoids also seem to be involved in the regulation of *Lep* gene expression.[15] Daily treatment of rats with glucocorticoids results in an increase in *Lep* mRNA levels, whereas food intake and body weight gain decrease. The human *LEP* gene is also likely to be regulated by hormonal and metabolic factors other than a possible action of insulin. Pregnancy is a period of dramatic growth, weight gain, and hormonal and metabolic changes. The increased energy expenditure that is required for tissue synthesis and maintenance during pregnancy is achieved by small changes in other components of the energy balance equation. Food intake increases, intestinal absorption is augmented, maternal metabolism is altered—initially to lay down fat stores and then to encourage energy transfer to the fetus—and maternal energy expenditure of physical activity diminishes.[16] There is a diabetogenic effect on the maternal carbohydrate metabolism, which is manifested by an exaggerated insulin and glucose response following ingestion of a meal. This is due to the increased degradation of insulin that occurs in the placenta, as well as to increased peripheral insulin resistance.[17]

Considering leptin's connection to obesity and energy balance, it is interesting that an increase in circulating leptin levels is found in pregnant women,[18-20] as growth and weight gain characterize pregnancy. Available data indicate that expression of the *LEP* gene is not acutely affected by insulin; however, there may be a long-term effect. We therefore chose to compare leptin levels between insulin-dependent diabetes mellitus (IDDM) patients and healthy normal-weight women in the third trimester of pregnancy and up to 1 month after delivery. Furthermore, we studied whether leptin levels were changed during ingestion of a test meal, since it has been shown in the rat that *Lep* mRNA levels increase when the animals start to eat.[2]

RESEARCH DESIGN AND METHODS

Subjects

The study population was recruited from one antenatal care unit in the catchment area of the Karolinska Hospital (control patients) and from the special antenatal care unit for diabetic women at the hospital. Fourteen diabetic women and 11 women with normal pregnancies were included in the study. Subject characteristics are shown in Table 1. All of the diabetic women had a duration of diabetes of at least 5 years with no known complications. Metabolic control was achieved with intensive blood glucose testing performed at least six times per day. The insulin dose was adjusted according to preprandial and postprandial blood glucose levels. The pregnant women were highly motivated to control their blood glucose levels. Subjects in the control group had no family history of diabetes in close relatives and were tested with random blood glucose samples at least once monthly. None of the control subjects had blood glucose levels greater than 7.0 mmol/L during pregnancy. All of the women had healthy children after vaginal delivery at term. The study was approved by the local ethics committee, and informed consent was obtained from each participant.

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Table 1. Characteristics of Subjects (mean ± SD)

Characteristics	Control Group	Diabetic Group
No. of subjects	11	14
Age (yr)	29 ± 5.7	30 ± 5.0*
Height (cm)	168 ± 5.7	168 ± 4.9*
Prepregnancy weight (kg)	66 ± 5.7	67 ± 9.8*
Prepregnancy BMI (kg/m²)	23.4 ± 2.0	23.9 ± 4.2*
Maternal weight gain (kg)	16 ± 6.0	18 ± 6.7*
Weight before parturition (kg)	82 ± 8.6	85 ± 10.4*
BMI before parturition (kg/m²)	28.8 ± 2.4	30.2 ± 4.5*
Gestational week at lunch test	36 ± 1.1	36 ± 1.2*

*No significant difference between the 2 groups.

Experimental Design

The subjects ate breakfast at home and then fasted for 5 hours before the start of the experiment. In all subjects, three fasting blood samples were drawn at 15-minute intervals. The diabetic women took their prescribed insulin dose between the first and second blood sample. Immediately following the third blood sample, a 707-kcal lunch with calories from carbohydrate, protein, and fat representing 47%, 18%, and 35%, respectively, was served. Repeated blood samples were collected for 2 hours after the start of the lunch. Six of the diabetic mothers and six of the control mothers were examined a second time, 0.5 to 4 weeks after delivery. On this occasion, a single blood sample was collected 2 to 5 hours after breakfast.

Hormone and Glucose Analysis

The plasma levels of leptin (Linco, St Charles, MO), insulin, and C-peptide (DPC, Los Angeles, CA) were determined with commercial radioimmunoassay (RIA) kits. The sensitivity of the leptin assay is 0.5 ng/mL and the intraassay and interassay coefficients of variation (CV) are 3.9% and 4.7%, respectively. For the insulin assay, the detection limit is 1.2 μU/mL and the intraassay and interassay CVs are 5.0% and 7.1%, respectively. Finally, the sensitivity of the C-peptide assay is 16 pmol/L and the intraassay and interassay CVs are 3.0% and 1.9%, respectively. The glucose level was analyzed using a glucose oxidase-peroxidase kit.

Statistical Calculations

Results are expressed as means ± SD. Differences in leptin levels between the diabetic and control groups were evaluated by the Mann-Whitney *U* test, and differences between the two experiments within the same group were evaluated by the Wilcoxon signed-rank test. The Spearman rank-correlation test and partial correlation were used to calculate correlations within each group and within the whole sample.

RESULTS

Leptin Levels

There was no difference in plasma leptin levels between the diabetic and the control group (Fig 1). The leptin level was not affected by ingestion of the test meal. In the six women from each group who were tested a second time, the leptin level was lower 0.5 to 4 weeks postpartum compared with late pregnancy. In the diabetic group, the basal level (sample no. 1) of leptin decreased from 23.8 ± 12.0 to 8.2 ± 3.7 ng/mL ($P = .0277$), and in the control group from 18.8 ± 6.7 to 6.9 ± 1.1 ng/mL ($P = .0277$).

Insulin and Glucose Levels

The results of the insulin and glucose levels are reported in more detail elsewhere (S.M. Stock, E.K. Nordlander, K.A. Bremme, manuscript submitted) and are only summarized here (Table 2). In the control subjects, there was a peak in insulin levels 60 minutes after the start of ingestion of the test meal. In the diabetic group, insulin levels remained stable during the whole experiment. Measurement of the plasma level of C-

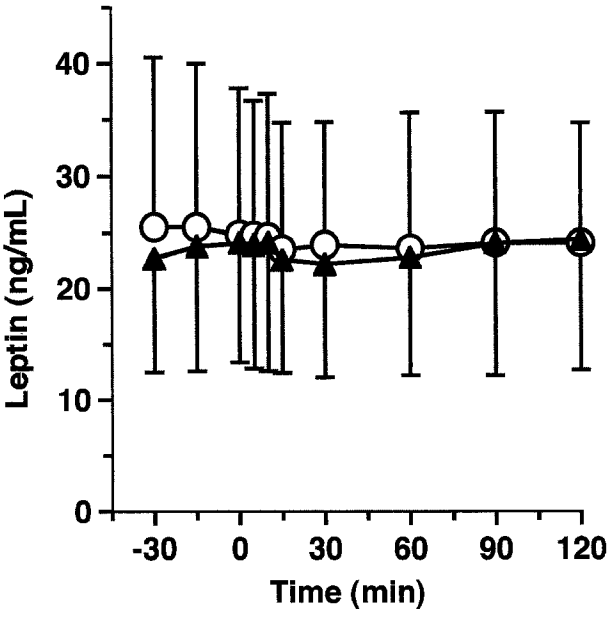

Fig 1. Plasma levels (mean ± SD) of leptin in connection with a 707-kcal test meal performed in late pregnancy (week 34 to 38) in IDDM patients (n = 14, ▲—▲) and healthy normal-weight (n = 11, ○—○) women. The diabetic women took their insulin dose between the first and second blood samples. All subjects started the meal at 0 minutes.

Table 2. Summary of Plasma Levels of Insulin, C-Peptide, and Glucose and the Insulin Treatment of the Diabetic Women (mean ± SD)

Diabetic Group	Control Group
Pregnancy, 34-38 weeks	
Insulin dose: 96.9 ± 38.5 IU	
Insulin, basal (−30 min): 55.7 ± 59.7 μU/mL	Insulin, basal (−30 min): 12.6 ± 14.9 μU/mL
Insulin, peak (60 min): 57.6 ± 57.8 μU/mL	Insulin, peak (60 min): 52.5 ± 30.9 μU/mL
C-peptide, basal (−30 min): 46 ± 29 pmol/L	C-peptide, basal (−30 min): 911 ± 684 pmol/L
C-peptide, peak (60 min): 56 ± 49 pmol/L	C-peptide, peak (60 min): 2024 ± 885 pmol/L
Glucose, basal (−30 min): 3.8 ± 1.7 mmol/L	Glucose, basal (−30 min): 4.0 ± 0.9 mmol/L
Glucose, peak (60 min): 5.2 ± 1.0 mmol/L	Glucose, peak (60 min): 5.8 ± 1.2 mmol/L
Postpartum, 0.5-4 weeks	
Insulin dose: 34.4 ± 12.8 IU	
Insulin, basal: 26.5 ± 15.5 μU/mL	Insulin, basal: 5.0 ± 3.5 μU/mL
Glucose, basal: 7.5 ± 3.4 mmol/L	Glucose, basal: 4.2 ± 0.2 mmol/L

NOTE. The basal and peak values during pregnancy refer to the levels registered during the test meal.

peptide confirmed that the secretion of insulin was almost eliminated in diabetic patients (Table 2). Glucose levels peaked at 60 minutes in both groups.

Correlations

In both groups, basal levels of leptin in late pregnancy correlated positively to BMI registered closely before parturition (diabetics: correlation coefficient, $r_s = .618$, $P = .0258$; controls: $r_s = .770$, $P = .0209$). Weight gain tended to correlate to basal leptin level, but only when data from the two groups were pooled ($r_s = .359$, $P = .0853$). There were no significant relationships between either basal or stimulated levels of leptin and levels of insulin or glucose. Only the insulin dose taken by the diabetic women was related to leptin level. This correlated positively in late pregnancy ($r_s = .686$, $P = .0133$), and there was still a tendency to correlation when controlled for BMI ($r = .445$, $P = .1275$). In breast-feeding mothers, the correlation did not reach significance ($r_s = .900$, $P = .0719$; not controlled for BMI) due to the small number of subjects ($n = 6$).

DISCUSSION

In the present study, there was no difference in the plasma level of leptin between IDDM patients and healthy normal-weight women, either in late pregnancy or postpartum. Our results support recent data demonstrating similar leptin levels in both IDDM and NIDDM patients compared with control subjects with corresponding BMI.[13,14] However Tuominen et al[21] described elevated fasting levels of leptin in a group of IDDM patients. The reason for these contradictiory results is unknown, but a possible explanation could be that different settings of the experimental groups interfered. We examined pregnant women and Dagogo-Jack et al[14] included both women and men in their study, whereas Tuominen et al[21] studied men only. As previously reported,[12-14] there was a positive correlation between leptin levels and BMI. However, we also found a tendency toward correlation between leptin levels and weight gain.

In both diabetic and control subjects, leptin levels were lower postpartum compared with late pregnancy. This agrees with data from recent studies showing an elevation of leptin levels during pregnancy[18-20] followed by a postpartum decrease to concentrations below those measured before pregnancy.[19] Throughout gestation, the fetal-placental unit secretes protein and steroid hormones into the mother's circulation, affecting the functions of the maternal endocrine glands, ie, the production of ovarian steroids is taken over by the placenta after the seventh week of gestation, and the serum concentrations of estrogens and progesterone exhibit a steady increase. Following parturition, steroid levels decline dramatically and estradiol reaches follicular-phase levels, ie, the nadir of the menstrual cycle.[22] Similarly, in obesity, there is an increase in the circulating levels of estrogens due to an enhanced conversion of androstenedione to estrone by the fat tissue.[23] It is possible that high estrogen levels could be responsible for the increase in plasma leptin levels. In support of this, it is known that the leptin-deficient *Lep/Lep* female mouse has decreased levels of reproductive hormones.[24]

Frequent measurements before, during, and after ingestion of a standardized lunch showed no variations in leptin levels. This confirms the results from an earlier study in which plasma leptin levels were unchanged 3 hours after food intake.[14] In rats, *Lep* mRNA levels increase in the evening when the animals start to eat and remain elevated during the rest of the night.[2] Whether these findings indicate that variations in the levels of *Lep* mRNA and leptin do not occur simultaneously, or that rodents and humans differ with regard to the activation of leptin, is unclear. No relationship was found between leptin and insulin or glucose, when either basal or postprandial (60-minute) levels were compared. This is in accordance with earlier reports that demonstrate the lack of an acute effect of insulin stimulation on circulating leptin levels.[11,12,14] On the other hand, there was a positive correlation between the insulin dose taken by the diabetic women and their leptin levels, suggesting that insulin might be involved in the long-term regulation of leptin. There are also previous data indicating that long-term stimulation with insulin may increase leptin levels.[12]

During pregnancy, there is an augmented energy expenditure and the maternal metabolism is altered to increase the fat stores. Our observation, like recent findings of elevated leptin levels in pregnant women, suggests an additional role for leptin in the accumulation of body fat.

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