Postnatal changes in concentrations of free and bound leptin

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Abstract

Aim—To evaluate the effect of maternal diabetes on the concentrations of free and bound leptin at birth and during postnatal adaptation.

Methods—Total, bound, and free leptin concentrations and the percentage of free leptin were measured in cord plasma and plasma at 3 days of age of 13 term infants of mothers with gestational diabetes mellitus (GDM) and 13 term infants of healthy mothers. Gestational age was 40.2 (1.4) weeks, and birth weight was 3693 (549) g (means (SD)).

Results—At birth, infants of mothers with GDM had significantly higher concentrations of total, bound, and free leptin and a higher percentage of free leptin (all p < 0.05). In all infants, these concentrations were significantly lower at 3 days of age than at birth (all p < 0.003), and the differences in concentrations of total, bound, and free leptin between the two groups were no longer significant. In infants of mothers with GDM, the percentage of free leptin remained unchanged, and was higher (p<0.05) than in infants of healthy mothers; in the latter group the percentage of free leptin significantly declined (p = 0.02).

Conclusions—GDM appears to influence fetoplacental leptin metabolism. This effect may be mediated through altered maternal glucose metabolism, or insulinaemia, or both.

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The adipocyte derived hormone leptin plays a role in a multiplicity of events in human physiology; it is involved in the regulation of energy balance and food intake, and it may also play a role in fertility and fetal development.[1–7] In human circulation, leptin is found both in a free form and bound to specific binding proteins, one of them being a soluble leptin receptor.[8 9] In vivo insulin increases total leptin concentration,[10 11] which appears to be mediated at the adipocyte level, at least in part, by insulin stimulated glucose uptake and metabolism.[12] However, at present, data on the regulation of free and bound leptin are sparse. In patients with impaired glucose tolerance, a significant correlation exists between free leptin and fasting insulin levels and HbA1c.[13] In healthy subjects and patients with insulin dependent diabetes mellitus (type 1), both maternal free and bound leptin concentrations increase during pregnancy. In addition, diabetic mothers have higher levels of soluble leptin receptor.[14] Taken together, these data suggest that insulin or altered glucose metabolism, or both, may affect concentrations of free and bound leptin.

Infants of diabetic mothers have higher cord blood leptin concentrations than infants of healthy mothers.[15–19] In addition, cord plasma leptin levels are influenced by the infant's birth weight,[20–24] type of fetal growth,[24–26] and gestational age.[26] After birth, serum leptin concentrations decrease: at 6 hours of age no difference has been observed, but by 16 hours, leptin concentrations are significantly lower than at birth and remain so up to the age of 7 days.[27–32]

At present, there are no data on to what extent leptin circulates in free and bound forms in newborn infants. Furthermore, whether maternal gestational diabetes mellitus (GDM) affects these variables remains unknown. During the early postnatal period, total leptin concentrations decrease,[27–32] but whether this decrease reflects a decrease in free or bound leptin concentrations has not been studied. This study was therefore undertaken to examine the cord plasma concentrations of free and bound leptin, and the early postnatal changes in these variables in infants of healthy mothers and those with GDM.

Subjects and methods

We studied 13 infants of normal mothers and 13 of mothers with GDM. Mean (SD) gestational age, corrected by ultrasound examination, was 40.2 (1.4) weeks and birth weight was 3693 (549) g. Relative birth weight (weight standard deviation score) was determined by reference to a Finnish newborn population of 74 766 singeltons born from 1978 to 1982.[33] Weight, length, and head circumference were measured at birth, and weight was determined when the control blood sample was obtained at 3 days of age. Body mass index (BMI; weight (kg)/length (m)^2) was calculated, using birth length for both BMI at birth and BMI at 3 days of age. None of the infants presented with signs of hypoglycaemia or were hypoglycaemic during the study period. As part of the clinical routine, blood glucose levels are monitored for the first 24–48 hours in infants of mothers with GDM. None of these infants were hypoglycaemic as defined by blood glucose below 2.5 mmol/l. All infants were clinically normal and well. There were no differences in clinical variables between these two groups (table 1).

Table 1 Data on infants born to either mothers with gestational diabetes mellitus (GDM) or healthy mothers

	Infants of GDM mothers	Infants of healthy mothers
Male/female	6/7	6/7
Gestational age (weeks)	40.2 (1.4)	40.3 (1.4)
Range	36.9–41.6	37.1–42.4
Birth weight (g)	3757 (614)	3630 (491)
Range	2570–4500	2760–4740
Birth weight (SDS)	0.3 (1.0)	0.0 (0.9)
Range	−2.0 to 1.9	−2.0 to 2.0
Birth length (cm)	50.3 (2.3)	51.0 (2.4)
Range	45.0–53.0	47.0–56.0
BMI at birth	14.7 (1.4)	13.9 (0.7)
Range	11.8–17.1	12.5–15.1
Weight at 3 days of age (g)	3543 (565)★	3415 (506)★
Range	2545–4245	2580–4600
BMI at 3 days of age	13.9 (1.3)★	13.1 (1.0)★
Range	11.7–16.3	11.7–14.7
Weight loss by 3 days of age (g)	214 (94)	215 (83)
Range	25–320	100–360
Weight of the placenta (g)	635 (135)	637 (111)
Range	360–850	390–880

Values are mean (SD) unless otherwise indicated.
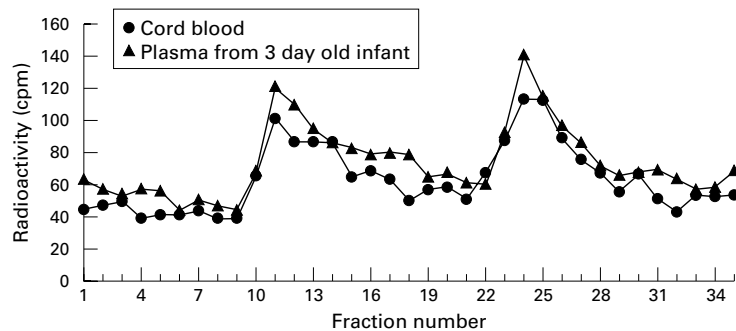★p < 0.001 compared with at birth.
BMI, Body mass index.

Figure 1 An example of the elution profile (high performance liquid chromatography) of the cord blood and plasma from a 3 day old infant of a mother with gestational diabetes mellitus. The first peaks show bound leptin, and the last peaks represent free leptin.

GDM was diagnosed after a 75 g oral glucose tolerance test according to recommendations by the Finnish committee on diagnosis and treatment of GDM.[34 35] None of the mothers had pre-eclampsia. In our patients, GDM was treated with diet only, and none of the mothers received treatment with insulin.

The study was approved by the ethics committee of the Helsinki City Hospitals. Written informed consent of the parents was obtained before participation.

METHODS
Blood samples were drawn at birth from the umbilical vein, and, at the postpartum age of 3 days (mean (SD) 62 (12) h, range 40–87 h), a sample was taken from each infant from a superficial vein, collected into an EDTA tube, and spun for 10 minutes at 2000 *g*. Plasma was stored at −20°C until analysis.

ASSAY OF LEPTIN
Leptin was determined with a radioimmunoassay (Linco Research, St Charles, Missouri, USA).[36] The detection limit of this assay is 0.26 µg/l in our laboratory as determined by calculating 2 SDs (mean of 13 assays) from a zero reference point. The intra-assay and inter-assay coefficients of variation at low concentration (2.8 (0.2) µg/l) are 6.1% and 3.0%, and at medium concentration (19.6 (1.4) µg/l) 6.7% and 2.2% respectively.

ASSAY OF FREE LEPTIN
Free and bound leptin concentrations were determined by high performance liquid chromatography (HPLC). In brief, 150 µl plasma was incubated with 150 µl [^{125}I]leptin (standard amount) at room temperature overnight. Samples were then diluted 1:10 with eluting buffer (0.1 mol/l sodium phosphate, pH 7.2), and filtered through a Millex-HV 0.45 µm filter (Millipore). Each cord plasma sample and three day sample was treated and eluted in parallel during the same day.

HPLC analysis was performed using LKB equipment which included a 2150 HPLC pump, 2152 controller, and 2212 Helirac (LKB, Bromma, Sweden). The column was an Ultropac column (TSK G 3000 SW; 7.5 × 300 mm; LKB) equipped with a sample injector with a 100 µl loop. Elution was performed with degassed 0.1 mol/l sodium phosphate buffer (pH 7.2). The flow rate was constantly 1.0 ml/min, and the fraction volume was 1.0 ml. Elution time was 50 min, and therefore we obtained 50 fractions per sample. Absorbance at 275 nm was monitored with a 2151 variable wavelength monitor (LKB). The radioactivity of the samples was measured with a 1282 Compugamma CS (LKB Wallac, Turku, Finland). On the elution profile, the first peaks shown are bound leptin and the last peak represents free leptin (fig 1). Peak areas were then estimated from the elution profiles.

The reproducibility of HPLC was confirmed using [^{125}I]leptin in 1% bovine serum albumin/phosphate buffer. Leptin was iodinated by the solid lactoperoxidase method.[37] The coefficient of variation of the assay was 12%. Amounts of bound and free leptin were calculated from the total leptin concentration assayed by radioimmunoassay for each sample. The percentage of free leptin was calculated as (free leptin concentration/total leptin concentration) × 100.

STATISTICAL ANALYSIS
Comparisons between groups were made by the Mann-Whitney U test. The Wilcoxon test was used for comparison of paired items. Leptin concentrations were logarithmically transformed when appropriate. Simple and multiple regression analysis were used. p < 0.05 was considered statistically significant. The patient data are given as median, SD, and range, and the results as median and range. All calculations were performed with StatView 4.1 (Abacus Concepts Inc, Berkeley, California, USA).

Results
Infants of mothers with GDM had higher concentrations of total leptin in cord plasma than did infants of healthy mothers (p < 0.05). Likewise, the cord plasma concentrations of free and bound leptin, as well as the percentage of free leptin, were significantly higher in infants of mothers with GDM (all p < 0.05).

A significant decrease from birth to 3 days of age was observed in the concentrations of total, free, and bound leptin in infants of mothers with GDM and infants of healthy mothers (table 2). The percentage of free leptin

Table 2   Plasma concentrations of total, free, and bound leptin, and percentage of free leptin at birth and at 3 days of age, in infants born to either mothers with gestational diabetes mellitus (GDM) or healthy mothers

	Infants of GDM mothers	Infants of healthy mothers
Leptin concentration in cord plasma (μg/l)		
Total	15.0 (3.2–43.8)*	7.2 (2.4–26.8)
Free	8.5 (1.8–27.2)*	3.4 (1.1–13.9)
Bound	5.5 (1.4–16.6)*	3.5 (1.4–12.9)
Percentage of free leptin	58.7 (49.1–70.0)*	52.6 (40.6–57.5)
Leptin concentration at 3 days of age (μg/l)		
Total	1.8 (1.0–3.7)†	1.7 (0.6–2.6)†
Free	1.0 (0.5–1.9)†	0.9 (0.5–1.2)†
Bound	0.8 (0.5–1.8)†	0.8 (0.6–1.4)†
Percentage of free leptin	55.3 (49.6–67.7)*	50.4 (44.5–56.2)††

Values are median (range).
*p < 0.05 compared with infants of healthy mothers.
†p < 0.005 compared with at birth.
††p < 0.05 compared with at birth.

remained stable from birth to 3 days of age in infants of mothers with GDM. In infants of healthy mothers, a significant decline from birth to 3 days of age was observed (p < 0.05).

At 3 days of age, no significant difference existed in leptin levels between infants of mothers with GDM and healthy mothers (table 2). Infants of mothers with GDM tended to have somewhat higher concentrations of free leptin, but this difference did not reach statistical significance (p = 0.08). The percentage of free leptin was also higher in infants of mothers with GDM at 3 days of age (p < 0.05; table 2).

In all infants, cord plasma concentrations of total, free, or bound leptin correlated with none of the clinical variables presented in table 1 (data not shown). At 3 days of age, a correlation existed between the percentage of free leptin and BMI (r = 0.487, p = 0.0016). In multiple regression analysis, with the percentage of free leptin at 3 days of age as dependent, and GDM of the mother and BMI at 3 days of age as independent variables, the percentage of free leptin remained significantly dependent on GDM of the mother (partial r = 0.432, p = 0.028), but not on BMI.

Discussion

Although infants of mothers with GDM and those of healthy mothers were similar in terms of patient characteristics, the former had higher concentrations of total, free, and bound cord plasma leptin. These data therefore suggest that the differences in leptin metabolism between infants of mothers with GDM and those of healthy mothers are due to the effect of maternal glucose metabolism. As infants of diabetic mothers have higher insulin concentrations in the cord plasma,[15][17] this, or enhanced glucose metabolism, or both[12–14] may have stimulated leptin secretion in utero to a higher extent. It is also possible that some of the difference in leptin concentration between infants of healthy mothers and those with GDM is due to placental leptin production,[38] because it has been shown that placental leptin protein content is higher in insulin treated diabetic pregnant women than in healthy pregnant women.[15] This finding is in accordance with recent data showing that cord plasma leptin concentrations are elevated to the same extent in infants of mothers with either GDM or type

1 diabetes compared with concentrations in infants of healthy mothers.[17] They also concluded that the difference was explained mostly by enhanced placental leptin production.

In infants of mothers with GDM, we observed an increase in bound leptin levels at birth, suggesting that maternal GDM increases the concentrations of leptin binding proteins in the fetoplacental circulation. The situation appears to be analogous to that in pregnant women with type 1 diabetes, who have higher levels of soluble leptin receptor.[11] Taken together, these data show that insulin or maternal hyperglycaemia, or both, may participate in the regulation of levels of free leptin and leptin binding protein.

In the infants in our study, the concentration of total leptin decreased significantly from birth to 3 days of age, confirming previous observations.[27–32] Similarly, the concentrations of bound and free leptin decreased. Interestingly, in our infants, the decrease in free leptin concentration from birth to 3 days was similar to that in lean adults as a result of 24 hours of fasting,[8] suggesting that, during the early postnatal period when the infant adapts to extrauterine life, the decrease in total and free leptin may be a physiological adaptation to reduce the inhibitory actions of leptin on food intake.

By 3 days of age, the differences in concentrations of total and bound leptin between infants of mothers with GDM and healthy mothers were no longer significant. However, at 3 days of age, the concentration of free leptin tended to be higher in infants of mothers with GDM. This may reflect the significant decline observed in the percentage of free leptin in infants of healthy mothers, whereas in infants of mothers with GDM, the percentage of free leptin at 3 days of age did not differ from that at birth. In infants of mothers with GDM, the percentage of free leptin was significantly higher at both birth and 3 days of age. Although the number of infants studied is comparatively small, these results suggest an effect of maternal glucose metabolism on fetal leptin metabolism. Whether they merely reflect a short term metabolic difference between these infants resulting from abnormal glucose homoeostasis in utero or predict long term metabolic effects in infants of mothers with GDM warrants further study. It has been reported that the offspring of mothers with either GDM or type 1 diabetes are at increased risk of childhood obesity and later development of disorders of glucose metabolism.[39–41] A recent result showed that, at 1 day of age, the differences in leptin concentrations between infants of healthy mothers and those with either GDM or type 1 diabetes were no longer significant.[42] Therefore this result favours the idea that the differences in leptin concentration observed at birth are a transient phenomenon which normalises as fetal adaptation proceeds.

The leptin binding activity that is compatible with the affinity of the soluble leptin receptor is higher in prepubertal children than in infants and adults, but appears to be similar in infants and adults.[43] In all the infants in our study, the percentage of free leptin was about 55%, which

is comparable to that in lean adults.[8] Free and bound leptin appear to behave as different compartments, and physiological alterations such as fasting affect the percentage of free leptin.[8] In our study, it is of interest to note that, during the first few postnatal days, the infants of mothers with GDM had a higher, relatively constant, percentage of free leptin, whereas in infants of healthy mothers the percentage of free leptin decreased. Whether this indicates leptin resistance in infants of mothers with GDM, as in obese adults,[8] remains to be established. In adults, the percentage of free leptin is related to the degree of obesity and BMI[8–10]; accordingly we observed a correlation between the percentage of free leptin and BMI at 3 days of age. However, as indicated by multiple regression analysis, this correlation appeared to be a function of maternal GDM, and was not explained by BMI per se.

In conclusion, infants of mothers with GDM have higher concentrations of total, free, and bound leptin in cord plasma than do offspring of healthy mothers, suggesting that enhanced glucose metabolism or insulinaemia, or both, regulate fetoplacental leptin secretion. By 3 days of age, plasma concentrations of total, free, and bound leptin decrease in all infants.

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