

CLINICAL STUDY

Fetal leptin and insulin levels only correlate in large-for-gestational age infants

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Abstract

Objective: To determine whether fetal leptin levels correlate with fetal weight and whether such correlation is direct or indirect via insulin or human placental lactogen (hPL), respectively.

Design: Cross-sectional study of offspring at term ($n=175$) with over-representation of large-for-gestational age (LGA; $n=70$) and small-for-gestational age (SGA; $n=23$) cases in a population of Caucasian women with no pregnancy pathology.

Methods: Fetal cord blood was collected after delivery. In several cases ($n=62$) paired mother–fetus blood samples were obtained. Leptin, insulin and hPL levels were measured by RIA. Anthropometric data (birth weight, body mass index, placental weight) were recorded.

Results and Conclusions: Maternal insulin, hPL and leptin levels were higher than fetal concentrations. Cord blood leptin levels positively correlated with the anthropometric data with stronger correlations in female ($0.54 < r < 0.66$) than in male ($0.32 < r < 0.39$) neonates. Cord blood leptin levels did not differ between appropriate-for-gestational age (AGA; $n=82$) and SGA ($n=23$) neonates, but were higher ($P < 0.001$) by 83% in LGA ($n=70$) than in AGA neonates. Among the different weight classes the correlations between fetal leptin and anthropometric data were only observed in LGAs, but not in AGAs or SGAs. Fetal, but not maternal, leptin levels strongly correlated with fetal insulin ($r=0.56$; $P < 0.001$). After accounting for this close relationship insulin could no longer be used to predict birth weight ($r=0.15$, $P=0.051$).

We suggest that the correlation of cord blood insulin with neonatal weight in LGAs is, in addition to insulin's direct anabolic action, indirectly mediated via leptin. It is hypothesized that fetal insulin stimulates fetal adipocyte leptin production.

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Introduction

Normal intrauterine growth and development is the ultimate goal of reproduction. Any disturbance resulting either in large- or small-for-gestational age infants (LGA and SGA, respectively) may lead to elevated perinatal morbidity and mortality with the potential of long-term consequences for the fetus later in life.

The mechanisms regulating fetal growth are poorly understood. Chromosomal aberrations (1), nutritional and environmental factors or toxic exposition during pregnancy (2), as well as hormonal factors such as insulin (2) and insulin-like growth factors (3) have all been implicated in addition to genetic predisposition, the primary determinant of fetal weight.

In 1994 leptin, a 16 kDa hormone, was identified as the product of the obesity (*ob*) gene (4). Subsequent work

clearly demonstrated the role of leptin in the regulation of body weight and has led to hypotheses explaining the mechanism(s) of how leptin may affect body weight. Mouse leptin is secreted into the circulation by large adipocytes (5), crosses the blood–brain barrier (6) and binds to its hypothalamic receptor (7), where the expression of neuropeptide Y (NPY) is downregulated (7). Consecutively, a loss of food intake and increase in energy expenditure leads to a reduction of body fat and body weight (8, 9). Human obesity in children and adults is associated with elevated serum leptin levels (10, 11). A genetic defect in the *ob* gene leads to extreme adiposity (12). These data strongly indicate an important role for leptin in regulation of body weight in humans also.

The influence of this hormone on the developing fetus is still unknown. Leptin is regularly present in fetal serum at term (13–15). Its cord serum levels positively correlate with fetal birth weight, Kaup index and body

weight/body height ratio (15), whereas no such correlation has been found with maternal serum leptin. This suggests an independent production of leptin by the feto-placental unit, a notion recently supported by experimental evidence identifying trophoblasts and amnion cells as non-adipose tissue source (16). Therefore, available evidence indicates leptin's importance for intrauterine growth and development.

Maternal human placental lactogen (hPL) is an important hormone contributing to fetal growth by stimulating a catabolic state in the mother in the second half of gestation. Thus, circulating maternal nutrient levels increase resulting in an increased supply to the fetus (17). We hypothesized that maternal hPL may correlate negatively with fetal leptin levels, because maternal leptin levels are highest in the second trimester and drop later in gestation when hPL levels rise sharply (18).

Insulin has been shown to induce expression of the *ob* gene in rats and humans (19), although in the latter this effect appears to be indirect rather than direct (10, 20–22). Because insulin is also an important growth factor for the fetus the present study investigated whether cord blood insulin and leptin levels correlate. Particular focus was put on potential correlations between high birth weight-associated hyperinsulinemia and leptin levels.

Serum leptin levels were measured in the umbilical blood of 175 term infants in a cross-sectional manner. Additionally, leptin, insulin and hPL levels were measured in the sera of 62 mothers at term. The correlations between leptin, insulin and birth weight, body mass index (BMI) and placental weight, were determined over the full range of birth weights and in different gender and weight classes.

Subjects and methods

Subjects

The study population comprised 175 newborns (95 male, 80 female) born from uncomplicated, singleton pregnancies at term, i.e., between weeks 37 and 43 of pregnancy. In 62 cases (27 female neonates, 35 male neonates) paired blood samples were obtained from mother and neonate. The following parameters were recorded: gestational age, birth weight, BMI, placental weight, and insulin and leptin levels in cord blood and maternal venous blood were measured. Neonates were divided into three groups: term-AGA, term-LGA (birth weight > 90th centile) and term-SGA (birthweight < 10th centile) according to the birth weight charts of the Department of Obstetrics and Gynaecology, University of Innsbruck, Austria. Neonates and mothers with a notable disease such as gestational or overt diabetes, pre-eclampsia/hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome or thyroid

diseases were excluded. Nutritional intake of the women was unrestricted and reflected the average Austrian diet. The women did not receive any medication except multivitamins, iron supplementation or Mg^{2+} during pregnancy. The study was approved by the ethical committee of the University of Innsbruck, Austria. Informed consent was obtained from the mothers. The characteristics of the mothers and their newborns are summarized in Table 1.

Blood sampling

Maternal blood was obtained from a peripheral vein before delivery and mixed cord blood was collected within 2 min after delivery. In pilot studies paired venous and arterial cord blood samples ($n = 10$) were collected separately after delivery. No consistent differences were found in leptin and insulin levels in both samples. Sera were obtained by centrifugation at 4 °C and were analyzed immediately or stored frozen at – 80 °C.

Serum assays

Leptin levels were determined by RIA (Linco Research, St Charles, MO, USA) with an antiserum that did not cross-react with human insulin, proinsulin, rat insulin, C-peptide, glucagon, pancreatic propeptide or somatostatin. According to the manufacturer the detection range is 0.5 to 100 ng/ml. The intra- and interassay coefficients of variation determined in our laboratory for the concentration range observed were < 4 and < 6%, respectively.

Insulin levels were determined by RIA (Pharmacia, Uppsala, Sweden). The detection limit of the assay is < 2 μ U/ml, the measuring range 3–240 μ U/ml. The antibody does not cross-react with C-peptide, IGF-1 or IGF-2.

hPL levels were assayed by IRMA (Amersham, Little Chalfont, UK) with a measuring range of 0.06–15 μ g hPL/ml. The intra- and interassay coefficients of variation were < 4.3 and < 5.8%, respectively. hPL levels in the fetal circulation were too low to be reliably determined.

The assays were carried out following the instructions of the manufacturers. All samples were diluted so as to fall within the linear range of the assays.

Statistical analysis

SPSS 6.1.1 software was used for all statistical analyses. Data were analyzed for normal distribution using the Kolmogoroff–Smirnov test. Insulin and leptin values were skewed. After reciprocal (insulin) and log-transformation (leptin), respectively, they were normally distributed as indicated by Lilliefors statistics. Differences between means were tested using Student's two-tailed *t*-test. Multivariate linear regression analysis

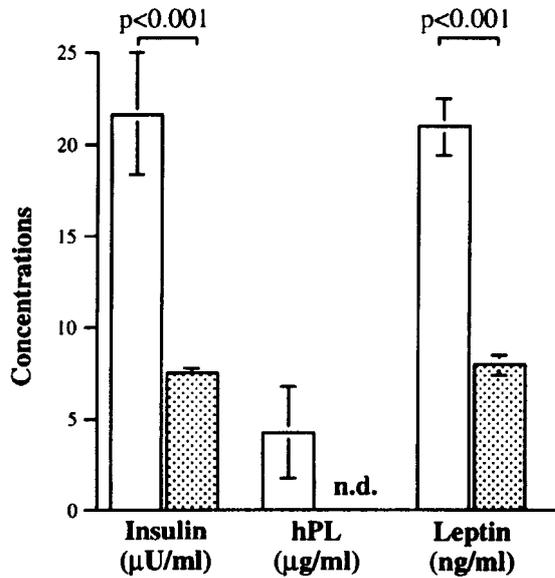


Figure 1 Concentration (mean \pm s.d.) of insulin (μ U/ml), hPL (μ g/ml) and leptin (ng/ml) in 62 mothers at term (open bars) and in their offspring (stippled bars). n.d., not detectable.

was performed using the stepwise method; identical results were obtained by forward and backward stepping. Univariate correlation analysis was used to test for interactions between insulin and leptin as birth weight predictors. $P < 0.05$ was considered significant.

Results

Maternal parameters

In general, maternal insulin, hPL and leptin levels were higher than the cord blood concentrations (Fig. 1). Leptin concentrations (mean \pm s.d.) in the cord blood of 175 neonates (95 male, 80 female) and in 62 maternal venous blood samples were 8.0 ± 7.4 and 20.8 ± 11.8 ng/ml, respectively ($P < 0.0001$). A paired analysis of maternal leptin or insulin levels with the respective cord blood leptin or insulin levels did not reveal a significant correlation between both compartments (data not shown). Maternal hPL was not

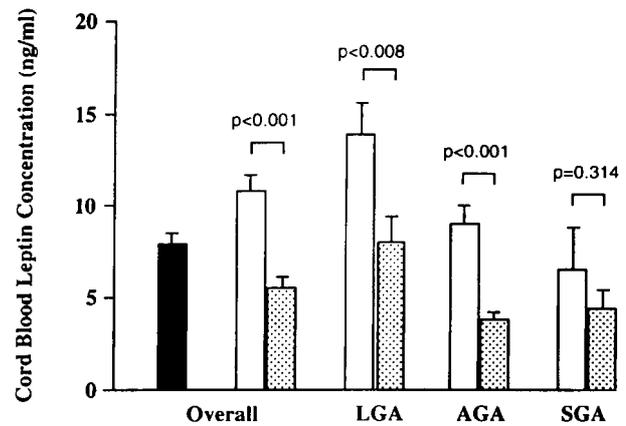


Figure 2 Cord blood leptin levels (mean \pm s.d.) in the total collective (full bar) and in female (open bars) and male (stippled bars) neonates of different weight classes.

linearly associated with fetal leptin or insulin levels, but correlated with the anthropometric data with the exception of the fetal-to-placental weight ratio ($P = 0.40$). The correlations of hPL with birthweight (female: $r = 0.59$; $P = 0.001$; male: $r = 0.32$; $P = 0.057$), BMI (female: $r = 0.66$; $P = 0.0001$; male: $r = 0.35$; $P = 0.036$) and placental weight (female: $r = 0.54$; $P = 0.003$; male: $r = 0.39$; $P = 0.018$) were stronger in female than in male neonates.

Fetal parameters

Cord blood insulin levels were 7.4 ± 4.3 μ U/ml. Cord blood leptin concentrations were lower by 51% ($P < 0.0001$) in male than in female neonates (5.5 ± 5.8 versus 10.8 ± 8.1 ng/ml) (Fig. 2), although the anthropometric data were similar in both groups (Tables 1 and 2). A similar, though less pronounced, gender-specific difference was observed with insulin. Cord blood insulin levels were lower ($P = 0.018$) in male than in female neonates (6.8 ± 4.0 μ U/ml versus 8.4 ± 4.6 μ U/ml).

Cord blood leptin levels positively correlated with birth weight ($r = 0.39$, $P < 0.0001$), BMI ($r = 0.34$, $P < 0.001$), placental weight ($r = 0.30$, $P < 0.0001$),

Table 1 Characteristics of study subjects.

	All neonates (n = 175)	Female (n = 80)	Male (n = 95)
Gestational age (weeks)	40.2 \pm 1.5	40.4 \pm 1.4	40.1 \pm 1.6
Birth weight (g)	3376 \pm 588	3403 \pm 552	3353 \pm 619
Fetal BMI (kg/m ²)	13.8 \pm 1.6	13.9 \pm 1.5	13.7 \pm 1.6
Placental weight (g)	555 \pm 160	567 \pm 123	545 \pm 185
Birth weight-to-placental weight ratio	6.36 \pm 0.99	6.34 \pm 0.87	6.37 \pm 1.03

Table 2 Characteristics of female and male neonates of different weight classes.

	LGA	AGA	SGA
Number of subjects			
Female	34	35	11
Male	36	47	12
Gestational age (weeks)			
Female	40.3 ± 1.5	40.6 ± 1.3	40.0 ± 0.6
Male	40.3 ± 1.3	40.0 ± 2.0	39.8 ± 1.2
Birth weight (g)			
Female	3781 ± 427 ^{\$\$\$}	3190 ± 294	2453 ± 257 ^{\$\$\$}
Male	3868 ± 302 ^{\$\$\$}	3192 ± 443	2580 ± 447 ^{\$\$\$}
BMI (kg/m ²)			
Female	14.8 ± 1.3 ^{\$\$\$}	13.3 ± 1.2	11.9 ± 0.8 ^{\$\$}
Male	14.8 ± 1.1 ^{\$\$\$}	13.4 ± 1.1	11.8 ± 1.5 ^{\$\$\$}
Placental weight (g)			
Female	624 ± 100 [§]	522 ± 93	376 ± 73 [§]
Male	647 ± 125 [§]	525 ± 199	399 ± 145
Birth weight-to-placental weight ratio			
Female	6.17 ± 0.85	6.54 ± 1.00	6.39 ± 1.04
Male	6.15 ± 0.96	6.66 ± 0.90	6.35 ± 0.89

^{\$\$\$} $P < 0.001$, ^{\$\$} $P < 0.01$, [§] $P < 0.05$ versus AGA.

and insulin levels ($r = 0.56$, $P < 0.0001$) (Fig. 3). These correlations were stronger in female ($0.54 < r < 0.66$) than in male ($0.32 < r < 0.39$) neonates.

When the total collective of neonates was subdivided into groups of different weight classes, cord blood leptin levels did not differ between AGA ($n = 82$) and SGA ($n = 23$) neonates, but were higher ($P < 0.001$) in LGA ($n = 70$) than AGA neonates by 83%. Cord blood insulin levels differed among all weight classes in the sequence SGA < AGA < LGA (Fig. 3). The increased levels in LGAs were observed in both sexes.

In LGAs fetal leptin correlated with birth weight and placental weight, but not with the BMI and the birth weight-to-placental weight ratio. In AGA only a

correlation of fetal leptin with birth weight and in SGAs no correlation was found (Table 3). Of note is the strong correlation of fetal, but not maternal, leptin levels with fetal insulin ($r = 0.56$; $P < 0.001$). As with the anthropometric parameters, the LGA group accounted for most of this effect ($r = 0.66$; $P < 0.0001$) (Fig. 4). Multiple linear regression analysis using birth weight as a dependent and leptin and insulin, respectively, as independent, explanatory variables revealed a highly significant positive correlation between leptin and birth weight ($r = 0.39$, $P < 0.001$). After accounting for this close relationship insulin could no longer be used to predict birth weight ($r = 0.15$, $P = 0.051$).

Interestingly, there was a clear break in the association between leptin and insulin levels. Whereas in SGA and AGA increases in leptin levels were associated with small increments in insulin levels, the slope was much steeper in LGAs.

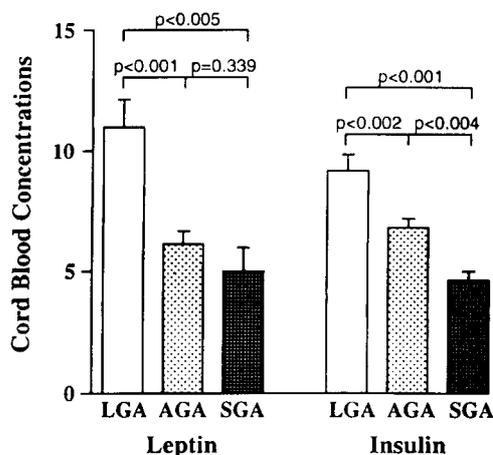


Figure 3 Cord blood leptin and insulin levels (mean ± s.d.) in LGA ($n = 84$; open bars), AGA ($n = 70$; stippled bars) and SGA ($n = 21$; hatched bars) neonates.

Table 3 Pearson's correlation coefficient (r) of cord blood leptin levels with anthropometric parameters and cord blood insulin levels in SGA ($n = 70$), AGA ($n = 82$) and SGA ($n = 23$) fetuses.

	LGA	AGA	SGA
Birth weight	$r = 0.25$ ($P = 0.035$)	$r = 0.26$ ($P = 0.018$)	$r = 0.28$ n.s.
BMI	$r = 0.22$ ($P = 0.065$)	$r = 0.13$ n.s.	$r = 0.15$ n.s.
Placental weight	$r = 0.25$ ($P = 0.022$)	$r = 0.01$ n.s.	$r = 0.21$ n.s.
Birth weight-to-placental weight ratio	$r = -0.22$ ($P = 0.065$)	$r = -0.06$ n.s.	$r = -0.01$ n.s.
Insulin	$r = 0.66$ ($P < 0.0001$)	$r = 0.18$ n.s.	$r = 0.10$ n.s.

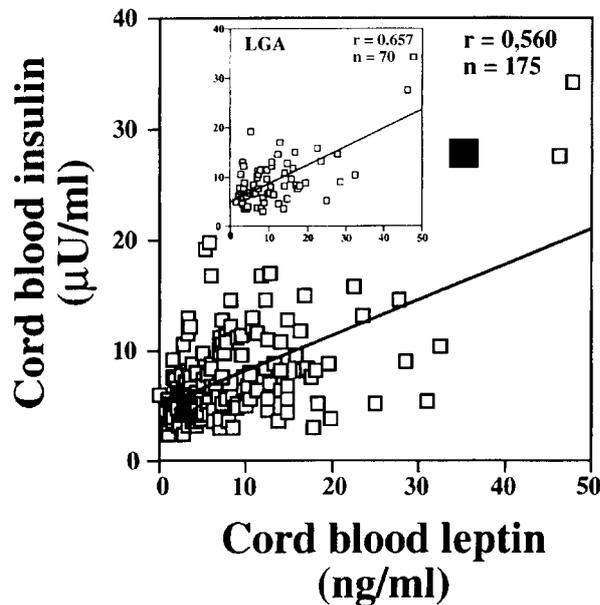


Figure 4 Correlation of leptin (ng/ml) with insulin (μ U/ml) levels in the cord blood of all fetuses and in LGAs (insert).

Discussion

The present study demonstrated considerable leptin levels in the cord blood of all 175 neonates, but maternal levels were about 2.5-fold higher than fetal leptin levels. In none of the mother–neonate pairs were higher fetal than maternal leptin concentrations found, in contrast to a recent study reporting 13% of newborns to have higher leptin levels than their mothers (15).

Blood samples were taken from non-fasted mothers at all times during the day. This may explain the variation in data, because leptin secretion follows a circadian rhythm (23) and is influenced by the metabolic status of the individuals (24).

The absence of correlation between maternal and fetal leptin levels despite this maternal-to-fetal concentration gradient suggests that maternal leptin is either not transported across the placenta to the fetus in a simple manner, or that the fetoplacental unit is the source of leptin found in the umbilical circulation. The placenta is one site of leptin synthesis within the fetal-placental unit (16, 25, 26) and the trophoblast, the placental epithelium bathing in maternal blood, has been identified as the tissue which produces leptin (16). This location makes a secretion of significant amounts of leptin into the fetal circulation unlikely, a notion, which is also supported by the absence of an arterial–venous concentration difference in the umbilical cord. Thus, the predominant source of the umbilical cord leptin may instead be the fetus proper, but this awaits demonstration in placental perfusion studies.

In contrast to a recent study on 27 mothers and 14 newborns, leptin levels in cord blood at term were

clearly different in neonates of different sexes. Concentrations in female were higher than in male neonates. This result observed here in a Caucasian population is in accordance with those in a Japanese cohort (27). Gender-specific differences with higher leptin concentrations in females than in males have also been recognized in children (11) and adults (10, 23, 28–30). Here the leptin levels seem to reflect the different proportion of body fat between females and males. However, in neonates total body fat is similar in both sexes (31) and, therefore, the gender-specific differences in leptin levels must be of other origin. It is also unlikely that the difference is due to a different reproductive hormonal status, because estradiol and testosterone concentrations are similar in male and female neonates (27). The stronger correlation of circulating fetal leptin with fetal weight in female than in male neonates is not without precedent albeit found in adults (28).

hPL stimulates lipolysis in the mother and, thus, may contribute to fetal growth by enhancing fat accumulation (17). This is supported by the correlation of maternal hPL with birth weight and BMI. The absence of any correlation between maternal hPL with insulin and leptin in the maternal or fetal compartment suggests that hPL is not involved in the regulation of either hormones' levels.

The most striking result of this study was the correlation of leptin concentration with insulin levels and the anthropometric data solely in LGA neonates, but not in lower weight classes. This result could have only been found because of the selective sampling resulting in an overrepresentation of LGA (40%) and SGA (13%) in the study collective. All previous studies either investigated smaller collectives or did not have this broad range of weight distributions with considerable representation of high and low birth weights. This might explain why other studies have missed this clear-cut result.

The correlation between leptin levels and birth weight in LGA but not in AGA or SGA may reflect distinct changes in heavier fetuses affecting regulation of fetal leptin levels. The major difference in fetal body composition between LGA and non-LGA fetuses at birth is the massive increase in fat mass but not in lean body mass in both normal (Dr P Catalano, personal communication) and diabetic (32) mothers. The major site of leptin synthesis is the adipose tissue. The proportion of fat and the size of the adipocytes are the major determinants of leptin production (5, 10, 33). Changes in weight are associated with altered serum leptin levels in adult human and mice, and this works in both directions (34, 35).

Insulin is an important regulator of fetal growth. Prolonged hyperinsulinemia is accompanied by a stimulation of fat deposition in the fetus and, hence, by an increased proportion of adipose tissue resulting in fetal overweight (36). Insulin also stimulates adipocyte leptin production *in vitro*. Hyperinsulinemia *in vivo*,

although not acutely leading to higher leptin levels (21, 37), has a long-term stimulatory effect on leptin production (20). It can be hypothesized that fetal hyperinsulinemia in LGAs is the primary cause of the almost doubling of leptin levels by a combination of two factors, which mutually potentiate each other: (1) increased fat deposition resulting in a greater potential for leptin synthesis, and (2) stimulation of adipocyte *ob* gene transcription by insulin. Future work in animals needs to establish that fetal adipocytes respond to insulin similar to adipocytes in adults, i.e. by enhancing leptin biosynthesis. A critical role for insulin in regulating leptin expression in fetal tissue was reported recently (38).

The consequences of the high fetal leptin levels in LGAs are unclear. In human adults and mice adipocyte-derived leptin acts as a signal to the brain where it affects satiety. It can hardly be imagined that this mechanism is operative in the growing fetus. Here the placenta is the limiting organ for nutrition and a regulation seems to be independent of fetal hunger and satiety. It can be speculated that high fetal leptin levels in LGAs may downregulate transplacental transport of nutrients similar to the small intestine (39) via placental leptin receptors (26). This would confer some protection to the fetus against overnutrition in the wake of hyperinsulinemia, which would resemble the downregulation of the placental GLUT1 system after sustained maternal hyperglycemia (40).

Collectively, the present study provides evidence for a direct association between fetal leptin levels and birth weight in LGAs and AGAs. The correlation of cord blood insulin with birth weight is the result of the correlation between insulin and leptin. The data may suggest that, in addition to the direct anabolic action, part of insulin's effect on birth weight is mediated by leptin. It is speculated that insulin stimulates fetal adipocyte leptin synthesis.

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