Placental Leptin: An Important New Growth Factor in Intrauterine and Neonatal Development?

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ABSTRACT. *Background.* **Leptin, the protein product of the *ob* gene, is produced by the adipocyte and seems to function as a link between adiposity, satiety, and activity. Leptin has also been found to be necessary for pubertal development, conception, and pregnancy in mice, and is increased in prepubertal children, independent of adiposity, suggesting a role in childhood growth and development. This study investigated 100 mother/newborn pairs to determine the role of leptin in neonatal development. Placental tissue was assayed for leptin mRNA to evaluate it as a source of leptin production in utero.**

Methods. **One hundred mother/newborn pairs were enrolled in this study. Radioimmunoassay was performed for leptin on maternal venous and newborn cord blood. Leptin concentrations were measured in 43 children in Tanner stages 1 and 2 as a control group. Placental tissue was obtained from five mothers and assayed for leptin mRNA by reverse transcription/polymerase chain reaction (RT/PCR). Human placental cell lines JAR and JEG-3 were also assayed for leptin mRNA expression.**

Results. **Leptin was present in all newborns studied at a mean concentration of 8.8 ng/mL (±9.6 standard deviations). Leptin concentrations in cord blood correlated with newborn weight (r = .51), body mass index (BMI) (r = .48), and arm fat (r = .42). There was no correlation between leptin and insulin. When statistically covarying for adiposity for newborns and Tanner stages 1 and 2 children, newborns had greater concentrations of leptin (mean, 10.57 ng/mL) than children (mean, 3.04 ng/mL). Leptin was present in all mothers at a mean value of 28.8 ng/mL (±22.2 standard deviations). Leptin concentration correlated with prepregnancy BMI (r = .56), BMI at time of delivery (r = .74), and arm fat (r = .73). Maternal leptin correlated with serum insulin (r = .49). There was no correlation between maternal and newborn leptin concentrations. Thirteen percent of newborns had higher leptin concentrations than their mothers. Placental tissue from five separate placentas expressed leptin mRNA at comparable or greater levels than adipose tissue. Two human trophoblastic placental cell lines, JAR and JEG-3, also expressed leptin mRNA.**

Conclusions. **The correlation between leptin and adiposity found in children and adults was also found in newborns. Serum leptin concentrations in newborns were increased more than three-fold compared with children in Tanner stages 1 and 2 when controlling for adiposity, suggesting that leptin concentrations in the newborn are not explained by adiposity alone. Maternal leptin concentrations correlated with measures of adiposity at delivery but did not correlate with newborn adiposity or leptin. Leptin mRNA was expressed both in placental tissue and in two human placental cell lines. These data suggest that leptin has a role in intrauterine and neonatal development and that the placenta provides a source of leptin for the growing fetus.** ***Pediatrics*** **1997; 100(1). URL: http://www.pediatrics.org/cgi/content/full/100/1/e1; *leptin, newborn, fetus, placenta.***

ABBREVIATIONS. RT/PCR, reverse transcription/polymerase chain reaction; BMI, body mass index; SD, standard deviation; PCR, polymerase chain reaction; bp, base pairs.

Leptin, the adipocyte-specific protein product of the *ob* gene,[1] has been linked in mice to the body's system of energy regulation. It has been hypothesized that leptin is integral in the feedback loop from adipose tissue stores to satiety centers in the hypothalamus, resulting in a decrease in appetite and an increase in energy expenditure.[2] *Ob/ob* mice, which lack leptin, are obese and lose weight with exogenous leptin administration.[3–5] In humans, serum leptin concentrations have been found to correlate directly with measures of adipose tissue stores.[6] Increased serum leptin concentrations in obese adults and children[6,7] suggest that obesity may be associated with an alteration in the feedback loop between central appetite and satiety centers.

Hassink et al[7] hypothesized that leptin serves an additional role in the child's dynamic energy needs necessary for growth and development. In a previous study,[7] leptin concentrations were found to vary with Tanner stage in children independent of adiposity. Higher leptin levels were found at Tanner stages 1 and 2 as compared with Tanner stage 5. The suggestion that leptin is central to development in animals was provided by Barash et al,[8] who treated infertile *ob/ob* mice with leptin and found that leptin-treated females had significantly elevated serum levels of luteinizing hormone and increased ovarian and uterine weights. Chehab et al[9] corrected the sterility

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defect in female *ob/ob* mice using human recombinant leptin administration.

The role of leptin in the neonatal period, a time of rapid growth and development,[10] is poorly understood. Frazer-Llado et al[11] reported low serum leptin concentrations in critically ill infants during their stay in the intensive care unit. Leptin concentrations in these infants did not correlate with weight. In contrast, Schubring et al[12] using a small sample of full term infants (n = 15), reported moderate significant correlations between leptin concentrations in cord blood and newborn weight. There were no correlations between maternal leptin concentrations and birth weight.

In the present study, a representative sample of 100 healthy newborns was studied to determine the association between serum leptin concentrations and adiposity. Serum leptin concentrations were then compared between newborns and children at Tanner stages 1 and 2 by controlling for levels of adiposity. Mother and newborn serum leptin concentrations were also studied. Subsequently, placental tissue was assayed for leptin mRNA to evaluate possible sources of leptin production because of the lack of association between mother and newborn leptin levels.

METHODS

Study Subjects

One hundred consecutive pregnant women and their newborns were enrolled in this study. Anthropometric measurements and blood sampling of mother and newborn were performed at the time of delivery. Table 1 presents demographic and anthropometric data for mothers and newborns. This was the first pregnancy for 31 of the mothers, the second for 28, the third for 19, and the fourth or greater pregnancy for 20 mothers with information on two pregnancies not available. Mean maternal BMI (body mass index) before pregnancy was 26.17 [±7.55 (SD)]. Three percent of the newborns had gestational ages of 32 weeks or less. Twenty-seven deliveries were by caesarean section. Informed consent was obtained from the mothers for themselves and their newborns. This study was approved by the Institutional Review Boards of the Alfred I. duPont Institute and the two birthplace hospitals, Thomas Jefferson University Hospital and the Medical Center of Delaware.

Forty-three children in Tanner stages 1 and 2 were used as a comparison to the newborns. This control group included 25 males and 18 females (n = 43), 35 white, 7 black, 1 other, with an overall mean age (10.25 yr, ±1.67 SD), and mean BMI (17.67 ± 3.46 SD).

Procedures

Maternal height was measured to the nearest cm by stadiometer; weight was measured to the nearest .1 kg on a balance beam scale, and BMI was calculated by dividing the weight in kilograms by the height in meters squared. Triceps skinfold was measured at the mid-arm distance to the nearest millimeter using Lange calipers. Arm fat was calculated from the measured mid-arm circumference and triceps skinfold according to the formulas provided by Must and colleagues.[13] Prepregnancy weight was obtained by history.

Newborn lengths were obtained to the nearest mm and weights to the nearest .1 kg. Triceps skinfold measurements and mid-arm circumference were measured as described above. Arm fat was calculated as for mothers, because there is not yet an accepted standard for determining adiposity in newborns.

A venous blood sample was collected from the mother and a cord blood sample from the newborn at the time of delivery. Serum was frozen at −70°C until analysis. Radioimmunoassay (Linco Research, St. Charles, MO) for serum leptin was performed as previously described by Considine et al.[6] Serum insulin was measured by radioimmunoassay (Linco Research, St. Charles, MO), and serum glucose was measured by the glucose oxidase method with a glucose analyzer 29 (Beckman, Brea, CA).

TABLE 1. Means and Standard Deviations for Demographic and Anthropometric Variables in Mothers and Newborns

Variable Units	Mothers (n = 100) × (SD)	Newborns (n = 100) × (SD)
Age	28 y, 9 mo (6 y, 7 mo)	39 wk, 1 d (2 wk, 1 d)
Gender		
Male	—	51
Females	100	49
Race		
White	63	63
Nonwhite	37	37
BMI wt/ht^2	31.5 (7.3)	12.9 (1.5)
Arm fat mm^2	3556.5 (2211.9)	237.0 (77.4)

Cell Lines and Tissues

Biopsies of both subcutaneous and abdominal fat were obtained from children undergoing orthopedic surgery. A biopsy of abdominal fat was obtained from an obese pregnant woman. Human placental cell lines, JAR[14] and JEG-3,[15] were obtained from the American Type Culture Collection (Rockville, MD). JAR cells were grown in RPMI medium 1640 with 10% (vol/vol) fetal calf serum and penicillin-streptomycin, and JEG-3 cells were cultured in α-MEM medium with 15% (vol/vol) fetal calf serum and penicillin-streptomycin.

RNA Isolation and RT/PCR Analysis

After cells reached confluence, total RNA was extracted using the RNeasy kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Placental tissues were frozen in liquid nitrogen, pulverized with a mortar and pestle, and total RNA was extracted as described above. Total RNA from two additional human placentas was obtained from Clontech (Palo Alto, CA). RNA concentration and purity were determined by absorbency at 260 and 280 nm. One microgram of total RNA was reverse-transcribed using avian myeloblastosis virus and oligo (dT) primer (Promega, Madison, WI), according to the manufacturer's instructions. The reverse transcription reaction was performed at 42°C for 15 min. Subsequent amplification of the cDNA sequence was performed with 10 μL of the reverse transcription reaction in 1X *Taq* buffer, 5% dimethylsulfoxide, 25 pmol each of leptin exon 2 forward (5′CATTGGGGAACCCTGTGCGGATTC3′) and exon 3 reverse (5′TGGCAGCTCTTAGAGAAGGCCAGC3′) PCR primers, and 1.25 U *Taq* polymerase in a 50-μL reaction volume. For assessment of the relative levels of leptin to β-actin, a multiplex RT/PCR approach was used as described by Dukas et al.[16] The PCR reaction was done as described above except that the β-actin primers (forward: 5′TGTATGCCTCTGGTCGTACCAC3′; reverse: 5′ACAGAGTACTTGCGCTCAGGAG3′) were added at 6.25 pmol/μL. The temperature profile for the PCR reactions consisted of a 2-min melting step at 95°C, then 30 cycles of 30 s at 94°C, 30 s at 55°C, and 90 sec at 65°C, followed by a final extension step of 6 min at 65°C. Independent experiments established that 30 cycles were within the linear range of the multiplex PCR assay. RT/PCR products were separated by size on a 4% agarose gel and stained with ethidium bromide. Images were transferred to an Apple MacIntosh Quadra 800 via an Eagle Eye still video imaging system, and the relative band intensities analyzed with National Center for Supercomputing Applications Gelreader Version 2.07 software.

Statistical Analysis

Because of variation in the distribution of serum leptin concentrations and estimates of percentage body fat, the relations between continuous variables were evaluated by Spearman rank correlations. A log (Ln) transformation of serum leptin levels was performed to normalize the distribution of values to meet the assumptions for subsequent analyses. All analyses were performed using the general factorial analyses of covariance model, controlling for the effects of both BMI and upper arm fat. Arm fat

and BMI were selected as covariates because of their high correlations with serum leptin concentrations.[6,7] Post hoc (Tukey) analyses were performed to determine differences between males and females, and between newborns and Tanner stage 1 and 2 children. Post hoc power contrast yielded power estimates ≥.80 for all comparisons. Data are presented as the mean and (±) standard deviation, and a $P < .05$ was used as a determinant of statistical significance. All analyses were two tailed and conducted with the SPSS software (version 6.1.3 for Windows, SPSS Inc, Chicago, IL).

RESULTS

Newborn Data

Table 1 presents newborn demographic and anthropometric data. Leptin was present in all newborns studied. Leptin concentrations in cord blood correlated with newborn weight ($r = .51$; $P < .001$), BMI ($r = .48$; $P < .001$), and arm fat ($r = .42$; $P < .001$). Correlation between newborn leptin, insulin, or glucose levels failed to reach significance (Table 2).

Newborn Childhood Comparisons

A 2 (group) × 2 (gender) general factorial analyses of covariance model was performed between newborns and children at Tanner stages 1 and 2 with BMI and arm fat as covariates. Newborns had greater concentrations of leptin (mean, 10.57 ng/mL) than Tanner stage 1 and 2 children (mean, 3.04 ng/mL; $P < .001$). A significant main effect was found for gender, with females (mean, 7.11 ng/mL) demonstrating higher leptin concentrations than males (mean, 4.52 ng/mL; $P < .001$).

Maternal Data

Table 1 presents demographic and anthropometric data for mothers. Leptin was present in all mothers studied. Leptin concentrations correlated with prepregnancy BMI ($r = .56$; $P < .001$), BMI at time of delivery ($r = .74$; $P < .001$), and arm fat ($r = .73$; $P < .001$). Maternal serum leptin concentrations correlated with serum insulin levels ($r = .49$; $P < .001$) but had no relationship to serum glucose (Table 2).

Mother/Newborn Dyad

Correlations between maternal and newborn serum leptin concentrations, insulin, and glucose failed to reach significance. Figure 1 and Figure 2 depict the relationship between arm fat, BMI, and log leptin concentrations in newborns and mothers. Thirteen percent of newborns had higher leptin concentrations than did their mothers.

Placental Tissue

Placental tissue from five women was examined for expression of leptin mRNA by RT/PCR analysis. Placental tissue showed high expression of leptin mRNA (Fig 3, lane 1). PCR primers that flanked the entire leptin coding sequence were also used to amplify leptin mRNA from placenta and abdominal fat. A PCR product of the appropriate size [~600 base pairs (bp)] was obtained (data not shown). Sequence analysis proved that the RT/PCR product amplified from placenta was identical with the reported human leptin cDNA sequence (GenBank accession no. D49487).

Comparison of the relative levels of leptin to β-actin mRNA by multiplex RT/PCR revealed that placenta contained from 1 to 2.5 times the amount of leptin mRNA as abdominal fat tissue (Fig 3, lanes 5 to 7). Similar results were obtained when comparing leptin to glyceraldehyde-3-phosphate dehydrogenase mRNA levels. Independent experiments established that the multiplex RT/PCR assay was within the linear range.

Leptin mRNA was expressed in the human choriocarcinoma cell lines, JAR (not shown) and JEG-3 (Fig 3, lane 4). To establish that leptin expression is not a characteristic of transformed cell lines, CACO2 cells (American Type Culture Collection) were used as a negative control. No leptin mRNA was detected in these cells.

TABLE 2. Means and Standard Deviations for Laboratory Variables in Mothers and Newborns

Variable Units	Mothers (n = 100) × (SD)	Newborns (n = 100) × (SD)
Leptin ng/mL	28.8 (22.2)	8.8 (9.6)
Insulin uU/mL	24.2 (18.6)	10.2 (5.0)

DISCUSSION

The significant correlation between leptin and adiposity previously found in children[7] and adults[6] was also found in this study of newborns but at a more modest level of association. The moderate correlations between newborn leptin, arm fat ($r = .42$), and BMI ($r = .48$) in this study are consistent with the correlation found between leptin concentrations and birth weight ($r = .57$) in a sample of 15 newborns studied by Schubring et al.[12] When statistically correcting for adiposity, serum leptin concentrations in the newborns were increased more than three-fold, compared with children in Tanner stages 1 and 2. Thus, leptin concentrations in the newborn cannot be explained on the basis of weight or adiposity alone.

Several lines of research have suggested that leptin may play an important role in growth and development. Barash et al[8] reported an increase in ovarian and uterine weight in female *ob/ob* mice and testicular weight in male *ob/ob* mice independent of nutritional status. Chehab et al[9] corrected the sterility defect in female *ob/ob* mice by using human recombinant leptin administration. Treatment resulted in ovulation, pregnancy, and parturition in the treated female mice. Consistent with findings of infants having increased leptin concentrations compared with prepubescent children, Hassink et al[7] reported that children at earlier stages of development have higher leptin concentrations than do children at more advanced Tanner stages, when statistically correcting for levels of adiposity. The concept of leptin resistance[6,17] was extended to account for these increased leptin concentrations in a growing child.[7] In a further illustration of the importance of leptin in growth, poorly growing, critically ill infants were reported to have absent to low leptin concentrations (mean, 2.3 ng/mL).[11] In contrast, healthy newborns in this study had mean leptin concentrations of 8.8 ng/mL.

Lastly, modeling of human leptin[18] reveals that the

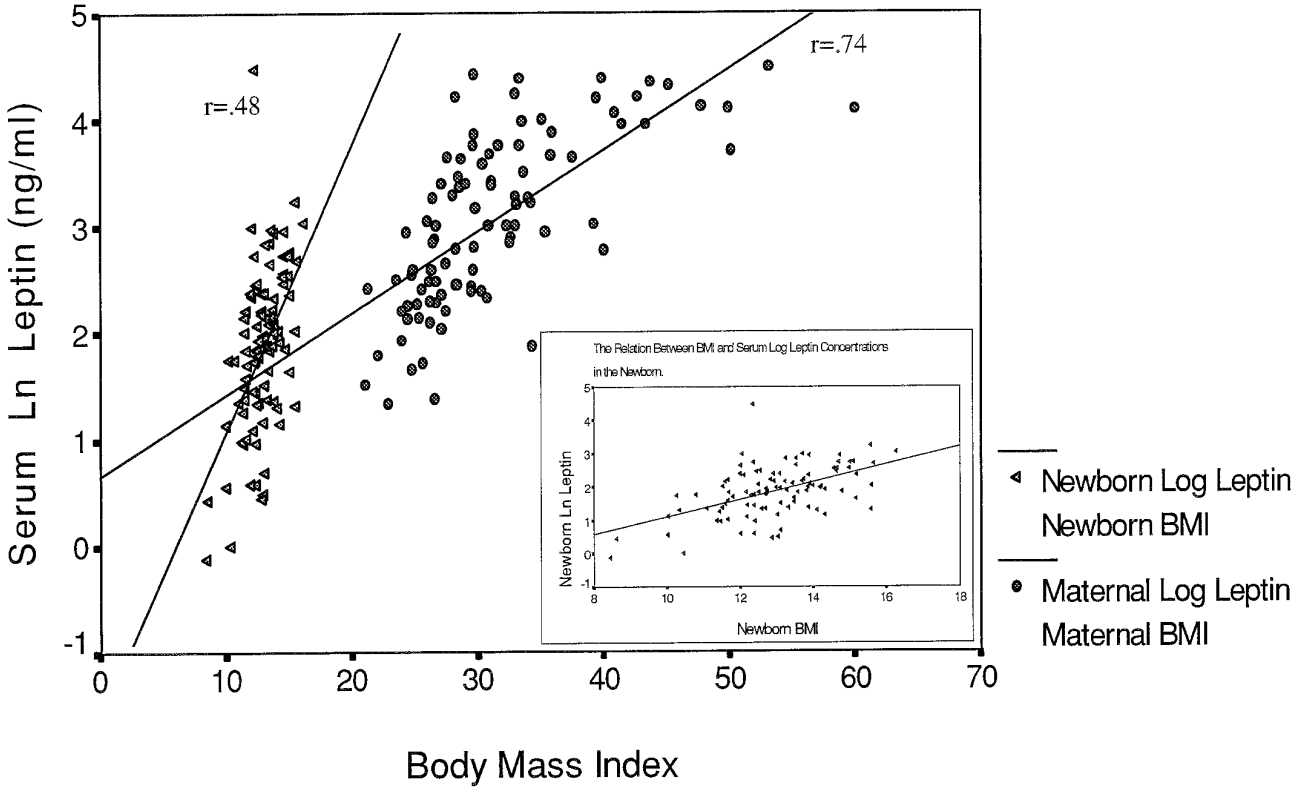

Fig 1. The relation between BMI and serum log leptin concentrations in newborns and mothers.

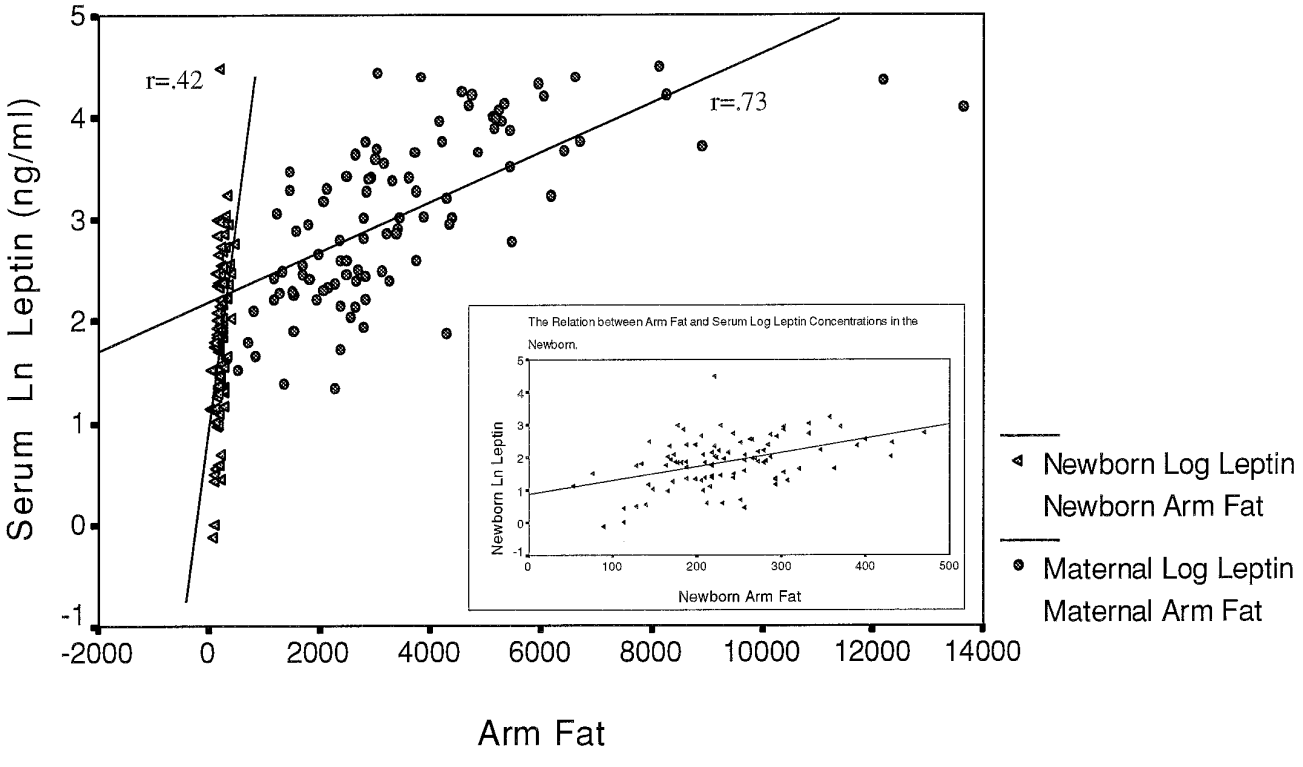

Fig 2. The relation between arm fat and serum log leptin concentrations in newborns and mothers.

molecule is a globular protein with a tertiary structure similar to that of helical cytokines, which include IL-2 and growth hormone. The long form of the leptin receptor functions similarly to cytokine receptors and has been detected in human lung, kidney, liver and skeletal muscle,[19] as well as heart, placenta, spleen, thymus, prostrate, testis, ovary, small intestine, and colon.[20] The finding of leptin receptors in numerous tissues supports the hypothesis that leptin is important for growth and development.

Although maternal leptin concentrations corre-

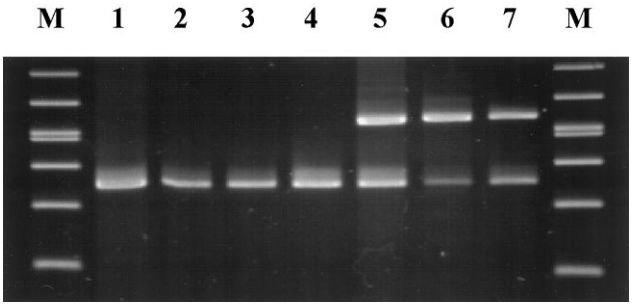

Fig 3. RT/PCR analysis of leptin mRNA in placenta and fat tissue. Total RNA was reverse-transcribed and amplified with either leptin PCR primers (lanes 1 to 4) or both leptin and β-actin PCR primers (lanes 5 to 7) as described in Methods. Lanes 1, 5: placenta; lanes 2, 6: child abdominal fat; lanes 3, 7: obese pregnant adult abdominal fat; lane 4: JEG-3 placental cells. Markers (M) are 1000, 700, 525, 500, 400, 300, and 200 bp. The size of the leptin RT/PCR product is 348 bp; the size of the β-actin RT/PCR product is 592 bp.

lated with measures of adiposity both at prepregnancy and at delivery, there was no association between leptin concentrations in mother/newborn dyads. Interestingly, 13% of newborns had higher serum leptin concentrations than their mothers. These findings make it difficult to see leptin exclusively in light of its putative role in satiety and activity management. The independence of maternal and infant leptin concentrations and the high infant leptin concentrations suggest: 1) that the fetus produces its own leptin with associated leptin resistance,[7] and/or 2) that the placenta is the major source of leptin production for the fetus.

Placental tissue was assayed for leptin mRNA to evaluate possible sources of leptin production. The placenta was found to express leptin mRNA at comparable or greater levels than adipose tissue. Green et al[21] using Northern blot analysis, also found leptin mRNA in polyA+ RNA from placental tissue but at a lower level than in total RNA from adipose tissue. It is possible that a comparison of leptin expression from total RNA of fat tissue to that of polyA+ RNA from placenta underestimated placental tissue as a major source of leptin production. It should be noted that adipocytes have not been found in placental tissue,[22] eliminating the adipocyte as a source of placental leptin. Trophoblastic cells, which are the predominant cell type in the placenta, are the most likely source of placental leptin. In support of this hypothesis, human trophoblastic cell lines, JEG-3 and JAR, were found to express leptin. These cell lines have also been shown to synthesize and secrete human chorionic gonadotrophin, human somatomammotrophin, and progesterone, all hormones produced by the human placenta.[14,15] Our results indicate that leptin is another placental hormone.

As this is the first study to report on the role of the placenta as a source of leptin production in a representative sample of newborns, findings need to be treated cautiously. It is not possible to report on leptin concentrations at different gestational ages because of the small number of preterm babies studied. Although it is tempting to speculate that leptin differentially affects growth and development in females based on the finding of higher leptin concentrations in female as opposed to male newborns, leptin gender differences in newborns and children[7,23] remain unexplained. As well, it is unclear if leptin has a central or supporting role in fetal growth or in growth and development, in general. Whether there is differential expression of leptin on the maternal or fetal side of the placenta also remains to be established.

Previous studies in animals and humans have stressed the role of leptin in satiety and activity regulation. Several animal studies[8,9,24] have also indicated an important role for leptin in development and reproductive function. This study and previous work involving infants and children[7,11,12] have suggested a role for leptin in growth and development. A unifying hypothesis integrating leptin's roles in satiety, activity, growth, and development may center around leptin's role in energy expenditure and conservation.

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