

Leptin in Maternal Serum and Breast Milk: Association With Infants' Body Weight Gain in a Longitudinal Study Over 6 Months of Lactation

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ABSTRACT: The adipokine leptin has been detected in human breast milk, but its effect on postnatal growth and development remains largely unclear. We hypothesized that leptin could affect infant's body weight gain during early lactation in the first 6 mo of life. Therefore, we evaluated leptin levels in maternal serum and breast milk of 23 healthy, lactating mothers and their neonates in a prospective, longitudinal study. Leptin concentration was quantified by a commercially available human leptin RIA. Our results showed that leptin levels in breast milk were 22-fold lower than in maternal serum, but both parameters were positively correlated to each other ($r = 0.431$, $p = 0.001$) and to maternal BMI (serum: $r = 0.512$, $p < 0.001$; milk: $r = 0.298$, $p < 0.001$) over 6 mo of lactation. A negative association was found between breast milk leptin levels during the first week after delivery and the infant weight gain from the end of the first to the sixth month ($r = -0.681$, $p = 0.007$). This suggests that milk-borne leptin provides a link between maternal body composition and infant growth and development and plays a critical role in regulating appetite and food intake during early infancy. (*Pediatr Res* 70: 633–637, 2011)

Since the discovery of leptin in human breast milk (1,2), the role of leptin in controlling neonatal development has come to the fore of pediatric research. Originally, leptin was identified as the product of the *obese* gene (*ob*). It is synthesized in proportion to adipose tissue mass and involved in regulating energy homeostasis, appetite, and food intake (3–5). Leptin is also produced by the placenta, the stomach, and the mammary epithelium (6) and plays a role in processes such as blood pressure regulation and angiogenesis as well as in the hematopoietic system (7). At the level of the CNS, leptin exhibits anorexigenic effects by signaling satiety and decreasing appetite (8). Recent findings have shown that during brain development, leptin regulates the activity of neurons in the arcuate nucleus of the hypothalamus and represents a powerful neurotrophic agent (5). Over the past years, research groups are engaged in supporting the fact that

breastfeeding has beneficial effects later in life. Moreover, prolonged breastfeeding appears to be associated with a lower risk of obesity than formula feeding (5,9–15).

Breast milk contains various hormones such as leptin, ghrelin, and adiponectin as well as growth factors that are absent in milk formulas (15). This makes leptin an interesting candidate to explain anthropometric differences and changes in dietary habits between breastfed and formula-fed infants later in life. Previous studies demonstrated that suckling rats, which received physiological doses of leptin during lactation, were programmed for lower body weight and became more protected against fat accumulation in adult life. In addition, the leptin-mediated short- and long-term regulation of food intake seemed to be more sensitive (4). The role of leptin among the complex network of factors controlling infant development is still incompletely understood. In umbilical cord blood of newborns, leptin levels have been reported to be related to fetal weight and BMI (16). Breast milk leptin and maternal leptin levels appeared to be positively correlated to each other (1,17). However, the levels in breast milk were found to be distinctly lower than in maternal plasma (1,2,17–19) and revealed to be positively associated with maternal BMI (1,17). Furthermore, different studies reported a clear association between breast milk leptin levels and neonatal weight gain (17,20). All these results suggested that the infant body weight may be influenced by milk leptin during the first stages of lactation.

The knowledge that much of this evidence comes from animal studies and the lack of information about breast milk leptin levels during all stages of lactation prompted us to evaluate leptin concentrations in maternal serum and breast milk in relation to different stages of lactation. Therefore, we took samples of colostrum, transitional, and mature milk from nonobese women and aimed to determine whether there is a correlation of leptin levels with anthropometric parameters of mothers and their infants within the first 6 mo of lactation.

METHODS

Subjects. Twenty-three healthy, lactating women at the age of 21 to 42 y (median, 28 y; interquartile range [IR], 25–33 y) with a normal BMI (median, 20.9 kg/m²; IR, 19.3–22.6 kg/m²) and their newborn infants (median, 3530 g; IR, 3060–4020 g) were enrolled in this longitudinal study, expanding over 6 mo. All infants were born appropriate for GA (AGA) (median, GA 40.1 wk;

Received January 4, 2011; accepted June 23, 2011.

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Supported by the Deutsche Forschungsgemeinschaft KFO, the LIFE program (Leipzig Interdisciplinary Research Cluster of Genetic Factors, Clinical Phenotypes and Environment), the "Kompetenznetz Adipositas-Verbund LARGE (TP01)" and the IFB Adiposity Diseases.

The authors report no conflicts of interest.

S.S. and C.H. contributed equally to the work.

Abbreviation: IR, interquartile range

IR, 38.5–41 wk). Infant weight and weight gain were documented at each time point (first, second, third, and fourth week followed by the second, third, fourth, fifth, and sixth month postpartum). To the best of our knowledge, none of the infants suffered from any infections such as gastroenteritis, urinary tract infection, or respiratory airway infection during the study period. Subjects who were under 18 y or had a metabolic disease, such as diabetes, were excluded from this study. Only 15 women completed the measurements over 6 mo. The anthropometric data of mothers and infants are shown in Table 1. The protocol followed in this study was reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Leipzig (No. 188-2005). The written informed consent of all participants was obtained.

Sample collection. Milk samples were obtained from mothers at the end of the first, second, third, and fourth week, followed by the second, third, fourth, fifth, and sixth month postpartum. BMI of the mother and child weight were documented at every time point in this study as mentioned above. Maternal blood samples were obtained after the first week and the third and sixth month, collected in serum tubes, and centrifuged at 1500 g for 10 min at 4°C. Breast milk and serum samples were stored at –20°C until analysis.

Sample preparation. Before analyzing the leptin concentration in maternal breast milk, we performed sample preparation steps. First, 1 mL of breast milk sample was homogenized three times for a 5-s burst with an ultrasound stick

(ultrasonic processor UP100H, 100 W, 30 kHz, 100% amplitude; Hielscher Ultrasonics GmbH, Teltow, Germany). After sonication, the milk samples were centrifuged for 30 min at 14,000 rpm, and the lower phase of skimmed milk was aspirated by a cannula for measurement in the leptin assay. If the sample was still cloudy, the centrifugation step was repeated.

Leptin assay. Leptin concentrations in maternal serum and breast milk samples were determined by a commercially available human leptin RIA (Human-Leptin-RIA-sensitive, Lep-R40; Mediagnost, Reutlingen, Germany). We performed the high-sensitive protocol of the assay, which included two extra overnight incubation steps and additional use of low concentration standards. This increased the sensitivity of the assay to 0.01 ng/mL as stated by the manufacturer. In addition, we performed tests to assess the accuracy (mean recovery for leptin spiking of 1.6, 3.2, and 4.8 ng/mL was 70.2 ± 8.7%, *n* = 10) and linearity (mean recovery for dilution steps 1:2 and 1:4 was 82.2 ± 11.3%, *n* = 11) of the assay after our sample preparation.

Statistical analysis. Data were analyzed using the statistical analysis software package SPSS (SPSS Statistics 17.0, IBM). Descriptive statistics were calculated and reported as median and IR. The Kolmogorov-Smirnov test was used for normality and the Wilcoxon test for values to detect longitudinal patterns. Nonparametric, independent sample values were examined using the Mann-Whitney *U* test. Correlations between two variables were tested by linear regression analysis. Simple correlations were assessed by Pearson’s correlation coefficients. Nonparametric data were analyzed using the Spearman’s rank correlation coefficient. In all cases, threshold of significance was defined as *p* < 0.05.

Table 1. Anthropometric and laboratory data of lactating mothers and their infants

	Median (IR)	Mean ± SD
Mothers		
Age (yr)	28 (25–33)	30 ± 5.9
BMI before pregnancy (kg/m ²)	20.9 (19.3–22.6)	21.4 ± 2.6
Serum leptin at first week (ng/mL)	3.83 (2.55–8.64)	5.32 ± 4.2
Serum leptin after 6 mo (ng/mL)	4.67 (3.46–7.83)	5.39 ± 3.0
Breast milk leptin at first week (ng/mL)	0.17 (0.08–0.25)	0.21 ± 0.19
Breast milk leptin after 6 mo (ng/mL)	0.15 (0.05–0.32)	0.18 ± 0.15
Infants		
Sex (female/ male)	10/13	
Birth weight (g)	3530 (3060–4010)	3480 ± 708
Birth length (cm)	50 (48–51)	49.4 ± 2.9
Gestational age (wk)	40.1 (38.5–41)	39.4 ± 2.4

Data are shown as median and IR and mean ± SD.

RESULTS

Breast milk leptin versus maternal serum leptin levels and anthropometric data. Over the first 6 mo after delivery, we documented anthropometric changes of all mothers and their infants. In addition, serum and breast milk samples were withdrawn at different time points during the whole lactation period, as described in Methods section.

Course of breast milk leptin levels. One week postpartum, the leptin levels showed a median value of 0.17 ng/mL (IR, 0.08–0.25 ng/mL). During the next 3 wk this level remained nearly constant (median, 0.17–0.18; Fig. 1A), whereas the individual leptin values varied between minimum and maximum values of 0.01–0.65 ng/mL after the first week, 0.01–0.56 ng/mL after the second week, 0.01–0.87 ng/mL after the third week, and 0.01–0.42 ng/mL after 1 mo. At the second month postpartum, the leptin levels in milk (0.11, ng/mL; IR,

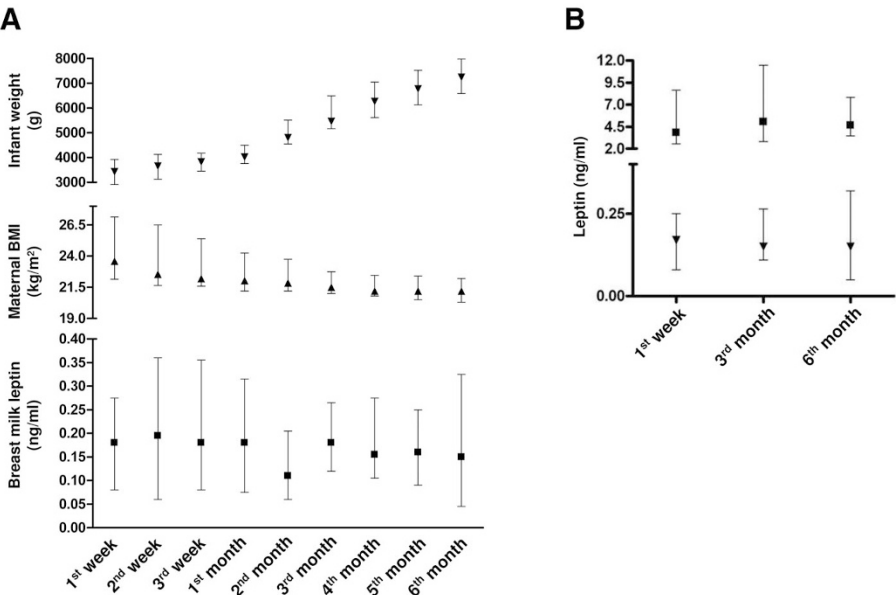


Figure 1. (A) Determination of breast milk leptin concentrations and anthropometric parameters. The shapes in (A) demonstrate data of breast milk leptin (ng/mL) (■), maternal BMI (kg/m²) (▲), and infant weight (g) (▼) over a period of 6 mo. Although infant weight increased significantly, no significant changes were seen in maternal BMI over the period. The breast milk leptin levels at the second month postpartum were significantly (*p* = 0.01) lower compared with the data at the first and third month postpartum. Values are shown as median and IR. (B) Determination of leptin concentrations in breast milk and maternal serum. Leptin concentrations (ng/mL) were determined in maternal serum (■) and breast milk (▼) at the first week and the third and the sixth month postpartum and are depicted as median and IR. The leptin levels in breast milk were significantly (*p* < 0.001) lower than in maternal serum during the whole lactation period.

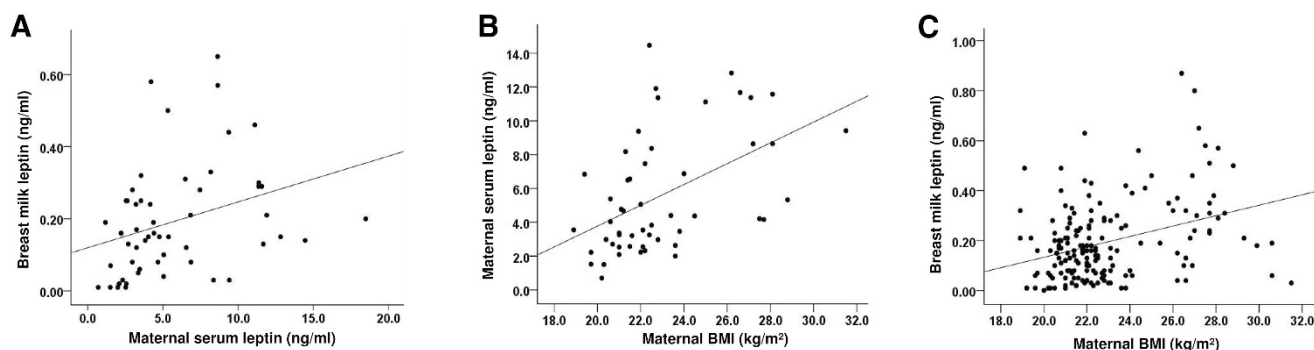


Figure 2. (A) Comparison of maternal leptin levels (ng/mL) in serum and breast milk. Due to the nonparametric distribution of the data, the correlation was assessed by Spearman's rank correlation coefficient. A significantly positive correlation between both groups was observed throughout the whole lactation period ($r = 0.431$, $p = 0.001$, $n = 55$). (B) Comparison of maternal BMI (kg/m^2) and maternal serum leptin levels (ng/mL). Due to nonparametric distribution of the values, the correlation was assessed by Spearman's rank correlation coefficient. A significantly positive correlation between both groups was observed throughout the whole lactation period ($r = 0.512$, $p < 0.001$, $n = 53$). (C) Comparison of maternal BMI (kg/m^2) and breast milk leptin levels (ng/mL). Due to nonparametric distribution of the values, the correlation was assessed by Spearman's rank correlation coefficient. A significantly positive correlation between both groups was observed throughout the whole lactation period ($r = 0.298$, $p < 0.001$, $n = 174$).

0.06–0.20 ng/mL) were significantly ($p = 0.01$) lower compared with the data at the first and third month postpartum (Fig. 1A). Thereafter, the levels increased slightly and remained almost unchanged until 6 mo (0.15, ng/mL; IR, 0.05–0.32 ng/mL).

Maternal serum versus breast milk leptin levels. In the first week after delivery the maternal serum levels of leptin showed a median value of 3.83 ng/mL (IR, 2.55–8.64 ng/mL). At the third month, the serum leptin concentrations increased not significantly (5.06 ng/mL; IR 2.09–14.47 ng/mL) and showed a slight decrease at the sixth month postpartum (4.67 ng/mL; IR 3.55–7.47 ng/mL) (Fig. 1B).

Compared with maternal serum, leptin levels in breast milk were significantly ($p < 0.001$) lower (22-fold) during the whole lactation period, as shown in Figure 1B. Maternal serum and breast milk leptin correlated significantly over the 6-mo lactation period ($r = 0.431$, $p = 0.001$, $n = 55$; Fig. 2A). Further, we observed a positive correlation between serum leptin levels and maternal BMI ($r = 0.512$, $p < 0.001$, $n = 53$) over the 6 mo (Fig. 2B). In addition, breast milk leptin levels (Fig. 2C) also demonstrated a significant correlation with maternal BMI ($r = 0.298$, $p < 0.001$, $n = 174$). Maternal BMI before pregnancy did not correlate with postnatal serum and breast milk leptin levels.

Leptin levels and infant growth. To find an association between the postnatal changes of infant weight and maternal anthropometric data or leptin concentrations, we compared infant weight gain from birth to 6 mo to maternal BMI and to breast milk leptin concentrations (Fig. 1A). Although the median infant weight significantly ($p < 0.001$) increased from 3530 g (IR, 3060–4020 g) to 7240 g (IR, 6580–7980 g) during the lactation period, we observed no significant changes of maternal BMI or breast milk leptin levels.

GA, birth weight, length, and gender of infants did not significantly correlate with leptin levels in maternal serum and breast milk. Further, our data showed that the infant weight correlated neither with breast milk leptin nor with serum leptin levels at every measured time point. As expected, we observed

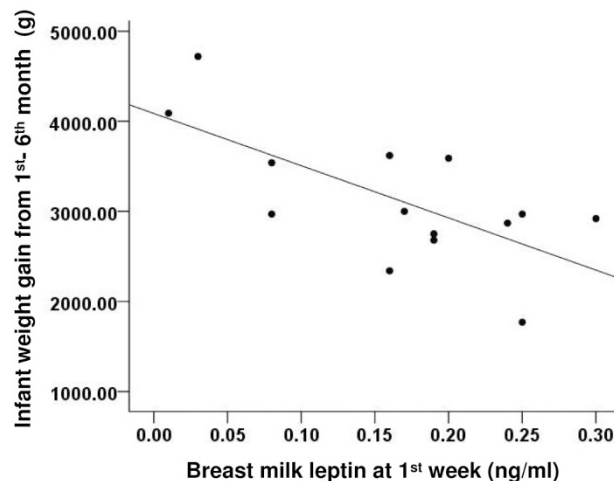


Figure 3. Association of breast milk leptin (ng/mL) at the first week and infant weight gain (g) from the end of the first to the sixth month of lactation. Data were distributed parametrically. Correlation analysis was performed using Pearson's correlation coefficient. A significantly negative correlation was observed ($r = -0.681$, $p = 0.007$, $n = 14$).

that the median value of neonate weight gain during the first 4 wk of lactation was 570 g (IR, 452–840 g; $n = 15$) and significantly lower compared with 2970 g (IR, 2759–3544 g; $n = 15$) within the last 5 mo of lactation. Interestingly, we found a negative association between breast milk leptin levels at the first week after delivery and the infant weight gain from the end of the first to the sixth month ($r = -0.681$, $p = 0.007$, $n = 14$; Fig. 3). In contrast, no correlation was observed between breast milk leptin, detected in the first week, and the infant weight gain during the first 4 wk of life ($r = 0.154$, $p = 0.51$).

DISCUSSION

This study presents data of leptin levels from maternal serum and breast milk samples and its association with infant weight

gain at critical points during the first 6 mo in neonatal life. Furthermore, our study is strengthened by the fact that we enrolled a relative homogenous group of lactating mothers and their infants. Thus, we have avoided possible pathophysiological influences that could confuse the normal relationship between leptin concentrations and anthropometric parameters. There are only a few longitudinal studies about the relationship between early stages of lactation and infant growth pattern during the first 6 mo postpartum (17,20). In addition, the group of Savino *et al.* reported that infants' serum leptin concentration was positively correlated with infants' body weight and BMI as well as maternal BMI (19,21,22), but they found no association between breast milk and serum leptin levels in infants and mothers (19). The cross-sectional design could explain the differences compared with findings in our study. We obtained longitudinal data from breast milk samples starting in the first week including colostrum and transitional milk and ending with mature milk after 6 mo lactation. Our findings were supported by previous data about the presence of leptin in breast milk during the whole lactation period (1,2,6,18,23–30) and suggest that maternal leptin could regulate body weight gain during early infancy. We observed quite low, but nearly constant breast milk leptin levels over 6 mo with distinct interindividual differences. Leptin, acting as a bioactive compound, could be responsible for the effect of breastfeeding in lowering the risk of childhood obesity (5,9–15).

Smith-Kirwin *et al.* (6) showed that the leptin gene is expressed in the mammary gland of lactating women and that leptin is produced by mammary epithelial cells. Thus, there are two sources of leptin in maternal breast milk: leptin can be secreted into the milk by the mammary gland and transferred from the bloodstream (6,31). Because leptin receptors have been identified in gastric epithelial cells, as well as in the absorptive cells of mouse and human small intestine (32), leptin might also activate signaling pathways involved in the development of the infant gastrointestinal system.

Leptin concentration in milk and serum has been assayed mainly by RIA. We used a RIA with a sensitivity of 0.01 ng/mL to detect minimal changes in leptin concentrations. Houseknecht *et al.* (1) found higher leptin levels in whole than in skim milk. These data are in accordance with our findings and support the hypothesis that milk fat could interfere with the antigen-antibody interaction of the immunoassay or that leptin may be associated with milk fat globules (1,6,24). Therefore, we centrifuged and sonicated the milk samples prior analysis to separate leptin from milk fat globules and achieved acceptable values in linearity and spiking experiments.

Considering the presence of leptin in breast milk, we evaluated maternal serum leptin levels in lactating women and found a positive correlation between leptin levels in breast milk and maternal serum, with a 22-fold lower concentration in breast milk than in serum. This could be explained by a barrier-conditioned transfer process from blood to milk, which has been observed from serum to cerebrospinal fluid previously (33). A lower production of leptin in mammary glands could also be a reason. These results are in accordance with the literature, particularly with the Weyermann *et al.* (29) study. In contrast to Uçar *et al.* (23), we demonstrated that leptin levels in breast milk were positively correlated with the maternal BMI during the whole

lactation period. We found a positive correlation between mothers' serum leptin levels, weight, and BMI. This has also been reported by Savino *et al.* (19). This may reflect a relationship with maternal adipose tissue mass, suggesting that infants nursed by mothers with significant overweight are exposed to higher amounts of leptin in milk than infants nursed by normal-weight mothers. This result raised the question whether milk-borne leptin provides a link between maternal body composition and infant growth and development.

We found that breast milk leptin during the first week after delivery is negatively correlated with the infant weight gain from the end of first to the sixth month of infant age, suggesting an influence of maternal leptin on infant weight gain. The first week is a period where the child switches from a placental to an oral energy and nutrient supply and breast milk represents the only food and nutrient source. Our result is in line with two previous studies. First, Miralles *et al.* (17) found a significantly negative correlation between milk leptin levels and infant weight gain during 24 mo of infant life, despite measuring the hormone in the somewhat different sample matrix of whole milk. Second, Doneray *et al.* studied skim milk samples of 15 women and serum samples of their infants during the first month postpartum. Although this study included only two points of measurement and was restricted to the first month of lactation, the authors found a significantly negative correlation between mature milk leptin levels and infant weight gain (20). However, they detected a wide range of milk leptin levels in colostrum and mature milk, a finding that we could not replicate, possibly due to different sample preparations in both studies. Moreover, Savino *et al.* (21) reported differences in breastfed and formula-fed infants for the relation between serum leptin and infant body composition during the first 6 mo of life.

Studies on suckling rats demonstrated that the production of endogenous leptin in the neonatal stomach was still low, but that gastric cells were able to absorb leptin from maternal breast milk (34). The fact that leptin in maternal milk from nursing rats could be absorbed by the gastric mucosa of neonatal rats and transferred to infant rat circulation (2) implicates that milk-borne leptin could be the main source of leptin during the suckling period, when the appetite regulatory system is also immature (35). Interestingly, animals treated with leptin during lactation were programmed for lower body weight, fat content, and took up fewer calories (4,36). Moreover, our study is supported by data of Priego *et al.* (37) who showed that leptin intake during the suckling period of neonatal rats improves the leptin sensitivity and metabolic response later on in life as evaluated by the expression of the leptin receptor (*OB-Rb*) and genes related with energy intake. Therefore, our results support the hypothesis that leptin during early lactation could have long-term effects on regulating body weight and appetite (15,38). Another possible explanation for the negatively associated weight gain demonstrated in our study might be the formation of neural circuits induced by leptin, controlling food intake, and weight gain later in life (4,5,15). Appetite is controlled by neurons in the arcuate nucleus of the hypothalamus and is regulated by leptin. During the development of the hypothalamus, leptin plays a neurotrophic role, primarily during a defined neonatal period. Therefore, this

adipokine is involved in mediating cellular events such as neurogenesis, axon growth, and synaptogenesis (5). Leptin, ingested by the newborn infant, may program hypothalamic organization during early life. Probably, it represents a hormonal mediator of the environmental nutrient-sensing system that directs metabolic programming.

A limiting factor of our study could be of methodical nature. The mean recovery values of our analysis were around 70% and therefore somewhat lower than expected. We assume that a minor part of the added leptin remained associated with fat globules during the centrifugation step leading to an overall underestimation of leptin levels. The acceptable variance for the recovery experiments of below 12.5% supports this assumption and suggests that a qualitative interpretation of our results is justified. In addition, we investigated formula milk as a comparable matrix for endogenous milk to ensure that the assay did not recognize matrix-related leptin-like immunoreactivity. The measured "zero" level revealed that a putative matrix effect was not detectable. Furthermore, we also cannot exclude an influence of family heredity, involving genetic and environmental components, which could mask the effects of leptin, because they are strong determinants of childhood obesity (39,40).

In conclusion, human milk contains growth factors, hormones, and adipocytokines, of which the function in infant growth and development is still unknown. Our results led to the hypothesis that milk leptin during the first stages of lactation, in which the gastric mucosa is still immature and the absorption of intact leptin may be facilitated (34), may control mechanisms that influence infant weight gain from the first to the sixth month. This suggests a critical relevance for the action of this adipocytokine during early life.

Acknowledgments. We thank all women who participated in this study. We very gratefully appreciate the help of the nurses, technical assistants, and physicians who performed the clinical examinations and data collection.

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