Serum leptin concentrations in infants: effects of diet, sex, and adiposity[1,2]

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ABSTRACT

Background: Leptin, the product of the obese (*ob*) gene, is a regulator of food intake and energy metabolism. Immunoreactive leptin was detected recently in breast milk and it has been hypothesized that leptin may be absorbed and may contribute to differences in body composition between breast-fed and formula-fed infants.

Objective: The objective was to evaluate whether diet, adiposity, or sex affect plasma leptin in breast-fed and formula-fed infants.

Design: Venous blood samples were drawn from healthy, exclusively breast-fed or formula-fed Swedish infants at 1, 4, and 6 mo of age (n = 193) and from 12-mo-old Finnish infants (n = 79). Anthropometric measurements were made and plasma samples were analyzed for leptin, insulin, and glucose.

Results: There were no significant differences in plasma leptin between formula-fed and breast-fed infants at 1 and 4 mo of age, whereas formula-fed infants had significantly higher (≈5%) leptin concentrations at 6 mo of age. Similar results were observed after correction for BMI. Plasma leptin was 15–25% higher in female than in male infants at 1, 4, and 12 mo of age ($P < 0.05$), also after correction for BMI. When all infants were analyzed together, a positive correlation ($r = 0.34$, $P < 0.0001$) was found between plasma leptin and BMI. Very low leptin concentrations were found in breast milk after centrifugation and the high concentrations reported previously were likely due to interference in the assay by milk fat.

Conclusions: Plasma leptin concentrations are not higher in breast-fed than in formula-fed infants; however, sex and adiposity affect leptin concentrations even at this early age. *Am J Clin Nutr* 2000;72:484–9.

KEY WORDS Leptin, infants, milk, formula, breast-feeding, adiposity, sex

INTRODUCTION

The growth pattern of formula-fed infants has been shown to be different from that of breast-fed infants (1–4). Most studies showed that weight gain of the former group is lower than that of the latter, although not consistently so (5). Weight-for-length z scores are significantly higher in formula-fed than in breast-fed infants from 7 to 18 mo of age and the sum of skinfold thicknesses was significantly higher in formula-fed infants from 5 mo of age, suggesting that breast-fed infants are leaner (6). The reasons for the observed differences in body composition are not known, although it has been speculated that differences in the volume of milk or formula ingested, the amount of complementary foods eaten, or the hormonal responses to diet may be involved (4).

Differences in the endocrine response to feeding may contribute to the differences in body composition between formula-fed and breast-fed infants (7). The endocrine signal may be a response to the diet fed, such as the difference in serum insulin concentrations observed between breast-fed and formula-fed infants (8). It may also be a direct consequence of hormones being absorbed by breast-fed infants because it is known that many biologically active hormones are present in human milk (9). Leptin, a product of the obese gene (*ob*), is synthesized by adipocytes and is known to be a regulator of food intake and energy metabolism (10). Circulating leptin concentrations are highly correlated with adiposity in adults (11, 12) and children (13–15) and are markedly elevated in obese individuals (12). However, adiposity is not the sole determinant of circulating leptin concentrations because plasma leptin decreases after fasting (16, 17) and energy restriction (18) and increases after refeeding (16). Circulating leptin concentrations (24 h) are lower when subjects consume high-fat meals than when they consume high-carbohydrate meals (19). Changes in leptin in response to fasting, refeeding, and dietary macronutrients are likely to be related to insulin-mediated glucose metabolism in adipose tissue (20). In adults, plasma leptin concentrations are higher in women than in men, even after correction for the higher body fat in women (21).

It is believed that leptin is integral in the feedback loop from adipose stores to the hypothalamus because leptin administration decreases food intake in rodents and nonhuman primates and, in addition, increases energy expenditure in mice and activates the sympathetic nervous system (22–24). Furthermore, decreases in leptin during energy restriction in humans are related to increased sensations of hunger, suggesting that leptin also regulates appetite in humans (25). Recently, immunoreactive leptin was reported to be present in human milk (26, 27). Concentrations of leptin in milk were lower than in plasma, but were still

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TABLE 1
Characteristics of study infants[1]

	Boys				Girls			
	1 mo (n = 29)	4 mo (n = 33)	6 mo (n = 33)	12 mo (n = 39)	1 mo (n = 29)	4 mo (n = 35)	6 mo (n = 34)	12 mo (n = 40)
Weight (kg)	4.45 ± 0.14	7.01 ± 0.13	8.08 ± 0.15	10.56 ± 0.17	4.27 ± 0.11	6.49 ± 0.14[2]	7.56 ± 0.16[3]	10.06 ± 0.22[4]
Length (m)	0.55 ± 0.01	0.64 ± 0.01	0.68 ± 0.01	0.78 ± 0.01	0.54 ± 0.01	0.63 ± 0.01	0.67 ± 0.01	0.76 ± 0.01
BMI (kg/m^2)	14.6 ± 0.3	16.8 ± 0.2	17.3 ± 0.2	17.4 ± 0.2	14.4 ± 0.2	16.3 ± 0.2	17.0 ± 0.3	17.3 ± 0.2

[1] $\bar{x}$ ± SEM.
[2–4] Significantly different from boys at a given age (unpaired t test): [2] $P < 0.005$, [3] $P < 0.02$, [4] $P < 0.05$.

within the range of normal plasma leptin concentrations (26). The possibility that milk-derived leptin has an effect on infant growth and metabolism was therefore raised (26, 27).

In the present study, we explored whether plasma leptin concentrations in breast-fed and formula-fed infants are different and whether the body mass index (BMI; in kg/m^2) of infants, regardless of the diet fed, affects circulating leptin concentrations. We further investigated whether the sex differences in plasma leptin that were observed in children and adults are present at this early age.

SUBJECTS AND METHODS

Subjects

We used 2 cohorts of healthy infants in this study: *1*) exclusively breast-fed or formula-fed infants at 1, 4, and 6 mo of age who had participated in a clinical study in Umeå, Sweden (28), and *2*) 12-mo-old infants from a cross-sectional study in Helsinki (L Lope, B Lönnerdal, unpublished observations, 1996). The formula used was a whey-predominant, powdered, commercially available product (Baby Semp 2; Semper AB, Stockholm) containing 13 g protein/L. To study the consequences of breast-feeding compared with formula-feeding, these infants were exclusively breast-fed or formula-fed up to 6 mo of age. (In Sweden, the recommendation is to not introduce solid foods before 6 mo of age.) Minimal taste portions (<2 tsp/d, or ≈10 mL/d) of fruit purée were allowed between 5 and 6 mo of age, but no other foods, drinks, or supplements were allowed.

Detailed anthropometric measurements were available from the data sets from the clinical studies. Sex, weight, length, BMI, and the number of infants at each age are given in **Table 1**. Venous blood samples were drawn 2–3 h postfeeding. Plasma samples were stored at −80°C until analyzed. The human subjects committees at the 2 sites approved the respective studies and informed consent was obtained.

Assays

Plasma leptin was measured by radioimmunoassay (Linco Research, St Louis) (29), as was insulin (30); glucose was measured with a glucose analyzer (model 2300; Yellow Springs Instruments, Yellow Springs, OH). Human milk was obtained from healthy women in midlactation (2–4 mo) and was stored frozen. Whole milk and skim milk were centrifuged at 1100 × g for 30 min at 4°C and samples were assayed for leptin by using the plasma leptin assay. We added 100 μg human leptin/L to the samples to study the recovery of leptin. We also added Intralipid (Baxter, Deerfield, IL) or cow milk fat to human skim milk samples to obtain a fat concentration similar to that of whole human milk (4%) to study the possible interference of lipids.

Statistical analyses

The statistics package STATVIEW (Abacus Concepts, Inc, Berkeley, CA) was used for linear regression analysis. Unpaired t tests were used for comparisons between male and female infants and between breast-fed and formula-fed infants. Multivariate statistical models were also used to analyze the data. Results are expressed as means ± SEMs. Statistical significance was set at $P < 0.05$.

RESULTS

There were no significant differences in birth weight between the breast-fed and formula-fed infants (**Table 2**). Breast-fed infants weighed more did than formula-fed infants at all ages, but the BMI was higher in breast-fed than in formula-fed infants only at 1 mo of age. Concentrations of absolute and adiposity-corrected plasma leptin, plasma insulin, and plasma glucose are also given in Table 2. There were no significant differences in plasma leptin concentrations between formula-fed and breast-fed infants at 1 and 4 mo of age, whereas formula-fed infants had slightly, but significantly, higher leptin concentrations at 6 mo of age. Similar results were obtained after correction for adiposity (BMI). Insulin concentrations were higher ($P < 0.03$) in breast-fed than in formula-fed infants at 1 mo of age, but at 4 and 6 mo of age, formula-fed infants had considerably higher concentrations than did breast-fed infants. Insulin-glucose ratios were significantly higher in formula-fed than in breast-fed infants at 4 mo of age.

Male infants weighed more than did female infants at 4, 6, and 12 mo of age, whereas BMI was similar in male and female infants at all ages. Plasma leptin concentrations were significantly higher in female than in male infants at 1, 4, and 12 mo of age, whereas no significant difference was found at 6 mo of age (**Table 3**). In an analysis of all infants, regardless of age, girls had significantly higher plasma leptin concentrations than boys ($P < 0.003$). This difference remained after correction for adiposity (leptin:BMI) ($P < 0.002$). The plasma insulin concentration ($P < 0.03$) and the insulin-glucose ratio ($P < 0.02$) were significantly higher in boys than in girls at 6 mo of age.

Plasma leptin concentrations were significantly correlated with BMI when all infants were analyzed, regardless of age, sex, or diet group (**Figure 1**). There was no significant correlation between plasma leptin and plasma insulin concentrations or between plasma leptin and plasma glucose concentrations (**Figure 2**). Surprisingly, plasma insulin was marginally, but negatively, correlated with BMI ($r = -0.15$, $P < 0.03$).

TABLE 2
Effect of diet on morphometric indexes and absolute and adiposity-corrected plasma leptin and insulin and glucose in infants[1]

	Formula-fed				Breast-fed			
	Birth (n = 38)	1 mo (n = 30)	4 mo (n = 38)	6 mo (n = 38)	Birth (n = 31)	1 mo (n = 31)	4 mo (n = 31)	6 mo (n = 30)
Weight (kg)	3.43 ± 0.09	4.11 ± 0.15	6.55 ± 0.12	7.61 ± 0.16	3.54 ± 0.09	4.55 ± 0.12[2]	6.95 ± 0.15[3]	8.02 ± 0.16[3]
BMI (kg/m^2)	—	14.1 ± 0.2	16.5 ± 0.2	17.0 ± 0.2	—	14.9 ± 0.2[3]	16.7 ± 0.3	17.3 ± 0.3
Leptin (μg/L)	—	4.4 ± 0.3	5.0 ± 0.2	5.1 ± 0.3	—	5.0 ± 0.4	5.6 ± 0.4	4.4 ± 0.2[3]
Leptin:BMI	—	0.31 ± 0.02	0.30 ± 0.01	0.30 ± 0.02	—	0.34 ± 0.03	0.31 ± 0.03	0.25 ± 0.01[3]
Insulin (pmol/L)	—	99.6 ± 13.8	135.0 ± 15.0	96.6 ± 12.6	—	155.4 ± 29.4[3]	60.6 ± 10.2[4]	69.6 ± 19.2
Glucose (mmol/L)	—	4.4 ± 0.1	4.7 ± 0.1	4.5 ± 0.2	—	4.4 ± 0.1	4.0 ± 0.2[4]	4.7 ± 0.2
Insulin:glucose	—	21.6 ± 2.2	28.1 ± 2.2	20.5 ± 2.2	—	34.6 ± 6.5[3]	15.1 ± 2.2[4]	13.0 ± 3.2[3]

[1] $\bar{x}$ ± SEM.
[2–4] Significantly different from formula-fed infants at a given age (unpaired t test): [2] $P < 0.01$, [3] $P < 0.05$, [4] $P < 0.001$.

In a multiple regression analysis, formula feeding was found to be a significant predictor of only leptin at 6 mo of age, which was 15% higher in formula-fed infants.

Insulin was not a predictor of leptin in a multiple regression analysis either in the 1–6-mo data set or in all infants ≤12 mo of age. In the 1–6-mo data set, only BMI ($P < 0.0001$), sex ($P < 0.005$), and age ($P < 0.01$) were independent predictors of leptin in a multiple regression analysis including diet, BMI, insulin, glucose, sex, and age. In the 1–12-mo data set, only BMI ($P < 0.0001$) and sex ($P < 0.002$) were independent predictors of leptin in a multiple regression analysis including BMI, insulin, glucose, sex, and age, but not diet (because the 12-mo-olds were consuming varied diets). Values were normally distributed and log transformation did not affect any of our conclusions.

Earlier reports suggested relatively high concentrations of leptin in human milk. Our first assay results also indicated leptin concentrations in human milk that were high enough to be considered of potential physiologic relevance. However, results after centrifugation suggested either that the leptin segregated with the lipid fraction of the milk or that milk fat interfered with the assay method and that the measured values were an artifact. As suggested by the assay manufacturer, 100 μg human leptin/L was added to 4 pools of human milk. Samples for leptin assay were collected before, immediately after, and 24 h after addition of human leptin. Because we suspected interference by lipids, we also centrifuged the milk at 1100 × g for 30 min at 4°C ("skimming") and at 12 000 × g for 30 min at 4°C (high-speed centrifugation). As shown in **Table 4**, whole human milk appeared to contain 13.3–74.4 μg leptin/L. Addition of human leptin to the samples did not lead to any significant increase in the leptin concentration of whole milk. However, when leptin was added to defatted milk, nearly all of the added leptin was recovered, although the assayed values did decrease after 24 h of additional incubation. It therefore appears that lipids in human milk interfere with the leptin assay and that actual leptin concentrations are relatively low. This finding was also supported by the observation that rat milk appeared to contain substantial concentrations of human leptin when assayed with the human antiserum (Linco), which does not cross-react with rat leptin. In contrast, defatted milk samples from this species contained very low or undetectable amounts of leptin when measured with the human leptin assay. When lipids were added to human skim milk in the form of Intralipid or cow milk fat (neither containing human leptin), the measured leptin concentrations increased substantially, which also suggests lipid interference with the assay.

DISCUSSION

The leptin concentrations we found in infant plasma were similar to those of the 5–9-y-old children in the study by Garcia-Mayor et al (15). Male infants had mean leptin concentrations of ≈4 μg/L, whereas female infants had significantly higher concentrations (≈5.5 μg/L). Thus, during the first year of life, sex differences in circulating leptin concentrations are already apparent, although the differences are not as great as those observed in adults. A recent study by Jaquet et al (31) suggested that there are sex differences in serum leptin concentrations in human fetuses (37 wk of gestation) and in newborns, although these differences are not significant. Helland et al (32) found recently that there are sex differences in leptin concentrations in umbilical cord plasma (10.8 and 7.6 μg/L in girls and boys, respectively) and at 0.5 mo of age (3.9 and 3.2 μg/L in girls and

TABLE 3
Effect of sex on absolute and adiposity-corrected plasma leptin and insulin and glucose in infants[1]

	Boys				Girls			
	1 mo (n = 29)	4 mo (n = 33)	6 mo (n = 33)	12 mo (n = 39)	1 mo (n = 29)	4 mo (n = 35)	6 mo (n = 35)	12 mo (n = 40)
Leptin (μg/L)	4.6 ± 0.3	4.5 ± 0.3	4.8 ± 0.3	5.0 ± 0.2	5.8 ± 0.3[2]	5.4 ± 0.3[2]	4.8 ± 0.3	5.7 ± 0.3[3]
Leptin:BMI	0.28 ± 0.02	0.27 ± 0.01	0.27 ± 0.02	0.28 ± 0.01	0.36 ± 0.03[3]	0.35 ± 0.02[2]	0.28 ± 0.02	0.33 ± 0.01[3]
Insulin (pmol/L)	141.0 ± 30.6	111.6 ± 15.0	108.6 ± 18.6	90.0 ± 9.0	109.8 ± 12.0	107.4 ± 17.4	67.8 ± 10.8[3]	41.4 ± 9.6
Glucose (mmol/L)	4.4 ± 0.1	4.6 ± 0.2	4.7 ± 0.29	3.9 ± 0.1	4.4 ± 0.2	4.4 ± 0.1	4.5 ± 0.1	3.7 ± 0.1[3]
Insulin:glucose	31.3 ± 6.5	23.8 ± 2.2	21.6 ± 3.2	8.6 ± 1.1	24.8 ± 2.2	23.8 ± 3.2	14.0 ± 2.2[3]	10.8 ± 2.2

[1] $\bar{x}$ ± SEM.
[2,3] Significantly different from boys at a given age (unpaired t test): [2] $P < 0.01$, [3] $P < 0.05$.

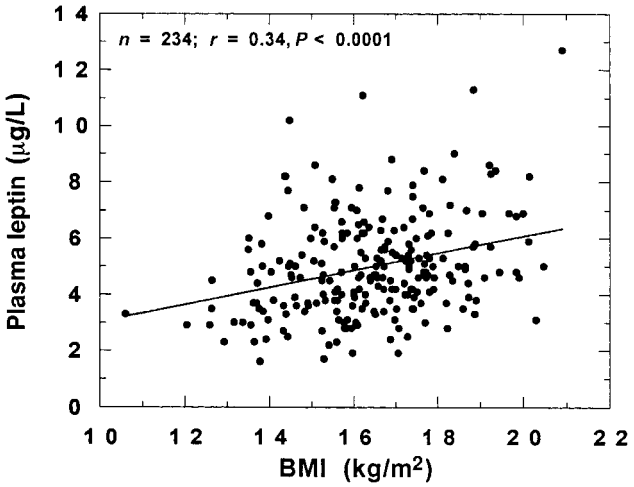

FIGURE 1. Correlation between plasma leptin concentrations and body mass index (BMI) in all infants.

boys, respectively). Taken together, these observations suggest that sex differences are apparent very early postnatally.

Serum leptin concentrations were examined previously in newborns (31–33), children (14, 15), adolescents (13, 15), and adults (11, 12, 18, 21). In males, serum leptin concentrations increased between the ages of 5 and 10 y, decreased from 10 to 15 y (15), and then increased very modestly to reach adult concentrations of ≈3.5 μg/L in normal-weight men (21). The decrease observed between 10 and 15 y of age correlated with the increased testosterone concentrations concomitant with the onset of puberty (15).

The prepubertal and pubertal rise in leptin concentrations in young females was found to correlate with increases in serum follicle-stimulating hormone, luteinizing hormone, and estradiol (15). However, plasma leptin concentrations are not significantly different between premenopausal and postmenopausal women or between postmenopausal women receiving and not receiving hormone replacement therapy (10). Therefore, normal concentrations of female reproductive hormones do not appear to be important determinants of circulating leptin concentrations in adult women. Furthermore, in adults, the sex difference in circulating leptin concentrations cannot be explained by increased adipose mass in women (10). Because overall body adiposity and fat distribution are not significantly different between male and female infants, and because the hormonal profile of male and female infants is unlikely to be different at this young age, the reason for the sex difference in serum leptin concentrations in infants remains unknown.

Serum leptin concentrations were not significantly higher in breast-fed than in formula-fed infants; at 1 and 4 mo of age there was no significant difference between the groups and at 6 mo of age, breast-fed infants had significantly lower serum leptin concentrations. This difference remained after correction for adiposity (leptin:BMI). Thus, it appears unlikely that leptin in breast milk is absorbed in physiologically relevant amounts, which, in turn, would contribute to differences in growth and body composition in late infancy. If there was an effect on body composition of exogenous leptin from breast milk, differences in serum leptin between the breast-fed and formula-fed groups would have been expected at 4 and 6 mo of age because body fat (sum of skinfold thicknesses) is significantly different at 5 mo of age and weight-for-height is significantly different at 7 mo of age between these 2 groups (6). Dietary macronutrient composition was shown to influence circulating leptin concentrations over a 24-h period in adults (19). Whether this also occurs in infants is not known; however, the percentage of the diet being provided as fat and carbohydrate in our study was similar in breast-fed and formula-fed infants and, therefore, it was unlikely to have affected leptin concentrations.

We found that the breast-fed infants had significantly higher body weights than did the formula-fed infants at 1, 4, and 6 mo of age. This was most likely due to the initially higher weights of the breast-fed infants at 1 mo of age. We believe that this was a coincidence because the infants could not be randomly assigned to groups for ethical reasons. As can be seen in Table 3, weight gain between 1 and 4 mo and between 4 and 6 mo of age was similar in the 2 groups. In our previous longitudinal study of growth and body composition of infants (the DARLING study), several of the skinfold-thickness measurements of breast-fed infants tended to be higher than those of formula-fed infants at 1 mo of age, although not significantly so (6). Two recent studies (31, 33) showed that serum leptin concentrations in newborns are ≈2 μg/L, which is considerably lower than the concentrations we found at 1 mo of age. Serum leptin concentrations are also lower than those reported as 0.5 mo of age (32). Therefore, it appears that serum leptin concentrations increase significantly during the first weeks of life, most likely as a consequence of the early postnatal gain in adipose tissue. Marchini et al (33) showed that serum leptin decreased by ≈26% during the first 4 d after birth, which was associated with a 3–6% weight reduction. A subsequent rapid weight gain is likely to result in increased adipose tissue.

There appear to be analytic problems associated with measuring leptin in whole milk with a radioimmunoassay routinely used for measuring serum and plasma leptin concentrations. This interference by milk fat is likely to explain some previous reports of high leptin concentrations in milk. Skimming of the breast milk led to analyzed leptin concentrations in breast milk of <0.5 μg/L, which is considerably lower than those reported by Houseknecht et al (26). These authors reported that whole breast milk contained 10.1 ± 2.6 μg/L, whereas one of their figures clearly showed that most of their whole milk samples

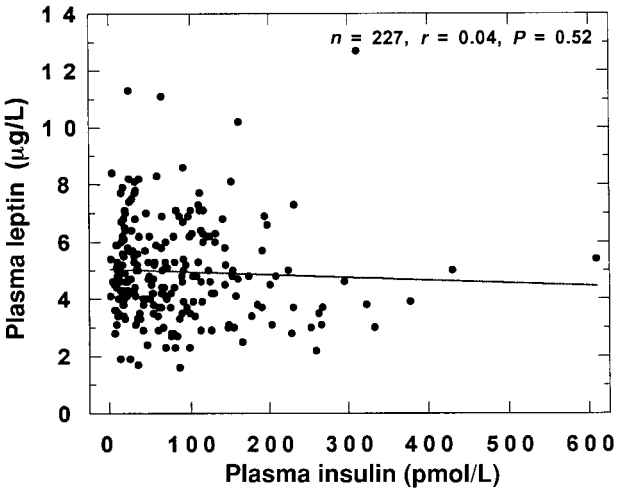

FIGURE 2. Correlation between plasma leptin and plasma insulin concentrations in all infants.

TABLE 4
Radioimmunoassay and recovery of human leptin in milk[1]

	Leptin
	μg/L
Whole milk	32.7 ± 14.1 (13.3–74.4)
Whole milk + 100 μg leptin/L	28.9 ± 8.5 (4.8–42.6)
Skim milk	0.2 ± 0.1 (0.1–0.4)
Skim milk + 100 μg leptin/L	92.4 ± 7.8 (76.6–108.9)
Skim milk + Intralipid (4%, wt:vol)[2]	2.9 ± 0.4 (1.1–6.1)
Skim milk + cow milk fat (4%, wt:vol)	3.8 ± 0.2 (0.4–10.5)

[1] $\bar{x}$ ± SEM; range in parentheses; $n = 4$.
[2] Intralipid (Baxter, Deerfield, IL).

contained ≤1 μg leptin/L. Similarly, they reported a leptin concentration in skim milk of 1.5 ± 0.8 μg/L, whereas the same figure showed that most analyzed values were ≈0.5 μg/L and that several samples contained no detectable leptin. Although we did not extensively analyze leptin in breast milk, the values reported for skim milk by Houseknecht et al (26) are similar to those that we found. Because these values are ≈10-fold lower than serum leptin concentrations in infants, it is unlikely that they would contribute significantly to circulating serum leptin concentrations (30).

A recent study by Smith-Kirwin et al (34) showed that human mammary epithelial cells express leptin and that they are the likely origin of leptin in breast milk. These researchers found concentrations of leptin in skim milk that were similar to those in our study, but found considerably higher concentrations in whole milk that had been sonicated. We attempted to use this method but consistently experienced interference by milk fat in the assay, giving what we believe were artificially elevated values. Furthermore, our observation that adding rat milk to the human leptin assay yielded high leptin concentrations (but leptin from this species does not cross-react with this antibody) and that addition of Intralipid or cow milk fat to human skim milk samples resulted in high milk leptin concentrations strongly suggests interference by fat in the assay. It is therefore evident that further work is needed to best determine how to accurately assess leptin concentrations in whole milk.

The breast-fed infants had significantly lower plasma insulin concentrations than did the formula-fed infants at 4 mo of age. This was reported earlier and it is believed to have been due to a considerably higher protein intake in the formula-fed infants (8). Despite the higher plasma insulin concentrations in the formula-fed than in the breast-fed infants, plasma glucose concentrations were significantly higher in the formula-fed than in the breast-fed infants at the same age. This suggests some insulin resistance in the formula-fed infants. Unlike in adults, we found no significant correlation between plasma leptin and plasma insulin concentrations. One possible explanation for this lack of correlation is the relative homogeneity of serum leptin concentrations in infants. Serum leptin ranged from only 2 to ≈12 μg/L, whereas in most studies in adults in which correlations between insulin and leptin were reported, the range of circulating leptin concentrations was much wider, eg, 5–70 μg/L in one study (20). Furthermore, much of the relation between leptin and insulin is due to their correlations with adiposity, such that the relation between leptin and insulin is not always significant independently of variations in BMI and adiposity (20). In the present study, no positive relation between BMI and insulin was observed. However, a time-course study examining circulating leptin over time in relation to insulin responses to feeding (19) would likely provide more relevant information on insulin-leptin relations in this population.

In summary, circulating leptin concentrations are not higher in breast-fed than in formula-fed infants. Serum leptin concentrations are higher in female than in male infants and are significantly related to BMI, but not to serum insulin. Thus, a small but significant sex difference and a relation with adiposity are already present in young infants.

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