The relation of serum ghrelin, leptin and insulin levels to the growth patterns and feeding characteristics in breast-fed versus formula-fed infants

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The differences in growth patterns in breast-fed (BF) and formula-fed (FF) infants remain poorly understood. The aim of this study was to examine the relation of serum ghrelin and leptin concentrations to the different growth patterns between the formula-fed and breast-fed babies.

Feeding behaviors and anthropometric data were noted at the 3rd and 6th months of age. Serum ghrelin and leptin levels in both groups and breast-milk ghrelin and leptin levels in the mothers of the BF group were determined at the 3rd month of age.

Body weight, length, TSF (triceps skin fold thickness), postnatal weight gain, and serum ghrelin levels were higher in BF babies than in the FF group. In BF babies, serum ghrelin was correlated to TSF, and serum leptin was correlated to weight, TSF and weight gain at three months of age. As the serum leptin increased, energy intake from supplemental foods decreased in the BF group at the 6th month.

Higher serum ghrelin in BF babies might have played a role in their faster growth rate during the first three months of age. On the other hand, lower energy intake from supplemental foods in correlation with higher serum leptin in BF babies may explain why these babies show marked decline in growth rate compared to FF babies after three months of age.

Key words: breast-milk, formula, ghrelin, leptin, infant, anthropometry.

In the first year of life, different growth patterns have been identified in breast-fed (BF) and formula-fed (FF) infants. BF infants have accelerated growth rate in the first 3-6 months of age[1,2]. However, in the second six months of life, the growth rate of these infants decreases progressively, while it does not decrease to the same extent in the FF group[3,4]. The differences in growth patterns in these two groups of infants remain poorly understood. The neuroendocrine regulation of food intake is complex and involves the brain receiving both neuronal and hormonal inputs. Gut hormones have been found to be important in appetite regulation[5]. Stimulating hormones originating from the gastrointestinal system are important in the initiation, cessation and frequency of eating[5]. Recently, the studies have focused on ghrelin, a novel peptide hormone that is isolated from the rat stomach[6-8]. It has been concluded that ghrelin stimulates hunger and food intake[7].

Ghrelin is a natural antagonist to leptin. Leptin is secreted mainly by adipocytes, decreases food intake and increases metabolic rate[8]. The central effects of leptin and ghrelin are mediated, at least partly, through inhibition and stimulation, respectively, of the neuropeptide

Y/Y1 receptor pathway in the arcuate nucleus neurones in the hypothalamus[9]. Insulin, another hormone, also regulates body weight and energy metabolism.

In this study, our goal was to investigate the effect of various hormones, including ghrelin, leptin and insulin, on growth patterns and feeding characteristics of BF and FF infants during the first six months of life.

Material and Methods

Healthy term babies of approximately three to four months of age (range: 80-135 days) were enrolled in this prospective study. Those babies who were breastfed only formed the BF group and those who were given formula only since birth formed the FF group. None of the babies in either group received supplemental foods before four months of age. Premature or small for gestational age babies and those having infection or immunization 10 days prior to the study were excluded. The study was approved by the Ethical Committee of Dokuz Eylül University Faculty of Medicine, İzmir, Turkey (Project No: 04.KB.SAG.027). Signed informed consent was obtained from legal guardians of each infant.

Birth weight, feeding patterns and bowel movement patterns of the babies were queried via a questionnaire. The questionnaire was performed by face-to-face interview between the investigator (U.Y.) and the caregivers.

At the onset of the study, when the infants were around three-to-four months old, physical examinations including anthropometric measurements [body weight, length, head circumference, and triceps skin fold thickness (TSF)] were performed and body mass indexes (BMI) were calculated. In addition, venous blood samples of all babies were obtained for plasma leptin, ghrelin, insulin, and glucose measurement between 10:00 a.m. to 12:00 noon after 3-4 hours of fasting. At the same time, pre-feeding breast-milk samples of the mothers of BF infants were obtained for leptin and ghrelin determination.

When the babies were re-evaluated three months later, at around six months of age, timing of the introduction of supplemental foods, feeding characteristics, and daily energy and protein intake only from supplemental foods were queried, and anthropometric measurements were repeated. Blood and breast-milk hormone levels were not studied at that time.

For plasma ghrelin assay, 1-2 ml whole blood was drawn into a centrifuge tube containing 1.8 mg of EDTA plus 500 U of aprotinin per ml of blood (Sigma-Aldrich, Germany). Samples were transferred on ice, cold centrifuged, and plasma was separated and stored at -70°C until the assay.

For serum leptin, insulin and glucose, 3-4 ml whole blood was allowed to clot and then centrifuged at room temperature. The serum samples were transferred and stored at -70°C until the assay.

Breast-milk samples were taken into plain test tubes for leptin and to tubes containing 500 U of aprotinin per ml of milk for ghrelin assay. Milk samples were stored at -70°C until the assay. For leptin and ghrelin analyses, milk samples were thawed overnight at 4°C, and whole milk was sonicated for 3-10 second burst with 20-second cooling intervals.

Plasma and breast-milk ghrelin were measured by using a "peptide enzyme immunoassay" kit (Peninsula Laboratories, Inc., Texas, USA) with intra-assay and inter-assay variations of coefficients (CV) of less than 5% and 14%, respectively, sensitivity limit of 0.08-1.0 ng/ml, and measuring range of 0-25 ng/ml.

Breast-milk and serum leptin concentrations were analyzed with a "two-site immunoradiometric assay" (IRMA) kit (Diagnostic Systems Laboratories, Inc, Texas, USA) with intra-assay and inter-assay CV of 2.6% to 4.9% and 3.7% to 6.6%, respectively, sensitivity limit of 0.1 ng/ml, and measuring range of 0-120 ng/ml.

Serum insulin levels were measured with a "solid phase enzyme amplified sensitivity immunoassay" kit (BioSource, Belgium), and the results were expressed as µIU/ml. Serum glucose concentrations were measured with a commercially available kit (Roche Diagnostics, Germany) by an auto-analyzer (Roche Diagnostics Modular Analytics D-P, Japan).

Statistical Analyses

Breastfed and FF infants were compared with respect to anthropometric measurements, feeding and bowel movement characteristics, and hormone levels at three to four months of age. The same comparisons with the exception of hormone levels were repeated at the last visit when they were six months old. In addition, daily energy and protein intake and weight gain were compared at this period of the study. Furthermore, correlation of hormone levels with the evaluated parameters was analyzed. The data were analyzed using software SPSS (version 10.0; SPSS, Chicago, IL). Results were expressed as percentages and as means ± standard deviation (SD). Statistical analyses were carried out by the Wilcoxon signed rank test for difference in two related groups of measures, Mann-Whitney U test in two independent groups of measures, and Spearman and Pearson rank correlation analysis for the relation between two variables. A p value of <0.05 was considered significant in all analyses.

Results

There were a total of 47 babies (male/female = 25/22), of which, 24 (51%; 13 male; 100±9 days old) were in only BF and 23 (49%; 12 male; 97±17 days old) in FF groups. There were no differences between the two groups with respect to age, sex, and birth weight (Table I) ($p>0.05$).

Anthropometric Data, Feeding and Bowel Movement Characteristics

Mean body weight, length, TSF, and postnatal weight gain of babies were higher in the BF group than in the FF group at the first visit at 3-4 months of age ($p=0.017$, $p=0.016$, $p=0.001$, and $p=0.022$, respectively; Table I). Frequency and duration of feeding ($p=0.006$, $p=0.01$, respectively), feeding frequency in the daytime ($p=0.004$), and the frequency of bowel movements ($p=0.006$) were also higher in the BF than FF group. Nineteen (79.2%) of the BF babies were reported to have bowel movements during feeding. However, timing of the bowel movements was variable in FF babies.

We did not determine any difference between the two groups in relation to body weight, length, and TSF during the second visit at around six months of age. However, mean weight gain between the first and second visits was higher in the FF group compared to the BF group ($p=0.013$). Difference in the bowel movement pattern between the two groups disappeared at that time ($p=0.088$; Table I). Although feeding frequency and duration remained higher in the BF group, daily energy and protein intake from supplemental foods was lower in these babies than in FF babies [363.7±205.2 (range: 72-730) vs 890.3±341.3 (range: 440-1700) kcal, $p=0.001$; and 12.5±6.3 (range: 2-25) vs 31.7- 11.8 (17-60) g, $p=0.001$, respectively].

Serum Ghrelin and Leptin Levels and Their Relation to Anthropometric Data, Daily Energy Intake, and Bowel Movement Patterns

Table II shows the results of serum hormonal analyses of babies in the BF and FF groups. In BF babies, mean serum ghrelin was higher than in the FF group. The mean serum leptin, although slightly higher in the BF group, was not significantly different between the two groups. In both groups, serum ghrelin was not correlated with serum leptin, glucose or insulin. On the other hand, there was a positive correlation between serum ghrelin and TSF at the 3rd month of age in the BF group ($r=0.511$, $p=0.011$; Fig. 1), but not in the FF group. Similarly, serum leptin was also correlated with body weight, BMI, TSF, and postnatal weight gain only in BF babies ($r=0.469$, $p=0.028$; $r=0.564$, $p<0.010$; $r=0.556$, $p<0.007$ and $r=0.471$, $p<0.027$, respectively). On the other hand, as the serum leptin increased, energy intake from supplemental foods at the 6th month of age decreased in the BF group ($r=-0.502$, $p=0.048$; Fig. 2).

Serum Glucose and Insulin Levels in the BF and FF Groups

Mean serum glucose was not different between the two groups (Table II). Although the mean serum insulin concentration in FF babies was higher than in the BF group, the difference did not show statistical significance (Table II).

Breast-Milk Ghrelin and Leptin Concentrations and Their Relation to the Anthropometric Data and Bowel Movement Patterns

The mean ghrelin and leptin concentrations in breast-milk in the BF group were 0.8±0.5

TABLE I. Comparison of Anthropometric Data, Feeding and Bowel Movement Frequency of Breast-Fed and Formula-Fed Babies at Three and Six Months of Age

	Breast-fed group (n=24)			Formula-fed group (n=23)			P-value
	Mean	SD	Range	Mean	SD	Range	
Birth weight (g)	3300	400	2500-4200	3230	437	2500-3900	>0.05
Body weight (g)							
3 month	6421	719	5200-8000	5858	1045	4500-8300	<0.05
6 month	7903	942	5950-9700	7896	1236	5870-10500	>0.05
Body length (cm)							
3 month	61.4	1.2	57-66	59.8	2.8	56-66.5	<0.05
6 month	67.8	2.2	63-72	66.6	3.2	62-73	>0.05
Head circumference (cm)							
3 month	40.7	1	39-43	40.1	1.2	38-43	>0.05
6 month	43.3	0.8	42-44.5	43.4	1.2	40.5-45	>0.05
Postnatal weight gain (g)							
3 month	3104	630	2000-4800	2525	974	700-4700	<0.05
6 month*	1145	619	300-2400	2036	693	870-3150	<0.05
Triceps skinfold thickness (mm)							
3 month	12	2	7-16	10	1.8	7-14.5	<0.05
6 month	14.6	2.72	10-19	16.7	2.2	13-21.5	>0.05
Daily feeding frequency							
3 month	9.9	1.8	6-12	6.6	2.3	3-11	<0.05
6 month	8.5	2.5	5-15	2.2	1.9	1-8	<0.05
Daytime feeding frequency							
3 month	7.8	1.3	5-10	5.4	1.7	3-8	<0.05
6 month	6.1	2.1	2-10	1.2	1.6	0-6	<0.05
Nighttime feeding frequency							
3 month	2.2	1.1	0-4	1.2	1	0-3	>0.05
6 month	2.4	1.3	0-5	0.9	0.5	0-2	<0.05
Daily feeding duration (min.)							
3 month	13.5	7.4	5-30	9.3	2.3	5-15	<0.05
6 month	10.2	5.1	5-30	8	2.1	5-12	<0.05
Daily bowel movement frequency							
3 month	2.2	1.3	0.5-4	1.4	0.8	0.5-4	<0.05
6 month	1.5	0.9	0.5-4	1.8	0.9	1-4	>0.05
Daily mean energy intake from supplemental foods (kcal)							
6 month*	364	205	72-730	890	341	440-1700	<0.05
Daily mean protein intake from supplemental foods (g)							
6 month*	12.5	6.3	2-25	31.7	11.8	17-60	<0.05

*Between 3rd and 6th months of life.
In evaluation of two independent groups, Mann-Whitney U test was used.

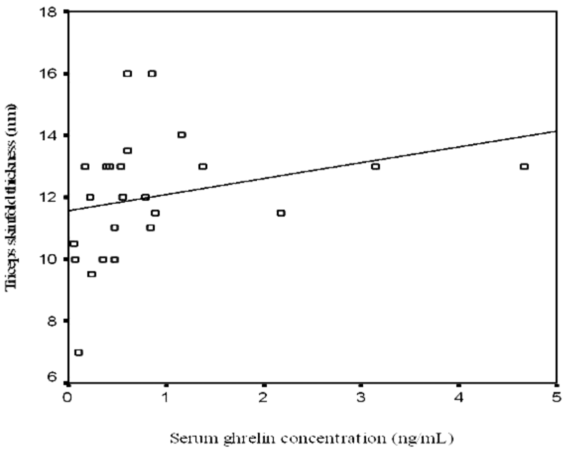

Figure 1. Positive correlation between serum ghrelin concentrations and triceps skinfold thickness of breastfed babies at three to four months of age (r=0.511, p<0.011).

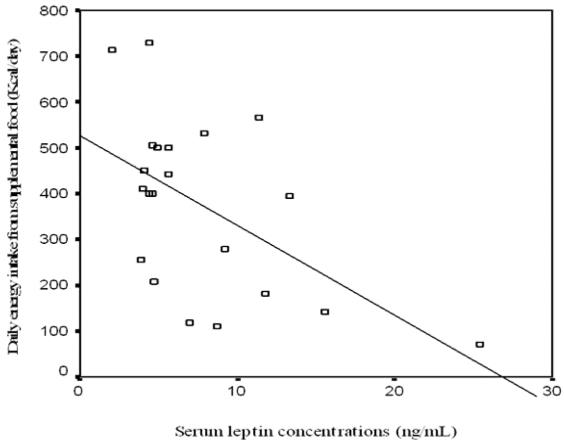

Figure 2. Negative correlation between serum leptin concentrations and energy intake from supplemental foods at six months of age in the breast-fed group (r=-0.502, p<0.048).

TABLE II. Comparison of Serum Ghrelin, Leptin, Insulin and Glucose Concentrations of Breast-Fed and Formula-Fed Babies at Three Months of Age

	Breast-fed group (n=24)			Formula-fed group (n=23)			P-value
	Mean	SD	Range	Mean	SD	Range	
Ghrelin (ng/ml)	0.9	1.1	0.05-4.7	0.6	1.0	1.1-4.1	<0.05
Leptin (ng/ml)	7.9	5.2	2.1-25.4	6.8	7.1	0.3-31.6	>0.05
Insulin (μIU/ml)	9.5	4.3	4.4-18.4	12.1	11.4	3.9-59.7	>0.05
Glucose (mg/dl)	81.7	9.3	59.0-96.0	80.0	15.5	46.0-114.0	>0.05

In evaluation of two independent groups, Mann-Whitney U test was used.

(range: 0.2- 2.5) ng/ml and 0.4±0.2 (range: 0.2-0.9) ng/ml, respectively. Neither breast-milk ghrelin nor breast-milk leptin was correlated with the respective serum ghrelin or leptin, anthropometric data or bowel movement patterns of the infants in the BF group.

Discussion

Although growth in early childhood is related to many factors, such as the intrauterine environment, birth weight, sex, parental height, and nutrition, the possible effects of other undiscovered regulatory mechanisms on growth have also been suggested[1,2,10]. Nutrition is one of the most important variables affecting growth, and the importance of nutrition on early childhood growth is further emphasized by the marked differences in growth rates between BF and FF infants during the first three months of life[1,2,4,11]. The babies in the BF group in our study also showed increased growth rate during the first three to four months of life. The lack of difference in size at birth excludes any initial size-based selection bias in forming the feeding groups.

Our results suggest that differences in growth patterns between BF and FF babies in early infancy may be related to the difference in serum ghrelin levels. Higher serum ghrelin in BF babies may stimulate appetite via faster gastric emptying so that BF babies feed more frequently and for a longer duration. As a result, BF babies grow more rapidly than FF babies during the first three to four months of life.

Additionally, more frequent bowel movements and moving their bowels during breastfeeding may be related to higher serum ghrelin in BF groups[12,13]. Furthermore, we speculate that breast-milk ghrelin can also contribute to increased gastrointestinal motility via affecting the gastrointestinal system directly.

Controversial relationships between ghrelin and leptin are reported in many studies[14,15]. Both of these hormones affect the same areas in the central neuronal system. While ghrelin stimulates appetite and food intake, leptin conducts satiety signals and increases energy consumption[5]. We did not find any correlation between leptin and ghrelin concentrations in our study population. In the literature, the relationship between ghrelin and leptin concentration was mainly studied in pathologic states like anorexia nervosa and obesity[16,17]. Further studies are needed to better understand the interaction between ghrelin and leptin in different physiologic conditions.

Lower energy intake from supplemental foods in correlation with higher serum leptin in BF babies may be the underlying cause of evident decline in weight gain in BF babies compared to FF babies. In our study, while serum leptin correlated with anthropometric data in BF babies, such correlations were not present in the FF group. FF infants ingested higher energy and protein from supplemental food and their serum ghrelin and leptin were lower than in the BF group. These findings suggest that the body weight regulatory function of leptin was more effective in BF infants. It is suggested that breast-milk leptin and/or high serum leptin concentrations of BF babies are important in food intake and appetite. In our study, the finding of increased fat tissue in BF babies at three months of age may have caused high serum leptin concentrations, which in turn decreases food intake and results in lower weight gain in the second three months of life.

The weakness of our study, on the other hand, is that there was only one spot measurement of ghrelin and leptin levels in BF and FF infants. Although the levels of these hormones seem to be related to feeding and bowel movement characteristics and growth in this population, serial measurement of ghrelin and leptin would be more informative in future studies.

In conclusion, differences in growth patterns between BF and FF babies in early infancy may be related to the higher serum ghrelin in BF babies. Lower energy intake from supplemental foods in correlation with higher serum leptin in BF babies may explain why BF babies show evident decline in weight gain compared to FF babies. BF infants self-regulate their food intake, which is likely controlled by hormonal mechanisms such as leptin and ghrelin. This physiologic state may be disturbed by formula feeding. Long-term prospective studies are needed to reveal the effects of various hormones on feeding patterns and growth from infancy to adulthood.

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