ORIGINAL ARTICLE

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The Role of Breastfeeding in Postpartum Weight Loss; Relationship between Maternal Plasma Ghrelin, Serum Leptin and Lipid Levels with Breast Milk

ABSTRACT
Objective: The aim of this study is to examine the relationship between blood ghrelin, leptin and lipid levels of breast milk and maternal weight loss in postpartum period.
Methods: Sixty-two mothers were subdivided according to babies dietary patterns; as breastfed (BF), BF plus formula-fed (BF+FF) and formula-fed (FF). All participating mothers were evaluated twice.
Results: On the first visit, in BF group total/active ghrelin ratio and serum leptin levels were lower than FF group ($p<0.05$). The milk leptin levels of BF group were higher than BF+FF group on both visits ($p<0.001$, $p<0.05$ respectively). On the second visit, in BF group, body mass index (BMI) were decreased when compared with first visit ($p<0.05$). On the first visit, there was no correlation between milk leptin levels and BMI in lactating mothers. However a negative correlation between milk leptin and total cholesterol levels in lactating mothers ($p<0.01$) and positive correlation between milk active ghrelin and triglyceride levels in BF group ($p<0.05$). There was no correlation between ghrelin, leptin and lipid levels in breast milk and maternal blood.
Conclusion: The low serum leptin levels and total/active ghrelin in lactating mothers than non-lactating are thought the role of these hormones, which passes to the baby through breast milk, on losing weight. Additionally, the higher milk leptin levels in only BF group than BF+FF group, shows that the role of protective effect of leptin in only breastfeeding infants. Breastfeeding should be prompted more to prevent obesity.
Keywords: Lactation, Breast Milk, Ghrelin, Leptin, Lipid

Doğum Sonrası Kilo Kaybında Emzirmenin Rolü; Anne Plazma Ghrelin, Serum Leptin ve Lipid Düzeylerinin Anne Sütüyle İlişkisi

ÖZET
Amaç: Bebeklerin beslenme şekline göre anne sütü (AS) ghrelin, leptin ve lipid düzeylerinin anne kan düzeyleri ve annenin antropometrisi ile ilişkisini araştırmak.
Gereç ve Yöntem: Çalışma grubunu oluşturan 62 anne bebeklerin beslenme şekillerine göre Anne Sütü (AS), AS + formül mama (AS+FM), formül mama (FM) olarak üç gruba ayrıldı. Tüm anneler laktasyonun ortalama ikinci ve beşinci ayında iki kez değerlendirildi.
Bulgular: İlk değerlendirmede AS grubunda formül mama (FM) grubu annelerine göre plazma total / aktif ghrelin oranı ve serum leptin düzeylerinin düşük olduğu saptandı ($p<0.05$). AS grubunun süt leptin düzeyi AS+FM grubuna göre ilk ve ikinci değerlendirmede yüksekti (sırasıyla $p<0.001$, $p<0.05$). AS grubu annelerinin ikinci değerlendirmede VKİ'lerinin ilk değerlendirmeye göre azaldığı saptandı ($p<0.05$). İlk değerlendirmede emziren annelerin süt leptin düzeyleri ile annelerin VKİ'leri arasında ilişki saptanmadı. Emziren annelerde süt leptin düzeyleri ile TK düzeyleri arasında negatif ilişki varken sadece AS grubunda süt aktif ghrelin ile TG düzeyleri arasında pozitif ilişki vardı (sırasıyla $p<0.01$, $p<0.05$). Anne kanı ile AS arasında ise G-HH, leptin ve lipid düzeyleri arasında herhangi bir ilişki saptanmadı.
Tartışma: Emziren annelerin plazma total / aktif ghrelin oranı ve serum leptin düzeylerinin emzirmeyenlere göre düşük olması emziren annelerin kilo vermesinde bu hormonların rol aldığını ve bu hormonların AS yoluyla bebeğe geçtiğini düşündürmektedir. Ayrıca sadece AS ile beslenen bebeklerde süt leptin düzeyinin AS+FM grubuna göre yüksek olması sadece AS ile beslenen bebeklerin obeziteden korunmasında rolü olduğunu göstermektedir. Anne ve bebeklerin obeziteden korunması için emzirme daha çok teşvik edilmelidir.
Anahtar Kelimeler: Laktasyon, Anne Sütü, Ghrelin, Leptin, Lipid

INTRODUCTION

Postpartum weight retention can contribute to maternal long-term obesity and associated adverse health sequellae of cardiovascular disease, hypertension, diabetes and degenerative joint disease, among others (1,2). It has been associated with many factors such as increased gestational weight gain, exercise frequency, dietary choices, smoking status, educational attainment (2-6). It is known that breastfeeding mothers give their weights more quickly than non-lactating, which gained during pregnancy. Since Rutishauser and Carlin (7) showed mothers' weight and their breastfeeding durations inversely related, many studies have been done to support them (8,9). In addition, breastfeeding protecting babies against later obesity and hormones and growth factors such as ghrelin, leptin, insulin, adiponectin, epidermal-growth factor, platelet-derived growth factor and insulin-like growth factor-1 are located which effect on energy metabolism in breast milk (10-14). Ghrelin, produced in the gastrointestinal system, is a peptide hormone discovered recently effective on eating behavior and body weight (BW) regulation with central effect (11,15). A portion of ghrelin possesses a unique fatty acid modification, an *n*-octanoylation, at Ser 3 (16,17). Of the two ghrelin forms, acylated and des-acylated, the acylated forms (known as active ghrelin) is thought to be essential for binding to the growth hormone secretagogue receptor 1a (16-18). Desacyl ghrelin, however, is no totally inactive, has influence on cell proliferation and adipogenesis and counteracts the metabolic action of active ghrelin (19,20). Total ghrelin is sum amount of desacyl and acylated ghrelin. Leptin is a hormone that provides information to the brain about body fat stores. Leptin reduces appetite and increases metabolic rate when reached to the hypothalamus (21).

The aim of this study is to examine the type of feeding babies, in lactating and non-lactating mothers, the relationship between blood ghrelin, leptin and lipid levels breast milk and maternal weight loss in postpartum period.

MATERIAL AND METHODS

This study was performed with 62 mothers (17-42 year-old) in postpartum period (30-91 days) in Eskisehir, Turkey. The study procedure was approved by Eskisehir Osmangazi University Local Ethical Committee, and informed consent was received from all participants. This study was financially endorsed by Eskisehir Osmangazi University Scientific Study Research Commission (200711033).

Mothers were subdivided according to feeding patterns of infants; as breastfed (BF), BF plus formula-fed (BF+FF) and formula-fed (FF). All participating mothers were evaluated twice (6th weeks and 6th months) in postpartum period. Prenatal BW of mothers and their total weight increments during pregnancy were recorded and anthropometric measurements [mid-arm circumference (MAC), triceps skinfold thickness (TST), body mass index (BMI)] were performed by the same physician (A.K.). BMI levels were calculated with the formula "Weight (kg) / Height $(m)^2$".

Exclusion criterias were complicated pregnancies (preterm delivery, any existing disease), cesarian delivery, any type of hormonal or drug therapy, smoking, BMI greater than 35.0% at enrolment and with changing feeding practices of infants between first and second visits.

Blood and serum samples (6 mL) were obtained at around 09:00 A.M. before breakfast. At the first visit 4 mL blood samples were taken from mothers were stored at -80°C for leptin, triglyceride (TG), total cholesterol (TC), HDL-C and LDL-C levels. Before storage, all samples were centrifuged at 4000 rpm for 5 minutes and serums were excluded. The remaining 2 mL blood samples were centrifuged at 4000 rpm for 5 minutes in Ethylene Diamine Tetra- Acetic Acid (EDTA); plasmas were excluded for total and active ghrelin and stored at -80°C. Before storage each sample (2 mL) were added in Eppendorf tubes. Breast milk samples (6 mL) were obtained at the beginning of nursing at both first and second visits. Each sample (2 mL) was stored in Eppendorf tubes for TG and TC levels. The remaining 4 mL breast milk samples were stored at -80°C for active and total ghrelin and leptin levels. Before storage, all samples were centrifuged at 4000 rpm for 15 minutes; supernatant lipid layers were excluded, and samples were centrifuged again. One set of milk and blood samples was acidified, by adding 1 N HCL (10% of volume; pH≈3-4), to stabilize the labile side chain of active ghrelin and prevent a rapid deacylation of ghrelin, as suggested (22). To protect peptides from proteolysis, 500 IU 10 μL of aprotinin was added per milliliter of sample (23).

Total and active ghrelin levels were measured by using a commercially available radioimmunoassay kit from Linco Research. Leptin levels were studied with enzyme-linked immunosorbent assay (ELISA) using the DRG-Human Leptin kit. Breast milk samples were diluated at 1/30 ratio after homogenization with vortex for analyzing TG and TC levels. Both serum and breast milk TG and TC, serum HDL-C and LDL-C levels were studied using homogen colorimetric methods with Roche Modular D-P and Triglycerides GPO and Cholesterol CHOD-PAP Biolabo kits.

Statistical analysis was performed using the SPSS package program version 13.0. The appropriately distributed (parametric) data are given as arithmetic means ± standard deviation (SD) and not appropriately distributed (non-parametric) data are given as median (minimum-maximum) for. The comparison between groups was determined by the *Student's t* test *or Mann-Whitney U* test (parametric data, non-parametric data respectively). Differences

in breast milk concentration of the parameters changes with time periods (e.g., first and second visit) were examined using *Paired-samples* test or *Mann-Whitney U* test (parametric data, non-parametric data respectively). Correlations were assessed by *Pearson's* test or *Spearman's* test (parametric data, non-parametric data respectively). The p-values less than 0.05 were considered significant in all tests.

RESULTS

Sixty two mothers (17-42 years old) were enrolled. Forty-two percent (n=26) of mothers were fully BF, 25.8% (n=16) were FF and 32.2% (n=20) were BF+FF. Numbers, ages, weight gain during pregnancy, first and second visit anthropometric values of mothers were similar between the groups (p>0.05, Table 1). On the second visit BW and BMI measurements were determined lower compared with first visit in only BF group (p<0.05, Table 2).

At the first visit total/active ghrelin ratio and serum leptin levels were determined lower in BF group compared with FF (p<0.05, Table 3). There was a positive correlation with serum leptin level and BMI only in BF+FF group (p<0.05). On first and second visit there were no differences between breast milk total and active ghrelin, TG and TC levels in BF and BF+FF groups. However breast milk leptin level were determined higher in BF group than BF+FF group on both visits (p<0.001, p<0.05, respectively, Table 4).

Breast milk total and active ghrelin levels were lower than plasma levels in both BF and BF+FF groups (In BF group: total and active ghrelin p<0.001; In BF+FF group total ghrelin: p<0.01, active ghrelin: p<0.001). In addition, breast milk leptin levels were lower than serum leptin levels in BF and BF+FF groups (p<0.001).

Table 1 Comparison of anthropometric measurements of mothers according to groups in first and second visit (n=62)

		BF Group (n=26)	***FF Group (n=16)***	***BF + FF Group (n=20)***	***P***
Age (year)		28.23±5.7	28.60±5.31	26.20±4.60	p1>0.05
Weight gain during pregnancy (kg)		14.26±5.14	12.93±5.86	12.85±3.78	p2>0.05
First Visit	***Body Weight (kg)***	65.74±9.74	64.50±11.49	65.26±14.44	p3>0.05
	Height (cm)	160.1±5.85	159.20±7.56	160.70±7.62	
	BMI (kg/m²)	25.74±4.45	25.64±4.07	25.16±5.15	
	TST (mm)	23.38±6.96	25.33±8.07	24.50±8.11	
	MAC (cm)	26.87±4.03	26.82±5.93	26.93±4.02	
Second visit	***Body Weight (kg)***	64.40±10.16	64.20±10.36	64.52±15.02	
	BMI (kg/m²)	25.24±4.68	25.34±4.03	24.99±5.72	
	TST (mm)	22.32±6.95	24.93±8.02	21.67±7.84	
	MAC (cm)	26.82±5.93	26.52±4.86	27.32±3.72	

BMI: Body Mass Index, TST: Triceps Skinfold Thickness, MAC: Mid-arm Circumference
p1: Between BF and FF, p2: BF and BF + FF, p3: FF and BF + FF groups.

Table 2 Comparison of anthropometric measurements of groups in first visit with second visit (n=62)

Group		***First visit***	***Second visit***	***p***
BF	***Body Weight (kg)***	65.74±9.74	64.40±10.16	**<0.05**
	BMI (kg/m^2)	25.74±4.45	25.24±4.68	
	MAC (cm)	26.87±4.03	26.82±5.93	>0.05
	TST (mm)	23.38±6.96	22.32±6.95	
BF+FF	***Body Weight (kg)***	65.26±14.44	64.52±15.02	
	BMI (kg/m^2)	25.16±5.15	24.99±5.72	
	MAC (cm)	26.93±4.02	27.32±3.72	
	TST (mm)	24.50±8.11	21.67±7.84	
FF	***Body Weight (kg)***	64.50±11.49	64.20±10.36	
	BMI (kg/m^2)	25.64±4.07	25.34±4.03	
	MAC (cm)	26.82±5.93	24.93±8.02	
	TST (mm)	25.33±8.07	26.52±4.86	

BMI: Body Mass Index, MAC: Mid-arm Circumference, TST: Triceps Skinfold Thickness,
p1: Between BF and FF, p2: BF and BF + FF, p3: FF and BF + FF groups.

Table 3 Comparison of blood ghrelin, leptin and lipid levels of mothers in first visit

	BF Group (n=26)	*FF Group (n=16)*	*B F+ FF Group (n=20)*	*P*
Total ghrelin (pg/mL)	391.70±101.52	461.66±17.12	422.19±125.85	p1>0.05
Active ghrelin (pg/mL)	29.08±11.17	29.07±2.20	31.26±16.95	p2>0.05 p3>0.05
Total/active ghrelin	15.79±8.29	26.08±1.92	18.58±1.42	**p1<0.05**
Leptin (ng/mL)	18.03±1.17	29.23±15.67	23.36±1.78	p2>0.05 p3>0.05
Total cholesterol (mg/dL)	175.57±39.71	164.53±30.05	168.35±20.76	p1>0.05
LDL-C (mg/dL)	103.96±33.30	92.86±22.40	104.35±17.09	p2>0.05 p3>0.05
HDL-C (mg/dL)	58.26±14.83	58.13±14.51	53.20±9.86	
Triglyceride (mg/dL)	106±65.69	97.6±32.45	101.85±35.19	

p1: Between BF and FF, p2: BF and BF + FF, p3: FF and BF + FF groups.

Table 4 Comparison of breast milk ghrelin, leptin and lipid levels according to groups in first and second visit (n=46)

	First visit			**Second visit**		
	BF Group	***BF+ FF Group***	***p***	***BF Group***	***BF + FF Group***	***p***
Total ghrelin (pg/mL)	288.9±63.3	319.65±10.70	>0.05	234.9±83.6	230.7±73.12	>0.05
Active ghrelin (pg/mL)	11.87±2.54	13.40±3.64		15.33±3.90	17.01±1.41	
Leptin (ng/mL)	0.33(0.28-0.50)	0.22±0.05	**<0.001**	0.26(0.14-0.48)	0.18(0.12-0.46)	**<0.05**
Triglyceride (g/dL)	4.54±1.49	4.38±1.72	>0.05	2.50±1.04	1.92±0.79	>0.05
Total cholesterol (g/dL)	0.14(0.12-0.18)	0.14±0.01		0.12(0.11-0.16)	0.12(0.11-0.15)	

The data in the first line show mean ± SD values for normal distribution, median for abnormal distribution, in the second line show minimum and maximum values.

When the breast milk samples were examined at the first evaluation; there were negative correlation between leptin and total ghrelin, TC levels in all lactating mothers ($p<0.05$, $p<0.01$ respectively). Whereas there was positive correlation between active ghrelin and TG only in BF group ($p<0.05$). When the relationship were examined between breast milk and plasma ghrelin with serum leptin and lipid levels; there was negative correlation between serum HDL-C and breast milk leptin levels in lactating mothers ($p<0.01$). In addition; negative correlation were determined between serum TC with leptin and TC levels in breast milk in BF group ($p<0.05$). We determined positive correlation between plasma total ghrelin with breast milk active ghrelin levels, nevertheless there is no relationship between mother serum with breast milk leptin and TG levels.

DISCUSSION

Lactation is a physiological state characterized by a large energy demand due to milk production. There have been reported that breast milk contains some hormones effecting on energy metabolism, appetite and food intake in recent studies (11,13). Aydin et al. (23) firstly have showed ghrelin in breast milk. The source of ghrelin in breast milk is not clear. There are some studies claiming to be due to maternal plasma (23) and the others claim synthesized in mammary gland (24,25). Dundar et al. (26) were found no relationship between colostrum and serum total ghrelin levels, however, higher active ghrelin levels than serum. In addition, source of ghrelin has been suggested due to both mammary gland and serum, because of positive correlation between colostrum and serum total and active ghrelin levels (26). According to our study results we think that ghrelin is synthesized only in mammary gland because of finding lower breast milk ghrelin than plasma and not showing relationship between breast milk and plasma ghrelin levels. Interaction becomes between ghrelin and hormones such as progesteron, prolactin and estrogen during lactation period in lactating mothers. Aydin et al. (23) showed that plasma ghrelin levels were lower in lactating mother than non-lactating. Although studies showing mostly negative relationship there are studies showing positive relationship or whether any relationship between plasma ghrelin and BMI (15,23). Aydin et al. (23) had suggested that the lack of relationship

between BMI with plasma and breast milk ghrelin levels are connected with higher BMI of lactating mothers and increase plasma ghrelin levels after birth (27).

According to our study results although BMI values of lactating and non-lactating mothers are similar; we determined plasma total/active ghrelin ratio of lactating mother were significantly and the plasma total and active ghrelin levels were not statistically significant than non-lactating. We found no significant relation between ghrelin levels in breast milk and BMI. In BF group, BW were decreased at second visit according to the first visit, there was no change in BF+FF and FF groups. Also we have determined decreased breast milk total ghrelin, leptin, TG and TC and increased active ghrelin levels of lactating mothers in second evaluation according to the first. Whereas in one study increased total ghrelin and decreased active ghrelin later days of lactation were demonstrated (25). This decrease in breastmilk hormones and lipids are thought that breast-feeding is important at loosing weight of mothers.

Leptin is an indicator of body fat tissue (21). Whereas the ghrelin is initiating the action of eating by stimulating the appetite, leptin terminates eating by reducing it. Leptin shows anti-obesity effect by reducing energy intake, as well as increasing energy expenditure by different mechanisms (28). It has been reported that leptin is synthesized in breast tissue and stored in fat globules of breast (29). Studies have shown that breast milk leptin levels are lower than maternal serum (30-32). Similarly, we found that leptin level in breast milk lower than maternal serum. This finding is an indicator of leptin in breast milk synthesized in breast.

Maffei et al. (33) had showed that leptin had been a heterogeneous distribution among people with similar BMI. Leptin resistance develops with the progression of age and weight gain (34). Although Ucar et al. (31) are not reported any correlation between maternal plasma leptin levels and their BMI; positive correlation between body fat percentage and BMI with serum leptin levels in many studies had been demonstrated (35,36). In our study, there were positive correlation between serum leptin levels and BMI of mothers both first and second evaluation only in BF+FF group; however no correlation in BF and FF groups was observed.

In some studies, positive correlation had been reported between breast milk leptin and maternal BMI, however in some this relationship have not been demonstrated (23,30,31). Furthermore some studies have shown positive correlation between breast milk leptin and maternal BW and BMI in early period; however this association disappears in later period (37). Additionally, diurnal rhythm of leptin is inhibited in lactating mothers. Maternal serum leptin level during lactation period was found low in some studies (38,39). Denis et al. have suggested that mothers ate more food in lactation period depending on hypoleptinemia (38). Yu et al. (40) have shown a dose-dependent association between leptin and increased prolactin in rats; whereas Butte et al. (41) showed negative correlation in women during lactation. In our study, BW were lower at second visit than first only in fully breastfeeding mothers and in BF group total/active ghrelin ratio and serum leptin levels were significantly lower than FF group. Therefore, to say the role of total/active ghrelin ratio as well as leptin level, losing weight despite eating more food seems to be possible. Ghrelin and leptin show opposite interaction with each other (Ying-Yang principle) in organism under physiological condition. However, the relationship between ghrelin and leptin in humans has not been fully clarified. Some studies show no correlation (15,42) and the others show negative correlation (43,44). We did not observe any relationship between plasma ghrelin and serum leptin levels, however, found negative correlation between breast milk total ghrelin and leptin levels. Nevertheless Aydin et al. (32) claim that there is no relationship between breast milk ghrelin and leptin levels.

Kierson et al. (24) have showed that total ghrelin amount in whole breast milk is more than skimmed breast milk and positive correlation between total ghrelin and amount of fat. In the present study, positive correlation between total ghrelin and TC, also active ghrelin and TG in breast milk. Although determining negative correlation between breast milk leptin and TC levels, we did not find with TG levels. This finding was similar to those observed in Houseknecht et al. (30). These results show that ghrelin and leptin in milk be associated with lipids. In our study, positive correlation between plasma active ghrelin with serum LDL-C and TC levels were determined in BF group. However, there was no relationship between total ghrelin and serum lipids. In FF group, there were negative correlations only between plasma active ghrelin and serum TC levels. Shiotani et al. (45) had showed negative correlation between plasma active ghrelin and LDL-C.

It has been reported that ghrelin transporting with HDL-C (15,46). As well as studies showing negative correlation between serum ghrelin and TG, positive correlation with TC; also there are some studies showing no association (47,48). However, we found positive correlation between total ghrelin with TC and active ghrelin with TG levels in breast milk. Because of not studied HDL-C in breast milk, we could not comment about it.

In the literature, positive correlation had been reported between maternal serum with total and active ghrelin in colostrum, also between leptin levels of maternal serum and breast milk (23,32). In our study, we found positive correlation only

between plasma total ghrelin and breast milk active ghrelin level and could not find any correlation between breast milk and serum leptin levels.

Ucar et al. (31) concluded that there is no relationship between breast milk leptin and serum TC, LDL-C levels; however, could not show any correlation between serum TG and HDL-C levels. In our study, we observed negative correlation between breast milk leptin and serum HDL-C in lactating mothers, between breast milk leptin and serum TC levels in BF group. We did not observe any association in BF+FF group. No association between serum leptin and lipid levels were found in several studies (31,48,49). Ucar et al. (31) observed negative correlation between plasma leptin and serum TG but not serum TC and LDL-C. We could not find any correlation between serum leptin and lipid levels in all groups. Nevertheless, we found negative correlation between serum and breast milk TC levels only in BF group.

CONCLUSION

The low total/active ghrelin ratio and serum leptin level of fully breastfeeding mothers suggest that these hormones have a significant effect on loosing weight of nursing mothers and passing through to the baby with breast milk. The higher breast milk leptin level in BF group than BF+FF and lower BMI values of mothers at second visit than first evaluation only in BF group show that breastfeeding protecting both mothers and babies from obesity. Breastfeeding should be prompted more to prevent obesity.

REFERENCES

1. Rooney BL, Schauberger CW. Excess pregnancy weight gain and long-term obesity: one decade later. Obstet Gynecol 2002;100(2):245-52.
2. Linne Y, Neovius M. Identification of women at risk of adverse weight development following pregnancy. Int J Obesity 2006;30(8):1234-9.
3. Brawarsky P, Stotland NE, Jackson RA, et al. Pre-pregnancy and pregnancy-related factors and the risk of excessive or inadequate gestational weight gain. Int J Gynecol Obstet 2005;91(2):125-31.
4. Gunderson EP, Quesenberry CP, Lewis CE, et al. Development of overweight associated with childbearing depends on smoking habit: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Obes Res 2004;12(12):2041-53.
5. Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. Am J Obstet Gynecol 2009;201(1):58.
6. Oken E, Taveras EM, Popoola FA, et al. Television, walking, and diet: associations with postpartum weight retention. Am J Prev Med 2007;32(4):305-11.
7. Rutishauser IHE, Carlin JB. Body mass index and duration of breast feeding: a survival analysis during the first six months of life. J Epidemiol Community Health 1992;46(6):559-65.
8. Oddy WH, Li J, Landsborough L, et al. The association of maternal overweight and obesity with breastfeeding duration. J Pediatr 2006;149(2):185-91.
9. Donath SM, Amir LH. Maternal obesity and initiation and duration of breastfeeding: data from the longitudinal study of Australian children. Matern Child Nutr 2008;4(3):163-70.
10. Li R, Fein SB, Grummer-Strawn LM. Do Infants Fed From Bottles Lack Self-regulation of Milk Intake Compared With Directly Breastfed Infants? Pediatrics 2010;125(6):1386-93.
11. Savino F, Fissore MF, Liguori SA, et al. Can hormones contained in mothers' milk account for the beneficial effect of breast-feeding on obesity in children? Clin Endocrinol 2009;71(6):757-65.
12. Petruchke T, Rotrig K, Hauner H. Transforming growth factor beta inhibits the differentiation of human adipocyte precursor cells in primary culture. Int J Obes Metab Disord 1994;18(8):532-6.
13. Hamosh M. Bioactive factors in human milk. Pediatr Clin North Am 2001;48(1):69-86.
14. Claud EC, Savidge T, Walker WA. Modulation of human intestinal epithelial cell IL-8 secretion by human milk factors. Pediatr Res 2003;53(3):419-25.
15. Van der Lely AJ, Tschöp M, Heiman ML, et al. Biological, Physiological, Pathophysiological, and Pharmacological Aspects of Ghrelin. Endocrine Reviews 2004;25(3):426-57.
16. Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005;85(2):495-522.
17. Hosoda H, Kojima M, Kangawa K. Biological, physiological, and pharmacological aspects of ghrelin. J Pharmacol Sci 2006;100(5):398-410.
18. Davenport AP, Bonner TI, Foord SM, et al. International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution and function. Pharmacol Rev 2005;57(4):541-6.
19. Bedendi I, Alloatti G, Marcantoni A, et al. Cardiac effects of ghrelin and its endogenous derivates des-octanoyl ghrelin and des-Gln14-ghrelin. Eur J Pharmacol 2003;476(1-2):87-95.
20. Broglio F, Gottero C, Prodam F, et al. Non-acylated ghrelin counteracts the metabolic but not the endocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab 2004;89(6):3062-5.
21. Bluher S, Mantzoros CS. The role of leptin in regulating neuroendocrine function in humans. J Nutr 2004;134(9):2469-74.

22. Hosoda H, Doi K, Nagaya N, et al. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem 2004;50(6):1077-80.
23. Aydin S, Aydin S, Ozkan Y, et al. Ghrelin is present in human colostrum, transitional and mature milk. Peptides 2006;27(4):878-82.
24. Kierson JA, Dimatteo DM, Locke RG, et al. Ghrelin and cholecystokinin in term and preterm human breast milk. Acta Paediatr 2006;95(8):991-5.
25. Ilcol YO, Hizli B. Active and total ghrelin concentrations increase in breast milk during lactation. Acta Paediatr 2007;96(11):1632-9.
26. Dundar NO, Dundar B, Cesur G, et al. Ghrelin and Adiponectin Levels in Colostrum, Cord Blood and Maternal Serum. Pediatr Int 2010;52(4):622-5.
27. Savino F, Liguori SA, Oggero R, et al. Maternal BMI and serum leptin concentration of infants in the first year of life. Acta Paediatr 2006;95(4):414-8.
28. Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regülation in ob/ob mice, Science 1995;269(5223):540-3.
29. Smith-Kirwin SM, O'Connor DM, De Johnston J, et al. Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 1998;83(5):1810-3.
30. Houseknecht KL, McGuire MK, Portocarrero CP, et al. Leptin is present in human milk and is related to maternal plasma leptin concentrations and adiposity. Biochem Biophys Res Commun 1997;240(3):742-7.
31. Ucar B, Kirel B, Bor O, et al. Breast milk leptin concentrations in initial and terminal milk samples: relationships to maternal and infant plasma leptin concentrations, adiposity, serum glucose, insulin, lipid and lipoprotein levels. J Pediatr Endocrinol Metab 2000;13(2):149-56.
32. Aydin S, Ozkan Y, Erman F, et al. Presence of obestatin in breast milk: relationship among obestatin, ghrelin, and leptin in lactating women. Nutrition 2008;24(7-8):689-93.
33. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1(11):1155-61.
34. Wang J, Obici S, Morgan K, et al. Overfeeding rapidly induces leptin and insulin resistance. Diabetes 2001;50(12):2786-91.
35. Petridou E, Mantzoros CS, Belechri M, et al. Neonatal leptin levels are strongly associated with female gender, birth length, IGF-I levels and formula feeding. Clin Endocrinol (Oxf) 2005;62(3):366-71.
36. Savino F, Costamagna M, Prino A, et al. Leptin levels in breast-fed and formula-fed infants. Acta Paediatr 2002;91(9):897-902.
37. Bielicki J, Huch R, von Mandach U. Time-course of leptin levels in term and preterm human milk. Eur J Endocrinol 2004;151(2):271-6.
38. Denis RG, Williams G, Vernon RG. Regulation of serum leptin and its role in the hyperphagia of lactation in the rat. J Endocrinol 2003;176(2):193-203.
39. Johnstone LE, Higuchi T. Food intake and leptin during pregnancy and lactation. Prog Brain Res 2001;133(1):215-27.
40. Yu WH, Kimura M, Walczewska A, et al. Role of leptin in hypothalamic-pituitary function. Proc Natl ACad Sci USA 1997;94(3):1023-8.
41. Butte NF, Hopkinson JM, Nicolson MA. Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab 1997;82(2):585-9.
42. Korbonits M, Goldstone AP, Gueorguiev M, et al. Ghrelin-a hormone with multiple functions. Front Neuroendocrinol 2004;25(1):27-68.
43. Ikezaki A, Hosoda H, Ito K, et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes 2002;51(12):3408-11.
44. Ng PC, Lee CH, Lam CW, et al. Ghrelin in preterm and term newborns: relation to anthropometry, leptin and insulin. Clin Endocrinol (Oxf) 2005;63(2):217-22.
45. Shiotani A, Miyanishi T, Uedo N, et al. Helicobacter pylori infection is associated with reduced circulating ghrelin levels independent of body mass index. Helicobacter 2005;10(5):373-8.
46. Beaumont NJ, Skinner VO, Tan TM, et al. Ghrelin can bind to a species of high density lipoprotein associated with paraoxonase. J Biol Chem 2003;278(11):8877-80.
47. Choi KM, Lee J, Lee KW, et al. The associations between plasma adiponectin, ghrelin levels and cardiovascular risk factors. Eur J Endocrinol 2004;150(5):715-8.
48. Park HS, Lee KU, Kim YS, et al. Relationships between fasting plasma ghrelin levels and metabolic parameters in children and adolescents. Metabolism 2005;54(7):925-9.
49. Kirel B, Tekin N, Tekin B, et al. Cord blood leptin levels: relationship to body weight, body mass index, sex and insulin and cortisol levels of maternal-newborn pairs at delivery. J Pediatr Endocrinol Metab 2000;13(1):71-7.