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ORIGINAL ARTICLE

Comparative measurement of ghrelin, leptin, adiponectin, EGF and IGF-1 in breast milk of mothers with overweight/obese and normal-weight infants

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BACKGROUND/OBJECTIVES: Obese infants are more susceptible to develop adulthood obesity and its related comorbidities. Previous studies have shown the presence of hormones and growth factors in maternal breast milk that may influence infant adiposity. The aim of this study was to investigate differences in concentrations of three hormones and two growth factors in the breast milk of mothers with obese and non-obese infants.

SUBJECTS/METHODS: In this cross-sectional study, 40 mothers with overweight or obese infants (weight for length percentile >97) and 40 age-matched mothers with normal-weight infant ($-10 <$ weight for length percentile < 85) who were between 2 and 5 months of age were enrolled. Anthropometric indices of infants and mothers were measured by routine methods. Breast milk concentrations of ghrelin and adiponectin, leptin, epithelial growth factor (EGF) and insulin-like growth factor-1 (IGF-1) were measured using enzyme-linked immunosorbent assay methods.

RESULTS: The mean breast milk concentration of ghrelin was higher in mothers with normal-weight infants, 137.50 pg/ml, than in mothers with obese infants, 132.00 pg/ml ($P=0.001$). This was also true regarding the concentration of EGF in mothers with (0/04 ng/ml) and without (0/038 ng/ml) normal-weight infants ($P=0.01$). No significant differences were observed in concentrations of leptin, adiponectin and IGF-1 between two groups ($P>0.05$). There was also a significant positive correlation between EGF and ghrelin in both groups.

CONCLUSIONS: This study revealed that there was a correlation between ghrelin and EGF level in breast milk of mothers with obese and non-obese infants, suggesting a possible regulatory effect of these two hormones on weight in infants.

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INTRODUCTION

Obesity and its comorbidities have been increasing in prevalence, including in children and infants.[1] It has been estimated that there are 43 million children <5 years of age globally with obesity and overweight.[2] A variety of factors including genetic susceptibility, high calorie intake, reduced energy expenditure, metabolic disorders and also other biological factors such as hormones and growth factors appear to contribute to the risk of obesity. Children with obesity are more likely to the develop obesity and its related comorbidities in adulthood, these include: hypertension, coronary artery disease, dyslipidemia, diabetes as well as physical injuries. The high treatment costs of these latter conditions further increase the imperative to identify individuals at risk of obesity and to develop new prevention strategies.[3] In early childhood, breast milk forms the principal food source, and weight change during infancy and sometimes childhood is therefore dependent on maternal breast milk intake. Human breast milk contains micronutrients, macronutrients, hormones and other growth factors that directly affect many aspects of infant growth.[4,5] They may also have long-term effects on the growth, development and overall health status of the infants.[6] Breast milk has been shown to contain various hormones including leptin, adiponectin, ghrelin, epithelial growth factor (EGF) and insulin-like growth factor-1 (IGF-1). These factors may have a role in the accrual of fat and lean body mass. Adiponectin, ghrelin and leptin may have a significant role in the regulation of appetite, energy balance and carbohydrate and lipid metabolism in infants.[7–10] These factors are related to the growth of infants during early postnatal life. EGF and IGF-1 are trophic growth factors that have been shown to induce cell growth, differentiation and proliferation and also have an important role in the development of the gastrointestinal tract.[11,12] The presence of these hormones in maternal breast milk could represent a link between early nutrition and the regulation of energy homeostasis.[13,14] There have been a very limited number of studies that have investigated the association between obesity in infants and the concentration of maternal breast milk hormone levels.

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The aim of the present study was to investigate the concentrations of several hormones in breast milk of mothers with obese and non-obese infants.

MATERIALS AND METHODS

Study population

This study was a cross-sectional study of a total of 80 mothers with infants referred to health-care centers of Mashhad city, and comprised 40 mothers with either overweight or obese infants (weight for length percentile >97) and an age-matched group of 40 mothers with non-obese infants ($10<$ weight for length percentile <85). Informed written consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Science and a standardized questionnaire was used to collect demographic information, physical characteristics of the infant such as age, weight at the time of birth, sex, height and other medical information. Demographic information including dietary intake, education level, method of delivery, number of children, specific disease and drug consumption was collected about the mothers and contraceptive history was also recorded in the questionnaire. Inclusion criteria included: apparently healthy infants who were 2–5 months of age and who were exclusively breast-fed up to 6 months. None of the infants included in the study were fed formula supplementation in the first 6 months of life. Pregnant women and mothers who were taking hormonal medications, or consumed alcohol or smoked tobacco were excluded. Mothers with gestational diabetes and infants with any evidence of hormonal or metabolic abnormalities were also excluded from the study.

Anthropometric measurements

The body weight of each infant was measured using a SECA Scale (SECA, Hamburg, Germany). Weight, body fat percentage and body mass index (BMI) of mothers were measured using a body composition analyzer (Tanita BC 418MA, Tokyo, Japan). Weight and height of infants were recorded at birth and at 2, 4 and 6 months.

Milk assay

Breast milk was collected from mothers after an interval of 2 h since the last breastfeeding between 0800 and 1000 hours. Mothers were fasted and the entire contents of one breast was evacuated using an electric breast pump (Spectra Dew 300, Selangor, Malaysia), and collected into a labeled plastic milk container and then samples were divided into eight aliquots. All aliquots were stored at −80 °C. Before analyses, samples were thawed at room temperature and vortexed continuously to ensure sample uniformity. Milk fat was separated from the aqueous phase by centrifugation at 3000 *g* for 10 min at 4 °C. The fat layer was removed by using a spatula and the liquid phase of the samples were used for the assay. The breast milk concentration of the study hormones was determined by enzyme-linked immunosorbent assay using IGF1 ELIZA kit (DE/CA40/00809/17), Mediagnoset, Reutlingen, Germany; Adiponecti ELIZA kit (DE/CA40/00809/18), Mediagnoset; leptin ELIZA KIT (DE/CA40/00809/17), Mediagnoset; ghrein ELIZAkit (E309Ra), Bioassay, Shanghai, China; and EGF ELIZA kit (E0144HU), Bioassay.

Statistical analysis

Population characteristics are summarized as mean ± s.d. Baseline demographics were compared among groups using student's *t*-test. To compare breast milk hormones' concentrations in two st udy groups, Mann–Whitney *U*-test were applied for non-normally distributed variables. Correlation analyses between hormones and other baseline demographics were assessed using the Pearson and Spearman correlation analysis.

Logistic regression was used to calculate odds ratios for association of breast milk hormones with infantile obesity. It is used to predict a binary response from several predictors, used for predicting the outcome of a categorical dependent variable such as obese and normal infants based on breast milk hormones levels and other predictor variables.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc.) version 18.0 software (Chicago, IL, USA). For all comparisons, a *P*-value <0.05 was considered as statistically significant.

RESULTS

The baseline characteristics of the study subjects, both mothers and their infants, are shown in Table 1. The mean age of mothers and their infants were 29 years and 3.7 months, respectively. Of the 80 infants, 58.8% were male and 41.2% were female and based on weight for length percentile $\geqslant 97$, 40 infants were overweight and obese and based on $10<$ weight for length percentile <85, 40 infants were grouped as normal. There were no significant differences in the distribution of gender and age between the obese- and normal-infant groups. No significant differences in BMI, body fat percentage and age were observed between the groups of mothers. However, there was a significant difference in infants' body weight at the time of birth and during the first 6 months of life between the two study groups ($P<0.0001$).

The breast milk concentrations of leptin, adiponectin, ghrelin, EGF and IGF-1 of the two groups of mothers are shown in Table 2. The results showed that concentrations of EGF and ghrelin in the milk of mothers with normal-weight infants were higher than those for the obese group ($P<0.05$). There was no significant difference in leptin, adiponectin and IGF-1 concentrations between the two study groups ($P>0.05$).

The data related to the correlations of hormone concentrations in breast milk of two groups are presented in Table 3. There was a significant positive correlation between EGF and ghrelin in both obese ($r=0.58$, $P<0.001$) and normal-weight ($r=0.93$, $P<0.0001$) groups, but the observed correlation was higher in later group.

The data related to the correlation between BMI and maternal body fat percentage with breast milk hormones and between breast milk hormone concentrations and infant weight at birth, 2nd, 4th and 6th months in the obese group are shown in Table 4. In the group of mothers with obese infant, there was a significant positive correlation between BMI and breast milk concentration of leptin ($r=0.48$, $P=0.004$), and also same result was observed as negative correlation between BMI and breast milk of IGF-1 ($r=-0.42$, $P=0.01$). Pearson and Spearman tests were applied to normal variables and non-normally distributed variables, respectively ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). In the normal infant group, there was a significant negative correlation between leptin and weight of 2nd month infants ($r=-0.35$, $P=0.01$). A significant correlation for the rest of the hormones was not observed.

Two logistic regression models were presented in Table 5. The variables in every model consist of breast milk hormones, and base line data were analyzed as backward. As shown in Table 5,

Table 1. Characteristic data from all subjects in each group

Characteristics	*Obese* (n = *40*)	*Normal* (n = *40*)	P-*value*
Maternal age (year)	28.84 ± 4.17	29.97 ± 5.52	0.310
Mothers' BMI	27.21 ± 4.5	26.00 ± 3.8	0.211
Delivery type (No. (%))			
Vaginal	17 (43.6)	14 (35.9)	—
Cesarean	22 (56.4)	25 (64.1)	0.488
Infant gender (No. (%))			
Male	24 (60)	23 (57.5)	—
Female	16 (40)	17 (42.5)	0.823
Infant age (month)	3.70 ± 1.20	3.73 ± 1.15	0.899
Infant weights			
Birth weight (kg)	3.73 ± 0.42	3.30 ± 0.44	< 0.001
2nd month weight (kg)	6.77 ± 0.79	5.44 ± 0.64	< 0.001
4th month weight (kg)	8.82 ± 0.71	6.90 ± 0.82	< 0.001
6th month weight (kg)	10.02 ± 0.88	7.73 ± 0.93	< 0.001

Abbreviation: BMI, body mass index. Quantitative variables are expressed as mean ± s.d. The statistically significant values are depicted as highlights.

Table 2. Comparison of breast milk hormone concentrations in two groups

Variable	*Obese*	*Normal*	P-*value*
Leptin (ng/ml)	1.78 (1.67–1.94)	1.81 (1.65–1.94)	0.757
Adiponectin (ng/ml)	323.48 (281.14–350.89)	330.05 (298.33–376.81)	0.393
EGF (ng/ml)	0.038 (0.037–0.039)	0.040 (0.038–0.045)	0.013
IGF-1 (ng/ml)	89.63 (64.30–104.79)	75.09 (55.35–117.41)	0.787
Ghrelin (pg/ml)	132.00 (130.75–136.25)	137.50 (133.00–156.00)	0.001

Abbreviations: EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1. All measurements are shown as median (interquartile range). Mann–Whitney *U*-tests were used for comparisons. The statistically significant values are depicted as highlights.

Table 3. Correlations of breast milk hormone levels together in two groups by Spearman's rank correlation matrix

	Leptin	Ghrelin	IGF-1	EGF
Ghrelin (pg/ml)				
Obese	0.03			
Normal	0.29			
IGF-1 (ng/ml)				
Obese	−0.13	0.04		
Normal	0.14	0.22		
EGF (ng/ml)				
Obese	−0.07	***0.58	−0.09	
Normal	0.3	***0.93	0.17	
Adiponectin (ng/ml)				
Obese	0.06	−0.26	−0.15	−0.29
Normal	−0.21	−0.08	0.12	−0.17

Abbreviations: EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1. The results are represented with the correlation coefficient. The statistically significant correlations are depicted as highlights (***$P < 0.001$).

Table 4. Correlation between maternal BMI and body fat percentage, and infant weight at birth, 2nd, 4th and 6th months after birth with breast milk hormones levels by spearman correlation analysis

	Adiponectin	*EGF*	*IGF-1*	*Ghrelin*	*Leptin*
Mother BMI					
Obese	0.48**	−0.06	0.08	−0.42*	−0.08
Normal	0.07	−0.01	−0.13	0.11	−0.007
Mother fat percentage					
Obese	0.41*	0.12	−0.12	−0.20	−0.10
Normal	0.07	−0.06	0.004	0.08	0.05
Birth weight					
Obese	−0.007	0.003	0.071	0.011	0.062
Normal	−0.286	0.169	−0.240	−0.108	−0.247
2nd month					
Obese	0.154	−0.274	0.071	0.200	0.295
Normal	−0.354*	−0.091	0.024	0.195	0.112
4th month					
Obese	0.164	−0.329	0.048	0.229	0.035
Normal	−0.177	0.075	0.092	0.012	0.169
6th month					
Obese	0.187	−0.336	0.194	0.004	0.071
Normal	−0.066	−0.027	0.096	−0.053	0.083

The results are shown with correlation coefficients. The statistically significant correlations are shown as highlights (*$P < 0.05$, **$P < 0.01$).

Table 5. Association of all variables and breast milk hormones' concentration on obese infants using logistic regression

	Coefficient (β)	*s.e.*	P-*value*	*OR*	*95% CI*	*OR*
(A)						
Ghrelin	−0.046	0.026	0.073	0.955	0.908	1.004
Mother current weight	0.060	0.030	0.043	1.062	1.002	1.126
(B)						
Mother current weight	0.068	0.031	0.030	1.070	1.007	1.137

Abbreviations: CI, confidence interval; OR, odds ratio. As ghrelin and EGF are highly correlated together, they separately entered with other variables in model A and model B, respectively. The variables in every model were analyzed as backward. Variables entered on step 1 is including infant age (month), delivery type, pre-pregnant mother weight, infant sex, mother age (year), mother BMI, mother fat percentage, mother current weight, leptin, adiponectin and IGF-1 for model A and infant age (month), delivery type, pre-pregnant mother weight, infant sex, mother age (year), mother BMI, mother fat percentage, mother current weight, leptin, adiponectin EGF and IGF-1 for model B. The statistically significant values are depicted as highlights.

mother current weight affects significantly obese infants (odds ratio 1.06 and 1.07 for model A and model B, respectively). On the basis of the models A and B, each unit increasing of mother current weight enhances 6 and 7% the odds of obese infant development, respectively.

DISCUSSION

In this study, a comparison of maternal breast milk hormones for groups of obese and normal-weight infants revealed that maternal breast milk ghrelin hormone concentrations of women with normal weight infants were higher than that of those with obese infants. Savino *et al*.[15] have previously reported a negative correlation between serum ghrelin concentration and weight gain of infants, but a positive correlation with age, weight and length. Kierson *et al*.[8] did not find any significant association between breast milk ghrelin and term and preterm infants. Shillina *et al*.[16] showed that mothers with high level of ghrelin in breast milk have higher-weight infants. Although ghrelin receptors located on gastric epithelial cells in man may be involved in ghrelin passing from milk to infant blood, there has been little work investigating the association of breast milk ghrelin concentration and growth of breastfed infants during early postnatal life.[17,18] Besides having a role in short-term regulation of food intake, ghrelin may also have a role in long-term regulation of energy balance. The ghrelin content of maternal milk may have an important regulatory role on appetite in the infant and may have a direct effect on milk intake. Plasma ghrelin concentrations are inversely related to the extent of adiposity, and in obese people ghrelin attains normal value after weight loss.[19] In the present study, the high levels of breast milk ghrelin in the normal infant group may be due to the putative role of ghrelin in regulation of appetite. In this study, concentration of EGF in the milk of mothers with normal infants was higher than that of those with obese infants. So far, no study has reported on the relationship between EGF and infant weight. The concentration of this factor in the human milk is high and has a potential to have a role in stimulating of cell growth, differentiation and cell

proliferation.[20] It is possible that EGF may have an important role in the regulation of growth and infant weight.

There was no significant difference in the mean concentration of milk leptin between the two infant groups. A positive correlation was observed between mothers' BMI and leptin in the obese infant group; however, this correlation was not significant in the normal infant group. Fields and colleagues reported that milk leptin concentration was positively associated with maternal BMI and negatively associated with infant BMI. Uysal *et al.*[21] showed that leptin concentrations of human milk are not different in the mothers of obese and non-obese infants. Khalili *et al.*[22] has reported that there is no significant difference between breast milk leptin of mothers with obese infants and those with normal weight. A few studies have investigated whether breast milk leptin can pass from mother to infant, and this has been supported by the demonstration of leptin receptors in the intestine of infants.[23] But in this study, breast milk leptin has no significant effect on weight during infancy. There was no significant difference between the two groups for breast milk adiponectin and IGF-1 concentrations. We found there was a significant negative correlation between milk adiponectin concentration and weight of the 4th-month-old obese infants. Woo *et al.*[24] found that high concentration of adiponectin is associated with weight loss and may be involved in the regulation of infantile growth. Cesur *et al.*[18] showed that there is no significant relationship between breast milk adiponectin and growth parameters of the infants.

We also found that there was a significant negative correlation between BMI and milk IGF-1 in the obese infant group; however, this was not found for the normal infant group. There was only a significant positive correlation between the EGF and ghrelin in both groups if infants. This correlation was stronger in the normal group and no significant correlation for other hormones was observed. Ghrelin has a regulatory role in the secretion of growth hormone by the pituitary gland, which in turn regulated the secretion of other growth factors including EGF.[25] Furthermore, ghrelin and EGF have an effect on the development of gastrointestinal tract.[26,27] We speculate that some of the variability in appetite regulation may result from a complex interaction between various substances within breast milk and, perhaps, the gastrointestinal tract. Ghrelin and EGF may potentially have an effect on behavioral and homeostatic processes beyond appetite regulation.

There was a significant difference in the body weights of infants at birth and at 2, 4 and 6 months of age. Also a significant difference regarding the growth was observed between the two groups. This difference is probably associated with the growth hormone content of breast milk, infant birth weight and feeding habits, such as frequency of drinking milk and milk volume, suggesting more studies in the future with larger sample sizes to control the effect of these three factors.

Despite having interesting outcomes and providing some evidence of the relationship between ghrelin concentration of breast milk and infant weight for the first time in an Iranian population, this study has several limitations. First, the sample size was relatively small. Second, the lack of data on the relationship between the hormone and growth factor content of maternal milk and feeding habits of the infants, such as frequency of drinking milk and milk volume. Third, a lack of data for serum ghrelin concentration in the blood of infant. Further studies are needed to investigate the role of ghrelin in breast milk and its potential interactions with growth factors and some hormones such as insulin in the regulation of body weight. It will be important to investigate the composition and volume of mother's milk in obese and normal infants, and their feeding habits to better understanding the etiology of obesity in infant.

In conclusion, the present study has shown for the first time that ghrelin and EGF concentrations in breast milk of mothers with normal infants were higher than that of those with obese infants, and that there is a strong correlation between ghrelin and EGF concentrations in the normal weight infants, suggesting a possible regulatory effect of these two hormones on infant's weight and postnatal growth of infants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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