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Association Between Fat Content of Breast Milk and Maternal Nutritional Status and Infants' Weight in Tabriz, Iran

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ABSTRACT

The aim of this study was to investigate the relationship between breast milk fat content with maternal anthropometric status, energy and macronutrient intake and weight for age Z-score of exclusively breastfed infants. Breast milk samples and information on energy and macronutrients intake (using 24-hour recall method for 3 days) were collected from 182 lactating women. Weight and height of mothers and infants were measured and the body mass index (BMI) and weight for age Z-score (WAZ) were calculated. The fat content of the milk samples was measured by the Gerber method. The mean daily energy intake was 2390 ± 405 kcal which was lower than the recommended values for mothers. The mean fat content of the milk samples was 3.52 ± 1.41 g/dl, which was significantly associated with dietary carbohydrate consumption (β= 0.39, P<0.004) and BMI (β= 0.28, P<0.02). A significant association between the WAZ of infants and maternal BMI (β= 0.36, P<0.001) was recorded. The WAZ of infants whose mothers' breast milk lipid content was more than 3 g/dl was significantly higher than those whose mothers had lower breast milk lipid content (P<0.031). Further studies need to be done on the effect of fat content of breast milk on infant's weight and how maternal factors regulate the composition of breast milk.

INTRODUCTION

The American Academy of Pediatrics recommends exclusive breastfeeding for 6 months and a total duration of >1 year to obtain the "full benefits of breastfeeding" (Gartner *et al.*, 2005). In our country, the majority of infants (87%) are now being breastfed for > 6 month and many are breast-fed throughout the second half of infancy (Health Indices of the Islamic Republic of Iran, 2003).

The effect of nutritional status of mothers on the quality and quantity of their milk is a frequent topic of discussion (Picciano, 2003). Human milk is a complex mixture of lipids, carbohydrates, proteins and vitamins that should provide the infant with a nutri-tionally complete food (Trahms & Mackean, 2008). In term of its macro-nutrients, the lipid fraction is crucial in fulfilling the newborn's nutritional needs because almost 50% of dietary calories are supplied to the newborn infant as fat (Trahms *et al.*, 2005). Lipids in comparison with the other nutrients of human milk have the greatest within and between sample variability (Weber *et al.*, 2001).

Although extensive research on animals has demonstrated a clear effect of diet on the

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yield and macronutrient composition of milk (Chwen *et al.*, 2002) such relationships have been difficult to demonstrate in humans (Villalpando &Prado, 1999). The results of an animal study conducted in Malaysia showed that dams fed on a high lipid diet during pregnancy and lactation had a higher milk lipid concentration which improved the growth of pups (Chwen *et al*., 2002).

Studies on humans have shown mixed results about the effect of maternal diet and anthropometric status on total milk fat content (Pearez-Escamilla *et al*., 1995; Ruel *et al*., 1997; Mandel *et al.*, 2005). The objectives of this study were to determine the possible relationship of breast milk fat content with maternal nutritional status and WAZ of exclusively breastfed infants.

METHODOLOGY

Study population

Breast milk samples were collected from 91 lactating women from urban areas of Tabriz and another 91 lactating women from its rural counterparts constituting about 9% of a total of 2022 lactating women who exclusively breastfed their infants (56 boys and 35 girls in each area), aged 90-120 days, in April 2007. The research sample included all mothers in a defined population of five urban health centres and five rural health houses who were admitted for their infants' vaccination.

Health centres and health houses which were included in this study were chosen by geographical clustered sampling method and the samples were randomly recruited from these centres. All mothers were from a range of low, intermediate and high socio-economic groups. Relevant criteria for mothers were: having infants with these characteristics; exclusively breastfed infants aged 90-120 days, infants with normal birth weight, full term infants and infants with no chronic disease.

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Science. All subjects were made aware of the content of the study and on agreeing to participate, a written informed consent document was obtained.

Data collection from mothers

Information on food intake was collected by using a 24-hour recall method for 3 days (one week-end day included). Dietary intake of subjects was analysed by Nutritionist III software programme.

Socio-economic data (working status), demographic data (place of residence, age) and clinical data (health status) were obtained through an interview.

Height was measured with the subject standing barefoot with arms hanging freely at the sides using a mounted tape and recorded to the nearest 0.5 cm. Body weight was measured with a Seca scale to the nearest 0.1 kg with subjects barefoot and wearing light clothing. Body mass index (BMI) was calculated as weight in kilogramme(kg) divided by height in meter square (m^2).

Data collection from children

Child body weight was measured using accurately calibrated instruments (electronic scales: Soehnle, max wt 20 kg, accurate to 10 g). Weight for age Z-score (WAZ) was calculated according to the median value of the international reference population recommended by National Centre for Health Statistics (NCHS) / World Health Organization (1986).

Extraction of fat from breast milk

Breast milk samples (15ml) were collected into sterile glass bottles by self-expression before nursing the baby in the morning at the third interview. The samples were kept in a refrigerator at a temperature of +4ºC, ready for analysis.

The fat content of the milk sample was measured by the Gerber method (Roginski, 2002). The milk was incubated at 38^0C to just liquefy the fat and then cooled to 20^0C. Then 10 ml H_2SO_4 (SG = 1.820 – 1.825) was added into a Gerber butyrometer followed by 10.77 ml milk being added carefully down the side of the butyrometer to form a layer on the sulfuric acid. One ml of amyl alcohol was added onto the layer of milk. The sample and H_2SO_4 was mixed and centrifuged for 5 minutes at 1100 rpm. The percentage of fat was then read from the upper meniscus.

Statistical analysis

Statistical analysis was performed with SPSS version 11.5 software and included means and SDs. Between-group comparisons were made by independent *t*-test. Multiple linear regressions using the backward technique were used to analyse the association of each potential factor with lipid content of breast milk. Potential factors like maternal BMI, energy, carbohydrate, protein and fat intake were selected as independent variables while the mean fat content of breast milk was chosen as the dependent variable.

To study the effect of breast milk fat content on WAZ of infants, multiple linear regression analysis was performed taking breast milk fat content, maternal age, energy intake, BMI, infants' birth weight as independent variables and weight for age of the children as the dependent variable. All of the variables were entered in the model as continuous variables.

RESULTS

Descriptive data of 182 mothers and their infants are presented in Table 1. In both urban and rural areas, the average BMI was within the over-weight range (26.79 ± 4**).** In this study, 34.56%, 23.8% and 1.64% of subjects were overweight, obese and underweight respectively. About 40% of participants were in the normal BMI range.

The daily mean intake of energy and macronutrients in lactating women and breast milk fat content are shown in Table 2. The measured value for energy (2390 ± 405 kcal / d) was significantly (p<0.05) less than RDA recommendations (2730 kcal / d). Comparison of calculated energy values adjusted to height, weight, and physical

Table1. Maternal and child descriptive data

Variables		*Urban (n=91)*	*Rural (n=91)*	*Total (n=182)*
Mother's age (meanY ± SD)		28 ± 6*	25 ± 5	26.5 ± 6
Mother's weight (mean Kg ± SD)		65.55 ± 12	65.15 ± 12	65.5 ± 12
Mother's height (mean Cm ± SD)		157 ±6	156 ± 6	156.5 ± 6
BMI (mean kg / m^2 ± SD)		26.81 ± 4	26.77 ± 4	26.79 ± 4
Number of miscarriages		21 (23%)	21 (23%)	42 (23%)
Number of children	1	39 (42%)	36 (39.5%)	75 (42.2%)
	>1	52 (58%)	55 (60.5%)	107 (58.8)
Employed		5 (5.5%)	3 (3.3%)	8 (4.4)
Not employed		86 (94.5%)	88 (96.7%)	174 (95.6)
WAZ (mean ± SD)		0.66 ± 1.1	0.78 ± 1.3	0.72 ± 1.2
HAZ (mean ± SD)		0.14 ± 1.1	0.09 ± 0.9	0.11 ± 1.0

Table 2. Daily mean intake of energy and macronutrients in lactating women and breast milk fat content (mean ± SD)

Variables	*Urban (n=91)*	*rural (n=91)*	*Total (n=182)*
Total energy(kcal)	2334 ± 410	2447 ± 372	2390 ± 405
Lipid (kcal)	669.8 ± 22	616.6 ± 70	643.2 ± 22
% energy	28.7% ± 6	25.2% ± 6	26.9% ± 6
Carbohydrate (kcal)	1342 ± 71	1500 ± 77	1421 ± 79
% energy	57.5% ± 6	61.3% ± 7	59.4% ± 7
Protein (kcal)	322 ± 19	330 ± 21	326 ± 20
% energy	13.8% ± 2	13.5% ± 2	13.6% ± 2
Fat content of milk (%)	3.42 ± 1.42	3.62 ± 1.40	3.52 ± 1.41

Table3. Estimated correlation between different variables and breast milk fat content

Association of factors with breast milk fat content	*β*	*P- value*	*SE**
Maternal BMI	0.28	0.02	0.1
Daily energy intake	0.14	0.43	0.9
Daily fat intake	0.23	0.19	0.17
Daily carbohydrate intake	0.39	0.004	0.11
Daily protein intake	0.16	0.31	0.14

Multiple $R^2_{\text{milk fat content}} = 0.3$
* Standard Error

Table4. Association of factors with infants' WAZ

Association of factors with infants' WAZ	*β*	*P- value*	*SE**
Daily energy intake	0.10	0.33	0.06
Maternal BMI	0.36	0.001	0.13
Birth weight	0.41	0.07	0.18
Breast milk fat content	0.28	0.09	0.15
Maternal age	-0.12	0.11	0.06

Multiple $R^2_{\text{WAZ}} = 0.58$
* Standard Error

activity levels (2458 ± 258kcal) with the mean energy intakes also showed a significant difference (p<0.05). The consumption of carbohydrates was significantly higher than the RDA recommendation (P>0.05).

The mean fat content of breast milk was 3.52 ± 1.41 g / dl. The mean fat in breast milk of 62 (34%) lactating women was less than 3 g / dl but exceeded 3 g / dl in 120 (66%) subjects.

The association of several maternal factors with breast milk fat content is shown in Table 3. After adjusting for maternal energy intake, protein and fat intake, BMI (β = 0.28, P<0.02) and mean daily carbohydrate intake (β = 0.39, P<0.004) were significantly associated with fat content of breast milk.

Table 4 shows the association of different maternal factors with infants' WAZ. After adjusting for breast milk fat content, maternal age, energy intake, infants' birth weight, a significant association was found between the WAZ of infants and maternal BMI (β= 0.36, P<0.001). Also the WAZ of infants whose mothers' breast milk lipid content was more than 3 g / dl (WAZ= 0.97), was significantly (P<0.031) higher than for others (WAZ= 0.53) (not shown).

DISCUSSION

Under mothers' optimal nutritional and living conditions, breast milk alone provides enough energy to meet the infant's requirements for normal development, at least until 4-6 months of age (WHO, 2001). Fat is the main source of energy in human milk and appears to be the most variable of the macronutrients, in response to maternal nutrition (Weber *et al.*, 2001).

Although the results from this study showed a tendency for milk lipid content (3.52 ± 1.41 g / dl) values to be close to that reported for human milk (35-40 g / L), it was lower than values reported in 12-20 weeks post partum: 3.9 g / dl for Brazil (Cunha,Macedoda Costa & Kiyomi, 2005), 4.0 g / dl for Chile (Cunha *et al.*, 2005), 3.8 g / dl for Finland (Villalpando & Prado, 1999), 4.0 g / dl for USA (Villalpando & Prado, 1999) and that previously reported (3.8 g / dl) in Kermanshah- Iran (Bahrami *et al.*, 2005). It was higher than the mean lipid content of breast milk (2.87 g / dl) reported in the Congolese study (Rocquelin *et al.*, 1998) and 2.34 g / dl reported in Mexico (Villalpando *et al.*, 1992). Our observation concurs with that of the WHO collaborative study (WHO, 1985), namely that the lipid content of human milk in developing countries is generally lower than that in affluent populations. However, comparisons from one study to another are questionable because methods for collecting milk and extracting lipid may affect the lipid content of breast-milk samples.

In this study no significant correlation was found between energy intake and fat content of the milk. Similar results were also found in the studies conducted in Mexican (Villalpando *et al.*, 1992) and American women (Michaelsen *et al.*, 1994). Also Prentice,Roberts & Prentice(1983) found no significant difference in the total fat concentration of the milk between energy supplemented and not supplemented women. It may be possible, that the low energy intake of mothers (2390 ± 405) when compared with RDA recommendation, shows a lack of energy effect on milk fat concentration in this study. The relative lack of effect of energy intake on milk fat concentration is puzzling, especially in the light of the sensitivity of milk fat concentration to the amount of maternal adipose tissue. Besides, methods to assess dietary intake may not be sufficiently accurate to detect associations with milk fat concentrations. Many studies have compared one dietary assessment method with another, but, in the absence of external validation, it has not been possible to conclude which method, if any, measures the true or valid intake (Villalpando & Prado, 1999). It appears that further carefully controlled research is needed to confirm the

correlation between energy intake and breast milk lipid content.

In our study, the association between fat consumption and lipid content of milk was not significant (β= 0.23, P= 0.19). In the study carried out on Mexican women, no correlation was found among maternal intake of energy and macronutrients and milk fat concentration at 4 and 6 months of lactation (Villalpando *et al.*, 1992). But a positive correlation was found in an intervention study in American women by Michaelsen *et al*. (1994) who reported low milk fat concentration in low fat diet mothers.

Our results showed a positive correlation between carbohydrate consumption and milk fat content (β= 0.39, P<0.004). Our data are in contradiction with that of the WHO study that the lipid content of mature breast milk is little influenced by variations in the mothers' nutritional status or dietary intake (WHO, 1985). This correlation was also found in the study of Rocquelin *et al.* (1998) which concluded that high-carbohydrate, low-fat diets lead to higher breast-milk lipid content than low carbohydrate, high-fat diets. In our study, a positive correlation was found between BMI and lipid content of milk (β= 0.28, P<0.02). It may be due to the low fat diet consumption that results in body fat mobilisation. Lovelady *et al.* (1993) speculated that with a low fat diet, milk fat is maintained by mobilising body fat if there is sufficient body fat to mobilise. Similar relationships have been found in undernourished women in Gambia (Prentice *et al.*, 1983), Honduras (Pearez-Escamilla *et al.*, 1995) and Mexico (Villalpando *et al.*, 1992). However, Mandel *et al.* (2005) indicated that fat content of breast milk was not influenced by maternal diet and BMI. The mean maternal BMI in Mandel's study was lower than the mean BMI of our subjects.

The results of this study showed that infants' weight was positively and significantly associated with maternal BMI (β= 0.36, P<0.001). Similar results were found in Bangladesh which showed that mothers with higher values of BMI had better nourished children than those with lower values (Rahman *et al.*, 1993). The results of this study showed that the WAZ of infants, whose mothers' breast milk lipid content was more than 3 g/dl, was significantly higher than others whose mothers had lower breast milk fat. Other studies conducted in the United States (Heinig *et al.*, 1993), Honduras (Pearez-Escamilla *et al.*, 1995) and Mexico (Villalpando *et al.*, 1992) showed the association of breast milk fat content and energy density with lactation performance. Also, among the Otomi Indians of Mexico, lactation performance was reported to be significantly correlated with maternal body size and composition (Ettyang *et al.*, 2005).

CONCLUSION

This study shows the positive effects of maternal nutritional status on breast milk lipid content and weight of infants. However, as the mean energy intake of mothers was less than the RDA estimate of needs for energy and calculated energy intake, appropriate nutritional education and interventions are suggested for lactating women.

Studies on the mechanisms by which milk fat concentration is influenced by maternal anthropometric status and its interactions with maternal intakes of dietary energy and macronutrients should be undertaken. Also, more information is needed about how external factors (nutrition, seasons and socio-economic factors) and metabolic mechanisms regulate composition of breast milk and maternal lactation performance.

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