PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 8 (5): 629-634, 2009
ISSN 1680-5194

Influence of the Lactating Women Diet on the Concentration of the Lipophilic Vitamins in Human Milk

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Abstract: The aim of the study was to assess the intake of vitamins A and E by Polish breast feeding mothers and the correlation between the intake of these vitamins and their concentration in the maternal milk. Dietary intake was assessed by triple 24 h diet recall questionnaire. Milk samples were collected and the content of vitamin A and E was determined. The mean intake of vitamin E (7.7±3.4 mg/day) covered 54.7% of the recommended value. The mean vitamin A content in daily food rations (DFR) -1012±735 µg-allowed to cover 63.2% of the requirement. The mean concentration of vitamin A in milk was 57.07±29.3 µg/100 mL. There was a correlation of the content of vitamin A in milk and the intake of this vitamin (r = 0.371) and the intake of essential fatty acids (r = 0.455) also. The mean concentration of the vitamin E in the milk (413.1±194.4 mg/100 mL), statistically significant correlated with its intake in DFR (r = 0.483). DFR of breast-feeding mothers, were characterized by insufficient supply of vitamins A and E. The dietary intake of these vitamins correlated with their concentration in maternal milk.

Key words: Lactating women, nutrition, lipophilic vitamins, breast milk

INTRODUCTION

Human milk is supposed to fully cover the infant's demand for nutritional components in the first 4-6 months of life. Over 200 ingredients of mother's milk, including hormones, enzymes, growth factors, antibodies as well as anti-inflammatory and immunomodulating substances (Takeda *et al*., 2004; Lonnerdal and Lien, 2003; Ogbe, 2008), have been identified to significantly affect child's correct growth and psychomotor development. Among indispensable constituents of breast milk, there are also vitamins A and E (Schweigert *et al*., 2004). Their concentration in human milk is highly individual-specific depending on physiologic as well as environmental factors. In this respect, the diet of the lactating mother is of significant importance (Prentice, 1991). During pregnancy, not only deficiency but also excess of vitamins, particularly A and D, are considered dangerous for the fetus development. For this reason it is recommended to complement the diet not by vitamin A supplementation but by increased dietary supply of beta-carotene (Wagner and Elmadfa, 2003). Vitamin A and carotenoids-by influencing functions of the immune system-protect a breast-fed infant from infections of respiratory and alimentary systems. They are also indispensable for maintaining proper vision (Khachlik *et al*., 1997; Canfield *et al*., 1997). Presence in the human milk of the Polyunsaturated Fatty Acids (PUFA), especially Essential Fatty Acids (EFA)-integral constituent of cell membranes, source of energy and important element of metabolic changes-requires the appropriate supply of vitamin E, a natural antioxidant protecting the organism against effects of the oxidation stress (Hornstra, 2000).

Nutritional requirements of lactating women are highly differentiated. The basic requirement is an energetically balanced and containing all the indispensable nutritional components diet. Appropriate dietary intake determines nutritional status of mother and infant (Dewey *et al*., 2001).

The aim of the present study was to assess the mode of nutrition of breast feeding mothers taking into consideration their diet, in particular the vitamins A and E intakes. An attempt was also made, for the first time in our country, to determine possible correlation between the intake of these vitamins and their concentration in the mothers milk.

MATERIALS AND METHODS

The study involved 30 breast feeding women aged 25-37 (average age 28.7±3.0) participated in the inquiry studies. About 96% of the inquired women were breast feeding for 2.5 month on the average-this period ranged from 1-12 months. Women were recruited to the study in several hospital obstetric wards, in the Mother and Child Institute and also by publishing an invitation on the website. Volunteering participants were mainly

inhabitants of 2 big urban agglomerations (56%) in central Poland or smaller towns of this region (44%). Similar number of women had university (46%) or college (50%) education. Majority of them estimated their financial situation as good (50%) or relatively good (30%). Only 20% considered, it very good; none of participants defined it as bad. Almost 73% of participants were professionally active.

Samples of mature milk for analysis were obtained from 30 participating women during 2-4 month of breast feeding. All participants expressed their consent to take part in the research in writing on a special form. The study was approved by the Bioethical Committee at the Mother and Child Institute in Warsaw (No.4/2001).

Study design: Each participant was interviewed by a responsible research staff member who informed her about the aim of the study, instructed about the way of registering the kind, amount of foods consumed and asked to maintain the usual eating habits. Total 24 h diet recall repeated for 3 consecutive days served to assess the amount of food consumed by the lactating women. Maternal milk was sampled once from the mothers who expressed their consent.

Dietary assessment: Participants of the study were met in person, most often by visiting them at home. During those visits, a 24 h diet recall was made and samples of milk were collected. Volunteering women were asked to very precisely register the kind and amount of food and dishes consumed indicating also the preparation techniques applied. A questionnaire referring to the diet included also some close type questions concerning the social situation, health status and eating habits. The registered 3 days menu was essentially verified by a team of researches who completed the lacking information and clarified inaccuracies, personally or by phone. The collected questionnaires served as the basis for determining the intake of basic nutritional components, vitamins and minerals. In order to obtain this information, the forms were subjected to computer analysis making use of the data base elaborated following the tables of nutritional value of food products in force in Poland (Kunachowicz *et al*., 1998). The degree of covering the requirement was established following the national recommended norms for breast feeding women (Ziemlanski *et al*., 1998).

Milk sampling: Before starting the study, the mothers were advised as to the way (by hand or using a sterile pump) of sampling the milk to sterile polypropylene test-tubes or glass bottles 1-2 h prior to actual feeding of the baby. After collection, the samples were delivered to the laboratory in a cool-box and then deep frozen and stored at -70°C until carrying out the analysis.

Chemical analysis: A 20 g human milk sample was weighed into a 250 mL round flask. Then, 0.3 g ascorbic acid, 50 mL absolute ethanol and 10 mL 60% potassium hydroxide solution were added. The mixture was kept at 80°C for 30 min. After boiling, the flask was cooled in ice-water bath for 10 min. Afterwards, the saponified mixture was transferred to a extractor rinsed with 30 mL water. The extraction was performed with 4x 50 mL diethyl ether and n-hexane (1:1 v/v). The combined organic layers were washed with 50 mL fraction of water. The extract was dried with anhydrous Na_2SO_4 and evaporated in a rotary evaporator (40°C). The residue was dissolved in 1 mL n-hexane. The samples were filtered through filters with pore size of 0.22 µm and injected into the chromatograph (injection volume 200 µL) (Escriva *et al*., 2002). The quantitative determination of vitamin E (all forms of tocopherols) and vitamin A (retinol) were carried out by HPLC. The HPLC system used consisted of HPLC Waters pomp 600, detector UV-VIS (Waters 2487), fluorescence detector (Warers 474) and LiChrosorb Si 60 column (250× 4,6 mm; 5µm) and precolumn LiChrospher Si 60. As a mobile phase n-hexane - 1,4 dioxane (97:3, v/v) was used at a flow rate 1.5 mL/min. Vitamin E was monitored by a fluorimetric detector (emission wavelength: 295 nm; excitation wavelength:330 nm). Detection of vitamin A was performed at 325 nm. The chromatographic data were acquired and analyzed with software program Millennium[32] (Waters). Contents of vitamin E (individual tocopherols homologues) and vitamin A (retinol) in milk samples were calculated on the basis of calibration curves for pure forms of these compounds (Strobel *et al*., 2000). Tocopherol equivalents (alpha-TE) were calculated from alpha, beta and gamma-tocopherols with the following formula: alpha-TE (µg) = alpha tocopherol (µg) + 0.5 beta-tocopherol (µg) + 0.25 gamma - tocopherol (µg) (Olafsdottir *et al*., 2001). Vitamin A was expressed as all-trans retinol (µg).

Statistical analysis: Statistical analysis of the results was carried out using the Statistica 6.0 program. Basic descriptive statistics were determined as well as the Pearson correlation coefficient for the analyzed variables and its confidence interval. The adopted level of significance was <0.05.

RESULTS

Subjects' characterization: In the investigated group (n = 30), the age of women ranged from 25-37 years. The dominating group included mothers aged 25-30 (73%); further 2 groups (24 and 3%), respectively were those aged 31-35 and over 36 years. Majority of the subjects (56.7%), gave birth to their first child at the age of 26-30. This confirms a phenomenon-noted in Poland also by other authors-of postponing the procreation time to later years of life (Kozlowska-Wojciechowska and Markiewicz-Wujec, 2002; Weker *et al*., 1999).

Table 1: Energy and nutrients in daily rations of lactating women and the realizations of Recommended Dietary Allowances (RDA%)

Food component	Mean±S.D.	Min-Max	RDA (%)
Energy (kcal)	2576.0±803	1559-4441	93
Energy (kJ)	10793.0±3364	6532-18607	-
Total protein (g)	92.8±28.9	32.0-139.3	107.9
Animal protein (g)	54.7±19.2	10.7-94.1	-
Plant protein (g)	19.7±9.8	19.7-58.6	-
Total fat (g)	91.1±33.6	46.5-190.3	120
Saturated fatty acids (g)	34.8±13.1	15.8-77.8	-
Monounasturated fatty acids (g)	34.9±13.1	16.1-71.1	-
Polyunsaturated fatty acids (g)	13.1±8.0	5.4-47.7	-
Cholesterol (mg)	478.5±271	136-1329	160
Total carbohyrates (g)	366±132	232-736	135
Saccharose (g)	89.8±78	6.7-387	12.9 (energy%)
Fibre (g)	23.4±9.4	9.5-50.7	83
Vitamin E-equivalent (mg)	7.7±3.4	3.0-20.9	54.7
Vitamin A-equivalent (μg)	1012±735	242-3557	63.2
Beta-carotene (μg)	2096±2465	207-12019	-

Recommended Dietary Allowances (RDA): vit. A-1600 μg; vit. E-14 mg

Dietary assessment: The intake of the majority of basic food components (proteins, lipids and carbohydrates) and the energetic value of daily food rations (DFR) varied considerably. However, as a rule, the level of intake covered daily requirements of the investigated women, which were assessed with respect to recommended intake for lactating women in Poland. The ratio of plant protein to the animal one and individual groups of fatty acids confirms domination of foodstuffs of animal origin in the diet (Table 1). Also very high cholesterol supply points to this. The average daily intake of vitamin E (7.7±3.4 mg/day) covers 54.7% of the recommended norms ranging from 20-147%. The vitamin E/EFA ratio (Harris index) has the average value of 0.6 thus conforming to the nutritional recommendations (Trumbo *et al*., 2001). The average content of vitamin A in DFR amounting to 1012±735 μg allows covering of 63.2% of daily demand on the average. In extreme cases, it is only 33.7% or over 198%. Significant part of vitamin A measured as retinol equivalent is beta-carotene (34%).

Vitamins A and E in breast milk: Concentration of vitamin A measured as retinol and that of vitamin E (totals of individual homologues considering their biologic activity) is shown in Table 2. These data constitute the average obtained from 3 consecutive repetitions. Average concentration of vitamin A in human milk was 57.07±29.3 μg/100 mL ranging from 15.72-142.4 μg/100 mL. About 76.7% of samples of the analyzed milk fell within the mean value±1SD, 6.7%-below and 16.6%-above it. The content of vitamin A correlated with its intake ($r = 0.371$; $p<0.044$). Statistically significant correlation was also found between concentration of vitamin A in the milk and the intake of EFA ($r = 0.455$; $p<0.012$).

In human milk, the mean concentration of vitamin E measured as alpha - tocopherol equivalent (alpha - TE) was 413.1±194.4 mg/100 mL (min -121.8 to max 987 mg/100 mL). The greatest part of alpha-tocopherol equivalent constituted the alpha-T homologue (97.8%); contents of beta-T and gamma-T were smaller: 0.31 and 1.88%, respectively. TE concentration in most (80%) samples of breast milk fell within the mean value TE±1SD. About 6.7% of samples were below this limit and 13.3%-above it. Statistically significant correlation ($p<0.0069$) was found between the content of vitamin E (alpha-TE) in the milk and its intake in DFR ($r = 0.483$). This concerned particularly, the alpha-T homologue ($r = 0.476$; $p<0.008$) and to smaller extent beta-T ($r = 0.442$; $p<0.015$). There was also a statistically significant correlation found between concentration of vitamin E (alpha-TE) in breast milk and the total intake of fats ($r = 0.483$; $p<0.007$).

DISCUSSION

Dietary intake: For the purpose of studies a questionnaire was used. Its exactness and precision were determined in the way the questioning was evaluated. In order to obtain as reliable data as possible, the participants were subjected to a triple 24 h diet recall-the first one always carried out by a member of the research staff.

As shown by the data concerning the vitamins intake, the obtained values are vary considerably, which is confirmed by high values of standard deviations. Remarkable differences in the intake by one person during the successive days of dietary recalls as well as interpersonal differences can significantly affect the final results obtained. Degree of covering the vitamin requirements was assessed following the Polish nutritional recommended norms (Ziemlanski *et al*., 1998) referring to the obtained value to the recommended daily intake (1600 μg retinol equivalent and 14 mg tocopherol equivalent) remarkably higher than the so called safe level (950 μg and 8 mg, respectively). It was considered appropriate to the intake

Table 2: Vitamins content in human milk

	Vitamin E (µg/100 mL)				
Variables	Alpha-TE	Alpha-T	Beta-T	Gamma-T	Vitamin A (µg/100 mL)
Mean	413.09	411.22	2.54	30.74	57.07
Median	355.53	348.39	2.02	25.21	50.03
SD	194.41	182.49	1.66	22.91	29.38
Min.	121.81	151.60	0.74	4.80	15.72
Max.	987.02	947.40	7.25	115.40	142.41

alpha-TE: alpha-tocopherol equivalent; alpha-T: alpha-tocopherol; beta-T: beta-tocopherol; gamma-T: gamma-tocopherol

vitamins from the DFR in their optimal values and not the minimum required for health (Kappler and Gronowska-Senger, 2004). At the same time, the latest European recommendations concerning the intake of the retinol equivalent (1500 µg) were taken into consideration (GNS, 2002).

The average intake of vitamin A (as retinol equivalent) by the breast-feeding mothers covered 75% of their requirements. It was by ca 400 µg higher than that noted in western Poland (Wielkopolska region) at the end of 1990s in a group of 731 women aged 30-50. It can be assumed that this favourable increase resulted from the general greater consumption of fruit and vegetables rich in beta-carotene noted in Poland in recent years. This is also confirmed by the increased intake of this provitamin in the diet: from 777 µg in the 1990s (Przyslawski and Duda, 2001) to 2739 µg at present.

The proper supply of vitamins with food limits the need for pharmacological supplementation, which is of importance particularly for vitamin A that can have a toxic effects if consumed in excess (Gutteridge and Halliwell, 1994). Such a risk can occur for 16% of the lactating mothers under investigation, whose diet included excessive amounts of the vitamin A equivalent surpassing their daily requirement. Relative safety of such a big intake of vitamin A is connected with the fact that a considerable part of the retinol equivalent (34.6%) was beta-carotene.

Daily food rations of the investigated group of women supplied on average of 8.1 mg vitamin E covering 57.7% of the requirement. As far as this vitamin is concerned, the Polish nutritional recommendations for lactating women (14 mg) are visibly higher than those in other European countries and in the USA (11 mg) (Trumbo *et al*., 2001). According to some authors, still higher level of reference concerning the requirement for tocopherols should be adopted: 17-20 mg/day. Such a level would not only limit the risk of many diseases but also it would reduce it in the highest degree (Lanhance, 1993). Taking into consideration various functions of tocopherols in human organism, their participation in inhibiting oxidative processes as well as little possibility of overdosing, the above opinion seems not be groundless. The content of vitamin E in DFRs of the lactating mothers is higher than that noted in the diet of women in the 1980s and 1990s. This can be due to the changing structure of fats consumption during the last decade in Poland. More plant oils and fats of plant origin are being consumed and consumption of animal fats decreases (Daniewski *et al*., 2003).

Vitamins A and E in human milk: Amount of milk produced by the organism of a properly nourished mother varies from 600-1000 mL. Its composition clearly changes, even in the same woman, mainly with respect to the fat and vitamin contents. Most probably, this is related to the lactation period in which concentration of fat in mature milk decreases with respect to the colostrum, while that of lipophilic vitamins increases (Macias and Schweigert, 2001). The time of breast-feeding is also of importance and it is known that concentrations of components in the fore-and hind milk differ. Studies carried out in recent years have been pointing to inverse correlation between the mothers age and the level of vitamin E in their milk (Ortega *et al*., 1999). Diet during pregnancy and lactation and also the source of vitamins (natural, supplied with food or synthetic-supplemented) are of crucial importance (Stone *et al*., 2003).

In the examined group of women, supply of vitamin A in DFR fully covered requirement only 40% of mothers (Table 1). Due to the fact that in the first year of life an infant consumes, on the average, 800-850 mL of milk, it was stated that 95% of samples of the analyzed milk supplied a baby with the indispensable quantity of vitamin A Ie 450 µg (Ziemlanski *et al*., 1998). Low concentration of this vitamin, not adequate to the recommended norms, was noted only in 3% of milk samples (Table 2). One can suppose that in a high percentage of mothers deficiency of vitamin A in the diet was compensated for during milk production by activation of the organism's reserves. The vitamin A content in the Polish maternal milk was slightly higher than that noted in American women (Hornstra, 2000) and very similar to the level determined in mothers from Iceland and Fulani (Olafsdottir *et al*., 2001; Schmeits *et al*., 1999).

Human milk contains many components with antioxidant properties, such as vitamin E and beta-carotene, preventing -among other things-retinopathy in premature infants (Hylander *et al*., 2001). Considering that human milk is rich in long chain fatty acids, in this particularly, valuable polyunsaturated fatty acids (PUFA) (linoleic and arachidonic), proper content of vitamin E is very

important. This vitamin protects PUFA against oxidation, protecting at the same time the infant's organism against negative effects of oxidation products (Kaempf and Linderkamp, 1998). In the analyzed samples of milk average concentration of vitamin E measured as alpha-tocopherol equivalent (alpha-TE) was adequate to the Polish nutritional norms recommending the daily intake of 4 mg alpha-TE by an infant (Ziemlanski *et al*., 1998). Concentrations of vitamin E in samples of milk collected from individual mothers considerably varied. In 70% of samples, the level of this vitamin was lower than that required for infants and it positively correlated with the percentage of women (66%), whose diet did not supply sufficient quantities of vitamin E. This may confirm scarce reports suggesting that decrease of concentration of vitamin E in milk with elongation of the breast-feeding time is not accompanied by complementation of the deficiency by the maternal tissue reserves when the intake with food is not sufficient (Macias and Schweigert, 2001; Schweigert *et al*., 2004). Authors observations are in accordance with findings by Olafsdottir *et al*. (2001) pointing to the fact that often in lactating women the diet alone is not a sufficient source of this vitamin. The alpha-tocopherol homologue quantitatively dominated among individual forms of homologues. Apart from clearly smaller quantity of gamma-also slight amount o fbeta-tocopherol was noted. The presence of the latter seems to be important because of the lack of information concerning its occurrence in human milk in other publications on this subject (Kaempf and Linderkamp, 1998; Kaempf-Retzoll *et al*., 2003).

Conclusion: This study has revealed that daily food rations of examined breast-feeding women-despite of proper balance of basic components are deficiency of vitamins A and E, providing 63 and 54% daily intake, respectively. On the other hand, the intake of vitamins A and E by lactating women correlates with concentrations of these vitamins in milk. It is confirmed by the correlation coefficients that are 0.371 and 0.483, respectively.

Recommendations: It should be stated that in many cases, the diet of lactating women does not meet the nutritional recommendations and it has an influence on the quality of maternal milk. The change of the dietary intake should include higher consumption of plant origin products, such as plant oils, vegetables and fruits.

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