

## Moderate zinc and vitamin A deficiency in breast milk of mothers from East-Jakarta

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**Objective:** To gain information about the micronutrient status of urban, middle-income, breast-feeding mothers in relation to zinc and selected fat-soluble vitamins in plasma and breast milk and to assess possible interaction between the measured micronutrients.

**Design:** Cross-sectional study

**Subjects:** 91 mothers and their infants living in middle-income areas of Jakarta, Indonesia

**Results:** None of the measured anthropometric data of the mothers (e.g. BMI:  $22.0 \pm 3.1$  kg/m<sup>2</sup>) and their infants (birth weight:  $3.2 \pm 0.5$  kg) gave any indication of undernutrition. The mean concentrations in blood were  $124 \pm 18$  g/l for hemoglobin,  $385 \pm 111$  µg/l for retinol,  $34 \pm 23$  µg/l for  $\alpha$ -carotene,  $104 \pm 72$  µg/l for  $\beta$ -carotene,  $7.7 \pm 3.3$  mg/l for  $\alpha$ -tocopherol,  $0.57 \pm 0.23$  mg/l for  $\gamma$ -tocopherol,  $855 \pm 242$  µg/l for zinc, and the median concentration of lycopene was 29 µg/l. The median breast milk concentrations were 420 µg/l for retinol, 7.8 µg/l for  $\beta$ -carotene, and 2.7 mg/l for zinc. With increased duration of lactation, vitamin A and zinc concentrations significantly decreased in breast milk whereas plasma zinc concentration increased. Plasma  $\alpha$ - and  $\beta$ -carotene were positively correlated ( $P < 0.0001$ ) with each other and with plasma lycopene. Breast milk  $\beta$ -carotene was positively correlated with breast milk retinol and with plasma  $\beta$ -carotene ( $P < 0.0001$ ). There was no correlation between zinc and vitamin A in either breast milk or plasma. Forty per cent of the mothers were anemic, 29.1% had a low plasma zinc concentration, and 23.7% had a moderately low plasma vitamin A concentration. Breast milk from 70% of the women had a low concentration of vitamin A and that from 66% had a low concentration of zinc.

**Conclusions:** Multi-micronutrient intervention should be considered to provide a sufficient supply of zinc and vitamin A for growth of exclusively breast-fed infants.

**Descriptors:** breast milk; anemia; zinc; retinol;  $\alpha$ -carotene;  $\beta$ -carotene; infants

### Introduction

Deficiencies of iron and vitamin A are well recognized as public-health problems, particularly in developing countries. Although the vital role of zinc in human health and nutrition has received increasing attention in the past two decades, much less information is available on the prevalence, severity and causes of zinc deficiency (Shrimpton, 1993). In particular, mild zinc deficiency may be a potential public-health problem in developing countries. Zinc is employed in numerous biochemical pathways of human metabolism, and is known to be incorporated in more than two hundred enzymes (Jackson, 1989). Therefore, zinc deficiency has direct and indirect nutritional and health implications. Zinc deficiency in women of fertile age may lead to an increased incidence and severity of infectious diseases, pregnancy complications and poor pregnancy outcome, as well as increased infant mortality, impaired

infant and child growth and development (Good, 1989; Keen & Hurley, 1989; Gibson, 1990).

Zinc deficiency may also influence the status of several other micronutrients. In particular, the interaction between zinc and vitamin A has received much attention and may be of clinical significance (Smith, 1980; Mejia, 1986). Studies in rats (Brown *et al*, 1976; Smith *et al*, 1976) demonstrated that zinc deficiency was accompanied by low serum levels of vitamin A. The same observation was made in pregnant monkeys (Baly *et al*, 1984; Golub *et al*, 1984) and pregnant rats (Duncan and Hurley, 1978; Peters *et al*, 1986). Morrison *et al* (1978) observed such a zinc–vitamin A interaction also in nonpregnant humans. Palin *et al* (1979) reported that zinc supplementation improved vitamin A status of rats. Husted *et al* (1988) made the same observation in preterm infants. Furthermore, Shrimpton *et al* (1983) reported that zinc supplementation in breast-feeding Amazonian women increased retinol concentration in breast milk.

In Indonesia a successful child-targeted vitamin A supplementation program drastically reduced vitamin A deficiency and has virtually eliminated keratomalacia in

children (Muhilal *et al*, 1994). Nevertheless, subclinical vitamin A deficiency is probably still widely prevalent among population groups such as preschoolers (Muhilal *et al*, 1994) and women of fertile age (Tanumihardjo *et al*, 1994). In view of the available information on the association between zinc and vitamin A deficiencies, the role of zinc as a determinant of deficient vitamin A status in Indonesian population groups should be considered.

The aim of this study was to inquire whether the Indonesian economic growth and the health policy had abolished micronutrient deficiencies as a public-health problem in higher risk groups at least in the middle-income group of the better-off capital of the country. Information on the micronutrient status of breast-feeding mothers of a middle-income group was collected with particular emphasis on zinc and vitamin A and their association.

## Subjects and methods

### Study area

The study was carried out from March to May 1994 in a middle-income area in East-Jakarta called Kampung Tengah. The area is located in urban Jakarta and comprises 203 ha that in 1990 had a total population of about 24 000 inhabitants (Kantor Statistik, 1991). The area has only poor sanitation facilities and no real sewerage system. As in several other middle-income squatter areas of Jakarta, the environment was polluted with waste. During the rainy season the area is often flooded.

### Subjects

The subjects were breast-feeding women during the first six months *post partum*. Some additional information was obtained on their infants and on their households. In total, 101 women were chosen randomly from lists of ten local health posts (*posyandu*). The dates of birth and the birth weights of the children were collected from the child health card. With the help of the health workers (*cadres*), the mothers and their infants were invited to visit the health posts for the study, which was implemented over a period of two weeks. Nine of the randomly chosen women did not participate in the survey because two mothers had moved away and the others refused further measurements.

### Questionnaire

A questionnaire was used for assessing the demographic and socioeconomic situation of the household and the frequency of consumption of food. The questionnaire was based on the recommendations of Gross *et al* (1997). For a semiquantitative indication of the vitamin A content in food, the classification of IVACG (1989) was used. The women were interviewed by local members of the research team, without the presence of their husbands or other adult family members. One of the authors (H.H.) directly supervised more than half of the interviews.

### Anthropometric measurements

Mid-upper-arm circumference (MUAC) was measured with a tape according to the recommendation of Jelliffe *et al* (1989). Biceps and triceps skinfolds of the mothers were measured with a HOLTAIN caliper (CMS Weighing Equipment, London, UK). Body stature was measured to the nearest 0.1 cm using a Microtoise (CMS) and body weight was measured to the nearest 0.1 kg with an

electronic scale (Model 770 alpha; SECA, Hamburg, Germany). The infant's body weight was measured to the nearest 0.1 kg with a mechanical baby scale (Misaki Kubota, Japan) and the body length was obtained using a wooden baby length board (Gross *et al*, 1997).

### Sample collection

Mothers came to their health posts to give their breast milk sample. From each woman milk from one breast was taken by a manual pump during five consecutive days in the morning between 09.00 and 11.00. Each pump was sterilized with boiling water and alcohol before usage. The entire content of one breast was taken from the breast that was not last suckled. The samples were then carefully transported and stored in the dark at  $-20^{\circ}\text{C}$  until analysis.

Within one week after breast milk sampling the mothers were gathered a second time. A 10 ml venous blood sample was taken from each woman, and her health status was checked by a trained doctor. The mothers were asked to fast before blood collection. Blood samples were all taken in the morning between 09.00 and 11.00 and immediately protected from sunlight and stored on ice on the way to the laboratory. The largest part of the blood was allowed to clot, after which the blood samples were centrifuged and the separated serum was then stored at  $-20^{\circ}\text{C}$  until analysis. Breast milk samples were sent one week after the last sample collection to Nestlé Research Center, Lausanne, Switzerland for zinc, vitamin and carotene determinations. Serum samples were sent to Hoffmann-LaRoche Vitamin Laboratory, Basel, Switzerland.

### Biochemical analyses

Hematocrit was determined directly in the field using a microcentrifuge and a capillary reader. Venous blood was put into heparinized microcapillary tubes, three measurements were made, and the mean value was taken. Hemoglobin concentrations were measured with the cyanmethemoglobin method (INACG, 1984) using a commercial kit (Boehringer Mannheim, Germany) and a spectrophotometer (PCP 6121, Eppendorf, Germany). If the hemoglobin measurements indicated that a woman was anemic, iron folate supplements were provided for treatment.

Determinations in serum were carried out by Hoffmann-LaRoche Vitamin Laboratory, Basel, Switzerland according to a method that simultaneously separates retinol,  $\alpha$ - and  $\gamma$ -tocopherol,  $\alpha$ - and  $\beta$ -carotene, lycopene and  $\beta$ -cryptoxanthin in human plasma or serum by high-performance liquid chromatography (HPLC) on reversed phase using a single extract (Hess *et al*, 1990). Since it required little additional effort and owing to the lack of information on the tocopherol status in Indonesian mothers, these micronutrients were included into the biochemical analysis. Two detectors with programmable wavelength were used sequentially, a spectrophotometer (Stagroma LCD 501, Reno, NV, USA) for the detection of the carotenes in the visible region and a fluorometer (LS 40, Perkin-Elmer, Norwalk, CT, USA) for the assay of retinol and the tocopherols.

Milk fat concentration was determined from the milk samples immediately after sample collection using a microcentrifuge and a capillary reader. From each milk sample three capillary tubes were measured and the mean value was calculated. The retinol and  $\beta$ -carotene concentrations in the breast milk were determined in the Nestlé Research Center, Lausanne, Switzerland. Sample preparations,

saponification and extraction were performed for retinol and  $\beta$ -carotene automatically on a robotized system (robot ORCA, system integrator SCITEC, monitored by the scheduling program WINCLARA from SCITEC) (Tagliaferri and Bonnetti, 1995).

The extraction step was performed with Extrelut-3 cartridges (Merck, Darmstadt, Germany). Sample portion was  $1 \pm 0.01$  g. Separation was performed on a normal-phase silica column with an isocratic HPLC system using a reversed phase column, Kontron double piston pump combined with Gilson 231 autosampler. The detection was performed with a Merck-Hitachi spectrophotometer and the evaluation of the peaks was obtained using a Hewlett Packard ChemStation Version A03.34 (de Leenheer *et al*, 1992). Recovery was measured through retinol spiking. Values were corrected in relation to the recovery factor (70% for retinol and 85% for  $\beta$ -carotene). The determinations were performed in duplicate.

Plasma zinc concentrations were analyzed by Microgen Laboratories, Rapperswil, Switzerland using flame atomic absorption spectroscopy (AAS). Maternal milk zinc concentrations were analyzed in the Nestlé Research Center following the procedure described by Anderson (1992). Samples were diluted at a 1:10 ratio with ultrapure water, and 0.1% suprapure nitric acid was added. Two measurements were done within 2 h by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Spectro-flame-D (Spectro, Kleve, Germany) sequential instrument. Zinc was measured at the 213.856 nm analytical line using single-sided peak background correction. Reference milk samples made from NIST 1549 low-fat milk powder (Zn  $46.1 \pm 2.2$  mg/kg) were measured at regular intervals, that is, after injection of six unknown samples. Results of human milk Zinc determinations were accepted only if the reference values were in the certified range.

#### Statistical analysis

Data entry and analysis were done using EPI INFO 5.1 (USD, Stone Mountain, GA, USA) and SPSS/PC 4.0 (SPSS, Chicago, IL, USA). To check for normal distribution, the Kolmogorov–Smirnov goodness of fit test and the Lilliefors test were employed. Analysis of variance was performed with ANOVA and Kruskal–Wallis one-way ANOVA for normally and non-normally distributed continuous values, respectively. Significance of differences for independent variables were tested by means of Student's *t*-test and Mann–Whitney *U*-test. Linear and nonlinear correlations were determined with Pearson's product moment correlation coefficient and Spearman/Kendall's tau-b coefficient, respectively. Significance of differences in prevalence among groups was checked with the chi-square test.

For the calculation of prevalences of different nutritional deficiencies, several cut-off points were used. Malnutrition in adult women was defined as a body mass index (BMI)  $< 18.5$  kg/m<sup>2</sup>. (James *et al*, 1988). To identify undernutrition in infants, the Z-scores of the three indicators weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) were calculated using the National Center for Health Statistics (NCHS) reference data. A Z-score below  $-2$  standard deviations (s.d.) was used as a cut-off point below which a child was considered to be undernourished (WHO, 1995). To identify anemia among the mothers, a cut-off point of 120 g/l as recommended by INACG (1984) was employed.

#### Ethical considerations

The guidelines of the Council for International Organizations of Medical Sciences were followed (CIOMS, 1990). The collected data were used for study purposes only. Before the study the mothers were informed about the purpose of the study and the research institution by the first author. Assurance was given that participation was voluntary and that no negative consequences would result for those who decided not to participate in the study. The study protocol was approved by the Ethical Committee of SEAMEO-TROPED Center.

## Results

#### Demographic and socioeconomic data

The mean age of the women examined was 25.4 years, with a range between 18 years and 40 years (Table 1). Households consisted on average of five members. Thirty-eight per cent of mothers had one child, 46% of mothers had two children, and 31% of the households had more than two children. Twenty per cent of the mothers were in the first month of lactation, 47% in the second and third months, and 33% in the fourth and fifth months. As shown in Table 2, the mean age of the infants was 2.4 months and ranged from 0.1 to 5.2 months.

Of the fathers, 31% were occupied as retailers, 18% were unskilled laborers, 13% were civil servants, 7.0% were members of the police or army, and 31% had some other occupation. Most of the mothers and fathers had attended primary (49% and 51%, respectively) and secondary (44% and 41%, respectively) education and only 7% of the mothers and 8% of the fathers had had no schooling. Only 16% of the mothers had their own income. The person who took care of the child was the mother in 85% of the households, and both parents in 13%. In 2% of the households the grandmother took care of the child.

Drinking and bathing water were always from the same source, and the majority of people got the water from a manual pump (37%) or a cemented open well (30%). Practically all used a private (54%) or a community latrine (44%) for defecation.

**Table 1** Ages and selected anthropometric data of breast-feeding women

Variable	n	Mean $\pm$ s.d.	Min	Max
Age (y)	92	25.4 $\pm$ 5.2	18	40
Height (cm)	91	150.1 $\pm$ 4.4	137	160
Weight (kg)	92	49.8 $\pm$ 7.7	35.7	79.4
BMI (kg/m <sup>2</sup> )	92	22.0 $\pm$ 3.1	16.2	33.9
Biceps (mm)	87	7.1 $\pm$ 2.8	3.3	18.5
Triceps (mm)	87	12.5 $\pm$ 3.5	6.8	26.1
MUAC (cm) <sup>a</sup>	87	26.2 $\pm$ 3.2	19.8	37.0

<sup>a</sup>Mid-upper-arm circumference.

**Table 2** Ages and selected anthropometric data of infants

Variable	n	Mean $\pm$ s.d.	Min	Max
Age (months)	92	2.4 $\pm$ 1.4	0.1	5.2
Birth weight (kg)	75	3.2 $\pm$ 0.5	1.7	4.3
Duration of pregnancy (months)	91	9.0 $\pm$ 0.5	7.0	9.9
Length (cm)	91	58.3 $\pm$ 4.9	49.0	68.4
Weight (kg)	92	5.18 $\pm$ 1.23	2.55	7.25
Weight-for-age (Z-score)	92	0.04 $\pm$ 0.89	-2.02	2.80
Height-for-age (Z-score)	91	0.09 $\pm$ 0.95	-2.31	1.94
Weight-for-height (Z-score)	91	0.13 $\pm$ 0.79	-2.15	1.41

### Nutritional habits

Almost all mothers (95%) stated that their babies had received colostrum, but nearly half of the children (45%) had received additional food/fluid during the first month of life. Most mothers (79%) reported that they avoided specific foods during lactation, whereas none reported avoidance during pregnancy. Almost 60% excluded fruits like pineapple, jackfruit, lemon, orange, banana, papaya, mango and melon from their diet and 13% did not eat vegetables such as cucumber and chili.

The majority of mothers reported that daily or at least several times per week they ate vegetables with low (< 500 RE/100 g), medium (500–1000 RE/100 g), or high (> 1000 RE/100 g) carotene content (51%, 84%, and 54%, respectively), such as green leafy vegetables and carrots. Almost 40% said that several times per week they ate fruits with medium and high carotene content such as papaya and mango. More than half of the mothers reported having never eaten beef liver and 76% had never eaten margarine. Eggs were consumed several times per week by 61% of the mothers. Consumption of traditional processed food was frequent. Tofu (soybean cheese) and tempe (fermented soybean) were eaten by 45% every day and by an additional 52% several times a week. Animal foods reported to be eaten daily or at least several times per week were chicken or duck eggs, dried-salted fish, and chicken (73%, 60%, and 53% of mothers, respectively).

### Anthropometric indices

Table 1 shows the anthropometric data of the mothers. The mean stature was 150 cm and the mean weight 49.8 kg with a mean BMI of 22.0 kg/m<sup>2</sup>. Six per cent of the mothers had a BMI higher than 27.5 kg/m<sup>2</sup> and 9% lower than 18.5 kg/m<sup>2</sup>.

The average age of the infants, and therefore average length of lactation of the mothers, was 2.4 months (Table 2). The mean birth weight of the infants was 3.2 kg, and only 4% of the newborns had a birth weight below 2.5 kg. Birth weight data were not available for 18% of the infants. The mean Z-scores of height-for-age, weight-for-height, and weight-for-age of the children were close to zero, and their distribution was similar to that of the NCHS reference population.

Maternal BMI was positively associated with the birth weight of their children ( $r=0.28$ ;  $P=0.013$ ) but was not correlated with any of the infant anthropometric indicators weight-for-age, height-for-age or weight-for-height calculated at the time of the data collection.

### Blood data

With the exception of lycopene, all blood parameters shown in Table 3 were normally distributed. The prevalence of anemia, as indicated by a Hb < 120 g/l, was 40%, whereas 29.1% of the women had an inadequate zinc status with plasma zinc < 700 µg/l. A serum retinol concentration < 200 µg/l occurred only in 3.9% of the women, and 23.7% of the women may have had a retinol level < 300 µg/l. As shown in Figure 1, plasma zinc concentration increases with time after delivery ( $r=0.36$ ;  $P<0.001$ ).

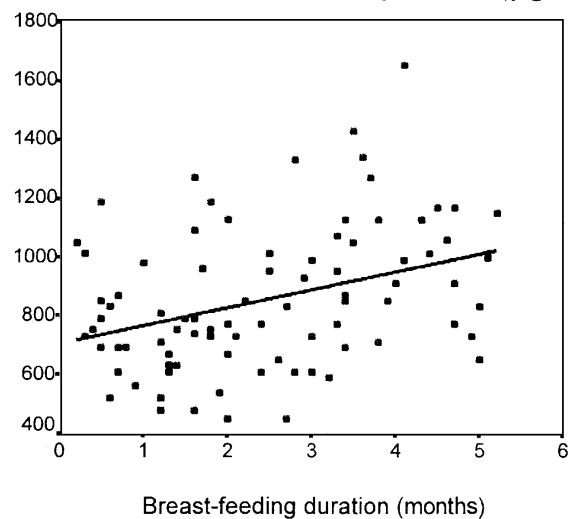
### Breast milk data

None of the measured breast milk nutrients, except fat, were normally distributed. According to Table 4, the median retinol concentration was 420 µg/l, the median

**Table 3** Selected blood parameters of breast-feeding women

Variable	n	Mean±s.d.	Centile			
			10	90	Min	Max
Hemoglobin (g/l)	85	124±18			70	162
Hematocrit (%)	86	37.4±3.3			23.5	43.6
Retinol (µg/l)	76	385±111			128	677
α-Carotene (µg/l)	76	34±23			8	142
β-Carotene (µg/l)	76	104±71			27	475
α-Tocopherol (mg/l)	76	7.71±3.25			1.00	22.12
γ-Tocopherol (mg/l)	76	0.57±0.23			0.09	1.26
Lycopene (µg/l)	76	29 (median)	14	66	11	183
Zinc (µg/l)	86	855±242			450	1650

**Zinc concentration in plasma (µg/L)**



**Figure 1** Plasma zinc concentration (µg/l) related to time after birth (months) ( $r=0.36$ ;  $P<0.001$ ). Solid square, observations; solid curve, calculated.

β-carotene concentration 7.8 µg/l, and the median zinc concentration 2.7 mg/l.

### Correlations

Table 5 shows correlations between the observed blood and breast milk parameters. As well as the correlation between hemoglobin and hematocrit, there was a cluster of significant correlations between fat-soluble substances in the blood: α-carotene correlated significantly with β-carotene and lycopene, and β-carotene correlated with lycopene and α-tocopherol. If the correlation between α- and β-carotene in serum was controlled for lycopene, the correlation was  $r=0.74$  ( $P<0.001$ ). However, the correlation between lycopene and α- or β-carotene disappeared if controlled for the other carotene by partial correlation. In addition to

**Table 4** Selected breast milk parameters of lactating women

Variable	n	Mean ± s.d. or median	Centile			
			10	90	Min	Max
Fat (%)	91	6.4±3.0			1.0	14.4
Retinol (µg/l)	81	420	164	971	75	1891
β-Carotene (µg/l)	80	7.8	3.3	16.6	1.8	58.0
Zinc (mg/l)	91	2.7	1.3	4.7	0.5	12.8

**Table 5** Correlations between selected blood and breast milk parameters

Code		Blood parameters							Breast milk parameters				
		Ht	A	$\alpha$ -c	$\beta$ -c	Lyc	$\alpha$ -t	$\gamma$ -t	Zn	Fat	A	$\beta$ -c	Zn
Hb	Hemoglobin <sup>a</sup>	0.76*	-0.01	0.07	0.10	0.19	0.10	0.01	-0.25	-0.01	0.14	0.16	-0.07
Ht	Hematocrit <sup>a</sup>	1.00	0.11	0.03	0.13	0.23	-0.05	-0.16	0.12	0.06	0.12	0.11	0.05
A	Retinol <sup>a</sup>		1.00	0.10	0.18	0.10	0.26	0.18	-0.18	-0.04	0.32*	-0.12	0.05
$\alpha$ -c	$\alpha$ -Carotene <sup>a</sup>			1.00	0.80*	0.40*	0.35	-0.01	-0.19	-0.01	-0.06	0.17	0.18
$\beta$ -c	$\beta$ -Carotene <sup>a</sup>				1.00	0.61*	0.47*	-0.02	-0.03	0.04	-0.04	0.35*	0.08
Lyc	Lycopene <sup>b</sup>					1.00	0.29	0.02		-1.23	-0.17	0.13	-0.08
$\alpha$ -t	$\alpha$ -Tocopherol <sup>a</sup>						1.00	0.38	-0.40*	0.04	0.30	0.09	0.29
$\gamma$ -t	$\gamma$ -tocopherol <sup>a</sup>							1.00	-0.19	0.07	0.23	-0.04	0.29
Zn	Zinc <sup>a</sup>								1.00	-0.06	-0.05	-0.08	-0.10
Fat	Fat <sup>a</sup>									1.00	0.59*	0.46*	0.37*
A	Retinol <sup>b</sup>										1.00	0.42*	0.24
$\beta$ -c	$\beta$ -Carotene <sup>b</sup>											1.00	0.13
Zn	Zinc <sup>b</sup>												1.00

<sup>a</sup>Pearson correlation.<sup>b</sup>Spearman correlation.\* $P < 0.0001$ .

correlation between fat-soluble components, blood  $\alpha$ -tocopherol was significantly inversely correlated with blood zinc.

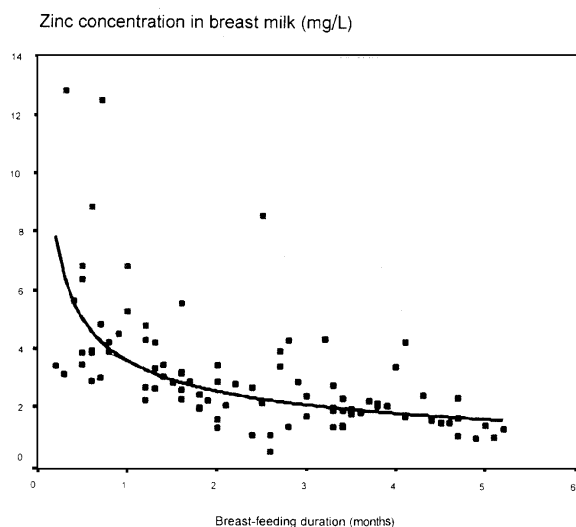
In breast milk, the percentage fat correlated significantly with the retinol and  $\beta$ -carotene concentrations, and retinol correlated also with  $\beta$ -carotene. If the correlations between retinol and fat, retinol and  $\beta$ -carotene, and fat and  $\beta$ -carotene were controlled for  $\beta$ -carotene, fat, and retinol, respectively, the three correlation factors decreased to  $r = 0.37$  ( $P = 0.001$ ),  $r = 0.37$  ( $P = 0.001$ ), and  $r = 0.25$  ( $P = 0.02$ ), respectively. There was a significant positive correlation between the  $\beta$ -carotene concentration in mothers' serum and breast milk, and between retinol in plasma and breast milk.

As shown in Figure 2, the zinc concentration of breast milk declined exponentially with the duration of lactation ( $r = 0.68$ ;  $P < 0.001$ ). The vitamin A concentration of breast milk also decreased with increased time of lactation ( $r = 0.48$ ;  $P < 0.001$ ) (Figure 3).

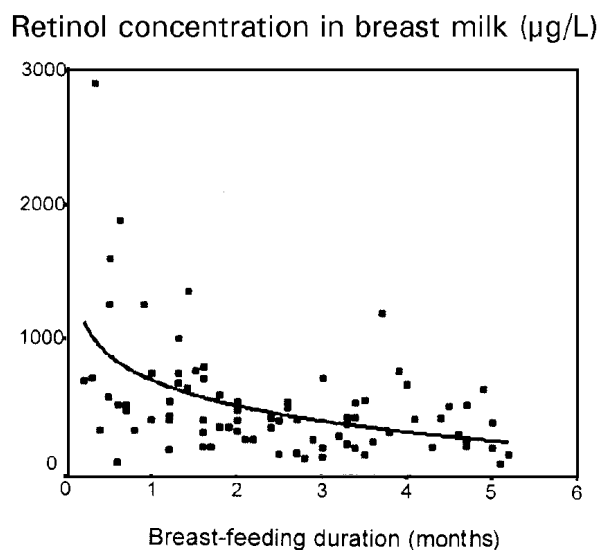
To investigate the main determinants of  $\beta$ -carotene and retinol concentration in breast milk, a stepwise multiple regression was carried out. The concentration of  $\beta$ -carotene in the milk was explained by  $\beta$ -carotene concentration in blood, and by retinol concentration in milk and blood (combined  $r = 0.72$ ), and the factors zinc concentration in blood and milk, duration of breast feeding, and fat in milk were excluded from the model. The concentration of retinol in milk was explained by  $\beta$ -carotene concentration in milk and blood, retinol concentration in blood by milk fat and by duration of breast feeding (combined  $r = 0.71$ ), and the factors zinc concentration in blood and milk were excluded from the model.

## Discussion

The socioeconomic data, such as housing conditions and education of mothers and fathers, indicate that the



**Figure 2** Plasma zinc concentration ( $\mu\text{g/l}$ ) related to length of lactation (months) ( $r = 0.68$ ;  $P < 0.001$ ). Solid square, observations; solid curve, calculated.



**Figure 3** Retinol concentration (RE/l) in breast milk related to length of lactation (months) ( $r = 0.48$ ;  $P < 0.001$ ). Solid squares, observations; solid curves, calculated.

socioeconomic situation of the observed households was above the Indonesian average (UNICEF, 1995). Therefore, in the interpretation of mothers' blood and milk data it must be considered that the families did not belong to low-income households.

The mean serum retinol concentration of the women was higher than the values that were reported by Tarwotjo (1990) for pregnant (130 µg/l) and lactating (200 µg/l) Indonesian women, and by Stolzhus *et al* (1993) for breast-feeding mothers. The plasma retinol concentrations measured in this group were also higher than observed in Gambian pregnant and lactating women (Bates *et al*, 1993) but were similar to the average found in developing countries in general (Newman, 1994). The plasma carotenoid level of the Indonesian mothers was comparable to that of the Gambian women during the season with high-carotene food intake (Bates *et al*, 1993). Nearly one-third of the mothers (31%) had retinol concentrations < 300 µg/l, which is seen as the threshold for an adequate vitamin A status (IVACG, 1982).

The retinol concentration in breast milk declined with the duration of lactation. This finding is in agreement with results obtained in Ethiopian women (Gebre-Medhin *et al*, 1976), and was also mentioned in a literature review by Newman (1994). The present cross-sectional study also showed an association between the retinol concentration in blood and milk, stressing the importance of the vitamin A status of the mother to ensure an adequate vitamin A uptake by the child. Assuming that mothers provide approximately 750 ml breast milk per day for the first 4–6 months of full lactation, between the third and fourth months of lactation (Garza, 1993) the daily calculated median vitamin A supply for an infant would be 315 RE/day. The FAO/WHO (1988) recommends a minimum of 180 RE per day to meet the basal needs of infants. None of the studied women showed vitamin A concentrations in their breast milk that would be below this minimum. However, according to the National Research Council (1989), children should receive 375 RE/day during the first year of life to ensure normal stores of vitamin A in the liver. With this cut-off point, the milk of 70% of the mothers did not meet these requirements.

The zinc status of the mothers was less adequate than their vitamin A status, considering the fact that about 29% had low plasma zinc levels. The plasma zinc level was a little lower than the level of 960 µg/l reported for healthy North American adults (Halsted & Smith, 1970) but higher than the level of 253–773 µg/l reported for Zairian mothers (Arnaud *et al*, 1994). Ohtake & Tamura (1993) found that zinc plasma concentration increased with time after birth, which was confirmed in this study. According to Shrimpton (1993), after birth the catabolism of uterine tissue contributes to circulating zinc levels.

This study confirms previous observations (Moran *et al*, 1983; Moser & Reynolds, 1983; Simmer *et al*, 1990) that zinc concentration in human breast milk declines with the duration of lactation. According to Nyazema *et al*, (1989), the zinc concentration decreased from 820 ± 100 µg/l in colostrum to 380 ± 30 µg/l in mature milk. The calculated zinc intake of infants from breast milk was found to be less than 50% of the RDA.

In Bangladeshi rural women, zinc levels in breast milk, which were comparable with those found in developed countries, fell with increasing time after birth (Simmer *et al*, 1990). As a result, the calculated daily intake decreased

from 17.7 to 8.0 µmol. Lehti (1990) also found in women living in the Brazilian Amazon basin that, with decreasing zinc levels in breast milk, three months after birth all women provided insufficient zinc through exclusive breast-feeding.

According to the National Research Council (1989), infants who receive 2 mg zinc daily from breast milk do not show any signs of zinc deficiency. Assuming a daily average breast milk intake of 750 ml in the studied group, one-third of the mothers provided less than this recommended amount of zinc. The present study confirms findings from other studies from several parts of the world that often the zinc concentration in breast milk of women is too low to provide an adequate amount of zinc for the fast-growing infant if it is exclusively breast-fed for 4–6 months.

The normal range of birth weight of the children indicates that the nutritional status of the mothers was sufficient for normal fetal growth. However, owing to the physiological decrease in the concentrations of zinc and vitamin A in breast milk, it seems very likely that if the infants are exclusively breast-fed and/or receive inadequate complementary food above the age of 4 months, the supply of both micronutrients may well be insufficient. Considering that anthropometric data of the surveyed mothers do not indicate signs of undernutrition and that they belong to a better-off urban community whose food pattern is more varied than in rural areas, one might expect that the breast milk of rural mothers in terms of vitamin A and zinc might be even more deficient. As a result, despite the impressive economic growth Indonesia has undergone since the 1980s, it must be considered that subclinical micronutrient deficiencies are still prevalent. More information on daily micronutrient intake and intervention trials are needed to determine whether zinc supply can be achieved through Indonesian diet alone or whether supplemental zinc should be given to infants before they reach the age of 4 months and/or whether after 4 months complementary infant food should be fortified with zinc.

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