CALCIUM, MAGNESIUM AND PHOSPHORUS CONCENTRATIONS IN HUMAN MILK AND IN SERA OF NURSING MOTHERS AND THEIR INFANTS DURING 26 WEEKS OF LACTATION

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ABSTRACT

Longitudinal calcium, magnesium and phosphorus concentrations were measured in milk and sera of 20 nursing mothers and their infants during a period of 26 weeks. Within this period, progressive increase in serum calcium, magnesium and total protein concentrations were observed in breast-fed infants, in association with decreasing phosphorus content of breastmilk. Maternal serum calcium, magnesium and total protein concentrations also increased during this period. It is speculated that a decrease in serum phosphorus in breast-fed infants can cause rickets. It is also speculated that the physiological rise in serum calcium concentrations in maternal serum may be related to the physiological increase in serum total protein.

Key words: Breast milk, Magnesium, Calcium, Phosphorus.

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The World Health Organization has accepted international law encouraging the feeding of infants in the first 4-6 months of life with breast milk as the sole source of nutrition; all the member states have decided to comply with this law. It is a known fact that breast milk is the most suitable means of nutrition for infants during the first months of life and that it meets all the needs of infants during this period. Also, almost every healthy woman, except for psychological reasons, is able to secrete sufficient milk for her child(1-4). However, very few studies have examined the effect of factors such as inadequate maternal nutrition and frequent delivery upon the composition of breast milk. The results of these studies, moreover, are inadequate(5).

With the widespread use of breast milk, it is important to obtain accurate information concerning the composition of breast milk as well the nutritional and metabolic status of nursing mothers and their children(6). This study was organized in the Sivas area of Turkey where the winters are long and without sunshine, the socio-economic conditions are poor, the mothers have inadequate nutrition and frequent deliveries. The objective was to examine whether there is adequate bone mineralization in the breast-feeding mothers given 400 IU vitamin D for a period of 26 weeks by measuring the calcium (Ca), magnesium (Mg), phosphorus (P) and total protein (TP) concentrations in the breast milk and sera of the mothers and their breast-fed infants.

Material and Methods

For this study, 20 infants without congenital malformations or gastrointestinal disease were selected. All infants were born after 38-40 weeks gestation calculated
from the first day of mother’s last menstrual period and confirmed clinically by Dubowitz table. All mothers were more than 18 years old. The infants as well as mothers living in poor socio-economic conditions, were followed up for a period of 26 weeks. Two of the infants were born in summer and 18 at the end of summer. The infants’ weight and head circumference were measured on the first day and 3rd, 6th, 12th and 26th weeks of life; they were classified as per Harvard(7) values, taking into account the studies of Fomon et al.(8).

The mothers received no supplemental vitamin D during their pregnancies, having usual diets during nursing. The types and amounts of maternal diets for 3 days were recorded on 3-day nutrition forms and evaluated according to the food exchange tables. The daily intake of elements and protein intake were then calculated. It was observed that Ca (370 mg), Mg (405 mg) and protein (59.8 g) intake were low but P (1009 mg) intake was high. In addition 400 IU vitamin D was given. The breast-fed infants received 400 IU vitamin D per day. The mothers were asked to collect samples during these periods. Three times a day.

After collecting the milk, 2.5 ml of blood was taken from the mother and the infant and studied following centrifugation. The breast milk and sera total protein concentrations were determined by the Biuret method(9) and the phosphorus concentration by the Fiske and Subbarow method(10). The remaining breast milk and serum samples were kept at -20°C and later the calcium and magnesium concentrations were determined by the atomic absorption spectrophotometric method (Perkin Elmer 107)(11).

The results obtained were interpreted by the Student’s t test, Variance analysis and Linear regression analysis(12).

Results

The Ca, P, Mg and total protein (TP) concentrations in the mothers’ sera are shown in Table I & Fig 1. Between the 3rd to 6th weeks of nursing, a significant decrease in the Ca, Mg and TP concentrations was observed with a gradual rise after the 6th week (p < 0.001).

The Ca, P, Mg and TP concentrations in breast milk are shown in Table II & Fig. 2. Between the 3rd to 6th weeks of nursing, a significant rise in the Ca, Mg and TP concentrations was observed. There was a sig-

<table>
<thead>
<tr>
<th>TABLE I-- Comparison of Difference Between the Ca, P, Mg and TP Concentrations in Maternal Sera in the 3rd and 26th Weeks</th>
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<tbody>
<tr>
<td>Parameter</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
</tr>
<tr>
<td>P (mg/dl)</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
</tr>
<tr>
<td>TP (g/l)</td>
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n = Number of cases, Av = Average, SE = Standard error.
significant decrease in the Ca and TP concentrations after the 6th week, while Mg values gradually rose \((p < 0.001)\), and p values showed a dramatic decrease \((p < 0.001)\).

**Figs. 3 & 4** show that during the entire 26 weeks, there was a negative relationship between the P and Ca in mothers' serum and breast milk; when P and Ca in the mothers' serum rose concentrations in breast milk fell \((r = -0.36, r = -0.63; p < 0.01, p < 0.01)\) respectively. There was no relation between the Mg concentrations in maternal serum and breast milk.

During lactation, the P in breast milk and infants' sera showed a reduction whereas Ca and Mg in the infants' sera increased. On linear regression analysis, there was a strong relation between the P, Ca and Mg in infants' sera and the P in the breast milk \(r=0.69; p (<0.01), r=0.38; p (<0.01), r=0.24; (p <0.05)\) (Figs. 5-8). There was a relation between the Ca in infants' sera and in breast milk also \((r = -0.050, p < 0.01; Fig. 9)\). There was a relation between the Ca and P values in
TABLE II--Comparison of Difference Between the Ca, P, Mg and TP Concentrations in Breast Milk in the 3rd and 26th Weeks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3rd week</th>
<th></th>
<th></th>
<th></th>
<th>26th week</th>
<th></th>
<th></th>
<th></th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Av</td>
<td>SE</td>
<td></td>
<td>n</td>
<td>Av</td>
<td>SE</td>
<td></td>
<td>t</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>20</td>
<td>26.8</td>
<td>0.26</td>
<td></td>
<td>20</td>
<td>23.6</td>
<td>0.35</td>
<td></td>
<td>13.4</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>20</td>
<td>13.3</td>
<td>0.30</td>
<td></td>
<td>20</td>
<td>8.29</td>
<td>0.21</td>
<td></td>
<td>17.1</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>20</td>
<td>4.16</td>
<td>0.15</td>
<td></td>
<td>20</td>
<td>4.67</td>
<td>0.15</td>
<td></td>
<td>8.53</td>
</tr>
<tr>
<td>TP (g/l)</td>
<td>20</td>
<td>8.39</td>
<td>0.38</td>
<td></td>
<td>20</td>
<td>7.09</td>
<td>0.35</td>
<td></td>
<td>6.53</td>
</tr>
</tbody>
</table>

n = Number of cases, Av = Average, SE = Standard error.

Fig. 3. The relationship between the P concentration in maternal sera and the P concentrations in the 3rd, 6th, 12th and 26th weeks of lactation and the calculated regression line.

Fig. 4. The relationship between the Ca concentrations in maternal sera and the Ca concentrations in breast milk in the 3rd, 6th, 12th and 26th weeks of lactation and the regression line.
Discussion

It is known that the essential factors contributing to bone mineralisation in full-term breast-fed infants are the mother's diet, providing the infant with Ca, P, Mg and Vitamin D through breast milk and the degree of sunlight(13,14), received by both. While the milk of mothers consuming adequate vitamins during pregnancy and lactation contains adequate concentrations of Ca, P and Mg for bone mineralisation and skeletal development of a full-term infant, it contains less than the minimal daily requirement of vitamin D (4-22 IU/L) and is consequently inadequate(12-15). In cases of insufficient sunlight, supplemental vitamin D gains importance(14-18). Adequate Ca, P, and Mg concentrations have been observed at the end of 26th week(6,19) in infants who have been only breast-fed for 6 months and given a supplemental 400 IU Vitamin D in suitable environmental and socio-economic conditions, where the
**Fig. 7.** The relationship between the P concentration in breast milk and the Mg concentration in infant sera in the 3rd, 6th, 12th and 26th weeks of lactation and regression line.

**Fig. 8.** The relationship between the Ca concentration in infant sera and the P concentration in breast milk in the 3rd, 6th, 12th and 26th weeks of lactation and regression line.

**Fig. 9.** The relationship between the Ca concentration in breast milk and infant sera in the 3rd, 6th and 26th weeks of lactation and regression line.
by us for the first time supports this. In our study, a strong relationship ($r = 0.94$) between Ca and TP displayed a marked and significant increase ($p < 0.001, p < 0.001$) from week 6 to week 26.

The increases in the maternal serum P concentrations observed by us, which were different from those in the study of Greer et al. (6), can be attributed to the excess amount of P in the mother's diet when compared with Ca and Mg as well as maternal supplementantion of vitamin.

In our study there was a significant decrease in the P, Ca and TP and significant rise in Mg concentrations in breast milk ($p < 0.001$; Fig. 2). There are very few studies showing the composition of breast milk in monthly follow-up (19). Winikoff (31) in a study in Australia showed that with longer lactation period there was a decrease in the Ca concentration of breast milk. Some other studies also reported a similar gradual decrease in the Ca concentration of milk with prolonged lactation (19,32). Likewise, it has been reported that as lactation is prolonged, the P concentration in milk decreases (19,20,31).

In our study in mothers with very low intake of Ca, the negative mineral balance during lactation may have been compensated by decreased Ca and P secretion into breast milk.

In some studies no change was shown in the Mg in milk as lactation was prolonged (6,19,31), however, an increase was observed in our study from the 3rd week to the 6th week ($p < 0.001$). In our study the breast milk TP concentrations in the 26th week were significantly lower than in the 3rd week (7.09-0.359/L and 8.389/L, $p < 0.01$). For many years it has been considered that the quality of breast milk is dependent upon the nutri-
tional status of the mother; various studies have been undertaken to obtain information on this subject. In developing countries the diet of nursing mothers is often deficient in protein; the high incidence of kwashiorkor in children in these countries suggests that the protein content of breast milk may be inadequate(33). In various studies uniform results have not been obtained; some found that the mother's protein intake affected the concentration of protein in milk(34-35) while others observed no such effect(36,38). Karmarkar et al.(33) reported that the protein concentration of breast milk was directly affected by the protein concentration in the mother's diet. Thus, the low values of TP concentrations in milk in our study may be attributed to a poor intake of protein. This indicates that increased dietary protein will increase protein in breast milk, and emphasizes the necessity for sufficient maternal dietary protein especially in our country.

It is possible that the fall in the P concentration in infant sera during nursing is related to an increased ratio of glomerular filtration(39), or to the intake of supplemental vitamin D(5). Theoretically increased P in the infant's diet will increase the serum P and thus the Ca and Mg concentrations(40,41). This is achieved by the accumulation of Ca in the bones and soft tissues, with decreased Ca concentration outside the cells(42). On the contrary, decreased intake of P leads to hypercalcemia by mobilisation of Ca from the bones by physiochemical influences(43).

We consider that the intake of P decreases during lactation because we observed that over the 26 weeks of lactation, the P concentration decreased significantly (p <0.001). As the infant's intake of milk is decreased compared to that in the first 3 weeks, the P intake per unit of body weight also decreases(44). The strong positive relation (r = 0.69, p <0.01) of the infant serum P level with milk P level during the 26 weeks clearly indicated the effect of the milk P level in altering the infant P level.

The Ca and Mg concentrations of infant serum showed a marked rise throughout the period of lactation (p <0.001), with a strong negative relation with the p concentration of breast milk (r = 0.38, p < 0.01; r = 0.24, p <0.05). This can be interpreted as physiological increases in serum Ca and Mg concentrations of breast-fed infants decreasing the intake of phosphorus from the milk. This phenomenon, achieved by the mobilization of Ca and Mg from osseous tissues, can therefore adversely affect the formation of bones in childhood.

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