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 socioeconomic 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 come for follow-up in the 3rd, 6th, 12th and 26th weeks following delivery. For every nursing a total of 9 ml of breast milk was collected immediately after suckling, in the middle and at the end of the feed. This was repeated 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 I-- *Comparison of Difference Between the Ca, P, Mg and TP Concentrations in Maternal Sera in the 3rd and 26th Weeks*

Parameter	3rd week			26th week			Significance	
	n	Av	E	n	Av	SE	t	p <
Ca (mg/dl)	20	8.39	0.63	20	9.84	0.17	8.28	0.001
P (mg/dl)	20	3.58	0.96	20	3.95	0.93	3.7	0.010
Mg (mg/dl)	20	2.41	0.57	20	3.16	0.41	14.6	0.001
TP (g/l)	20	5.79	0.91	20	6.36	0.10	5.49	0.001

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

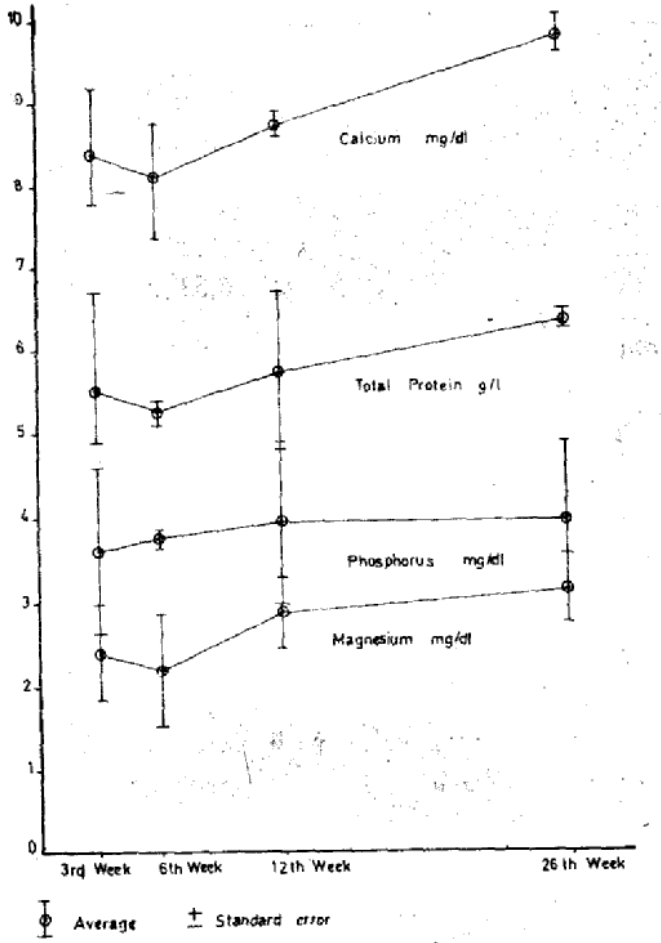

Fig. 1. Variations in serum concentrations of P, Ca, and TP in nursing mothers from the 3rd to the 26th weeks of lactation.

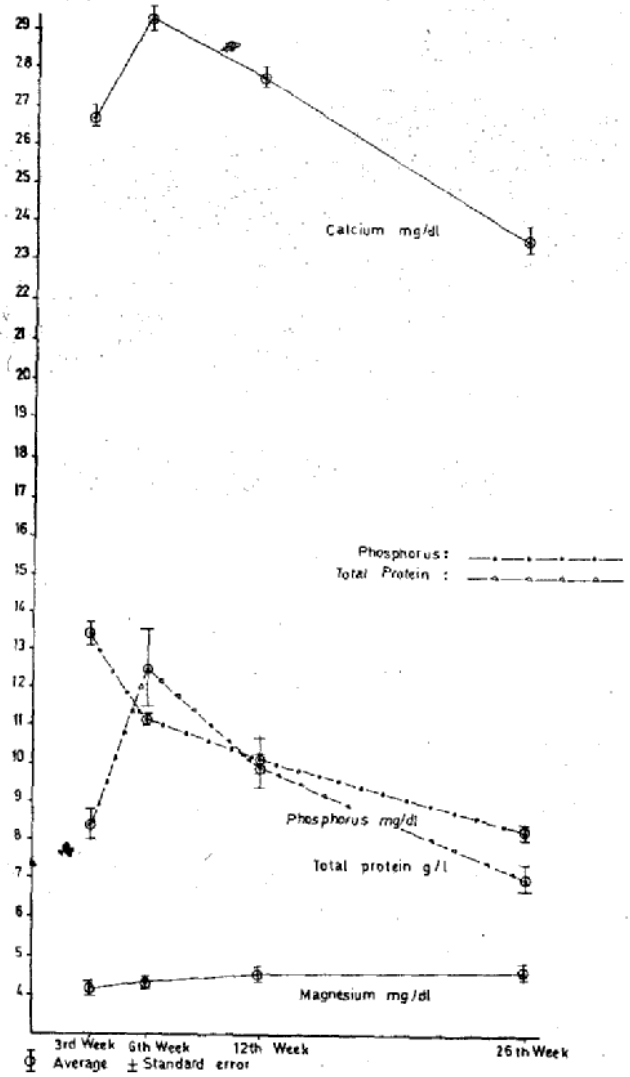

Fig. 2. The course shown by P, Ca, Mg, and TP in breast milk in the 3rd, 6th, 12th and 26th weeks of lactation.

nificant 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*

Parameter	3rd week			26th week			Significance	
	n	Av	SE	n	Av	SE	t	p <
Ca (mg/dl)	20	26.8	0.26	20	23.6	0.35	13.4	0.001
P (mg/dl)	20	13.3	0.30	20	8.29	0.21	17.1	0.001
Mg (mg/dl)	20	4.16	0.15	20	4.67	0.15	8.53	0.001
TP (g/l)	20	8.39	0.38	20	7.09	0.35	6.53	0.001

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

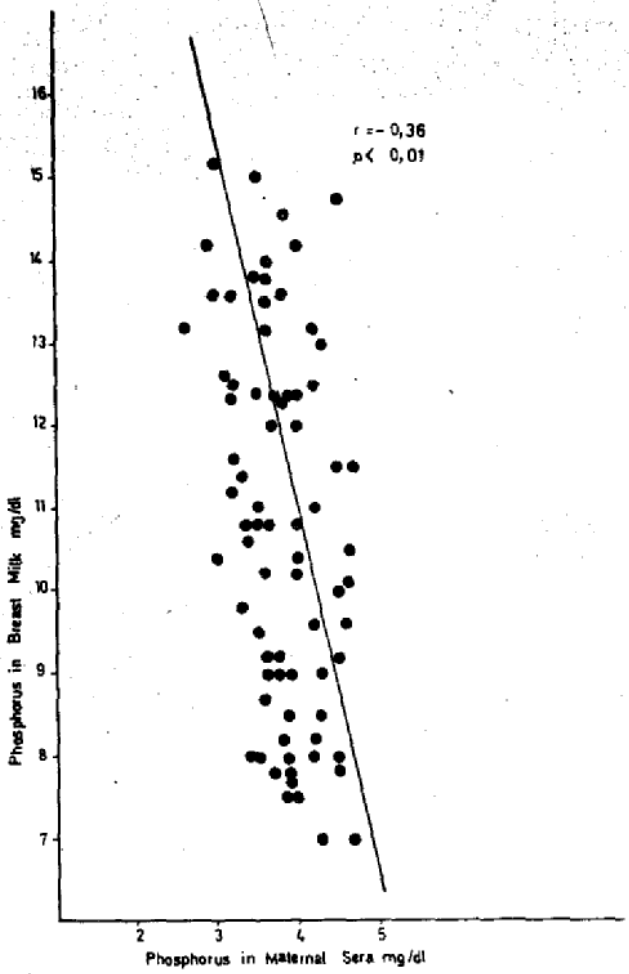

Fig. 3. The relationship between the P concentration in maternal sera and the P concentrations in the 3rd, 6th, 12th and 26th weeks of lactation and the calculated regression line.

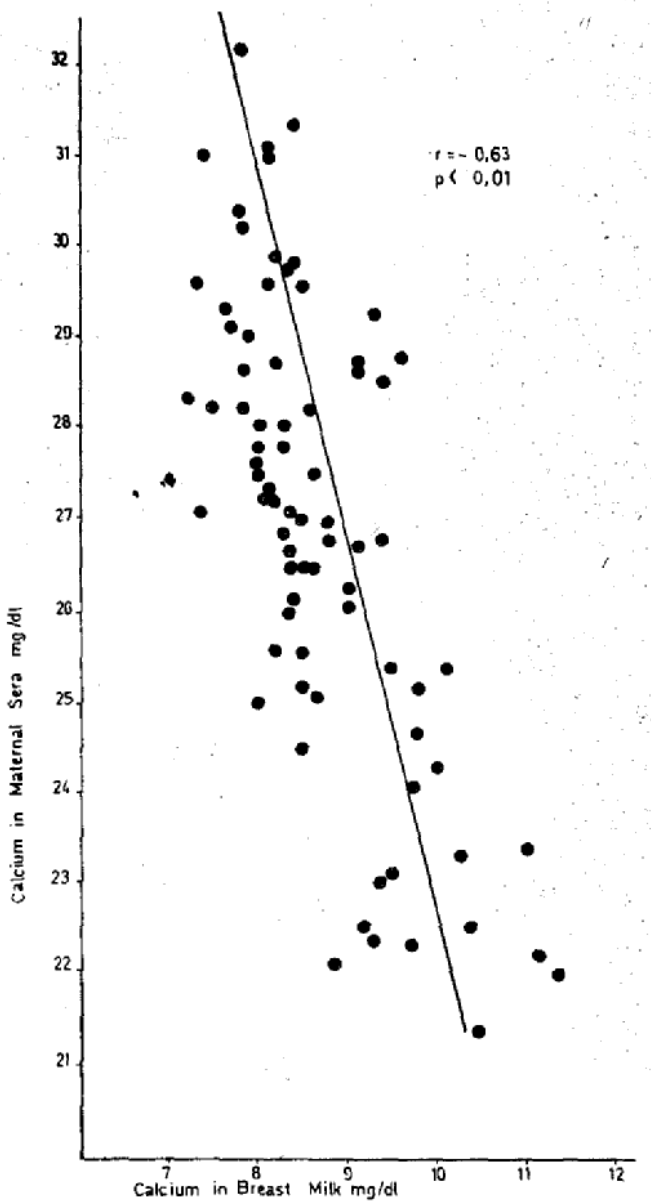

Fig. 4. The relationship between the Ca concentrations in maternal sera and the Ca concentrations in breast milk in the 3rd, 6th, 12th and 26th weeks of lactation and the regression line.

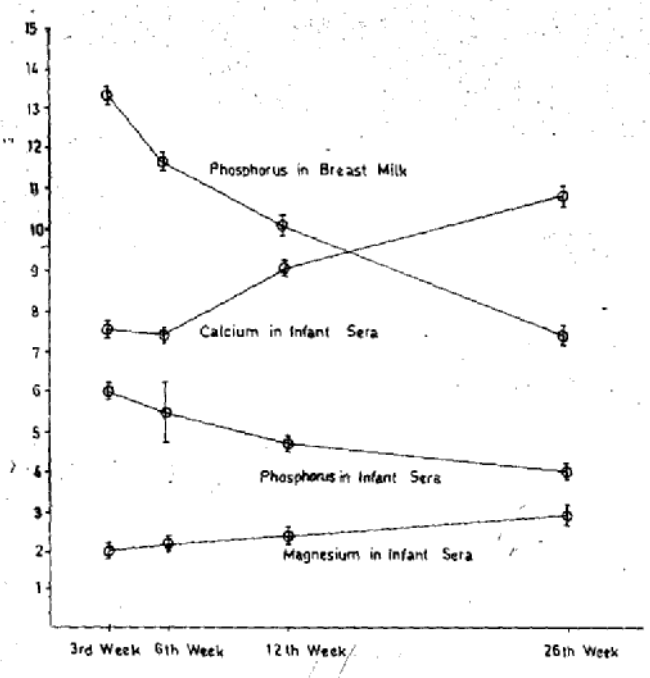

Fig. 5. The course of P, Ca and Mg shown in infant sera and the P in breast milk for the duration of lactation.

infants' sera (r= –0.53, p <0.01, *Fig. 10*) but none between the Mg and P values.

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

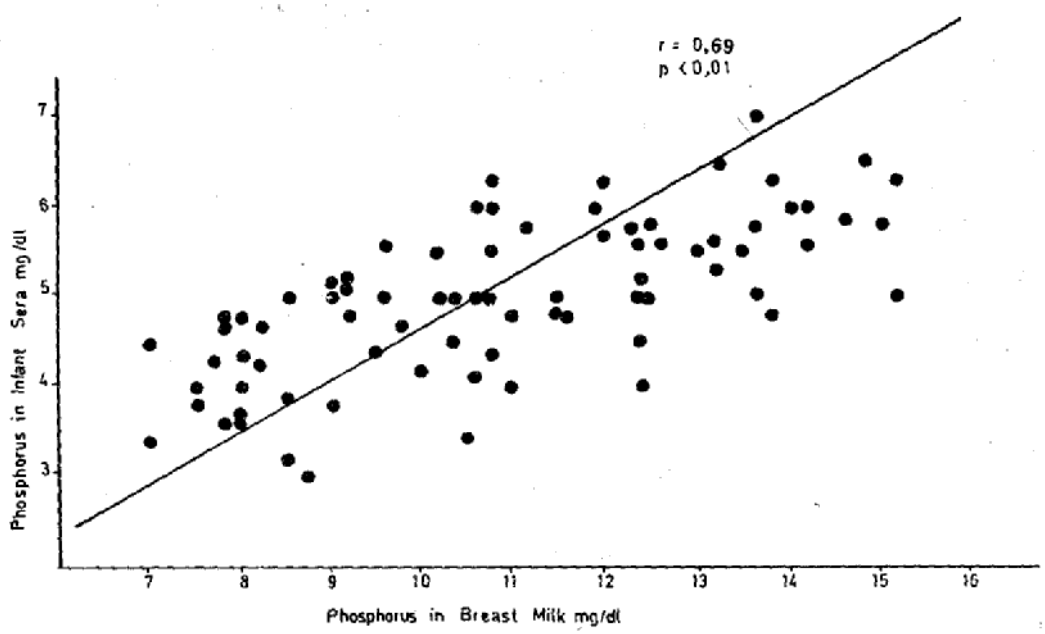

Fig. 6. The relationship between the P concentration in breast milk and infant sera in the 3rd, 6th, 12th and 26th weeks of lactation and the regression line.

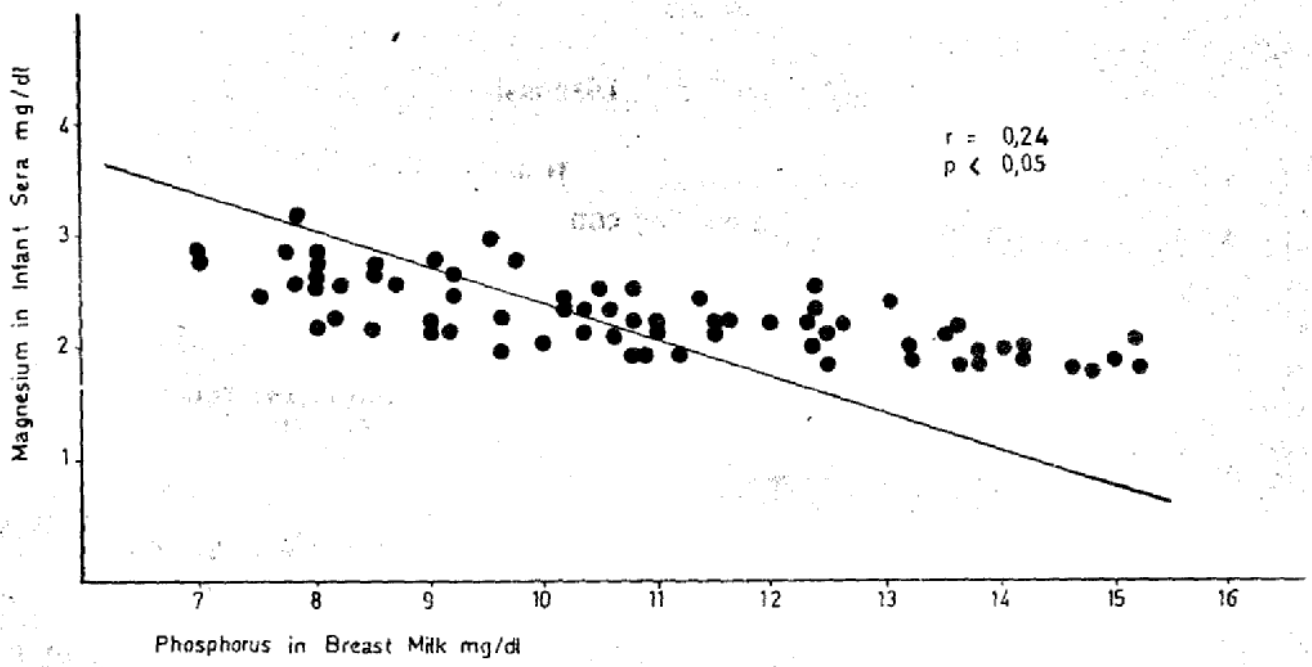

Fig. 7. The relationship between the P concentration in breast milk and the Mg concentration in infant sera in the 3rd, 6th, 12th and 26th weeks of lactation and regression line.

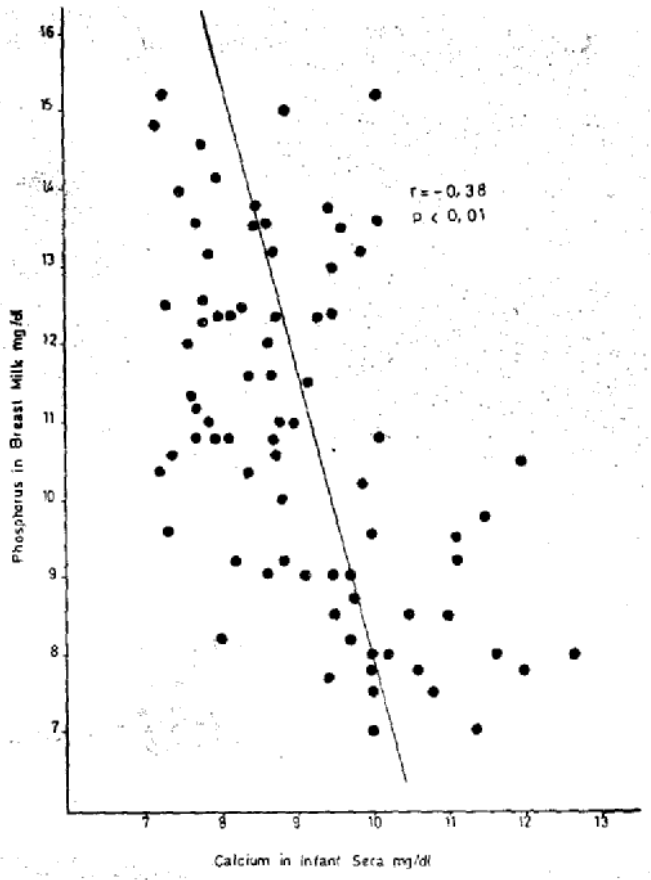

Fig. 8. The relationship between the Ca concentration in infant sera and the P concentration in breast milk in the 3rd, 6th, 12th and 26th weeks of lactation and regression line.

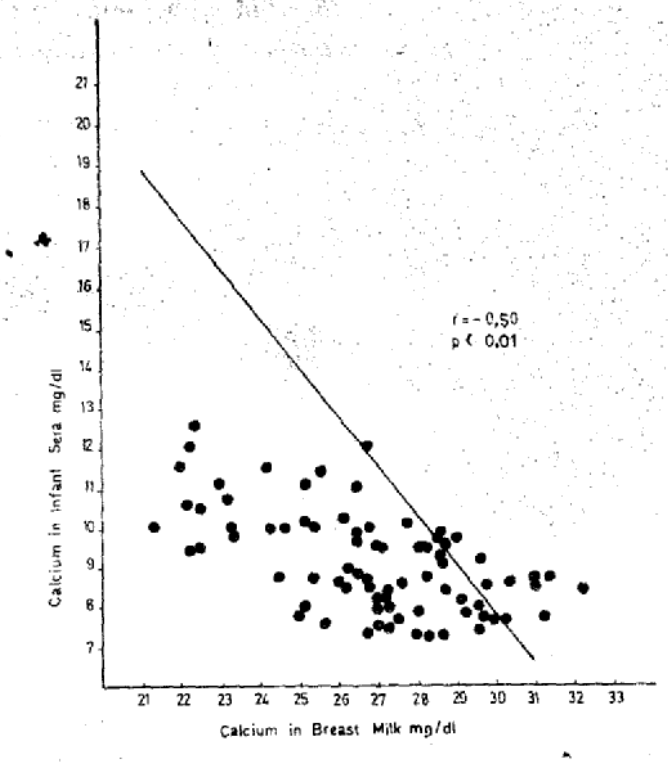

Fig. 9. The relationship between the Ca concentration in breast milk and infant sera in the 3rd, 6th and 26th weeks of lactation and regression line.

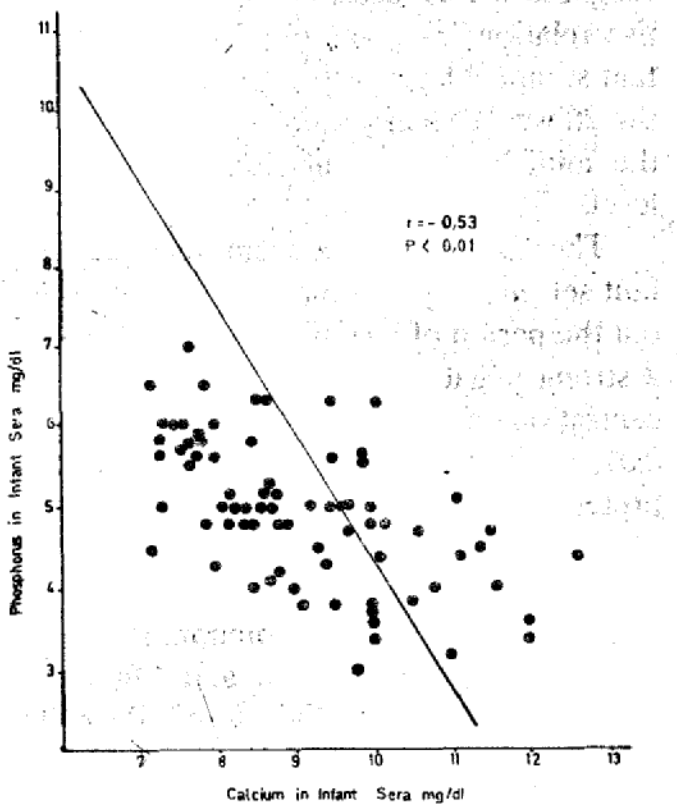

Fig. 10. The relationship between the Ca and P concentrations in infant sera in the 3rd, 6th, 12th and 26th weeks of lactation and regression line.

mother's diet is sufficient in Ca, P, Mg and Vitamin D.

Various workers have attempted to explain the decrease in serum protein and total Ca levels during pregnancy(20-27). The most valid explanation is that the physiological decrease in serum albumin results in a decrease in total Ca level(28). Some authors have reported that these decreased Ca and Mg values remained unchanged during lactation(19,29), others that they decreased further(30), and still others that they gradually increased(6), as in this report. The return of Ca to normal indicated that serum protein gradually returned to normal and more Ca was bound. The rise in the TP concentrations in the mother's serum during lactation shown 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 supplemantation 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 similiar 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 breastmilk.

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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REFERENCES

1. World Health Organization. Breast Feeding. Maternal and Child Health Unit, Geneva, WHO, 1979.
2. Cameron M, Hofvander Y. Manual of feeding infants and young children. 3rd edn. Delhi, Nairobi, Oxford Medical Publication, 1983, 19: pp 81-91.
3. Tanzer F, Gumuser C. A study of the growth of 200 newborn babies for a period of 6 months according to the type of nutrition. Ann Trop Pediatr 1989, 9: 54-58.
4. Ferris AG, Laus MJ, Hosmer DW, *et al.* The effect of diet on weight gain in infancy. Am J Clin Nutr 1980, 33: 2635-2640.
5. Jeliffe DB. World trends in infant feeding. Am J Clin Nutr 1976, 29: 1227-1232.

6. Greer FR, Isang RC, Levin RS, *et al.* Increasing serum calcium and magnesium concentrations in breast-fed infants—Longitudinal studies of minerals in human milk and in sera of nursing mothers and their infants. J Pediatr 1982, 100: 59-64.

7. Nelson WE. Assessment of physical growth and development. *In:* Textbook of Pediatrics. Eds Behrman RE, Victor VC, Nelson WE. Philadelphia, WB Saunders Co, 1976, pp 38-50.

8. Fomon SJ, Ziegler EE, Filler, *et al.* Growth and serum chemical values of normal breast-fed infants. Acta Pediatr Scand 1978, 273 (suppl 1): 1-17.

9. Fister HJ. Standard Scientific Supply, 1950, p 50.

10. Natelson S. Techniques of Clinical Chemistry. Springfield, Charles C Thomas, 1971, pp 576.

11. Sundesman FWJ, Carrol JE. Measurement of serum calcium and magnesium by atomic absorption spectrometry. Am J Clin Path 1965, 43: 302-310.

12. Sumbuloglu K. Saglik bilimlerinde arastirma teknikleri ve istatistik. First edn. Turkey, Ankara Matis Publication-3, 1978.

13. Tsang RC, Steichen JJ, Brown DR. Perinatal calcium homeostasis, neonatal hypocalcemia and bone demineralization. Clin Perinatol 1977, 4: 385-392.

14. Greer FR, Greer MD, Searcy E, *et al.* Bone mineral content and serum 25-hydroxyvitamin D concentration in breast-fed infants with and without supplemental vitamin D. J Pediatr 1981, 98: 696-701.

15. Roberts CC, Chan GM, Folland D, *et al.* Adequate bone mineralization in breast-fed infants. J Pediatr 1981, 99: 192-196.

16. Weisman Y, Bawnik JC, Eisenberg J, *et al.* Vitamin D metabolites in human milk. J Pediatr 1982, 100: 745-748.

17. Rudolf M, Arulanantham K, Greenstein RM. Unsuspected nutritional rickets. Pediatrics 1980, 66: 72-76.

18. Greer FR, Seichen JJ, Tsang RC. Effects of increased calcium, phosphorus and vitamin D intake on bone mineralization in very low birth weight infants fed formulas with polycose and medium-chain triglycerides. J Pediatr 1983, 100: 951-955.

19. Vaughan LA, Weber CW, Kemberling SR. Longitudinal changes in the mineral content of human milk. Am J Clin Nutr 1979, 32: 2301-2306.

20. Atkinson PJ, West RR. Loss of skeletal calcium in lactating women. J Obstet Gynecol 1970, 77: 555-560.

21. Lamke B, Brundes J, Moberg P. Changes of bone mineral content during pregnancy and lactation. Acta Obstet Gynecol Scand 1977, 56: 217-219.

22. Pitkin RM. Calcium metabolism in pregnancy. A review. Am J Obstet Gynecol 1975, 121: 724-734.

23. Newman RL. Further observations on serum calcium and phosphorus during pregnancy. Am J Obstet Gynecol 1953, 65: 796-802.

24. Newman RL. Calcium and phosphorus balance in pregnancy. Am J Obstet Gynecol 1956, 8: 561-564.

25. Goss DA. Renal conservation of calcium during pregnancy. Am J Obstet Gynecol 1962, 20: 199-203.

26. Heaney RP, Skilman TG. Calcium metabolism in normal human pregnancy. J Clin Endocrinol 1971, 33: 661-669.

27. Root AW, Harrison HE. Recent advances in calcium metabolism. J Pediatr 1976, 88: 1-8.

28. Kerr C, Loken HF, Glendening MB. Calcium and phosphorus dynamics in pregnancy. Am J Obstet Gynecol 1962, 83: 2-8.

29. Fairney A, Naughten E, Oppe TE. Vitamin D and human lactation. Lancet 1977, 2: 739-741.

30. Fenuku RI, Eorl-Quncoo SN. Serum calcium, magnesium and inorganic phosphate

during lactation. Trop Georgr Med 1978, 30: 495-501.

31. Winikoff D. Calcium, magnesium and phosphorus in the milk of Australian women. Med J Aust 1944, 2: 660-667.
32. Kader MM, Hay AE, El Satari S, *et al.* Clinical, biochemical and experimental studies on lactation. Am J Obstet Gynecol 1969, 105: 978-984.
33. Karmarkar MG, Ramakrishan CV. Studies on human lactation. Acta Pediatr 1960, 49: 599-604.
34. Deem HE. Observation on the milk of New Zealand women. Arch Dis Child 1931, 6: 53-61.
35. Forsum E, Lonnerdal B. Effect of protein intake on protein and nitrogen composition of breast milk. Am J Clin Nutr 1980, 33: 1809-1813.
36. Jeliffe DD, Jeliffe EFP. The volume and composition of human milk in poorly nourished communities. Am J Clin Nutr 1978, 31: 492-515.
37. Anderson HG. Human milk feeding. Pediatr Clin North Am 1985, 2: 335-354.
38. Gopalan C. Studies on lactation in poor Indian communities. J Trop Pediatr 1958, 4: 87-91.
39. Wiel WB. The evaluation of renal function in infancy and childhood. Am J Med 1955, 229: 678-681.
40. Oppe TE, Redstone D. Calcium and phosphorus levels in healthy newborn infants give various types of milk. Lancet 1968, 1: 1045-1048.
41. Gitlaman IF, Pinens JB. Influence of diet on the occurrence of hyperphosphatemia and hypocalcemia in the new born infant. Pediatrics 1951, 45: 778-788.
42. Tsang RC, Chen I, Henas W, *et al.* Neonatal hypocalcemia in infants with birth asphyxia. J Pediatr 1974, 84: 428-433.
43. Kleeman Cr, Massry S, Coburn JW. The clinical physiology of calcium hemostasis, parathyroid hormone and calcitonin. Calif Med 1971, 114: 16-18.
44. Nancy IG, Hunscher HA, Donelson E, *et al.* Human milk flow. Am J Child 1980, 38: 1186-1191.