

## Calcium levels in maternal milk: relationships with calcium intake during the third trimester of pregnancy

Rosa M. Ortega<sup>1\*</sup>, Rosa M. Martínez<sup>2</sup>, M. Elena Quintas<sup>1</sup>, Ana M. López-Sobaler<sup>1</sup> and Pedro Andrés<sup>3</sup>

<sup>1</sup>*Departamento de Nutrición, Facultad de Farmacia, Universidad Complutense, Madrid, Spain*

<sup>2</sup>*Servicio de Análisis Clínicos, Servicio de Obstetricia y Ginecología, Hospital INSALUD, Cuenca, Spain*

<sup>3</sup>*Laboratorio de Técnicas Instrumentales, Facultad de Farmacia, Universidad Complutense, Madrid, Spain*

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The aim of the present study was to investigate the relationship of Ca intake and serum Ca levels during the third trimester of pregnancy with levels of the same mineral in transition milk (days 13–14 of lactation) and mature milk (day 40 of lactation). The study subjects were a group of fifty-seven healthy, lactating mothers aged between 18 and 35 years (mean 27 (SD 3.7) years) whose pregnancies and labour were attended by the Department of Obstetrics and Gynaecology of Cuenca INSALUD Hospital, Spain. Ca intake during the third trimester was determined by recording the consumption of foods over a 5 d period and by registering Ca provided by dietary supplements. The same method was used to investigate the intake of protein, vitamin D, fibre and Fe, nutrients that could affect the use of dietary Ca. Ca levels in maternal serum during this stage of pregnancy, during lactation and in transition and mature milk samples, were determined using 2-cresolphthalein complexone. During pregnancy 70.2% of subjects showed Ca intakes below 1100 mg/d (75th percentile). The consumption of Ca supplements was very small and hardly modified the mean quantity supplied by the diet. Subjects with an intake < 1100 mg/d showed no fall in Ca levels in serum, either during pregnancy or lactation, nor were decreased levels found in transition milk. However, these subjects showed lower Ca levels in mature milk (5.95 (SD 1.56) mmol/l) than did subjects with greater Ca intakes (6.82 (SD 1.31) mmol/l). This may suggest that breast-fed babies of mothers with lower Ca intakes during pregnancy also receive less Ca.

### Calcium: Pregnancy: Lactation

Recommendations for Ca intake during pregnancy and lactation differ around the world, reflecting the inadequacy of knowledge about Ca requirements in human reproduction (Prentice, 1994).

In theory, insufficient Ca supply during pregnancy and lactation could result in maternal bone loss, reduced breast-milk Ca secretion or impaired infant bone development (Prentice, 1994; Prentice *et al.* 1994; Kalkwarf *et al.* 1997). However, although Ca intakes vary widely, no specific problems associated with dietary Ca deficiency have been identified. Alterations in absorption, metabolism and excretion may conserve Ca when requirements increase (King & Weininger, 1991; Prentice, 1994; Repke, 1994; Prentice *et al.* 1995). Bone changes have been observed during pregnancy and lactation (Prentice, 1994; Gambacciani *et al.* 1995; Kalkwarf *et al.* 1997), but their relationship with diet is controversial. Similarly, the effects

of maternal Ca intake on breast-milk Ca and infant bone growth are not understood (Prentice, 1994).

In order to test the hypothesis that women with greater Ca intakes show higher milk concentrations of this mineral, the relationships between intake and serum levels of Ca during the third trimester of pregnancy with levels of the same mineral in transition milk (days 13–14 of lactation) and mature milk (day 40 of lactation), were investigated.

### Methods

The study subjects were a group of fifty-seven healthy, pregnant (later lactating) women between 18 and 35 years of age (mean 27 (SD 3.7) years) whose labour was attended by the Department of Obstetrics and Gynaecology at Cuenca INSALUD Hospital, Spain. All subjects volunteered to take part.

\*Corresponding author: Prof. Dr Rosa Ortega, fax +34 1 394 1732, email rortega@eucmax.sim.ucm.es

The study protocol was approved by the Comité de Investigación de la Facultad de Farmacia, Universidad Complutense de Madrid and by the Comité Etico del Hospital del INSALUD de Cuenca. At the time of the study (May 1990–March 1991), the annual number of births in Cuenca and its Province was 1400. Of these 400 corresponded to city residents. In order to facilitate contact with subjects during their pregnancies and later during lactation, participants were chosen from the city area. A total of 100 subjects in their third trimester of pregnancy were selected. The criteria for inclusion were that subjects should be free of diabetic disease or pre-eclampsia, be between 18 and 35 years of age, be carrying a single fetus, live in the city area and have declared the desire to breast-feed their baby. After being informed of the characteristics of the study, 82% accepted the offer to participate. During their third trimester (between weeks 32 and 36) dietary, anthropometric and biochemical studies were made.

After giving birth the study was continued. The composition of subjects' (now lactating mothers) milk was analysed at days 13–14 (transition milk) and 40 (mature milk) (Patton *et al.* 1990; Institute of Medicine, 1991). A biochemical study of the serum of lactating subjects was also performed at day 40.

Those subjects with babies of low birth weight (< 2500 g; 4.9%) were excluded from the study, as were those who could not continue to provide maternal milk (8.5%). Fourteen subjects were lost from the study because they either moved out of the area, could not be located or lost interest. The final number of subjects was therefore fifty-seven of the eighty-two initially studied. Milk samples were taken between 10.00 and 11.00 hours by manual expression of a 5 ml sample from each breast at the beginning and end of a feed. The experimental details have been described previously by Ortega *et al.* (1997*a,b*).

#### *Dietary survey*

Subjects kept a 5 d dietary record. Kitchen scales were provided to all subjects in order to facilitate the weighing of food. After the questionnaire was completed, the booklets were returned in person. A qualified nutritionist inspected the records to ensure that they were complete and that sufficient detail had been recorded. In the same interview a food-frequency questionnaire was completed in order to contrast subjects' answers with the results of their 5 d dietary record and an explanation was requested if answers were inconsistent.

The intakes of Ca and of other nutrients that might interfere with Ca use (protein, vitamin D, fibre, Fe) were calculated using tables of food composition published by the Instituto de Nutrición (1994).

The intake of supplements was recorded by asking subjects what, and how much, they had taken during their pregnancy. This was then added to the quantity of nutrients provided by the diet.

Estimates of 24 h energy expenditure were made using equations proposed by the WHO (1985) multiplied by an activity ratio in accordance with the criteria of several expert groups (WHO, 1985; Departamento de Nutrición,

1994) and by adding 1255 kJ (because subjects were pregnant women; WHO, 1985).

The percentage of discrepancy in reporting was established in accordance with Johnson *et al.* (1994) using the following formula: (energy expenditure – energy intake) × 100/energy expenditure. When this method is used, a negative value indicates a reported energy intake greater than the predicted total energy expenditure (over-reporting) and a positive value denotes a reported energy intake less than the predicted total energy expenditure (under-reporting) (Johnson *et al.* 1994; Ortega *et al.* 1996*b*).

#### *Biochemical study*

Blood samples were taken first thing in the morning from night-fasted subjects. Blood was drawn from the cubital vein and stored in mineral-free, glass vacutainers. Serum Ca was measured using 2-cresolphthalein complexone (Lorentz, 1982) (CV 3.9%). Albumin was measured by using dye-binding with bromocresol green after a standard reaction time of 1.5 min (Rodkey, 1965) (CV 2.1%). Albumin-corrected Ca values were established using the formula of Payne *et al.* (1990): adjusted Ca = total Ca – (0.025 × albumin) + 1, with Ca in mmol/l and albumin in g/l. Alkaline phosphatase (EC 3.1.3.1) was determined by the phenyl phosphate method (Wootton, 1974) (CV 4.8%). Breast-milk Ca levels were also measured using 2-cresolphthalein complexone (Lorentz, 1982). Samples were treated according to Laskey *et al.* (1991). Therefore, before analysis, small portions of whole-milk were lyophilized, subjected to combustion at 500°, and digested with 0.3 M-HCl. The standards were diluted to contain the same concentration of acid as the samples. Before determinations, a quality control check was performed using pooled serum divided into fifty portions. Ca levels were determined in these fifty samples at a rate of ten per day over five consecutive days. The CV was then calculated (CV 3.7%). Accuracy and precision were monitored by including reference material (Reference Bovine Milk Powder, National Bureau of Standards, Gaithersburg, MD, USA), and a portion from a pooled breast-milk sample, with each batch of assays. The methods used were seen to be sufficiently accurate since the values obtained for the controls were within 1 SD of those quoted.

#### *Anthropometric study*

Data were collected in the morning. Weight and height were determined for subjects without shoes and wearing only underwear, using a digital electronic weighing scale (Seca alpha; Rue Lavoisier 91430, Igmy, France; range: 0.1–150 kg) and a digital stadiometer (Harpender Pfiffer 450; Badem, Padum Aveny, Carlstadt, NJ, USA; range 0.70–2.05 m) respectively. BMI (kg/m<sup>2</sup>) was calculated from these data. All data were collected by trained personnel following norms set out by WHO (1976).

In order to see how anthropometric values changed over pregnancy, the values of these variables at the beginning of gestation were taken from subjects' clinical records.

Weight and length of the newborn were measured immediately after birth.

#### Other data

The date of each subject's last period plus data from their first ultrasound scan were used to establish the point of gestation. Data such as age, number of children previously borne and use of tobacco were recorded in a questionnaire during the first interview.

#### Statistical analysis

Mean values and standard deviations are shown. Where the distribution of results was homogeneous, the degree of significance of differences between means was calculated using the Student's *t* test. Where the distribution of results was not homogeneous, the Mann-Whitney test was applied. The relationship between dietary and biochemical data plus the relationship between these and the concentration of Ca in maternal milk was established by calculating the corresponding coefficients for linear correlation. ANOVA was used to investigate the differences in breast-milk Ca levels and to analyse those of alkaline phosphatase with respect to Ca intake (25th, 50th, 75th and 100th percentiles). Differences were considered significant if  $P < 0.05$  (Wonnacott & Wonnacott, 1977).

#### Results

For the presentation of results, subjects were grouped according to whether their Ca intakes were greater or lower than 1100 mg/d (75th percentile of the Ca intake distribution). Table 1 presents both the mothers' and newborns' personal and anthropometric data and shows that there were

no differences between the groups for any of the variables recorded.

The percentage of discrepancy in reporting was positive (9.9 (SD 16.9)%) and no significant differences were observed between women with Ca intakes below 1100 mg/d and those with Ca intakes  $\geq 1100$  mg/d.

Taking into account the criterion of Goldberg *et al.* (1991) for the evaluation of energy intake data, energy intake was expressed as a multiple of estimated BMR. The value obtained was 1.23 (SD 0.24). No significant differences were seen between women with Ca intakes below 1100 mg/d (1.2 (SD 0.2)) and those with Ca intakes  $\geq 1100$  mg/d (1.3 (SD 0.2)).

Ca intake was lower than 1100 mg/d in 70.2% of subjects. The consumption of supplements was very small and hardly modified the dietary mean when taken into account (Table 2).

An adequate intake of vitamin D is of fundamental importance in the improvement of the use of dietary Ca (Seely *et al.* 1997). However, high intakes of protein (Hagsted & Linkswiler, 1981), Fe (Repke, 1994) or fibre (Frolich, 1995) can interfere with Ca absorption. The intakes of protein, vitamin D, fibre and Fe (dietary and supplementary), which can alter the use of dietary Ca, were therefore recorded (Table 2). The only differences between the groups were those of protein and fibre intakes which were significantly higher in pregnant subjects with Ca levels  $\geq 1100$  mg/d (Table 2).

With respect to the results obtained for serum and milk, it can be seen that the only significant difference between groups was the Ca content of mature milk which was greater in subjects with higher Ca intakes (Table 3). A relationship was found between transition and mature milk Ca levels ( $r$  0.4876). When Ca intake rose from below the 25th percentile in the third trimester (712 mg/d) to between the 25th and 50th percentiles (712–880 mg/d), the 50th and

Table 1. Personal and anthropometric data for subjects and their newborns

	(Mean values and standard deviations)			
	Calcium intake < 1100 mg/d*		Calcium intake $\geq 1100$ mg/d*	
	Mean	SD	Mean	SD
<i>n</i>		40		17
Age (years)	27.2	3.6	28.2	4.2
Initial anthropometric data				
Weight (kg)	56.0	6.1	60.1	11.7
Height (m)	1.60	0.06	1.61	0.06
BMI (kg/m <sup>2</sup> )	21.8	2.1	22.3	2.5
Anthropometric data in 3rd trimester				
Weight (kg)	64.9	6.5	68.4	11.1
Height (m)	1.60	0.05	1.61	0.05
BMI (kg/m <sup>2</sup> )	25.2	2.3	25.5	2.7
Weight gain in first two trimesters (kg)	8.9	2.9	7.7	2.2
No. of children previously borne	0.61	0.68	0.53	0.74
Length of pregnancy (weeks)	39.2	1.1	39.8	1.0
Weight of newborn (g)	3289.0	396.7	3315.3	426.4
Length of newborn (m)	0.501	0.014	0.500	0.016
Smokers (%)		27.5		23.5
No. of cigarettes/d	2.3	4.3	1.1	2.4
Takers of Ca supplements (%)		2.5		5.9

\*1100 mg Ca/d is the 75th percentile of Ca intake for study subjects.

**Table 2.** Calcium intake during the third trimester of pregnancy in fifty-seven women (Mean values and standard deviations)

	Calcium intake < 1100 mg/d† (n 40)		Calcium intake ≥ 1100 mg/d† (n 17)	
	Mean	SD	Mean	SD
Ca supplied by supplements (mg/d)	0.31	1.93	6.08	23.55
Ca supplied by supplements + diet (mg/d)	778.8	168.5	1545.0*	677.0
Ca density (mg/4.2 MJ)	380.2	104.7	630.6*	248.2
Provision by supplements + diet of:				
Protein (g/d)	82.1	12.4	111.5*	27.9
Vitamin D (µg/d)	5.8	11.8	7.0	9.2
Fibre (g/d)	17.2	5.4	20.3*	4.3
Fe (mg/d)	61.7	85.5	33.6	54.8

Mean values were significantly different from those for the low-Ca group, \* $P < 0.05$ .

†1100 mg Ca/d is the 75th percentile of Ca intake for study subjects.

**Table 3.** Levels of calcium in maternal serum during the third trimester, in lactation (day 40) and in transition (days 13–14) and mature milk (day 40) in fifty-seven women (Mean values and standard deviations)

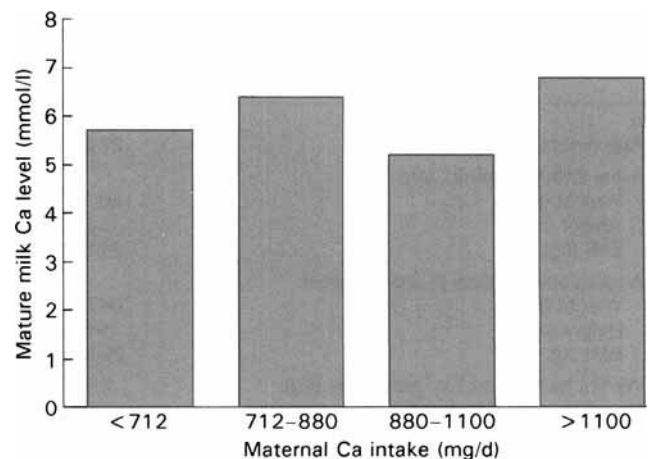
	Calcium intake < 1100 mg/d† (n 40)		Calcium intake ≥ 1100 mg/d† (n 17)	
	Mean	SD	Mean	SD
Serum data, third trimester of pregnancy				
Serum albumin (g/l)	3.6	0.2	3.7	0.2
Serum Ca (mmol/l)	2.21	0.11	2.23	0.13
Albumin-corrected Ca values (mmol/l)	3.12	0.12	3.13	0.12
Alkaline phosphatase (U/l)	169.5	51.1	157.2	59.6
Serum data, lactation				
Serum albumin (g/l)	3.9	0.3	4.0	0.2
Serum Ca (mmol/l)	2.36	0.12	2.42	0.14
Albumin-corrected Ca values (mmol/l)	3.32	0.10	3.30	0.12
Alkaline phosphatase (U/l)	167.3	34.8	113.3*	11.7
Ca in milk				
Transition (mmol/l)	6.44	2.20	6.37	1.96
Mature (mmol/l)	5.95	1.56	6.82*	1.31

Mean values were significantly different from those for the low-Ca group, \* $P < 0.05$ .

†1100 mg Ca/d is the 75th percentile of Ca intake for study subjects.

the 75th (880–1100 mg/d), or even higher (1100 mg/d), mature milk Ca levels changed from 5.7 (SD 1.2) mmol/l to 6.4 (SD 1.6) mmol/l, 5.2 (SD 1.3) mmol/l, and 6.8 (SD 1.3) mmol/l respectively (Fig. 1). When ANOVA was used to analyse milk Ca levels with respect to Ca intake, an almost significant difference ( $P < 0.1$ ) was found. The correlation coefficient for Ca intake and milk Ca levels was not significant.

No significant differences were found for alkaline phosphatase between pregnant subjects with Ca intakes greater than or below 1100 mg/d, although there was a tendency to register higher levels in pregnant subjects with lower Ca intakes. However, in lactating mothers, a significant difference was found for the levels of this enzyme between subjects who, during their pregnancies, took < 1100 mg Ca/d, and those with higher intakes ( $P < 0.05$ ; Table 3). When Ca intake rose from below the 25th percentile to between the 25th and 50th, 50th and 75th, or higher, the alkaline phosphatase values recorded were 169.4 (SD 20.6) U/l, 156.0 (SD 37.2) U/l, 182.3 (SD 31) U/l and 113.3 U/l respectively. ANOVA of alkaline



**Fig. 1.** Change in mature milk calcium levels with respect to calcium intake in the third trimester of pregnancy, from below the 25th percentile (712 mg/d) to between the 25th and 50th percentiles (712–880 mg/d), the 50th and the 75th percentiles (880–1100 mg/d), or higher (>1100 mg/d).



phosphatase levels with respect to Ca intake showed a significant difference ( $P < 0.05$ ). The correlation coefficient for the relationship between breast-milk Ca levels and alkaline phosphatase was significant, both for transition ( $r = -0.3337$ ), and mature milk ( $r = -0.4129$ ).

### Discussion

The duration of pregnancy and the anthropometric data for the mothers and their newborns (Table 1) were similar to those reported in other studies (Ortega *et al.* 1994, 1996a; Ash, 1995).

Since energy intake expressed as a multiple of estimated BMR (Goldberg *et al.* 1991) was low (1.23 (SD 0.24)), and the percentage of discrepancy between energy intake and energy expenditure (Johnson *et al.* 1994; Ortega *et al.* 1996b) was positive, subjects were probably guilty of under-reporting. Nevertheless, the WHO (1985) data are based on weight gains of 12.5 kg during pregnancy, whereas the pregnant women of the present study showed a weight gain of only 8.8 (SD 2.8) kg in the first two trimesters. They also declared that they had considerably diminished their physical activity during pregnancy. It is, therefore, possible that the energy output of these subjects was lower than that established by the WHO (1985). But even if there was some degree of underestimation, there were still no differences in the percentage of discrepancy between energy intake and energy expenditure between women with Ca intakes below 1100 mg/d (75th percentile of the Ca intake distribution) and those with Ca intakes  $\geq 1100$  mg/d.

Ca intake was lower than that reported in other studies (1009 (SD 336) mg/d in pregnant women in Guadalajara, Ortega *et al.* 1994; 1002 (SD 324) mg/d in pregnant women with serum cholesterol  $< 7.55$  mmol/l and 1030 (SD 363) mg/d in similar subjects with higher cholesterol levels, Ortega *et al.* 1996b). However, the intake recorded in the present study was similar to that reported by Haste *et al.* (1991) (840 (SD 37) mg/d in pregnant smokers and 990 (SD 32) mg/d in pregnant non-smokers) and by Borrud *et al.* (1993) (963 mg/d, 500 mg/4.2 MJ), and much greater than that observed by Prentice *et al.* (1995) in populations of The Gambia (283 mg/d).

The consumption of Ca supplements was very low (Table 2), and lower than that of another Spanish population (Guadalajara) investigated by Ortega *et al.* (1994). In that population, 71 % of subjects showed lower than recommended Ca intakes if Ca supplements were ignored. However, if these were included, this value fell to 52 %.

Transition and mature milk Ca levels (Table 3) were similar to those observed in other studies (Fransson & Lonnerdal, 1984; Neville *et al.* 1984; Committee on Nutrition, 1985; Harzer *et al.* 1986; Butte *et al.* 1987; Prentice & Barclay, 1991; Dagnelie *et al.* 1992; Garza *et al.* 1993; Prentice, 1994; Prentice *et al.* 1995; Itriago *et al.* 1997). No significant differences were seen in the Ca levels of transition and mature milk. These results are in agreement with those of Yoneyama *et al.* (1995) and

Harzer *et al.* (1986) who indicate that Ca concentration remains steady during the first five months of lactation.

Some studies have found no accompanying increase in milk Ca when Ca intake has been increased (Feeley *et al.* 1983; Butte *et al.* 1987; Karra *et al.* 1987; Institute of Medicine, 1991; Garza *et al.* 1993; Prentice *et al.* 1995; Kalkwarf *et al.* 1997). Others, however, have shown the existence of low milk Ca concentrations in parts of the world where Ca consumption is low (Laskey *et al.* 1990; Prentice & Barclay, 1991; Prentice, 1994; Prentice *et al.* 1994; Yoneyama *et al.* 1994).

In the present study, greater Ca concentrations were found in the milk of subjects who, during pregnancy, had Ca intakes above the 75th percentile (1100 mg/d). The results agree with those of Yoneyama *et al.* (1994) who indicated that a lower frequency of dairy milk intake, or no intake at all, appeared to affect the Ca content of maternal milk.

Similarly, Laskey *et al.* (1990), who compared the Ca content of the milk of women from The Gambia with that of British women, recorded that the former showed lower Ca concentrations throughout lactation. Taking into account the amount of milk imbibed by each child per day, these authors showed that the total Ca intake of Gambian children was also inferior.

Prentice *et al.* (1995) found Ca supplementation to have no effect on breast milk Ca concentration. However, these authors studied Gambian women with very low Ca intakes (283 mg/d; even with supplementation the value is still low at 714 mg/d). Further, there may be differences in Ca utilization between races (DeSimone *et al.* 1989). Although these authors indicate that in women with low Ca intakes, physiological mechanisms operate to furnish Ca for breast-milk production, the limits of this capability have not been established. Neither has the minimum tolerable Ca intake been clearly established, and whether it is the same in all races, nor has the optimum level for the protection of mother and newborn (either during pregnancy or lactation) been determined.

In disagreement with Chan (1982), and Moser & Reynolds (1983), no relationship was found between maternal serum Ca levels and those of milk. In accordance with Neville *et al.* (1984), Butte *et al.* (1987), and Prentice & Barclay (1991), the number of children previously borne was found to have no influence on milk Ca levels. As reported by Butte *et al.* (1987), no relationship was found between milk Ca levels and maternal anthropometric variables.

Although subjects with Ca intakes  $\geq 1100$  mg/d also showed greater intakes of fibre and protein, which could reduce absorption of the mineral (Hagsted & Linkswiler, 1981; Frolich, 1995), their mature milk Ca levels were higher compared with those who took  $< 1100$  mg/d during pregnancy.

Marya *et al.* (1981) found elevated levels of alkaline phosphatase in lactating subjects. This was taken to indicate a deterioration of subjects' bone. These authors showed that the elevation of enzyme level was significantly lower in women with milk intakes of more than 0.5 litres per d. Bearing in mind that in the lactating mothers of the present study a significant difference was found for the

levels of this enzyme between subjects who, during their pregnancies, took < 1100 mg Ca/d and those with higher intakes ( $P < 0.05$ ; Table 3), these results may indicate bone deterioration in subjects with low Ca intakes during pregnancy. The existence of significant, inverse correlations between alkaline phosphatase and transition and mature milk Ca levels, and the fact that it is subjects with Ca intakes of  $\geq 1100$  mg/d who show the highest milk Ca levels and lowest alkaline phosphatase levels, may indicate that higher intakes of Ca provide better bone protection and lead to higher milk Ca levels.

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