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Ascorbic acid levels in maternal milk: differences with respect to ascorbic acid status during the third trimester of pregnancy

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The aim of the present investigation was to study the relationship between ascorbic acid status during the third trimester of pregnancy and levels of this vitamin in transition milk (days 13–14 of lactation) and mature milk (day 40 of lactation). To this end, the pregnancies and lactation periods of fifty-seven healthy women between 18 and 35 years of age (27 (SD 3·7) years) were monitored. Vitamin intake during the third trimester was determined by recording the consumption of foods over 5 d, and by registering the quantities provided by dietary supplements. Ascorbic acid levels in maternal serum during this stage of pregnancy, and in transition and mature milk samples, were determined by spectrophotometry. Those subjects with ascorbic acid intakes below that recommended (80 mg/d) (group L) showed lower consumption of fruit and vegetables than did those with greater intakes (group H). The consumption of ascorbic acid supplements was very low, and was only seen in three group H subjects. The difference in ascorbic acid intake was reflected at serum level. Group L subjects showed significantly lower serum values than did group H subjects (30·1 (SD 36·3) μmol/l compared with 101·1 (SD 168·1) μmol/l). Vitamin intake also influenced the composition of transition milk. Group L subjects showed significantly lower levels of ascorbic acid in milk than did group H subjects (255·5 (SD 220·3) μmol/l compared with 437·8 (SD 288·4) μmol/l). The results of the present study reveal the need to increase the consumption of fruits and vegetables during pregnancy and to monitor maternal ascorbic acid intake and vitamin C status.

Ascorbic acid: Pregnancy: Lactation

Adequate nutrition during pregnancy and lactation is extremely important to both maternal and fetal health (Zeman & Ney, 1988). As nutritional requirements are high, the effects of malnutrition can be severe and long lasting in pregnant and lactating mothers. Nutritional status during these periods may, therefore, be a contributing factor in maternal and infant morbidity and mortality (González-Cossio & Delgado, 1991).

Specific nutrient deficiencies may affect a woman's functional capacity and increase her susceptibility to unfavourable perinatal processes (Antal *et al.* 1997). With regard to ascorbic acid, some studies on pregnant women have revealed that ascorbic acid deficiency is associated with increased risk of infections, premature rupture of the membranes (Casanueva *et al.* 1993; Pfeffer *et al.* 1996), prematurity (Tlaskal & Novakova, 1990; Casanueva *et al.* 1993) and eclampsia (King & Weininger, 1991; Jendryczko & Tomala, 1995).

The aim of the present investigation was, therefore, to study the relationship between ascorbic acid status during the third trimester of pregnancy and levels of this vitamin in maternal milk.

Materials and methods

The pregnancies and lactation periods of fifty-seven women were followed. The characteristics of the subjects and criteria of inclusion or exclusion have been reported in previous papers (Ortega *et al.* 1997*a*,*b*).

Abbreviations: MTT, 3-(4,5-dimethylthiazoyl-2)-2,5 diphenyltetrazolium bromide.

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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.

During the third trimester (between weeks 32 and 36), dietary, anthropometric and biochemical studies were made. After subjects gave birth the study continued. The composition of subjects' (now lactating mothers) milk was analysed at days 13–14 (transitional milk) and 40 (mature milk) (Patton *et al.* 1990).

Diet survey

Food intake was recorded by keeping a 'food record' questionnaire for 5 d, including a Sunday. Kitchen scales were provided to all the 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. Details of the diet survey method used have been published previously (Ortega *et al.* 1997*a,b*).

The ascorbic acid content of foods consumed was calculated using tables of food composition published by the Instituto de Nutrición (1994). The recommended intake of ascorbic acid accepted in this study (80 mg/d) was that for women in the second half of pregnancy as established in the tables of recommended energy and nutrient intakes for the Spanish population (Departamento de Nutrición, 1994).

The intake of supplements was recorded by asking subjects what, and how much, they had taken during pregnancy. This was then added to the quantity of ascorbic acid provided by the diet. The adequacy of the diet with respect to ascorbic acid was determined by comparing this value with that recommended.

Estimates of 24 h energy expenditure were made using equations proposed by WHO (1985) multiplied by an activity ratio in accordance with the criteria of several expert groups (WHO, 1985; Departamento de Nutrición, 1994).

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. Since ascorbic acid is unstable in storage (Anderson & Pittard, 1985), the serum component was separated and ascorbic acid levels determined colorimetrically (Henniger, 1981) (Boehringer Mannheim Biochemicals, Mannheim, Germany) immediately after extraction. Two samples were prepared from the collected serum. In one sample the oxidation of all reducing compounds, including L-ascorbic acid, was performed in the presence of methylsulphate-5-methylbenzene, reducing the tetrazolium salt 3-(4,5-dimethylthiazoyl-2)-2,5 diphenyltetrazolium bromide (MTT) to give dehydroascorbic acid plus MTT-formazan. In the presence of O_2, ascorbic acid oxidase (*EC* 1.10.3.3) was added to the blank sample, to form dehydroascorbic acid exclusively. The colour due to ascorbic acid was therefore eliminated. The difference between absorbance of the test sample and that of the blank was taken to be the quantity of ascorbic acid in the sample. The quantity of MTT-formazan was used as the measurement variable, and was determined by the absorption recorded at 578 nm (CV 4·8 %).

Milk samples were taken at 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 protocol for both collection and subsequent handling of milk has been described previously (Ortega *et al.* 1997*a,b*). After acidification of milk samples with citric acid to a pH of 3·5–4·0, followed by filtering (Beutler & Beinstingl, 1980) (CV 4·9 %), milk ascorbic acid levels were determined by the method described earlier.

In order to establish normality limits for ascorbic acid in serum, the criteria of the following authors were taken into account: Kübler (1988) and Keller & Salkeld (1988), who consider values between 11·4 and 142 μmol/l to be acceptable; Bates *et al.* (1983), who consider 17 μmol/l as the lower normal limit; and Dostálová (1984), who regards < 22·7 μmol/l as an indicator of high risk of deficiency, 22·7–34·1 μmol/l as a moderate risk, > 34·1 μmol/l as a low risk, and > 45·4 μmol/l as a very low risk (optimal situation). With respect to ascorbic acid levels in maternal milk, Byerley & Kirksey (1985) have established 250 μmol/l as the lower normal limit.

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, Igmy, France; range 0·1–150 kg) and a digital stadiometer (Harpenden Pfifter 450; Badem, Padum Aveny, Carlstadt, NJ, USA; range 0·70–2·05 m) respectively. BMI (kg/m^2) values were 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 during pregnancy, the values of these variables at the beginning of pregnancy were taken from subjects' clinical records. Weight and length of the newborn were measured immediately after birth.

Other data

Gestational age at delivery was calculated from the agreed delivery date recorded by the attending obstetrician, using

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last menstrual period and early ultrasound examination data. Data such as age, number of children previously borne and use of tobacco were recorded in a questionnaire during the first interview. Apgar scores (a neonatal condition score) at 1 and 5 min were measured.

Statistical analysis

Mean values and standard deviations are shown. Where the distribution of results was homogenous, the degree of significance of differences between means was calculated using Student's *t* test. Where the distribution of results was not homogeneous, the Mann–Whitney test was applied. Differences were considered significant if $P < 0.05$ (Wonnacott & Wonnacott, 1977).

Results

For the presentation of data, subjects were grouped with respect to whether their ascorbic acid intakes were below (group L), or above (group H) that recommended (80 mg/d) (Tables 1–3). Table 1 shows both the mothers' and newborns' personal and anthropometric data, and reveals that there were no differences between the groups for any of these variables.

The discrepancy between energy intake and energy expenditure was positive, but no significant differences were seen between group L and group H subjects (15·6 (SD 20·8) % compared with 9·4 (SD 19·1) %).

Group L subjects showed significantly lower intakes of fruit (148·8 (SD 78·9) g/d) and vegetables (145·5 (SD 44·9) g/d) than did group H subjects (398·1 (SD 221·9) g/d) and 275·1 (SD 104·9) g/d respectively). These differences are probably the reason for the greater intake of ascorbic acid seen in group H subjects (Table 2).

During the third trimester, only three group H subjects took a supplement containing ascorbic acid, at quantities of 60–300 mg/d. The dietary results were, therefore, hardly modified when supplements were taken into account (Table 2).

Serum and transition milk ascorbic acid levels were significantly higher in group H subjects, whilst the proportion of subjects with serum levels below the normal limit was greater amongst group L subjects (Table 3).

Discussion

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

Since the percentage discrepancy between energy intake and energy expenditure (Johnson *et al.* 1994; Ortega *et al.* 1996*b*) 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 lower weight gains in the first two trimesters (Table 1). They also declared that they had considerably diminished their physical activity during pregnancy. It is possible, therefore, that the energy output of these subjects was lower than that established by WHO (1985). However, even if underestimation did take place, it can be seen that there were no differences in this respect between subjects in groups L and H.

The intake of ascorbic acid observed during the third trimester was similar to that reported by other authors (Picone *et al.* 1982 (105 mg/d); Hunt *et al.* 1987 (83 mg/d); Borrud *et al.* 1993 (102 mg/d); Ortega *et al.* 1994 (174 (SD 81) mg/d); Job *et al.* 1995 (145 (SD 26) mg/d); Antal *et al.* 1997 (109·4 mg/d).

Table 1. Personal and anthropometric data for subjects and their newborns*
(Mean values and standard deviations)

	Ascorbic acid intake < RI		Ascorbic acid intake ≥ RI		
	Mean	SD	Mean	SD	
n	12		45		
Age (years)	27·3	4·1	27·5	3·8	H
Initial anthropometric data					
Weight (kg)	56·4	6·8	57·4	8·6	H
Height (m)	1·590	0·049	1·604	0·058	H
BMI (kg/m^2)	22·1	2·5	21·9	2·2	H
Anthropometric data in 3rd trimester					
Weight (kg)	64·4	6·3	66·3	8·5	H
Height (m)	1·590	0·048	1·604	0·057	H
BMI (kg/m^2)	25·0	2·1	25·3	2·4	H
Weight gain in first two trimesters (kg)	8·0	3·3	8·7	2·6	H
No. of children previously borne	0·73	0·79	0·55	0·67	H
Length of pregnancy (weeks)	39·2	1·3	39·4	1·0	H
Weight of newborn (g)	3300·9	501·8	3295·1	378·5	H
Length of newborn (mm)	501	18	500	14	H
No. of cigarettes/d	3·1	5·2	1·6	3·5	NH
Apgar score at 1 min	8·4	0·9	8·5	0·8	H
Apgar score at 5 min	9·9	0·3	10·0	0·2	H

RI, recommended intake (80 mg/d); H, homogeneous distribution of results (Student's *t* test applied); NH, non-homogeneous distribution of results (Mann–Whitney test applied).
* There were no significant differences between the established groups.

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

	Ascorbic acid intake < RI (*n* 12)		Ascorbic acid intake ≥ RI (*n* 45)	
	Mean	SD	Mean	SD
Ascorbic acid supplied by supplements (mg/d)	0		10·7	48·5
Ascorbic acid supplied by supplements + diet				
Total intake (mg/d)	55·3*	13·9	174·8	70·9
Coverage of RI (%)	69·1*	17·3	218·5	88·6
Intake < 70 % of RI (%)	41·7		0	
Ascorbic acid density (mg/1000 kJ)	6·8*	1·8	18·2	6·8
INQ of ascorbic acid	0·85*	0·22	2·33	0·95
INQ < 1 (%)	75·0		0	

RI, recommended intake (80 mg/d); INQ, index of nutritional quality (true density/recommended; recommended ascorbic acid density = RI × 1000 kJ/estimated expenditure (kJ)).
Mean values were significantly different from those for the high intake group, *$P < 0·05$ (Mann-Whitney test).

Table 3. Levels of ascorbic acid in maternal serum during the third trimester of pregnancy and in transition (days 13–14 and mature breast milk (day 40) in fifty-seven women
(Median values, mean values and standard deviations)

	Ascorbic acid intake < RI (*n* 12)			Ascorbic acid intake ≥ RI (*n* 45)			
	Median	Mean	SD	Median	Mean	SD	
Serum data, third trimester of pregnancy							
Serum ascorbic acid (μmol/l)	11·2	30·1*	36·3	98·1	101·1	168·1	NH
Low serum levels (%)							
< 11·4 μmol/l		50·0			35·6		
< 17·0 μmol/l		58·3			37·8		
< 22·7 μmol/l		58·3			37·8		
Ascorbic acid in milk							
Transition (μmol/l)	227·2	255·5*	220·3	407·9	437·8	288·4	H
Low breast milk levels (< 250 μmol/l) (%)		50·0			26·7		
Mature (μmol/l)	452·5	471·3	385·0	438·6	433·2	337·8	H
Low breast milk levels (< 250 μmol/l) (%)		33·3			28·9		

RI, recommended intake (80 mg/d); H, homogeneous distribution of results (Student's *t* test applied); NH, non-homogeneous distribution of results (Mann–Whitney test applied).
Mean values were significantly different from those for the high intake group, *$P < 0·05$.

Borrud *et al.* (1993) reported that insufficient intake of ascorbic acid is uncommon, although many women take less than the recommended quantities of fruits and vegetables. In the present study, despite the fact that average values for ascorbic acid intake were 88 % higher than those recommended, 21·1 % of subjects showed ascorbic acid intakes lower than those recommended, and 9·3 % showed intakes of below 70 % recommended.

The concentrations of ascorbic acid in serum (Table 2) were similar to those obtained by other authors for the third trimester of pregnancy (García *et al.* 1974 (55·1 (SD 21·6) μmol/l); Knight *et al.* 1994 (51·1 (SD 22·7) μmol/l). The present values are, however, somewhat higher than those found by Bates *et al.* (1982; 27·3 μmol/l), Bates *et al.* (1983; 14·2–40·9 μmol/l), Dostálová (1984; 34·6 (SD 15·3) μmol/l), and West *et al.* (1994; 37·5–61·9 μmol/l).

The percentages of deficient serum values were also similar to those found by Bates *et al.* (1983; 79·8 % of subjects with ascorbic acid intakes of 34·3 mg/d and 5·9 % of those with intakes of 103·5 mg/d), Dostálová (1984; 46·2 % with moderate risk and 15·4 % with high risk of deficiency), and Tlaskal & Novakova (1990; low ascorbic acid levels in 23·3 % of women who gave birth to mature, normal neonates, and 67 % in those who gave birth to premature infants).

Subjects in group H showed significantly higher serum ascorbic acid levels than did those in group L (Table 2). The influence of ascorbic acid intake on serum levels of the vitamin has been reported by other authors. Bates *et al.* (1983) found that plasma ascorbate increased from 14·2 to 40·9 μmol/l as intake increased from 34·3 to 103·5 mg/d. West *et al.* (1994) found higher serum levels of ascorbic acid in women who took vitamin–mineral supplements during the third trimester of pregnancy (61·9 μmol/l compared with 37·5 μmol/l in those who took no supplements). Bates *et al.* (1983) also showed that a fasting plasma ascorbate level of at least 17 μmol/l in 97·5 % of the population of lactating women in Keneba (a West African rural community) would require a daily ascorbic acid intake of about 117 mg. Levels of 28·4 μmol/l would require intakes of 150 mg/d and levels of 39·7 μmol/l would require 172 mg/d.

The mean concentration of ascorbic acid in maternal milk (Table 3) was similar to that reported in other studies

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Table 4. Milk ascorbic acid levels found in other studies

Point during the lactation period when analysis was performed	Milk ascorbic acid levels (μmol/l)	Breast milk ascorbate : serum ascorbate	Reference
Mature milk	596·2		Anderson & Pittard (1985)
Intakes forty times that recommended			
Mature milk			
Maternal intake (mg/d):			
34·3	193·1	13·6	Bates *et al.* (1983)
58·4	269·1	11·85	
81·1	301·5	11·06	
103·5	312·9	7·65	
Mature milk			
Maternal intake (mg/d):			
virtually nil	113·6	10	Bates *et al.* (1994)
100	340·7	5	
7–13 weeks	249·8–897·1		Byerley & Kirksey (1985)
Maternal intake (mg/d):			
< 100	473·0		
100–199	613·2		
200–399	550·2		
400–999	607·5		
≥ 1000	653·0		
Mature milk (> 21d)	227·1 (SD 56·8)		Committee on Nutrition (1985)
Mature milk	244·2–494·0		Fomon (1974)
0–6 months	295·3		Schwartz (1985)
5–7 d			
Maternal intake (mg/d):			
83	301·5 (SD 97·1)	6·90	Sneed *et al.* (1981)
152	346·4 (SD 56·8)	7·01	
43–45 d			
Maternal intake (mg/d):			
202	369·1 (SD 113·6)	6·44	
193	408·8 (SD 113·6)	6·16	
Mature milk	244·2–494·0		Thomas *et al.* (1979)

(Fomon, 1974; Thomas *et al.* 1979; Sneed *et al.* 1981; Byerley & Kirksey, 1985; Schwartz, 1985). However, it was greater than that recorded in still others (Bates *et al.* 1983, 1994; Committee on Nutrition, 1985) (Table 4).

In agreement with Sneed *et al.* (1981) and Udipi *et al.* (1985), a tendency was seen for milk ascorbic acid levels to rise as lactation progressed. Ascorbic acid levels in mature milk were slightly higher than those of transition milk, although the difference was not significant (Table 3). Subjects in group H showed significantly higher transition milk ascorbic acid levels than did those in group L (Table 3).

The influence of ascorbic acid intake on maternal milk concentrations has been recognized by many authors (Bates *et al.* 1983, 1994; Anderson & Pittard, 1985; Institute of Medicine, 1991) (Table 4). Bates *et al.* (1983) studied the relationship between breast milk ascorbic acid levels and intake in European women, and concluded that intakes of 80–100 mg/d are needed to achieve levels of 284–340·7 μmol/l.

It would appear that the changes produced in breast milk ascorbic acid levels are less pronounced than those seen in serum (Bates *et al.* 1982, 1983; Byerley & Kirksey, 1985). The breast milk ascorbate : serum ascorbate ratio decreased with increased intake and serum vitamin C levels (Table 4). This shows that mammary tissue becomes saturated with the vitamin when intake is high (Anderson & Pittard, 1985; Byerley & Kirksey, 1985). Byerley & Kirksey (1985) indicated the probable existence of a regulatory mechanism in mammary cells to prevent elevation of milk vitamin C concentrations beyond a certain saturation level.

Some authors dispute the influence of intake on breast milk levels. Salmenpera (1984) has suggested that the ascorbic acid status of breast-fed infants of mothers with inadequate status may remain optimal. Others have reported that when intake is low, breast milk ascorbic acid levels are sensitive to supplementation. However, when intake is high, breast milk levels are much less sensitive (Thomas *et al.* 1979; Sneed *et al.* 1981).

The results of the present study show that there is a relationship between ascorbic acid intake and serum and milk levels. Although the mean intake of ascorbic acid was 188 % of that recommended, 21·1 % of subjects showed lower than recommended intakes, 38·6 % showed serum levels below 11·4 μmol/l, 33·3 % showed < 250 μmol/l in transition milk and 31·5 % showed < 250 μmol/l in mature milk.

In agreement with Borrud *et al.* (1993), we consider that efforts to improve the nutritional status of pregnant and lactating women would be well served if all women of child-bearing age were encouraged to maximize the nutritional quality of their diets. It is especially important to recommend an increase in the consumption of fruit and vegetables during pregnancy, not only to raise ascorbic acid intake, but also that of carbohydrates, fibre, and other

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vitamins, especially folates. Folate deficiency has been associated with many maternal and fetal complications including low birth weight, detachment of the placenta and neural-tube defects (O'Connor, 1994; Keizer *et al.* 1995).

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