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Thiamin status during the third trimester of pregnancy and its influence on thiamin concentrations in transition and mature breast milk

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Thiamin deficiency remains an important public health problem in some populations. The aim of the present investigation was to study thiamin status during the third trimester of pregnancy and its influence on the concentration of this vitamin in transition (days 13–14 of lactation) and mature breast milk (day 40 of lactation) in a group of Spanish women. The pregnancies and lactation periods of fifty-one healthy women 18–35 (mean 26·7 (SD 3·7)) years old were monitored. Vitamin intake during the third trimester was determined by recording the consumption of foods over 5 d and of the quantities provided by dietary supplements. Thiamin status during this stage of pregnancy was determined by measuring the activation coefficient of erythrocyte transketolase (α-ETK). Milk thiamin content was estimated (in 41% of the subjects) by oxidizing thiamin to thiocrome and measuring fluorescence. Subjects with thiamin intakes above that recommended (group H) had more satisfactory serum α-ETK coefficients (1·01 (SD 0·19)) than did those with lower intakes (group L) (1·21 (SD 0·30); $P<0·05$). Mature milk thiamin concentrations were significantly higher in group H subjects (0·59 (SD 0·44) μmol/l) than group L subjects (0·25 (SD 0·07) μmol/l). Subjects with α-ETK coefficients $>1·25$ in the third trimester had significantly lower mature milk thiamin concentration (0·31 (SD 0·10) μmol/l) than did subjects with more satisfactory α-ETK levels at this time (0·55 (SD 0·42) μmol/l; $P<0·05$). The thiamin status of women can be improved since 25·5% of subjects took less than that recommended and 13·7% showed signs of severe deficiency (α-ETK $>1·25$). The influence of maternal thiamin intake on α-ETK coefficients and on mature breast milk thiamin concentration is confirmed.

Thiamin intake: Pregnancy: Lactation: α-Erthythrocyte transketolase: Thiamin in milk

Gestation imposes metabolic stress on the mother, and this increases as pregnancy progresses (Baker *et al.* 2002). Growth and development of the fetus (Ramakrishnan *et al.* 1999; Ladipo, 2000), the health of the mother and child (Antal *et al.* 1997; Godfrey & Robinson, 1998) and the composition of breast milk (Prentice *et al.* 1983; King & Weininger, 1991; Bohm *et al.* 1997) depend on the maternal supply of essential nutrients.

Thiamin deficiency remains an important public health problem in some populations, with pregnant women and young children at the highest risk of deficiency (Sánchez *et al.* 1999; McGready *et al.* 2001). Baker *et al.* (2002) indicated that during pregnancy approximately 50 % of women develop a biochemical thiamin deficiency; in the remainder, thiamin concentrations fall, but stay within normal limits.

Thiamin acts as a co-enzyme in the metabolism of carbohydrates and branched-chain amino acids (Food and Agriculture Organization/World Health Organization, 2001). During pregnancy this deficiency can cause problems such as alterations in glucose tolerance and intrauterine growth, thereby increasing the risk of low birth-weight in the offspring (Baker *et al.* 2000). Deficiency has also been associated with an increased risk of viscerocranial malformations, especially cleft alveolus and palate (Bienengraber *et al.* 1997). Moreover, thiamin deficiency has been associated with toxaemia in pregnancy (Rees *et al.* 1997) and is thought to be a possible cause of sudden infant death syndrome (the leading cause of death between 1 and 6 months of age in the developed world; Bernshaw, 1991). Attaining a good thiamin status during pregnancy and the first stages of infancy should therefore be a priority.

The aim of the present investigation was to study thiamin status during the third trimester of pregnancy and to analyse its relationship with the concentration of this vitamin in transition and mature maternal milk.

Materials and methods

The initial sample was a group of eighty-two healthy pregnant women aged 18–35 (mean 26·9 (SD 3·8)) years, whose

Abbreviations: H, thiamin intake equal to or greater than recommended levels; L, thiamin intake below recommended levels; , α-ETK: activation coefficient of erythrocyte transketolase.

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deliveries were attended by the Department of Obstetrics and Gynaecology at Cuenca INSALUD Hospital, Spain. All the subjects volunteered to take part. Of this initial group, the biochemical analysis of thiamin status was possible in fifty-one subjects.

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. Cuenca is an ethnically homogeneous urban town, made up of white individuals whose socio-economic level is close to the Spanish average. The characteristics of the subjects and criteria of for inclusion or exclusion have been reported in previous papers (Ortega *et al.* 1998, 1999*a*,*b*). The study subjects had normal pregnancies and were free of diabetes, renal disease, and cardiac and liver dysfunction.

During the third trimester of gestation (weeks 32–36) dietary, anthropometric and biochemical studies were made. After subjects gave birth, the study was continued. The composition of the subjects' (now lactating mothers) milk was analysed at days 13–14 (transitional milk; *n* 20) and day 40 (mature milk; *n* 21) (Patton *et al.* 1990).

Dietary survey

Food intake was recorded by keeping a food record booklet for 5 d, including a Sunday. Kitchen scales were provided to all subjects in order to weigh the foods. 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 intake questionnaire was completed in order to compare the subjects' answers with the results of their 5 d dietary records: an explanation was requested if answers were inconsistent. The details of the dietetic methods used have been published previously (Ortega *et al.* 1997*a*,*b*, 1998, 1999*a*,*b*).

The thiamin contents of consumed foods were calculated using the tables of food composition published by the Instituto de Nutrición (1994). The recommended intake of thiamin accepted in the present study 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 (0·4 mg/4184 kJ + 0·1 mg, with a minimum provision of 1 mg/d; Departamento de Nutrición, 1994). This recommended intake is lower than that recommended in the USA, where the Institute of Medicine (2001) set a recommended intake for gestating women of 1·4 mg thiamin/d.

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 thiamin provided by their diet. The adequacy of the diet with respect to thiamin was determined by comparing this value with that recommended.

Biochemical study

Venous blood (10 ml) was taken in the morning from the subjects, who had fasted overnight. Samples were collected in heparinized tubes and maintained at 4–6°C until thiamin analysis was performed (always within 48 h). For the determination of packed cell volume, 5 ml heparin-free blood was transferred to another test-tube and analysed at room temperature within 1 h of collection. Thiamin status was determined by measuring the activation of erythrocyte transketolase (α-ETK) before and after thiamin pyrophosphate stimulation (Vuilleumier *et al.* 1983). The activity of the enzyme was measured by determining the D-sedoheptulose-7-phosphate formed from D-ribose-5-phosphate and xylulose-5-phosphate (Sigma, St Louis, MO, USA) when incubated with haemolysed erythrocytes. Determinations were made with and without the addition of excess thiamin pyrophosphate (Sigma). The absorbances of the solutions were measured at 350 nm (absorbance minimum) and 405 (peak absorbance) in 10 mm pathlength cuvettes using a Shimadzu UV-1203 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The differences in absorbance were then calculated, corrected for the blank, and averaged. The relationship between enzyme activity before and after saturation is expressed by the saturation coefficient α. High α coefficients imply an unfavourable biochemical thiamin status (CV 5·4%). The packed cell volume, necessary for the calculation of the α-ETK coefficient, was determined using a Coulter S-Plus analyser (Coulter Diagnostics, Hialeah, FL, USA) (Cox *et al.* 1985). Values for non-stimulated and stimulated enzyme activity were obtained by calibration with sedoheptulose anhydride (Sigma) (0·3 μmol and 0·6 μmol in 0·5 ml buffer treated as though it were haemolysate). The enzyme activity was expressed as μmol sedoheptulose formed per min at 37°C per litre erythrocyte suspension (Vuilleumier *et al.* 1983).

To establish normality limits for the α-ETK coefficients, the criteria of McGready *et al.* (2001) and Sauberlich (1999) were taken into account. These authors regard coefficients of $<1{\cdot}20$ as indicating low risk, 1·20–1·25 marginal deficiency and $>1{\cdot}25$ severe deficiency. Bailey *et al.* (1994), Benton *et al.* (1997) and Sánchez *et al.* (1999) indicate that α-ETK values of $<1{\cdot}15\%$ are adequate, 1·15–1·25% are marginally deficient and $>1{\cdot}25\%$ are deficient.

Milk samples were taken between 10.00 and 11.00 hours in the morning by manual expression of a 5 ml sample from each breast at the beginning and end of feeds. Thiamin determination (AOAC International, 1993) was carried out in twenty samples of transition milk (days 13–14 of lactation) and twenty-one samples of mature milk (day 40 of lactation). The protocol for the collection and subsequent handling of milk has been described by Ortega *et al.* (1997*a*,*b*).

Samples were hydrolysed with 0·1 M-HCl (Merck, Darmstadt, Germany) in an autoclave at 121°C for 30 min. To purify the extract, the digest was adjusted to pH 3·5 with NaOH (Merck) and filtered, and a portion of the solution passed through an absorption column filled with a 100 mm bed of Bio-Rex 70 (H form; Bio Rad Laboratories, Hercules, CA, USA) to remove possible interfering compounds. For the oxidation of thiamin to thiochrome, a portion of the purified extract was oxidized with 1% potassium ferricyanide in 15% NaOH (Merck).

Thiochrome was extracted with isobutanol (Merck) and quantified fluorimetrically. Fluorescence was measured at 435 nm (emission) and 365 nm (excitation) using a fluorescence spectrophotometer (Perkin Elmer MPF-2A, Beaconsfield, Bucks., UK). The CV was 4·1%.

The lower normal limit of thiamin concentration in maternal milk has been established as 0·48 μmol/l (Committee on Nutrition, 1985).

Anthropometric study

All data were collected in the morning. Weight and height were measured with subjects in bare feet and underwear, using a digital electronic weighing scale (Seca alpha; Seca, Igmy, France; range 0·1–150·0 kg) and a digital stadiometer (Harpenden Pfifter 450; Badem, Carlstadt, NJ, USA; range 0·70–2·05 m) respectively. BMI (kg/m^2) was calculated from these data. All data were collected by trained personnel following norms set out by the World Health Organization (1976).

To measure changes in anthropometric values during pregnancy, the values of these variables at the beginning of pregnancy (4–8 weeks of gestation) were obtained from subjects' clinical records. The weight and length of the newborn infants were recorded immediately after birth.

Other data

The gestational age of the newborn infants at delivery was calculated from the time of the last menstrual period and early ultrasound examination data. Data such as maternal age, parity and the smoking habits were recorded in a questionnaire during the first interview.

Statistical analyses

Values are given as means and standard deviations. Where the distribution of results was normally distributed, the degree of significance of differences between mean values was calculated using Student's *t* test. Where the distribution of results was not normally distributed (length of newborn infant, number of cigarettes smoked, thiamin supplied by supplements, thiamin intake, coverage of recommended intake, thiamin density, index of nutritional quality of thiamin, α-ETK, thiamin in mature milk), the Mann–Whitney test was used. Analysis of covariance was used to eliminate the influence of variables such as carbohydrate and alcohol consumption, which could modify the results. Differences between qualitative variables were determined by the χ^2 test (percentage of smokers in Table 1, percentage of women with index of nutritional quality <1 for thiamin in Table 2 and percentage of women with inadequate figures for α-ETK and for thiamin in milk in Table 3, always comparing results of women whose intake of thiamin was less than recommended intake with those of women whose intake is greater than or equal to recommended intake). To test the correlation between quantitative variables, Pearson's product moment correlation coefficient *r* was calculated. Significance was set at $P<0·05$ (Wonnacott & Wonnacott, 1977).

Results

The subjects' results are presented with regard to whether intake was below (group L), or equal to or greater (group H) than that recommended (Tables 1–3). Table 1 shows both the mothers' and newborn infants' personal and anthropometric data; no differences were found between the groups in this regard.

Table 2 shows thiamin intake during the third trimester of pregnancy. Group L subjects had significantly lower intakes of cereal products (113·4 (SD 64·9) g/d) than did group H subjects (174·7 (SD 57·2) g/d; $P<0·05$). These differences are probably the reason for the greater intake of thiamin in group H subjects (Table 2).

Table 1. Personal and anthropometric results for subjects and their newborn infants
(Mean values and standard deviations)

	Thiamine intake < RI (*n* 13)*†		Thiamine intake ≥ RI (*n* 38)*†	
	Mean	SD	Mean	SD
Age (years)	25·5	4·0	27·2	3·6
Anthropometric data in first trimester				
Weight (kg)	57·8	8·1	57·8	7·7
Height (m)	1·603	0·074	1·612	0·053
BMI (kg/m^2)	22·3	2·8	21·9	2·3
Anthropometric data in third trimester				
Weight (kg)	67·3	8·0	66·0	8·2
BMI (kg/m^2)	25·6	2·3	25·3	2·8
Weight gain in first two trimesters (kg)	9·5	3·5	8·5	2·8
Parity (*n*)	0·62	0·77	0·51	0·65
Length of pregnancy (weeks)	38·9	1·6	39·4	1·4
Weight of newborn infant (g)	3252	428	3280	451
Length of newborn infant (m)	0·510	0·025	0·501	0·013
Smokers (%)	23·1		21·1	
Cigarettes smoked (*n* per d)‡	10·7	7·5	5·8	3·7

RI, recommended intake.
* RI 0·4 mg/4184 kJ + 0·1 mg, with a minimum provision of 1 mg/d (Departamento de Nutrición, 1994).
† There were no significant differences between groups.
‡ Mean cigarette consumption is given for smokers only.

Table 2. Thiamin intake during the third trimester of pregnancy†
(Mean values and standard deviations)

	Thiamine intake < RI (*n* 13)‡		Thiamine intake ≥ RI (*n* 38)‡	
	Mean	SD	Mean	SD
Thiamin supplied by supplements (mg/d)§	0		1·57	
Thiamin supplied by supplements + diet				
Total intake (mg/d)	0·87*	0·13	1·45	0·38
Coverage of RI (%)	86·6*	12·4	138·0	28·6
Thiamin density (mg/MJ)	0·14	0·04	0·16	0·03
INQ of thiamin (thiamin density/recommended thiamin density)	1·47	0·42	1·66	0·30
INQ<1 (%)	7·7‖		0·0	

RI, recommended intake; INQ, index of nutritional quality.
Mean values were significantly different from those of the group with thiamin intake ≥ RI (Mann–Whitney test): *$P<0·05$.
† For details of subjects and procedures, see Table 1 and p. 130.
‡ RI 0·4 mg/4184 KJ + 0·1 mg, with a minimum provision of 1 mg/d (Departamento de Nutrición, 1994).
§ Only one woman took supplements, which provided 1·57 mg/d.
‖ There was no significant difference between the values in the two groups (χ^2 test).

Only one H subject took a thiamin-containing supplement during the third trimester, and even then in very small amounts (1·57 mg/d). The dietary intakes are, therefore, hardly modified when supplements are taken into account (Table 2). Though the intake of this vitamin was less than that recommended in 25·5% of subjects, only 2% (one woman in group L) had an intake less that two-thirds of recommended intake.

Table 3 shows thiamin status during the third trimester and the concentration of thiamin in transition and mature milk. Group H subjects had more satisfactory serum α-ETK coefficients than did group L subjects; only 7·9% of group H subjects showed signs of severe deficiency (α-ETK >1·25) compared with 30·8% of group L subjects.

Mature milk thiamin concentrations were also significantly higher in group H subjects, while the percentage of subjects with thiamin concentration below the normal limit was greater amongst group L subjects (Table 3).

Subjects with α-ETK coefficients ≥1·2 in the third trimester had significantly lower mature milk thiamin concentration (0·32 (SD 0·08) μmol/l) than did subjects with more satisfactory α-ETK levels (0·56 (SD 0·43) μmol/l; $P<0·05$). In addition, subjects with α-ETK coefficients >1·25 in the third trimester had significantly lower mature milk thiamin concentration (0·31 (SD 0·10) μmol/l) than did subjects with more satisfactory α-ETK levels (0·55 (SD 0·42) μmol/l; $P<0·05$).

Discussion

Personal, anthropometric and dietetic results

The duration of pregnancy and the anthropometric results for the mothers and their newborn infants (Table 1) are similar to those reported in other studies (Ash, 1995; Ortega *et al.* 1996). The mean intake of thiamin (1·30 (SD 0·42) mg/d; Table 2) during the third trimester was similar to that reported by other authors (Haste *et al.* 1991; Borrud *et al.* 1993; Ortega *et al.* 1994; Antal *et al.* 1997; Erkkola *et al.* 2001).

Table 3. Thiamin status (determined by measuring the activation coefficient of erythrocyte transketolase) during the third trimester of pregnancy and concentration of thiamin in transition (days 13–14) and mature breast milk (day 40)†
(Mean values and standard deviations)

	Thiamin intake < RI (*n* 13)‡			Thiamin intake ≥ RI (*n* 38)‡		
	Mean	SD	*n*§	Mean	SD	*n*§
Blood α-ETK	1·21*	0·30		1·01	0·19	
Values indicating deficit (%)						
≥1·15	53·8‖			23·7‖		
≥1·20	38·5‖			21·1‖		
>1·25	30·8‖			7·9‖		
Thiamin in milk						
Transition (μmol/l)	0·90	1·03	3	0·88	0·57	17
Low breast-milk concentration (<0·48 μmol/l) (%)	66·7‖			31·3‖		
Mature (μmol/l)	0·25*	0·07	5	0·59	0·44	16
Low breast-milk concentration (<0·48 μmol/l) (%)	100‖			50‖		

RI, recommended intake; α-ETK, activation coefficient of erthyrocyte transketolase.
Mean values were significantly different from those of the group with thiamin intake ≥ RI: *$P<0·05$.
† For details of subjects and procedures, see Table 1 and p. 130.
‡ RI 0·4 mg/4184 KJ + 0·1 mg, with a minimum provision of 1 mg/d (Departamento de Nutrición, 1994).
§ Number of determinations.
‖ There were no significant differences between the values for each group (χ^2 test).

The low consumption of cereals observed could be one of the factors contributing to reduced dietary thiamin supply. Correlations were found between greater cereal consumption and thiamin intake (r 0·31, $P<0·05$) and lower (i.e. more adequate) α-ETK values (r −0·38, $P<0·05$). A high percentage of subjects said that they had reduced cereal consumption during pregnancy to try to gain better control over their weight; this could have impaired their thiamin status.

Thiamin deficiency is more probable if, as well as its intake being low, the intake of carbohydrates (Vir *et al.* 1980) or alcohol (Ba *et al.* 1996) is increased, for the metabolism of carbohydrates or alcohol increases the requirements for the vitamin. However, in the present study this was not a problem, since the consumption of carbohydrate and alcohol was low (38·0 (SD 7·9) % and 0·35 (SD 0·96) % energy intake respectively). In addition, the carbohydrate (g): thiamin (mg) ratio was 170·3 (SD 53·4) mg/g and a there was apositive, significant relationship between thiamin and carbohydrate intakes (r 0·48, $P<0·05$).

Since thiamin deficiency has been implicated in hyperemesis gravidarum, Rees *et al.* (1997) have stressed the importance of prescribing thiamin supplements to all women with prolonged vomiting during pregnancy. Other authors have highlighted the benefits of thiamin supplementation in pregnant women (Bienengraber *et al.* 1997; Baker *et al.* 2000). However, in the present population, the use of thiamin supplements was very uncommon (Table 2).

Blood analysis results

The prevalence of apparent thiamin deficiency in the present subjects was similar to that reported for pregnant refugee women at term (57·7% of whom had α-ETK ≥1·2 and 26·9% had α-ETK >1·25) (McGready *et al.* 2001), to the 62·6% deficient reported for Spanish women (Sánchez *et al.* 1999), and to the 28–39% reported for white mothers in the second trimester, third trimester and postpartum phases of pregnancy (Vir *et al.* 1980).

In contrast to the results of Vir *et al.* (1980), who found no significant correlation between thiamin intake and blood biochemical indices, the present results show that thiamin intake has a great influence on blood α-ETK levels. Subjects with adequate thiamin intakes had lower α-ETK levels (1·01 (SD 0·19)) than those with thiamin intakes below recommended intake (1·21 (SD 0·3); $P<0·05$) (Table 3). In fact, a significant inverse correlation was found between thiamin intake and α-ETK (r −0·34, $P<0·05$); analysis of covariance shows that this correlation is maintained after adjusting for carbohydrate and alcohol intake.

The influence of thiamin on birth weight has been reported by several authors (Heinze & Weber, 1990; Baker *et al.* 2000). Among the present subjects the correlation between birth weight and maternal α-ETK was significant (r −0·33, $P<0·05$). With respect to the length of the newborn infants, a correlation was found with the thiamin:carbohydrate intake ratio (r 0·46, $P<0·05$).

Thiamin content of maternal milk

The thiamin content of the subjects' milk (Table 3) was similar to values reported by Dostálova *et al.* (1988; 0·30–0·60 μmol/l in European mothers), Holland *et al.* (1995; 0·30 μmol/l transition milk and 0·60 μmol/l mature milk), Al-Othman *et al.* (1996; 0·80 μmol/l mature breast milk of Saudi women), Bohm *et al.* (1997; 0·80 μmol/l transition milk of thirty-five women aged 19–31 years with α-ETK values indicative of low or sufficient intake) and Scherz & Senser (2000; 0·60 μmol/l transition milk and 0·45 μmol/l mature milk).

There are large variations in the thiamin content of milk between individuals and over the course of lactation (Institute of Medicine, 1991). Some authors have indicated that milk from mothers with beriberi contains less thiamin than that of healthy women in the same country, and that infants nursed by mothers with beriberi develop the disease by 3 or 4 weeks of age (Institute of Medicine, 1991). Bohm *et al.* (1997) have expressed concern that the thiamin supply in maternal milk may be insufficient for newborn infants during the first weeks after parturition. Prentice *et al.* (1983) reported a maternal milk thiamin concentration of 0·48 μmol/l in 130 nursing mothers in Keneba before receiving a nutritionally balanced supplement (providing energy, nutrients, and in particular 1·36 mg thiamin/d), and 0·66 μmol/l after supplementation.

Other authors have reported no difference in the mean breast-milk thiamin concentration at 3 months postpartum between supplemented (0·38 μmol/l) and unsupplemented (0·35 μmol/l) groups (the supplemented group received a total mean dose of 0·9 mg thiamine/kg per d), suggesting that breast-milk thiamin content is independent of maternal intake (McGready *et al.* 2001).

In the present study, mature milk thiamin content was significantly greater in group H women than in group L women (Table 3), and a correlation was seen between transition milk thiamin content and α-ETK values (r −0·50, $P<0·05$). Therefore, thiamin intake during pregnancy would seem to affect milk composition.

Several studies have shown that maternal thiamin status determines that of the newborn infant (Sánchez *et al.* 1999; Baker *et al.* 2000). Although there seems to be preferential delivery of thiamin to the fetus at the expense of the pregnant mother, after delivery the mother recovers and the infant becomes depleted. Infant thiamin pyrophosphate status is usually better than that of the mother, but 85·2% of infants born to mothers with a biochemical deficiency of thiamin also have inadequate thiamin status (Sánchez *et al.* 1999). This must be taken into account since some studies suggest the existence of a possible link between thiamin deficiency and a higher risk of sudden infant death syndrome (Bernshaw, 1991). If the thiamin supply to the child via the milk is also insufficient, thiamin status could worsen, placing the child at even greater risk of sudden infant death syndrome.

In agreement with Borrud *et al.* (1993), it is probable that efforts to improve the nutritional status of pregnant and lactating women would be well served if all women of childbearing age were encouraged to maximize the nutritional quality of their diets. The tendency to restrict the consumption of cereals because of an erroneous association between their intake and an increase in body weight needs to be corrected. Further, the supplementation of women with low thiamin intakes should be studied.

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