

Riboflavin Levels in Maternal Milk: The Influence of Vitamin B₂ Status during the Third Trimester of Pregnancy

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Objective: The aim of the present investigation was to study the relationship between riboflavin status during the third trimester of pregnancy and levels of this vitamin in transition milk (days 13 to 14 of lactation) and mature milk (day 40 of lactation).

Methods: The pregnancies and lactation periods of 57 healthy women between 18 and 35 years of age (27 ± 3.7 years) were monitored, vitamin intake during the third trimester was determined by recording the consumption of foods over five days and by registering the quantities provided by dietary supplements. Riboflavin status during this stage of pregnancy was determined via the measurement of the activation of erythrocyte glutathione reductase (EGR) by flavine adenine dinucleotide (FAD). Milk riboflavin levels were determined by fluorometry.

Results: Those subjects with riboflavin intakes below recommended (1.6 mg/day) (Group L) showed lower consumption of milk products (305.2 ± 88.5 g/day) than did those with greater intakes (Group H) (507.9 ± 137.2 g/day). The consumption of riboflavin containing supplements was very low and was seen only in two H subjects. Transition and mature milk riboflavin levels were significantly higher in H subjects (948.1 ± 700.1 nmol/L for transition milk and 993.8 ± 436.6 nmol/L for mature milk) than L subjects (574.9 ± 258.7 nmol/L for transition milk and 725.4 ± 254.3 nmol/L for mature milk). Subjects with α -EGR coefficients over 1.2 in the third trimester showed significantly lower mature milk riboflavin levels (704.1 ± 241.8 nmol/L) than did subjects with more satisfactory α -EGR coefficients (996.4 ± 302.9 nmol/L).

Conclusion: The influence of maternal vitamin B₂ status during pregnancy on breast milk riboflavin levels was confirmed.

INTRODUCTION

Fetal growth and the secretion of breast milk are processes that require adequate supplies of nutrients [1]. It is possible that in well-nourished women the requirements of these processes are met by metabolic and physiological adjustments [1]. However, nutritional imbalances suffered during these periods could be harmful to the mother and the outcome of her pregnancy [1–3], and also impair the composition of her breast milk [4–6].

Given that riboflavin is an essential constituent of muscle

tissue [7] it is needed in considerably increased amounts during pregnancy for the growth of fetal tissues [5]. Haste *et al.* [8] report that riboflavin intake during pregnancy is related to the birthweight of the newborn. Riboflavin intake is of greater importance during the pre- and post-natal periods owing to its role as a co-enzyme in energy utilisation [9]. Further, when a child is born, a rapid change occurs from a relatively hypoxic to a relatively hyperoxic environment, with the concurrent risk of reactive oxygen species formation. Vitamin B₂ acts mainly as a cofactor of glutathione reductase, which maintains glutathione in the reduced state. It can therefore be considered an

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indirect antioxidative vitamin [10].

The aim of the present investigation was to study the relationship between vitamin B₂ status during the third trimester of pregnancy and levels of this vitamin in maternal milk.

MATERIALS AND METHODS

The pregnancies and lactation periods of 57 women were followed. The characteristics of the subjects and criteria of inclusion/exclusion have been reported in previous papers [11,12]. The study subjects had normal pregnancies and were free of diabetes, renal diseases or cardiac or liver dysfunction.

The study protocol was approved by the Comité de Investigación de la Facultad de Farmacia, Universidad Complutense de Madrid and by the Comité Ético del Hospital del INSALUD de Cuenca.

During their third trimester of gestation (between weeks 32 and 36), dietary, anthropometric and biochemical studies were made. After subjects gave birth, the study was continued. The composition of the now lactating subjects' maternal milk was analyzed at days 13 to 14 (transitional milk) and 40 (mature milk) [13].

Dietary Survey

Food intake was recorded by keeping a food record booklet for five days, including a Sunday. 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 intake" questionnaire was completed in order to contrast subjects' answers with the results of their five day dietary record and an explanation was requested if answers were inconsistent. The details of the dietetic method used have been published previously [11,12].

The vitamin B₂ contents of consumed foods were calculated using "Tables of Food Composition" published by the Instituto de Nutrición [14]. The recommended intake of riboflavin accepted in this 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" [15] (0.6 mg/1000 kcal + 0.2 mg, with a minimum provision of 1.6 mg/day). Given that no subject showed high energy intakes, recommended intake (RI) was established as 1.6 mg/day.

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

Biochemical Study

10 mL of venous blood was taken first thing in the morning from night-fasted subjects. Samples were collected in heparinized tubes and maintained at 4° to 6°C until riboflavin analysis was performed, (always before the elapse of 48 hours). For the determination of hematocrit, five mL of heparin-free blood was transferred to another test tube and analyzed at room temperature within an hour of collection. Vitamin B₂ status was determined by measuring the activation of erythrocyte glutathione reductase (EGR) (EC 1.6.4.2.) by flavine adenine dinucleotide (FAD) (Boehringer-Mannheim GmbH, Mannheim, Germany). The activity of the enzyme was measured in basal conditions and after the addition of excess FAD from hemolyzed blood samples, by spectrophotometric determinations at 340 nm of NADP formed in a spectrophotometer Shimadzu UV-1203 (Shimadzu Corporation, Kyoto, Japan). The relationship between enzyme activity before and after saturation is expressed by the saturation coefficient 'α'. High α coefficients imply an unfavorable biochemical riboflavin status [16] (C.V = 4.4%). The hematocrit index, necessary for the calculation of the α-EGR coefficient, was determined using a Coulter S. Plus analyser (Coulter Diagnostics, Hialeah FL 33014, USA) [17].

Milk samples were taken between 10 and 11 o'clock in the morning by manual expression of a five mL sample from each breast at the beginning and end of feeds. The protocol for both the collection and subsequent handling of milk has been previously described [11,12].

Milk samples were subjected to complete acid hydrolysis by autoclaving the sample in 0.1 N HCl (Merck, Darmstadt D-6100 Franfurter Strasse 250, Germany). The proteins were removed by adjusting the pH to 4.5 with NaOH (Merck) and centrifuging. Extract was acidified with glacial acetic acid (Merck) and oxidized with potassium permanganate (Merck); the excess of the oxidizing agent was destroyed with hydrogen peroxide (Merck). Riboflavin levels then determined by fluorometry (Perkin Elmer MPF-2A, Beconsfield, UK). Fluorescent measurements were made at the excitation and emission wave lengths of 440 and 565 nm, respectively, on sample solutions containing an internal standard (Riboflavine Merck) and on samples treated with 20 mg of sodium hydrosulphite (Merck) as a blank to correct for postoxidation remnant fluorescence [18]. The coefficient of variation was found to be 3.8%.

In order to establish normality limits for the α-EGR coefficients, the criteria of the following authors were taken into account: Vuillemier *et al.* [16] and Dostálová [19], who regard coefficients of <1.20 as an indicator of low risk, those between 1.20 and 1.29 as indicators of moderate risk and those above 1.29 as a high risk of deficiency; Bates *et al.* [4], who consider levels to be deficient when α-EGR is >1.30; Vir *et al.* [20], who consider the same when this coefficient is >1.20; Açkurt *et al.* [9] and Keller and Salkeld [21], who consider α-EGR

coefficients of >1.52 as indicators of high risk, those between 1.44 and 1.52 as indicators of moderate risk and <1.44 to represent low risk.

With respect to riboflavin levels in maternal milk, 800 nmol/L was established as the lower normal limit [22].

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; Rue Lavoisier 91430, Igmy, France; range: 0.1–150 Kg) and a digital stadiometer (Harpenden Pfifter 450; Badem, Padum Aveny, Carls-tadt, NJ, USA; range 70–205 cm), respectively. Body mass index (BMI) (kg/m²) was calculated from these data. All data were collected by trained personnel following norms set out by the World Health Organisation (WHO) [23].

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

Other Data

The maternal gestation age at delivery was calculated from the agreed delivery data recorded from the last menstrual period and early ultrasound examination data. Data such as age, parity and use of tobacco were recorded in a questionnaire during the first interview.

Statistical Analysis

Mean values and SD are shown. Where the distribution of results was normally distributed, the degree of significance of

differences between means was calculated using Student's *t* test. Where the distribution of results was not normally distributed, the Mann-Whitney test was applied. Analysis of covariance was used to eliminate the influence of variables, such as age, that could modify the results. Differences were considered significant if *p* < 0.05 [24].

RESULTS

Subjects' data are presented with respect to whether their intakes were below that recommended (Group L) or above (Group H) (Tables 1–3). Table 1 shows both the mothers' and newborns' demographic and anthropometric data and reveals that H subjects were older than L subjects. No other significant differences were found with respect to these parameters.

Table 2 shows the riboflavin intake during the third trimester of pregnancy. L subjects showed significantly lower intakes of milk products (305.2 ± 88.5 g/day) than did H subjects (507.9 ± 137.2 g/day). These differences are probably the reason for the greater intake of riboflavin seen in H subjects (Table 2).

Only two H subjects took a riboflavin-containing supplement during the third trimester and even then in very small amounts (2 mg/day). The dietetic results are, therefore, hardly modified when supplements are taken into account (Table 2).

Table 3 lists riboflavin status during the third trimester and levels of riboflavin in transition and mature milk. Though H subjects showed more satisfactory serum α-EGR coefficients than did L subjects, this difference was not significant. However, subjects with riboflavin intakes of <1.4 mg/day (25th percentile) showed less adequate α-EGR coefficients (1.32 ± 0.40) than did those with greater riboflavin intakes (1.07 ± 0.16) (*p* < 0.05).

Table 1. Demographic and Anthropometric Data of Subjects and their Newborn

	Riboflavin Intake < RI	Riboflavin Intake ≥ RI
n	25	32
Age, years	26.3 ± 3.2*	28.5 ± 4.0* H
Anthropometric data in 1st trimester		
Weight, kg	56.8 ± 7.1	57.4 ± 9.0 H
Height, cm	161.3 ± 5.9	159.3 ± 5.2 H
Body mass index, kg/m ²	21.6 ± 2.3	22.1 ± 2.1 H
Anthropometric data in 3rd trimester		
Weight, kg	65.0 ± 7.1	66.6 ± 8.9 H
Height, cm	161.3 ± 5.8	159.3 ± 5.1 H
Body mass index, kg/m ²	24.8 ± 2.1	25.6 ± 2.5 H
Weight gain in first two trimesters, kg	8.2 ± 2.8	8.8 ± 2.8 H
Parity, n	0.54 ± 0.66	0.62 ± 0.73 H
Length of pregnancy, weeks	39.3 ± 1.1	39.5 ± 1.1 H
Weight of newborn, g	3277 ± 458	3312 ± 357 H
Length of newborn, cm	50.0 ± 1.8	50.1 ± 1.2 NH
Smokers subjects (%)	24	31.3
Cigarettes smoked, n/day	6.4 ± 5.9 ¹	7.2 ± 4.1 ¹ H

Values are means ± SD, * *p* < 0.05: significant differences between the established groups. RI: Recommended intakes (1.6 mg/day), ¹Mean cigarette consumption is given for smokers only. H: Homogeneous distribution of results, NH: Non-homogeneous distribution of results.

Table 2. Riboflavin Intake during the Third Trimester of Pregnancy

	Riboflavin intake < RI (n = 25)	Riboflavin intake ≥ RI (n = 32)
Riboflavin supplied by supplements, mg/day	0	0.13 ± 0.50 ¹ NH
Riboflavin supplied by supplements + diet		
-Total intake, mg/day	1.37 ± 0.11*	2.52 ± 1.00* NH
-Coverage of RI, %	85.6 ± 6.9*	155.4 ± 61.6* NH
-Riboflavin density, mg/MJ	0.17 ± 0.03*	0.27 ± 0.10* NH
-INQ of riboflavin (true density/recommended)	0.86 ± 0.07*	1.57 ± 0.62* NH
-% of INQ < 1	100	3.1

Values are means±SD, INQ: Index of Nutritional Quality, RI: Recommended intakes, ¹The mean provision of supplements to H subjects is given: only two took supplements at levels of 2 mg/day. H: Homogeneous distribution of results, NH: Non-homogeneous distribution of results. *p < 0.05: significant differences between the established groups using the Mann-Whitney test.

Table 3. Riboflavin Status (Determined by Measuring the Activation Coefficient of Erythrocyte Glutathione Reductase, α-EGR) during the Third Trimester and Levels of Riboflavin in Transition (Days 13–14) and Mature Milk (Day 40)

	Riboflavin intake < RI (n = 25)	Riboflavin intake ≥ RI (n = 32)
Serum data, third trimester of pregnancy [α-EGR]	1.21 ± 0.35	1.06 ± 0.16 NH
% of values indicating deficit		
> 1.52	16.0	9.4
1.44–1.52	8.0	0
>1.29	24.0	12.5
>1.2	24.0	12.5
Riboflavin in milk		
-Transition, nmol/L	574.9 ± 258.7*	948.1 ± 700.1* NH
% low breast milk levels (<800 nmol/L)	24.0	6.3
-Mature, nmol/L	725.4 ± 254.3*	993.8 ± 436.6* H
% low breast milk levels (<800 nmol/L)	24.0	9.4

Values are means±SD, RI: Recommended intakes, * p < 0.05: significant differences between the established groups. H: Homogeneous distribution of results, NH: Non-homogeneous distribution of results.

Transition and mature milk riboflavin levels were significantly higher in H subjects, while the percentage of subjects with riboflavin levels below the normal limit was greater among L subjects (Table 3).

Analysis of covariance shows that the differences in transition and mature milk vitamin B₂ concentrations between L and H subjects are independent of the age difference between these groups (Table 1).

Subjects with α-EGR coefficients >1.2 in the third trimester showed significantly lower mature milk riboflavin levels (704.1 ± 241.8 nmol/L) than did subjects with more satisfactory α-EGR levels (996.4 ± 302.9 nmol/L).

DISCUSSION

The duration of pregnancy and the anthropometric data of the mothers and their newborn (Table 1) are similar to those reported in other studies [9,25–27]. The mean intake of vitamin B₂ (Table 2) observed during the third trimester was similar to that reported by other authors [2,8,26,28–30].

Though mean riboflavin intake is generally satisfactory

[6,26,29] given the increased intake of milk products usually seen during pregnancy [26,29], in the present study 43.9% of subjects showed riboflavin intakes lower than those recommended. This was higher than that recorded by Ortega *et al.* [26] in Guadalajara (Spain), where only 23% showed intakes below recommended. When supplements were taken into account this figure fell to only 3%.

Heller *et al.* [31] indicate that riboflavin supplementation is recommended to prevent subclinical metabolic disturbances of vitamin-dependent enzyme systems. However, in the present population, the use of riboflavin supplements was scarce (Table 2).

The α-EGR coefficients obtained (Table 2) were similar to those seen in similar subjects during the third trimester of pregnancy by Bates *et al.* [4] (1.19 ± 0.08 in pregnant women of Cambridge), Açkurt *et al.* [9] (1.4 ± 0.28) and Dostálová [19] (0.99 ± 0.12).

In the present study, although H subjects showed more adequate serum α-EGR coefficients than did L subjects, the difference was not significant. Neither was the correlation between riboflavin intake and α-EGR statistically significant. However, the influence of riboflavin intake on α-EGR coefficients is confirmed since those of subjects with intakes of <1.4

mg/day (25th percentile) showed less adequate serum α -EGR coefficients (1.32 ± 0.40 compared to 1.07 ± 0.16 in subjects with greater riboflavin intakes) ($p < 0.05$).

The mean concentration of riboflavin in breast milk (Table 3) was similar to that reported in other studies. Bamji *et al.* [32] found a concentration of 1095 nmol/L in the transition milk (6 to 30 days) of Indian mothers and 611 nmol/L in their mature milk. Further, the Committee on Nutrition [33] and the National Research Council [34] indicate that the milk of well-nourished mothers contains riboflavin concentrations of approximately 930 nmol/L. Similarly, Fomon [35] and Schwarz [36] report mature milk riboflavin concentrations of 957 nmol/L and 983 mol/L respectively.

In agreement with Ford *et al.* [37], the present results show riboflavin values to change little in the weeks following parturition (Table 3). Nail *et al.* [6] also report that riboflavin values did not change significantly as milk matured from one to six weeks postpartum.

Though Nail *et al.* [6] and Bamji *et al.* [32] report that levels of riboflavin in milk were generally satisfactory; in the present population, 14% of subjects showed values of <800 nmol/L in transition milk and 15.8% showed the same in mature milk. This should be improved, especially for L women.

Some authors dispute the influence of intake on breast milk levels. Bamji *et al.* [32] report a lack of clear-cut correlations between the enzymatic indices of vitamin status and milk levels of the corresponding vitamins. Neither does the Institute of Medicine [38] find any relationship between maternal intake of riboflavin and the concentration of vitamin B₂ in milk. However, Nail *et al.* [6] report that milk riboflavin values were significantly lower in a non-supplemented group of mothers, both at one and six weeks postpartum. Bates *et al.* [4] indicate that lower concentrations found in riboflavin-deficient populations can be increased by supplementation. In agreement with the latter authors, the results of the present study show a higher riboflavin concentration in the milk of H subjects (Table 3).

Though no data is available for riboflavin intake during lactation, it is probable that the mother's diet does not change drastically during after giving birth. This is confirmed by information that was possible to collect from some of the lactating subjects. Further, it is very likely that the composition of transition milk (day 13 to 14 postpartum) is greatly influenced by riboflavin intake at the end of pregnancy. In the present study, H subjects showed higher transition and mature milk riboflavin levels than did L subjects.

Breast milk riboflavin levels may have a significant influence on the nutritional status and health of suckling infants. Riboflavin is included among the antioxidative vitamins since it acts mainly as the essential cofactor of glutathione reductase, representing the most important cellular SH-bearing molecule [10]. At the moment of birth there is a rapid change from a relatively hypoxic to a relatively hyperoxic environment, with the risk of peroxidation reactions occurring which could damage the health of the newborn [10].

An adequate supply of riboflavin via the milk may be of extreme importance in certain clinical conditions. Light therapy for hyperbilirubinemia of the newborn is a common and readily employed means of therapy. This phototherapy has been recognized as a cause of riboflavin deficiency [39]; within 24 hours of phototherapy plasma riboflavin concentration decreases by about 50%. Low riboflavin concentrations indicate insufficient antioxidative protection through insufficient glutathione regeneration [10].

In agreement with Borrud *et al.* [29], it is probable 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 milk products during pregnancy, not only to raise riboflavin intake, but also that of calcium, the deficiency of which is associated with serious health problems in both mother and newborn [40–42].

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REFERENCES

1. King JC, Weininger J: Embarazo y lactancia. In Organización Panamericana de la Salud, Instituto Internacional de Ciencias de la Vida: "Conocimientos actuales sobre nutrición." Washington: ILSI-North America, pp 362–369, 1991.
2. Antal M, Regöly-Mérei A, Varsányi H, Biró L, Ságy K, Molnár DV, Zajkás G, Nagy K, Avar Z, Biró G: Nutritional survey of pregnant women in Hungary. *Int J Vit Nutr Res* 67:115–122, 1997.
3. González-Cossio T, Delgado H: Functional consequences of Maternal Malnutrition. *World Rev Nutr Diet* 64:139–173, 1991.
4. Bates CJ, Prentice AM, Paul AA, Sutcliffe BA, Watkinson M, Whitehead RG: Riboflavin status in Gambian pregnant and lactating women and its implications for recommended dietary allowances. *Am J Clin Nutr* 23:928–935, 1981.
5. Bates CJ, Prentice AM, Paul AA: Seasonal variations in vitamins A, C, riboflavin and folate intakes and status of pregnant and lactating women in a rural Gambian community: some possible implications. *Eur J Clin Nutr* 48:660–668, 1994.
6. Nail PA, Thomas MR, Eakin R: The effect of thiamin and riboflavin supplementation on the level of those vitamins in human breast milk and urine. *Am J Clin Nutr* 33:198–204, 1980.
7. Golden BE: Zinc in cell division and tissue growth: Physiological aspects. In Mills CF (ed): *Zinc in Human Biology*. Berlin: Springer-Verlag, pp 119–128, 1988.
8. Haste FM, Brooke OG, Anderson HR, Bland JM: The effects of nutritional intake on outcome of pregnancy in smokers and non-smokers. *Brit J Nutr* 65:347–354, 1991.
9. Açkurt F, Wetherilt H, Löker M, Hacibekiroglu M: Biochemical

- assessment and nutritional status in pre- and post-natal Turkish women and outcome of pregnancy. *Eur J Clin Nutr* 49:613–622, 1995.
10. Böhles H: Antioxidative vitamins in prematurely and maturely born infants. *Int J Vit Nutr Res* 67:321–328, 1997.
 11. Ortega RM, Andrés P, Martínez RM, López-Sobaler AM: Vitamin A status during the third trimester of pregnancy in Spanish women: influence on concentrations of vitamin A in breast milk. *Am J Clin Nutr* 66:564–568, 1997.
 12. Ortega RM, Andrés P, Martínez RM, López-Sobaler AM, Quintas ME: Zinc levels in maternal milk: the influence of nutritional status with respect to zinc during the third trimester of pregnancy. *Eur J Clin Nutr* 51:253–258, 1997.
 13. Patton S, Canfield LM, Huston GE, Ferris AM, Jensen RG: Carotenoids of human calostrum. *Lipids* 25:159–165, 1990.
 14. Instituto de Nutrición (CSIC): “Tablas de Composición de Alimentos españoles.” Madrid: Instituto de Nutrición, 1994.
 15. Departamento de Nutrición. “Ingestas Recomendadas de Energía y Nutrientes para la Población española.” Madrid: Departamento de Nutrición, 1994.
 16. Vuilleumier JP, Keller HE, Rettenmaier R, Hunziker F: Clinical chemical methods for the routine assessment of the vitamin status in human populations. Part II: The water-soluble vitamins B₁, B₂ and B₆. *Int J Vit Nutr Res* 53:359–370, 1983.
 17. Cox CJ, Haberman TM, Payne BA: Evaluation of the Coulter Counter Model S-Plus IV. *Am J Clin Pathol* 84:297–306, 1985.
 18. Association of Official Analytical Chemists: Methods 970.65: riboflavin (vitamin B₂) in foods and vitamin preparations, fluorimetric methods. In *Association of Official Analytical Chemists: “Official Methods of Analysis of the Association of Official Analytical Chemists,”* 15th ed. Arlington, VA: AOAC, 1990.
 19. Dostálová L: Vitamin status during puerperium and lactation. *An Nutr Metab* 28:385–408, 1984.
 20. Vir SC, Love AHG, Thompson W: Riboflavin status during pregnancy. *Am J Clin Nutr* 34:2699–2705, 1981.
 21. Keller HE, Salkeld RM: Bereichswerte von analysenparametern für den Vitaminstatus. *GCR B* 106:334, 1988.
 22. Souci SW, Fachman W, Kraut NH: “Food Composition and Nutrition Tables.” Stuttgart: Wissenschaftliche Verlagsgesellschaft, pp 9–16, 1994.
 23. World Health Organization (WHO): “Report of a joint FAO/WHO/ONU Expert Consultation. Methodology of Nutritional Surveillance.” Technical Report Series No. 53. Geneva: World Health Organization, pp 20–60, 1976.
 24. Wonnacott HW, Wonnacott RJ: “Introductory Statistics.” New York: John Wiley and Sons, 1977.
 25. Ash S: Dietary intakes of pregnant women in Sydney, New South Wales. *Aust J Nutr Diet* 52:149–153, 1995.
 26. Ortega RM, Gaspar MJ, Moreiras O: Dietary assessment of a pregnant spanish women group. *Int J Vit Nutr Res* 64:130–134, 1994.
 27. Ortega RM, Gaspar MJ, Cantero M: Influence of maternal serum lipids and maternal diet during the third trimester of pregnancy on umbilical cord blood lipids in two populations of Spanish newborn. *Int J Vit Nutr Res* 66:250–257, 1996.
 28. Anderson A, Whichelow MS: Constipation during pregnancy: dietary fibre intake and the effect of fibre supplementation. *Hum Nutr: Appl Nutr* 39:202–207, 1985.
 29. Borrud LG, Krebs-Smith SM, Friedman L, Guenther PM: Food and nutrient intakes of pregnant and lactating women in the United States. *J Nutr Educ* 25:176–185, 1993.
 30. Picone T, Allan LH, Schramm MH, Olsen D: Pregnancy outcome in North American women. 1. Effects of diet, cigarette smoking and psychological stress on maternal weight gain. *Am J Clin Nutr* 36:1205–1213, 1982.
 31. Heller S, Salkeld RM, Korner WF: Riboflavin status in pregnancy. *Am J Clin Nutr* 27:1225–1230, 1974.
 32. Bamji MS, Prema K, Jacob CM, Ramalakshmi BA, Madhavapeddi R: Relationship between maternal vitamins B₂ and B₆ status and the levels of these vitamins in milk at different stages of lactation. A study in a low-income group of Indian women. *Hum Nutr: Clin Nutr* 40:119–124, 1986.
 33. Committee on Nutrition: Composition of human milk: normative data. In “*Pediatric Nutrition Handbook,*” 2nd ed. Elk Grove Village, IL: American Academy of Pediatrics, pp 363–368, 1985.
 34. National Research Council (NRC): “Recommended Dietary Allowances,” 10th ed. (Report of the Subcommittee on the Tenth Edition of RDAs. Food and Nutrition Board, Commission on Life Sciences.) Washington, DC: National Academy Press, pp 284, 1989.
 35. Fomon SJ: “*Infant Nutrition,*” 2nd ed. Philadelphia: WB Saunders, pp 362, 1974.
 36. Schwarz KB: “*Vitamins in Nutrition in Pediatrics.*” Boston: Little Brown, pp 58–66, 1985.
 37. Ford JE, Zechalko A, Murphy J, Brooke OE: Comparison of the B vitamin composition of milk from mothers of preterm and term babies. *Arch Dis Child* 58:367–372, 1983.
 38. Institute of Medicine: “*Nutrition during Lactation.*” (Subcommittee on Nutrition during Lactation, Committee on Nutritional Status during Pregnancy and Lactation, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences.) Washington DC: National Academy Press, pp 57–219, 1991.
 39. Gromisch DS, López R, Cole HS, Cooperman JM: Light (phototherapy)-induced riboflavin deficiency in the neonate. *J Pediatr* 90:118–122, 1977.
 40. Marcoux S, Brisson J, Fabia J: Calcium intake from dairy products and supplements and the risks of preeclampsia and gestational hypertension. *Am J Epidemiol* 133:1266–1272, 1991.
 41. Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B, Fairweather-Tait S: Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. *Am J Clin Nutr* 62:58–67, 1995.
 42. Ortega RM, Martínez RM, López-Sobaler AM, Andrés P, Quintas ME: The influence of calcium intake on gestational hypertension. *Ann Nutr Metab* 43:37–46, 1999.

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