B Vitamins in Breast Milk: Relative Importance of Maternal Status and Intake, and Effects on Infant Status and Function[1,2]

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ABSTRACT

Infants should be exclusively breastfed for the first 6 mo of life. However, maternal deficiency of some micronutrients, conveniently classified as Group I micronutrients during lactation, can result in low concentrations in breast milk and subsequent infant deficiency preventable by improving maternal status. This article uses thiamin, riboflavin, vitamin B-6, vitamin B-12, and choline as examples and reviews the evidence for risk of inadequate intakes by infants in the first 6 mo of life. Folate, a Group II micronutrient, is included for comparison. Information is presented on forms and concentrations in human milk, analytical methods, the basis of current recommended intakes for infants and lactating women, and effects of maternal supplementation. From reports of maternal and/or infant deficiency, concentrations in milk were noted as well as any consequences for infant function. These milk values were used to estimate the percent of recommended daily intake that infants fed by a deficient mother could obtain from her milk. Estimates were 60% for thiamin, 53% for riboflavin, 80% for vitamin B-6, 16% for vitamin B-12, and 56% for choline. Lack of data limits the accuracy and generalizability of these conclusions, but the overall picture that emerges is consistent across nutrients and points to an urgent need to improve the information available on breast milk quality. *Adv. Nutr. 3: 362–369, 2012.*

Introduction

In 2001, after a debate ongoing for some years, the WHO concluded that infants should be exclusively breastfed for the first 6 mo of life rather than the previously recommended 4–6 mo (1). This recommendation was supported by evidence that extending exclusive breastfeeding would provide more protection against gastrointestinal infections. A review prepared as part of the background documentation evaluated the nutrient adequacy of exclusive breastfeeding for 6 mo (2). The authors identified maternal vitamins A and B-6 as the only maternal deficiencies that would lead to suboptimal concentrations in milk and adversely affect the infant. However, that review did not include deficiencies of other nutrients that can adversely affect the quality of breast milk, and the overall consultation concluded that apart from a possible negative effect of exclusive breastfeeding on iron status, "the available evidence is grossly inadequate to assess risks of deficiency in other micronutrients."

There has been relatively little research on the extent to which the nutrient content of human milk is affected by maternal status and intake and how low milk concentrations affect infant status. One reason for this may be fear of discouraging breastfeeding if milk is found to be lacking in nutrients. Also, it is generally assumed that poor breast milk quality does not play a major causal role in the widespread growth faltering that occurs in developing countries early in the first year of life, because the breast milk content of nutrients usually associated with growth, such as zinc and phosphorus, is unaffected by maternal status. This assumption remains to be tested, however. Although infant micronutrient status at 6 mo of age is often poor (3,4), this tends to be attributed to, without study, substitution of some breast milk by nutrient-poor fluids and foods, low birthweight and/or preterm delivery resulting in poor infant stores at birth, and/or infant morbidity. Investigation has also been limited by other uncertainties, including the range

[1] Published as a supplement to Advances in Nutrition. Presented as part of the symposium entitled "Impact of Maternal Status on Breast Milk Quality and Infant Outcomes: An Update on Key Nutrients," given at the Experimental Biology 2011 meeting, April 12, 2011, in Washington, DC. The symposium was sponsored by the American Society for Nutrition and supported by an unrestricted educational grant from Medela. The symposium was chaired by Laurie Nommsen-Rivers and Donna J. Chapman. Guest Editors for this symposium publication were Donna J. Chapman and Shelley McGuire. Guest Editor disclosure: Donna J. Chapman received travel support and compensation for editorial services provided for this symposium from the International Society for Research on Human Milk and Lactation. Shelley McGuire had no conflicts to disclose.

[2] Author disclosure: L. H. Allen, no conflicts of interest.

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of acceptable concentrations of nutrients in breast milk, the efficacy of maternal supplementation for increasing micronutrients in milk, and blood values that denote micronutrient deficiencies in the infant.

It is useful to categorize nutrients into 2 groups during lactation (5). Group I nutrients (thiamin, riboflavin, vitamin B-6, vitamin B-12, choline, retinol, vitamin A, vitamin D, selenium, and iodine) are of the most interest in public health nutrition, because their secretion into milk is rapidly and/or substantially reduced by maternal depletion. Conversely, maternal supplementation with these nutrients can increase breast milk concentrations and improve infant status. In contrast, the concentration of Group II nutrients (folate, calcium, iron, copper, and zinc) in breast milk is relatively unaffected by maternal intake or status; the mother gradually becomes more depleted when intake is less than the amount secreted in milk, and maternal supplementation benefits the mother rather than the infant.

This article focuses on the example of B vitamins, all of which except folate are Group I micronutrients. A literature review was conducted to summarize relevant information on B vitamins during lactation, including forms and concentrations in breast milk, methods of analysis, and values used when setting current recommended intakes in infants and lactating women. Articles were identified using the PubMed database and the terms "lactation," "human milk," "breast milk," "milk," "milk composition," and the name of each vitamin. Additional references were obtained from those cited in these articles. The search years were not restricted. Data from measurements made early in lactation were avoided, because concentrations of thiamin, vitamin B-6, and folate are lower and vitamin B-12 are higher in the first postpartum weeks. However, at least in well-nourished women, concentrations of all B vitamins are relatively stable after about wk 3 of lactation (6). Especially useful were reports of maternal and/or infant deficiency, where values were noted for concentrations of the vitamins in maternal blood, breast milk, and infant blood as well as any effects on infant function. Where breast milk concentrations were clearly inadequate based on poor maternal and/or infant status, these concentrations were used to estimate the gap between the infant's possible daily intake of the vitamin from breast milk and their recommended intake. Any reported effects of maternal supplementation on the mother, breast milk, and infant were also noted. This review updates a similar approach that the authors published nearly a decade ago (7) and expands the information on B vitamins.

Current status of knowledge

Thiamin

Thiamin is present in human milk as thiamin (~30%) and thiamin monophosphate (~70%) (8). The phosphorylated forms of thiamin cannot cross membranes, but the free thiamin is released by phosphatase in the upper small intestine. A common method of analysis is to oxidize the thiamin in milk to the thiochrome, which is extracted and measured by fluorescence spectrometry (9). In milk from well-nourished women, the concentration used in setting infant and maternal intake recommendations in the US and Canada and by the FAO is 0.21 ± 0.04 mg/L (0.62 mmol/L) based on a 1985 report, so the Adequate Intake (AI) recommended for infants is 0.2 mg/d in the first 6 mo, assuming that breast-fed infants consume 780 mL milk/d (10). Contrary to the expected effect for a Group I nutrient, thiamin supplementation (1.7 mg/d) did not increase milk concentration to a higher level (0.24 mg/L) than in a nonsupplemented group (0.22 mg/L) of U.S. women, but sample size was small (n = 12) and all had received supplemental thiamin during pregnancy. A range of thiamin in milk from ~0.02 to 0.35 mg/L was reported in well-nourished Russian women, with concentration strongly associated with maternal intake (from food and in some cases supplements) over the range of ~0.5–5 mg/d (11).

The global prevalence of thiamin depletion is uncertain but likely to be higher where diets are high in refined or polished unfortified grains and antithiamine factors or thiaminases and low in animal source foods and legumes (12). HIV-AIDS is associated with poor thiamin status (13), and in 1979 deficiency was prevalent in Ghanaian children aged 6 mo to 6 y (14). Certainly, severe deficiency is much less common than it was some 50 y ago. At that time, infantile beri-beri was prevalent in countries such as Malaysia where the staple diet was based on polished rice and animal source food consumption was low. In 1942, Clements (15) reported that when the breast milk thiamin concentration fell to <0.12 mg/L, infant growth was poor and <0.06 mg/L, growth was completely arrested. The infants became pale, screamed, and died rapidly from heart disease. He proved that the low concentrations in human milk produced these symptoms by feeding milk from thiamin-deficient women to dogs.

Maternal thiamin deficiency rapidly can result in very low concentrations of the vitamin in milk, which are improved with supplementation. Studies in The Gambia (16), India (17,18), and Karen women in Thailand (19) between 1964 and 2001 reported concentrations of 0.16 mg/L, 0.22 and 0.11 mg/L, and 0.12 mg/L, respectively, substantially lower than those used to set the AI in the US and Canada. Maternal deficiency was common in all locations. In India, with supplements of 0.2–20 mg/d (dosage was increased over an 8-mo period), milk thiamin increased from 0.11 to 0.27 mg/L. In The Gambia, maternal supplements of 2 mg/d improved maternal status within 3 wk and infant status in 1–9 d, with breast milk content increasing to 0.22 mg/L (16). Infants developed symptoms of deficiency earlier if their mothers were deficient during pregnancy. The functional consequences of mild infant depletion have scarcely been investigated, but in Thai Karen, in whom deficiency results from their dependence on polished rice and high intakes of antithiamine factors in betel nut, fermented fish, and tea leaves, maternal supplementation in pregnancy improved the visual alertness of infants at birth. Recently, Karen women in a refugee camp in Northern Thailand were assessed for thiamin adequacy. In light of the earlier observations of deficiency in Karen, which had been recognized as a major cause of infant mortality, the women had

been given thiamin supplements during pregnancy and lactation (92 mg/d, which is far higher than the recommended intake of 1.5 mg/d) as well as food rations with and without thiamin-fortified flour. Total breast milk thiamin did not differ before (0.27 ± 0.13 mg/L) or after (0.29 ± 0.83 mg/L) fortification (n = 180).

In summary for thiamin, breast milk concentrations and infant status are strongly dependent on maternal intake and status (with maternal deficiency during pregnancy likely an additional risk factor for infant deficiency). Maternal supplementation rapidly improves the vitamin concentration in milk and infant status. An older report of severe deficiency suggests it may reduce infant growth (15).

Riboflavin

Riboflavin is found in human milk as flavin adenine dinucleotide (54%) which is 48% riboflavin by weight, riboflavin (39%), and small amounts of other flavins (20). The range in concentration of 5 well-nourished women was reported to be 180–800 μg/L, values 50% higher than those found when using older methods that did not correct for fluorescence quenching (20). The Institute of Medicine assumes a mean of 0.35 mg/L (10) based on data from the 5 American women (average 0.39 mg/L) and samples measured by a valid microbiological assay from 2 participants and reported by WHO (average 0.31 mg/L); values from prior studies were not be used due to the underestimation caused by inappropriate analysis. The daily secretion in breast milk of 43 well-nourished Russian women was ~0.010–0.55 mg and strongly correlated with maternal intake from diet and supplements over the range of ~1–8 mg/d (11).

The global prevalence of riboflavin deficiency is uncertain but likely very high, because intake depends primarily on animal source food and green vegetable consumption. Reported prevalences include 77% in lactating urban Guatemalan women (LH Allen and MT Ruel, unpublished data), 85% in night-blind pregnant Nepali women (21), 14% in Ghanaian infants (4), 30% in Kenyan school children (22), and in 1982, nearly all pregnant and lactating women and their infants in The Gambia (23). Recently, it was reported that 66% of adults in the UK, if they are low milk-consumers, have marginal riboflavin status correctable by supplementation (24).

Maternal riboflavin deficiency rapidly results in low milk concentrations of the vitamin. Values in The Gambia and India (5 studies between 1964 and 1982) revealed concentrations of 0.16–0.22 mg/L (18,23,25). Maternal supplementation postdelivery in The Gambia (2 mg/d) increased the milk concentration to 0.22–0.28 mg/L compared to 0.12 mg/L in a placebo group, with the effect of supplementation similar in milk samples taken between 10 and 84 d postpartum (25). In this population where almost all mothers and infants were deficient at baseline, status (assessed from erythrocyte glutathione reductase activation coefficients) and clinical symptoms of deficiency improved as did the iron status of the women. A riboflavin-fortified weaning food also rapidly normalized the status of the infants (23). Thus, there is no doubt that milk riboflavin concentration was inadequate in this study.

Vitamin B-6

The predominant form of vitamin B-6 in breast milk is pyridoxal (75%), with smaller amounts of pyridoxal phosphate (9%), pyridoxamine, and pyridoxine, and mostly has been measured by HPLC for many years (26,27). The average concentration assumed when setting the AI for infants and lactation was 0.13 mg/L, based on a 1976 publication on a small group of U.S. women with intakes < 2.5 mg/d (28). The vitamin B-6 intake of mothers is a strong predictor of infant status (29).

The global prevalence of vitamin B-6 deficiency is very uncertain, because status is rarely measured. Estimates in a 2001 Indonesian study were 10% of children in urban areas and 40% of those in rural areas (30). In 1990 it was reported that ~40% of peri-urban Egyptian mothers had low concentrations of the vitamin in their milk [<415 nmol/L (0.10 mg/L), mean 435 (0.11 mg/L) nmol/L], although their status was not assessed. In the group with values < 415 nmol/L, birthweight of the infant was much lower. Lower milk concentrations were associated with abnormal behaviors in the women (less response to their infants' vocalizations and less effective intervention when their infant was distressed) and their infants displayed less consolability, inappropriate build-up to a crying state, and poorer response to aversive stimuli (31). Convulsive seizures were observed in infants fed breast milk containing <0.10 mg thiamin/L (32). Some older evidence suggested suboptimal perinatal vitamin B-6 status in industrialized countries. In the US, low maternal intakes were related to poorer maternal status in pregnancy and lactation and lower levels in breast milk at 14 d postpartum (32). Infant pyridoxal-5′-phosphate concentrations predicted infant growth among 20 mother-infant pairs in the US (29). Of 44 Finnish infants, 7 had low vitamin B-6 status and also slower growth (33). Lactating women in the US (n = 25) whose vitamin B-6 intake was greater than the group median value had higher vitamin B-6 concentrations in their milk and their infants' habituation and autonomic stability scores on the Brazelton Neonatal Assessment Scale were very strongly correlated with milk content of the vitamin (34). However, there is no evidence that any of the women were B-6 deficient and most took daily multivitamins during pregnancy; associations between the milk vitamin and infant behavior could have been due to other factors, including milk levels of other nutrients that were not measured.

Maternal supplementation with vitamin B-6 produces a rapid increase in milk concentrations. In U.S. women given a 15-mg dose of pyridoxine hydrochloride, there was a doubling of milk pyridoxal, pyridoxamine, and pyridoxal phosphate from 3 to 8 h later (26). All B-6 vitamers increased, although the proportional increase in pyridoxal was largest. A 2.5-mg dose increased milk concentration by ~50%. In a follow-up experiment, 47 mothers were supplemented throughout the first 6 mo of lactation with 2.5, 4.0, 7.5, or

10.0 mg of vitamin B-6/d, given as multi-vitamin/mineral supplements (35). Only those infants whose mother took the 10-mg/d supplement met their AI of 0.3 mg/d. Their plasma pyridoxal phosphate concentration paralleled that of maternal intake. However, the 2.5-mg/d supplement supported a milk concentration ranging from 0.89 to 1.31 nmol/L (0.22–0.32 mg/L) and normal infant growth and vitamin B-6 status. An older study in India is the only maternal supplementation trial that could be identified in a developing country (18). In this small study (n = 10), 0.4–40 mg/d (the dose was increased daily during the first 8 mo of lactation) in a multiple micronutrient supplement increased milk concentrations to 0.16 mg/L compared to 0.08 mg/L in placebo controls (18).

For vitamin B-6, we conclude that the limited evidence available suggests that breast milk concentrations and infant status definitely reflect maternal intake and status and that maternal supplementation rapidly increases the amount in milk. Associations between milk vitamin B-6 and infant size at birth or growth, and with infant behavior, may be important, but more data on this question are needed.

Vitamin B-12

Deficiency and depletion of vitamin B-12 is prevalent where the intake of animal source foods is low, because these foods contain almost all of the vitamin B-12 in the food supply, except for fortified foods. Reported prevalences of deficient plus low serum B-12 concentrations reported in Latin America are typically around 40% and reach 60 to 80% in some populations in Africa and Asia, affect all ages, and are associated with low animal source food consumption (36). Clearly, risk of deficiency is not limited to strictly vegetarian diets; intakes (37) and status (38) of lacto-ovo vegetarians are substantially lower than those of omnivores.

The neonatal period is thought to be a special period of vulnerability to vitamin B-12 deficiency (39). Maternal status prior to and during pregnancy is strongly associated with cord blood concentrations of vitamin B-12 and total homocysteine (40) as well as infant stores of the vitamin at birth (39). Severe maternal deficiency, caused by vegetarianism or undiagnosed pernicious anemia, is well recognized to result in clinical symptoms of deficiency within a few months of delivering an infant. A summary of case studies indicates that symptoms appear around 4–7 mo of age and include severe growth stunting (length, weight, and head circumference) and cerebral atrophy and a large number of muscular, behavioral, and other developmental problems, some of which are not reversed by treatment in ~40–50% of cases (41). Importantly, in all cases, the infants were exclusively breastfed, suggesting that the low vitamin B-12 concentration of breast milk was a contributory factor.

Vitamin B-12 in breast milk is tightly bound to haptocorrin, which is present in much higher amounts than in serum or plasma and has to be released from this binding to be measured accurately. Investigators have used a variety of approaches to remove the vitamin from haptocorrin, including pretreatment with papain or other enzymes. However, a method developed in 2009 using a cobinamide-sepharose column to remove apo-haptocorrin revealed that previous methods would have under- or overreported the amount of vitamin B-12 in breast milk depending on the method subsequently used for measuring the vitamin (42). The Institute of Medicine used a value of 0.43 μg/L (310 pmol/L) for the concentration in milk based on samples from 9 unsupplemented Brazilian women and an older assay method (43). However, using the new method, the median value in a group of 25 well-nourished Californian women, most of whom consumed supplements containing 5 μg/d during pregnancy, was 1.2 μg/L, whereas in 183 low socioeconomic status women in peri-urban Guatemala City, concentrations fell below the detectable limit of 50 pmol/L in 65% of the participants and <100 pmol/L in 77% (44). More data are needed to establish normal values in unsupplemented women.

There has been relatively little research on the effect of supplementing lactating women with oral doses of the vitamin; the usual therapeutic approach is to give high (500–1000 μg) i.m. doses of vitamin B-12 to the infant and mother once clinical symptoms are apparent. In a small pilot study, we supplemented Guatemalan lactating women with vitamin B-12 depletion (serum vitamin B-12, 150–221 pmol/L) with 3–1000 μg/d of the vitamin for 2 mo (K.L. Deegan, et al., unpublished data). Even at the highest doses, the concentration in breast milk increased from 67 to only 180 pmol/L, <1% of any dose was transferred to milk, and no dose reduced infant serum or urinary methylmalonic acid. Thus, maternal supplementation with vitamin B-12 in lactation may be too late to restore adequate milk concentrations and infant status. Further studies of this important question are needed.

Choline

In human milk, the majority of choline is found (using proton NMR spectroscopy) as phospho- and glycerophosphocholine, with smaller amounts of free choline, phosphatidylcholine, and sphingomyelin (45). HPLC and LC-MS analysis produce similar values. The total choline concentration doubles in the first week postpartum. Most formulas contain free choline, with a total concentration less than that of mature milk.

The AI for lactation and the infant assumes a milk choline concentration of 160 mg/L (1.5 mmol/L). Although choline is both consumed in the diet and endogenously synthesized, based on limited human data the choline concentration in milk is related to maternal intake. In 46 healthy women in the US, total dietary choline intake was significantly but weakly correlated with breast milk phosphatidylcholine but not other choline metabolites (46). In the same study, 48 women were randomly assigned to supplementation with 750 mg choline/d from mid-pregnancy to 45 d postpartum. Their milk concentrations of free choline, betaine, and phosphocholine were significantly higher. For the placebo and supplemented groups combined, correlations between total choline intake and milk concentrations of

free choline, phosphatidylcholine, betaine, and phosphocholine were all significant, and plasma choline was correlated with milk choline. A novel aspect of this study was the observation that common single nucleotide polymorphisms in genes for methylene tetrahydrofolate reductase and phosphatidylethanolamine *N*-methyltransferase, enzymes required for the endogenous synthesis of choline, altered the slope of the relationships between choline intake and the concentrations in milk and maternal plasma.

A relatively large study of Turkish women in 2005 measured choline components in the serum of lactating and nonlactating women and infants, and in breast milk, at stages from soon after delivery through >1 y postpartum, although samples were not collected longitudinally in the same participants (47). The total choline concentration in milk ranged from 1.35 to 1.60 μmol/L (144–170 mg/L) in samples from 142 women between 12 and 180 d of lactation. Maternal serum free choline, phosphocholine, and glycerophosphocholine were correlated with the free choline in milk and the free choline in infant serum was correlated with the milk concentration of free choline, phosphocholine, glycerophosphocholine, and total choline (r values 0.21–0.47). It was noted that lactating women had much higher serum free and phospholipid-bound choline than nonlactating women and that these values fell gradually during the first 6 mo postpartum.

In the only study identified from a developing country (48), the milk choline concentration was lower in Ecuador than in Boston. In 1982, 40% of a group of 55 women in Ecuador had concentrations < 1 mmol/L and 6% had values > 3 mmol/L. Comparative concentrations in a group of 11 women in the US were 0% < 1 mol/L and 55% > 3 mmol/L. To date, there are no supplementation data from developing countries and a lack of information on whether low choline in breast milk affects infant development.

Folate

5-Methyl tetrahydrofolate is the predominant form of folate in human milk. The vitamin is bound to folate binding protein and a tri-enzyme procedure increased measured concentrations by 85% compared to previous methods (49). The AI for lactation and pregnancy assumes that the average milk folate concentration is 85 μg/L (193 nmol/L), based on similar values among 4 reports between 1986 and 1991 (10). A value of 81 μg/L was reported in 2009 from 53 U.S. women who were not supplemented during lactation (50). Supplementation with 5-methyl tetrahydrofolate or folic acid did not affect milk concentrations of milk folate. Maternal folic acid supplements increased the amount of unmetabolized folic acid in milk, although this represented only 8% of total milk folate.

The global prevalence of folate deficiency and depletion is not well understood, but it appears that it may be a condition that is at least as common in industrialized countries as in poorer regions of the world (51,52). Folate is particularly lacking in refined cereals and intakes may be higher where the usual diet is higher in legumes, vegetables, and fruits (51). Unlike those B vitamins classified as Group I nutrients for lactation, folate is a Group II nutrient, because concentrations in human milk are maintained even when the mother is deficient in the vitamin and are unaffected by maternal supplementation (53). In rural Mexican Otomi, milk concentrations were ~100 nmol/L, unrelated to maternal status and unaffected by maternal supplementation with 400 μg folic acid provided from 22 to 138 d postpartum (54). As a consequence of maintaining the level of folate secretion in milk, women with low intakes will become more depleted as lactation progresses. Earlier reports that maternal iron deficiency may lower milk folate were not confirmed in a subsequent study in Mexico (54).

Serum folate was measured in a longitudinal study of 361 healthy Norwegian infants from birth through 2 y of age (55). Neither folic acid fortification of flour nor periconceptional folic acid supplementation was officially recommended at the time of the study. Serum folate concentrations averaged ~47–55 nmol/L in the first 6 mo of life, were substantially lower at age 12 mo, and fell to one-half the birth concentrations by 24 mo. There were no differences in serum folate between breast-fed and nonbreast-fed infants at any age. However, serum folate at 6 mo was positively correlated with the duration that the infant had been exclusively breastfed. Thus, exclusively breast-fed infants have good folate status when fed by higher income (56) or more poorly nourished (54,57) mothers, but the folate status of the mothers themselves may deteriorate. Maternal supplementation in lactation may therefore be important for preserving maternal stores, which are especially important for subsequent conception and pregnancy (58).

Inadequacy of intakes

From this review, studies were identified in which the milk concentration of the vitamin was insufficient to support adequate infant status and/or their mother was clearly deficient. Using the reported concentration of the nutrient in milk in the most appropriate studies and assuming intake was 780 mL/d during the first 6 mo of life (as was assumed when setting the AI for the US and Canada), the amount of the vitamin that the exclusively breast-fed infant could obtain from breast milk was calculated and compared to the AI recommendation (**Table 1**). Assuming that the AI recommendations are correct, it is clear that intakes of exclusively breast-fed infants fed by deficient mothers can be very inadequate. In our previous publication on this topic, we also found that estimated intakes compared to the AI could be 56% for vitamin A, 50% for vitamin C, 6–23% for iodine, and 52% for selenium (7).

Conclusions

Poor maternal status of Group I nutrients, including thiamin, riboflavin, vitamin B-6, vitamin B-12, and choline used as examples in this review, causes the concentrations of these nutrients to be low in breast milk and the infant to become deficient. In contrast, in maternal folate depletion, breast milk folate concentrations are maintained; if

TABLE 1 Milk concentrations assumed when setting the AI for the first 6 mo of life compared to value in milk of deficient infants and percent of AI this represents[1]

Nutrient	Milk concentration/L assumed for setting AI	Milk concentration reported for deficient infants (reference)	AI consumed by deficient infants, %
Thiamin, *mg*	0.21	0.16 (16)[2]	60
Riboflavin, *mg*	0.35	0.21 (16)[3]	53
Vitamin B-6, *mg*	0.13	0.10 (16,18,59)[4]	80
Vitamin B-12, μg	0.42	<0.05[5]	16
Choline, *mg*	160	90 (48)[6]	56

[1] Milk intake was assumed to be 780 mL/d.
[2] Study selected because of larger sample size. Note that breast milk concentration was higher than in India (18) and Thailand (19).
[3] Selected based on largest sample size and clear evidence of deficiency in mothers and infants.
[4] Similar values from these studies in India, The Gambia and Egypt.
[5] Selected because of large sample size and evidence of deficiency in mothers and infants (44).
[6] Based on data from the only available low income country (Ecuador). Forty percent of samples were lower than this value. Infant choline status was not assessed.

the mother's intake is inadequate, she will become more depleted in the vitamin as lactation progresses. The prevalence of maternal deficiency of these micronutrients is uncertain but likely to be high, especially for riboflavin and vitamin B-12 in the many poorer populations of the world that have a low intake of animal source foods. Based on the growth retardation that can accompany inadequate intakes of thiamin and vitamins B-6 and B-12 in breast milk, low milk concentrations of these nutrients could contribute to the widespread growth stunting that occurs in the first year of life. Maternal supplementation during lactation rapidly increases the concentrations of thiamin, riboflavin, and vitamin B-6 in milk, but increases in vitamin B-12 were small even when high doses were given to the mother for 2 mo.

The estimates of inadequate intake in Table 1 have definite limitations. The AI values are based on a very small number of samples in most cases and often collected from women whose intake from foods and supplements was not clear. In the case of vitamin B-12 milk, the AI values used were probably measured using invalid methods. There are also a very limited number of studies of breast milk concentrations in different locations and methods used for riboflavin and vitamin B-12 were not valid in older studies. Infant deficiency could have resulted in part from breastfeeding not being exclusive. Nevertheless, the general picture is consistent across all the Group I nutrients.

Clearly, much more needs to be known about the micronutrient composition of breast milk in different population groups with a range of nutritional status. Sample collection methods need to account for any diurnal and intra-individual variation and data are still limited concerning this question. Reference values need to be developed based on concentrations in the milk of well-nourished but unsupplemented women. Accurate analytical methods are now available for all vitamins in human milk, but for some nutrients these have been used in the analysis of very few samples. MS and other methods can simultaneously analyze concentrations of several vitamins, including most of the B vitamins, which should enable additional data to be more efficiently obtained. Much more information is also needed on the micronutrient status of exclusively breast-fed infants, including normal reference values for blood measures, and functional consequences of deficiency or depletion. Taken together, such data could enable the development of estimated adequate requirements for infants that provide a more valid basis for calculating the prevalence of inadequate intakes and requirements in this age group. The limited available data suggest that poor maternal vitamin B-12 status during pregnancy affects infant stores of the nutrient at birth. Inadequate maternal intake during lactation is most likely to lead to further maternal depletion, especially in the case of the Group II nutrients such as folate.

Taken together, these gaps in our information about the micronutrient content of breast milk, especially in populations with poor nutritional status, indicate that this should be a research priority. This does not imply that exclusive breastfeeding should not be recommended for the first 6 mo of life. Rather, the purpose of this review is to raise the issue that more attention needs to be paid in various locations concerning whether women with low stores or intakes of nutrients should be provided with supplements during lactation, the doses effective for increasing milk concentrations sufficiently to improve infant status, and the maternal and infant health benefits of this strategy.

Acknowledgments

The assistance of Dr. Setareh Shahab-Ferdows in identifying the literature used in this review is gratefully acknowledged. The sole author was responsible for all parts of the manuscript.

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