SUPPLEMENT

# Limitations of the Evidence Base Used to Set Recommended Nutrient Intakes for Infants and Lactating Women

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## ABSTRACT

Reported values for concentrations of micronutrients in human milk form the basis of the majority of micronutrient intake recommendations for infants and the additional maternal requirements for lactation. The infant recommendations may also be extrapolated to provide estimates for young children. The purpose of this review is to evaluate the adequacy of the milk micronutrient concentration data used by the Institute of Medicine to set recommendations for the United States and Canada, by FAO/WHO, the United Kingdom, and the European Food Safety Authority. The concentrations accepted by each agency are presented for each micronutrient accompanied by the source of information and comments on the number, location, status, and stage of lactation of the sample population, where known. These summaries show the small number of participants from which samples were collected in most studies, the wide range of concentrations within studies, the lack of longitudinal data, and the variability in collection methods. These factors contribute to the variability in nutrient intake recommendations among committees, although this variability is reduced by some committees that accept milk-composition values proposed by others. Values are also summarized from milk collected in studies in which mothers or infants were known to be deficient on the basis of clinical symptoms, biomarkers of inadequacy, or both, to show the extent to which milk micronutrients can be reduced by poor maternal nutritional status. We conclude that a new, multicenter study is needed to establish reference values for milk constituents across lactation. *Adv Nutr* 2018;9:295S–312S.

Keywords: lactation, milk, micronutrients, recommended nutrient intakes, reference values

## Introduction

In general, it is assumed that the amount of micronutrients secreted in breast milk (i.e., the product of the concentration of each micronutrient and an estimate of the volume of milk consumed by the infant) is adequate to meet the micronutrient requirements of exclusively breastfed infants. Thus, to derive recommended intake estimates for infants, such as the Adequate Intake (AI), valid data are required on the average concentration of each nutrient and the volume of milk consumed. The additional maternal micronutrient requirements for lactation are also typically based on the amounts that are secreted in milk, and estimates for young infants are often extrapolated on a body weight basis to derive estimates for older infants. This article shows that much of the available data on milk micronutrient concentrations are inadequate for defining infant and maternal requirements.

The purpose of this review is to summarize and compare the milk micronutrient values and assumptions used to set current recommended micronutrient intakes for infants and lactating women by the Institute of Medicine (IOM) for the United States and Canada, the WHO/FAO, the United Kingdom, and the European Food Safety Authority (EFSA). We also compare milk micronutrient concentrations defined as adequate with concentrations in samples collected from women, infants, or both who had clinical or biochemical signs of deficiency. This review shows the strengths and weaknesses of the studies accepted by the various committees

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Abbreviations used: AI, Adequate Intake; DRV, Dietary Reference Value; EAR, Estimated Average Requirement; EFSA, European Food Safety Authority; IOM, Institute of Medicine; RNI, reference nutrient intake.

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as being the best sources of information on the concentrations of nutrients in milk. It compares the milk concentration values used to set recommended intakes across agencies and shows their differences and similarities—the latter often explained by acceptance of a value proposed by another agency. Finally, we justify the need to develop reference values and describe a process for obtaining such values.

## Current Status of Knowledge

### United States and Canada

The DRIs for the United States and Canada are developed by the Food and Nutrition Board, IOM. **Table 1** shows the milk concentration accepted from published reports (in column 2) and the total amount consumed by exclusively breastfed infants, assuming a breast-milk intake of 780 mL/d for the first 6 mo of lactation (55), in column 3. The IOM states that estimates are based on "the average concentration of the nutrient from 2 to 6 mo of lactation using consensus values from several reported studies, if possible" (56). In reality, the values often differed widely among studies. This point is important because other agencies, especially FAO/WHO, have accepted many of the IOM's estimates.

For most micronutrients, the IOM's recommended intakes for infants aged 0–6 mo are based on the estimated intake of each nutrient from breast milk, except for vitamin D, and presented as AIs. From 7 to 12 mo, the recommendations are either unchanged, increased on a body weight basis or a factorial estimate of requirements, or assume a specified intake from solid foods. The increments in recommended intakes for lactating mothers are presented as Estimated Average Requirement (EAR) and AI values (56).

From Table 1 it is evident that the adequacy of the data used to derive the accepted values for concentrations in milk is poor for virtually all of the micronutrients. This is especially true for the vitamins, possibly because they are more difficult to analyze than minerals and because concentrations tend to be more affected by maternal status. Accepted values were often derived from the results of a single study with a small sample size. Samples were often collected by using an inconsistent protocol at various points in lactation and in some cases were analyzed with invalid methods. Supplementation status of the women was not always known. For iodine, accepted values were based on one study in which the actual reported mean (178 $\mu$g/L) differed from the value used by the IOM as the accepted mean (114 $\mu$g/L) (53), at a time when iodine intake was considered excessive (45). Few studies collected information on longitudinal changes in milk during lactation, and although most of the accepted values were measured in mature milk, there was a wide range of days postpartum. Only for zinc is the concentration in milk assumed to be different when setting recommendations for the first compared with the second 6 mo of lactation, whereas, in reality, concentrations of many micronutrients in milk do change during the postpartum period.

### FAO/WHO

The FAO/WHO recommendations were developed in 1998 and published in 2004 (**Table 2**) (64). For several nutrients, values from the 1985 Committee on Nutrition (1) were accepted, although the original source is not always clear. For the B-vitamins, except for niacin and folate, the IOM's concentrations and recommendations (2) were accepted (although some of those have weaknesses as discussed above).

### United Kingdom

The Dietary Reference Values (DRVs) for food energy and nutrients for the United Kingdom have not been updated since 1988 (65). Importantly, the panel agreed that breast milk is "best for babies and saw no value in setting DRVs for breast-fed infants." Thus most of the reference nutrient intake (RNI) values for infants are intended for those who are formula fed, resulting in the need to assume bioavailability from formula in some cases. These DRVs for infants who are not fully breastfed are, in most cases, at least as high as what would be obtained by an exclusively breastfed infant. To derive the DRVs, values for the concentration in breast milk are mentioned, and these are included in **Table 3**. The assumed milk volume is stated as 850 mL/d where mentioned, but the selection of this value, which is 90 mL/d higher than the IOM estimate (56), is not stated. In fact, combined data from studies in Sweden and the United Kingdom are cited as showing milk volume to be 750 mL/d from 3 to 6 mo and 650 mL/d from 6 mo onward in predominantly breastfeeding women.

Three values are provided within the DRVs. The Lower Recommended Nutrient Intake is calculated as the EAR minus 2 SDs, and is an amount that is almost certainly inadequate for most individuals. The RNI is set as 2 SDs above the EAR and will almost certainly be adequate. In Table 3 we use the RNI for infants, because this is theoretically most equivalent to the AI, and the RDA for lactating women. In addition, for purposes of comparison to the IOM data, values are provided in Table 3 for the EAR in lactation; although the United Kingdom recommendations do not state an EAR for lactating women, they do provide the RNI for nonpregnant, nonlactating women and the increment that should be added for lactation. The values for lactation represent the age range of 19–50 y and are sometimes higher for younger women.

### Europe—EFSA

The EFSA sets 4 types of DRVs, which it has published as a series of Scientific Opinions for each nutrient (75). The Population Reference Intake is equivalent to the IOM's RDA (i.e., the average requirement plus 2 SDs to meet the needs of 97.5% of individuals in the population). The Average Requirement is equivalent to the IOM's EAR, satisfying the specified criteria for adequacy in half of the population group. The AI is, like that of the IOM, the observed intake of healthy people when there is insufficient information to establish a Population Reference Intake. The reference intake range is

**TABLE 1** Recommended nutrient intakes for infants and lactating women: United States and Canada (Institute of Medicine)[1]

| | Milk | | Infant AI, units/d | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Concentration, units/L** | **Intake from milk (0–6 mo)[2]** | **0–6 mo** | **7–12 mo** | **Increment over NPNL RDA[3]** | **EAR** | **RDA** | **Data** | **Comments** |
| Thiamin, mg | 0.21 | 0.16 | 0.2 | 0.3 | +0.3 | 1.2 | 1.4 | 0.21 ± 0.4 mg/L (1). | Stated by IOM (2) but source of value not available.[4] |
| Riboflavin, mg | 0.35 | 0.27 | 0.3 | 0.4 | +0.5 | 1.3 | 1.6 | Mean of one study (mean: 0.39 mg/L; $n = 5$) (3) and old WHO report that used a microbiological assay (0.31 mg/L) (4). | No information on participants but intake data suggest unsupplemented (United States). Recognized FAD not detected before 1990 so older values likely underestimates. Source of data from WHO unknown. |
| Niacin, mg | 1.8 | 1.4 | 2.0 | 4.0 | +3 | 13 | 17 | 1.8 mg/L from one study ($n = 23$) (5). After 6 mo, contribution from food was >9 mg NE/d, which was assumed to be too high so an AI of 4 mg NE/d (extrapolated from adults) was used. For women, 1.4 mg/d in milk +1 mg/d to support energy used in milk production. | Supplementation unlikely but not stated (United Kingdom). Range: 1.2–2.8 at 16–244 d. Midfeeding, 4 times/d on 4 d between 16 and 244 d. Microbiological assay. Although tryptophan 210 mg/L, IOM assumed contribution to NEs (1 NE = 1 mg niacin or 60 mg tryptophan) of infants was negligible (2). |
| Vitamin B-6, mg | 0.13 | 0.1 | 0.1 | 0.3 | +0.7 | 1.7 | 2.0 | Value is for intake <2.5 mg/d ($n = 6$; 0.07–0.18 mg/L) vs. 0.24 for 2.5–5 mg/d ($n = 8$; 0.19–0.35 mg/L) and 0.31 for >5 mg/d ($n = 5$; 0.26–0.45 mg/L) (6). | Well-nourished, supplemented and unsupplemented with intakes near the RDA (United States). Samples for 3 wk to 30 mo; 5 samples foremilk/mother over 2 wk. Microbiological assay for total vitamin B-6. Some concern that 0.1 mg AI might be low but supported by maternal intake of 1.4 mg where infant PLP was normal and milk 0.12 mg/L (7). |
| Vitamin B-12, $\mu$g | 0.42 | 0.33 | 0.4 | 0.5 | +0.4 | 2.4 | 2.8 | 0.45 ± 0.26[5] $\mu$g/L; range: 0.01–1.49 $\mu$g/L; $n = 9$ (8). Also compared this with 0.31 $\mu$g/L in US vegan mothers with elevated MMA in infants (9) and even lower values where clinical deficiency was present in infants. | Well-nourished Brazilian unsupplemented mothers, EBF infants. Longitudinal samples from 4 d to 3 mo, 265 samples total with variable times of collection. Radioassay may be invalid. |

(*Continued*)

**TABLE 1** (*Continued*)

| Nutrient | Milk |  | Infant AI, units/d |  | Lactation, units/d |  |  | Data | Comments |
|---|---|---|---|---|---|---|---|---|---|
|  | Concentration, units/L | Intake from milk (0–6 mo)[2] | 0–6 mo | 7–12 mo | Increment over NPNL RDA[3] | EAR | RDA |  |  |
| Folate, $\mu$g DFE | 85 | 65 | 65 | 80 | +100 | 450 | 500 | 85 $\mu$g/L reported in 3 references from same laboratory since 1986 (10–12), but earlier values dubious depending on analytical method ($n = 42$). Age 7–12 mo recommendation extrapolated on body weight basis and supported by normal infant status. Assumes 50% bioavailability from diet in lactation. | Samples from both 3 and 6 mo of lactation (10) |
| Vitamin C, mg | 50 | 39 | 40 | 50 | +45 | 100 | 120 | Milk value from synthesis of 3 studies [$n = 200$ (13), $n = 118$ (14), $n$ unknown (15)] and stated to be in range of other studies on unsupplemented women (16–18). | Healthy Finnish nonsmokers, infants EBF for ≥3 mo, longitudinal study birth to 12 mo (13); United States, 1 and 6 wk postpartum (14). Very wide range of values. |
| Vitamin A, $\mu$g RAE | 485 | 400 | 400 | 500 | +400 | 900 | 1300 | 485 ± 85 $\mu$g/L (19); $n = 3$. Age 7–12 mo AI based on intake from milk (291 $\mu$g/d) + foods (244 $\mu$g/d) in United States and extrapolated from age 0–6 mo based on body weight. For women, CV = + 20% based on half-life of liver retinol. | Well-nourished US women, aged 23–36 y; 176 ± 101 d postpartum; EBF. Complete breast expression, including 2 midafternoon samples/mother. |
| Vitamin D, IU | — | — | 400 | 400 | 0 | 400 | 600 | Milk values not used because known to be very low; AI based on intake for maintaining serum 25(OH)D >50 nmol/L. | For lactation, observed no relation between maternal serum 25(OH)D and maternal BMD, milk, or infant 25(OH)D. |
| Vitamin E, mg | 4.9 | 3.8 | 4.0 | 5.0 | +4.0 | 16 | 19 | Composite of 5 studies with wide range of mean values: 2.3 mg/L (20), 3.5 mg/L (21), 3.7 ± 0.6 mg/L (22), 7.2 ± 3.9 mg/L ($n = 24$) (23), 8 ± 5 mg/L ($n = 13$) (24); age 7–12 mo AI increased based on body weight. | 12 d to 5 mo of lactation, all measured by HPLC; samples from milk bank after infant fed (23); midfeeding at 10–30 d (St. Lucia) (24). |
| Vitamin K, $\mu$g | 2.5 | 2.0 | 2.0 | 2.5 | 0 | — | 90 (AI) | Milk value based on 7 studies over wide age range and values of 0.85–9.2 $\mu$g/L (25–31). AI assumes high dose given at birth and principally breastfed. Age 7–12 mo increase is based on body weight. No increase for lactation due to low secretion in milk. | Colostrum through 26 wk but time postpartum (for mature milk) assumed to be unimportant. Maternal intake reported in one study and "no supplements" in one study. Remainder did not report. |

(*Continued*)

**TABLE 1** (*Continued*)

| | Milk | | Infant AI, units/d | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Concentration, units/L** | **Intake from milk (0–6 mo)[2]** | **0–6 mo** | **7–12 mo** | **Increment over NPNL RDA[3]** | **EAR** | **RDA** | **Data** | **Comments** |
| Iron, mg | 0.35 | 0.27 | 0.27 | 6.9 (RDA = 11) | +9 (19–50 y) | 6.5 | 9.0 | Milk value based on 9 studies; total *n* = 202 (32–40); range: 0.03–1.1 mg/L (the highest may be due to contamination). From age 7 to 12 mo, large increase due to use of factorial method. Lactation requirement for absorbed iron = 0.896 basal +0.27 mg in milk, with 18% absorption. 30% CV for lactation. | Mostly well-nourished women, 3 d to 31 mo of lactation. Unclear how mean was calculated for 0–6 mo. |
| Copper, $\mu$g | 250 | 200 | 200 | 220 | +400 | 1000 | 1300 | Table of several reports shows copper concentration in breast milk decreases with time. Not clear how 250 and 200 $\mu$g/L values were extracted but several references cited (41–43). *n* = 350 from ~15 studies. For age 7–12 mo, assumes 100 $\mu$g in complementary food. For lactation, assumes 70% absorption. | All stages of lactation. |
| Zinc, mg | 2.5 | 2.0 | 2.0 | 2.5 (EAR),<br>3.0 (RDA) | +4 | 10.4 | 12.0 | *n* = 270 from 12 studies. Marked decline through 6 mo. Milk value from early lactation is generous for age 4–6 mo. Consistent with factorial estimates. For age 7–12 mo, factorial method assumes milk provides only 0.5 mg and complementary foods provide 1.95 mg at 30% absorption. | All stages of lactation. |
| Calcium, mg | 259 ± 59 | 202 | 200 | 260 | 0 | 800 | 1000 | AI for infant based on review of milk calcium (44) but provides enough absorbed calcium to meet requirements estimated by factorial method. For age 7–12 mo, assumes 120 mg from milk + 140 mg from solid food. For lactation, evidence of no increase in calcium requirements. | Commented that studies on milk calcium are longstanding with little change. Does vary within a feeding and falls slightly in second 6 mo of lactation. |

(*Continued*)

**TABLE 1** (*Continued*)

| | Milk | | Infant AI, units/d | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Concentration, units/L** | **Intake from milk (0–6 mo)[2]** | **0–6 mo** | **7–12 mo** | **Increment over NPNL RDA[3]** | **EAR** | **RDA** | **Data** | **Comments** |
| Magnesium, mg | 34 | 30 | 30 | 75 | 0 | 255 (19–30 y), 265 (31–50 y) | 310 (19–30 y), 320 (31–50 y) | Milk value from review of studies (44). Increase at age 7–12 mo from complementary foods. No increment for lactation due to reduced urinary magnesium and increased bone resorption. | Across first year of lactation but concentration seems constant across this time. |
| Iodine, μg | 146 | 114 | 110 | 130 | +140 | 209 | 290 | 146 μg/L (45) but reported mean actually 178 μg/L (29–490 μg/L); $n = 37$. Within range of other studies (46, 47). For age 7–12 mo based on increase in weight. CV of 20% for lactation. | United States: 14 d to 3.5 y lactation; 61 samples. No correlation with age but correlated with intake. 143 μg/L if iodized salt intake low, 270 μg/L if high. |
| Selenium, μg | 18 | 14 | 15 | 15 | +15 | 59 | 70 | Milk value from 4 studies (48–51); $n \geq 500$. | Milk data provided for 14 studies, wide range of values and status. Value selected is for unsupplemented well-nourished women. |

[1] Data were obtained from the DRIs, IOM, and Food and Nutrition Board (2, 52–54) unless otherwise noted. AI, Adequate Intake; BMD, bone mineral density; DFE, dietary folate equivalent; EAR, Estimated Average Requirement; EBF, exclusively breastfed; IOM, Institute of Medicine; MMA, methylmalonic acid; NE, niacin equivalent; NPNL, nonpregnant, nonlactating; PLP, pyridoxal 5′-phosphate; RAE, retinol activity equivalent, where 1 RAE = 1 μg all-*trans* retinol; 25(OH)D, 25-hydroxyvitamin D.

[2] Calculation of infant intake assumes 0.78 L milk/d consumed from ages 0 to 6 mo.

[3] Data were calculated by the authors as the RDA for lactation minus the RDA for NPNL women.

[4] Data were cited from a secondary source, because the primary source was not available.

[5] Mean ± SD (all such values).

used for macronutrients and expresses recommendations as percentage of energy intake.

Similar to the United Kingdom decision, EFSA does not set recommended intakes for infants aged 0–6 mo (Table 4). Data on concentrations of micronutrients in breast milk are sometimes used for recommendations for infants aged 7–11 mo, but in the majority of cases requirements for this age group are extrapolated downward from adults on a body weight basis. Milk-composition values are used mostly for setting recommendations for lactating women. The estimated milk volume is 800 mL/d for the first 6 mo of lactation.

In Europe, breast-milk-composition data have also been used recently in a Scientific Opinion on nutrient requirements and dietary intakes of infants and young children (101). In addition, a review of data on breast-milk nutrient composition since 2010 has been commissioned and published, including weighted average concentrations across reports (102). Although useful to some of the panels developing DRVs, the weighted average values were not intended for this purpose.

## Comparison of milk micronutrient concentrations used among agencies

A comparison of milk micronutrient concentrations used by the 4 agencies to set recommended intakes for infants and lactating women is presented in Table 5. Values for thiamin, riboflavin, vitamins B-6 and B-12, and folate are identical or similar between the IOM and FAO/WHO, because FAO/WHO accepted the IOM recommendations (2, 63). However, because some of the IOM's values were taken from small studies (e.g., $n = 5$ for riboflavin, 23 for niacin, 19 for vitamin B-6, and 9 for vitamin B-12), both sets of values should be viewed with caution. Vitamin A values are also the same for these 2 organizations, although the IOM's data are from only 3 women and the source of the 1988 data used by FAO/WHO is uncertain (53, 63). EFSA's values for milk vitamin A are higher because they are derived from 5 European studies (101).

The values accepted by the United Kingdom tend to differ most from the other 4 sets of recommendations, especially for folate, iodine, and selenium, which are substantially lower, and vitamin K, which is much higher but based more on the need to prevent hemorrhagic disease in the infant than normal concentrations in breast milk. In part, the larger differences between the United Kingdom and the other 3 groups reflect the fact that the United Kingdom's DRVs were last published in 1991 (65).

No organization used the milk concentration of vitamin D to set recommendations because the amount of the vitamin in breast milk is too small to meet infant requirements. Iodine in milk is accepted as being much higher in the IOM's report (53) than by the United Kingdom (65) or EFSA (101).

**TABLE 2** RNIs for infants and lactating women: FAO/WHO[1]

| | Milk | | Infant AI | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | Data | Comments |
| Thiamin, mg | 0.21 | 0.16 | 0.2 | 0.3 | +0.4 | N/A | 1.5 | Used IOM value for milk (2). Increase for lactation accounts for increased energy cost. | See Table 1 |
| Riboflavin, mg | 0.35 | 0.26 | 0.3 | 0.4 | +0.5 | N/A | 1.6 | Used IOM data for milk and infant (2). Increase for lactation assumes efficiency for milk production = 70%. | See Table 1 |
| Niacin, mg and NE | 1.5 niacin +3.5 mg NE as tryptophan | 1.4 niacin, but total 4 mg NE | 2.0 mg niacin | 4 mg NE | +3 | N/A | 17 | No reference for 1.5 mg/L. Tryptophan value from (1). Increase for lactation supports additional energy expenditure. | — |
| Vitamin B-6, mg | 0.13 | 0.1 | 0.1 | 0.3 | +0.7 | N/A | 2.0 | Used IOM data (2). For age 7–12 mo, extrapolation based on body weight. For lactation, prudent to add 0.6 mg (although 0.7 mg added) to avoid poor status in infant (57). | See Table 1 |
| Vitamin B-12, μg | 0.4 | 0.3 | 0.4 | 0.7 | +0.4 | 2.4 | 2.8 | Used IOM data (2) | See Table 1 [FAO/WHO used the infant intake value (0.32 μg/d) as an EAR] |
| Folate, μg DFE | 85 | 65 | 80 | 80 | +130 (EAR) and +100 (RNI) | 450 | 500 | Used IOM data (2) | See Table 1 |
| Vitamin C, mg | 40 ± 10 | 20 | 25 | 30 | +25 | N/A | 70 | Not clear what study milk value is based on. Assumed 40 mg/L reflects maternal intake and exceeds infant needs. Value for 20 mg/d in milk inconsistent with concentration. Acknowledged that 25 mg/d for infant was arbitrary. In lactation, 20 mg/d in milk and 85% efficiency of absorption. | — |
| Vitamin A, μg RAE | 485 | 375 | 180 (safe intake ≤375) | 190 (safe intake ≤400) | +180 (basal), +350 (safe) | 450 | 850 (safe) | Found wide range of concentrations in reports but source of milk value not cited; no deficiency at 120–170 μg RAE/d so 180 is "mean" level but inadequate to build infant stores. After 6 mo of lactation mother's requirement falls since infant eats solid foods. | Only nutrient where estimate for >6 mo of lactation is reduced due to infant eating other foods |
| Vitamin D, IU | — | — | 200 | 200 | 0 | N/A | 200 | Milk D values not used. | Assumed that in lactation little purpose in recommending additional vitamin D for mother |

(*Continued*)

**TABLE 2** (*Continued*)

| Nutrient | Milk | | Infant AI | | Lactation, units/d | | | Data | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | | |
| Vitamin E, mg TE | 3.2 ± 1.8 (mean ± SD) | 2.7 | 3 | 3 | 0 | N/A | Insufficient data | Milk concentration from one study (23); $n = 24$. In lactation, no evidence that requirements differ from NPNL women, and needs met from increased energy intake. | Mature milk (12 d to 5 mo) from milk bank in Sweden, collected after infant fed |
| Vitamin K, $\mu$g | 2 | 1.6 | 5 | 10 | 0 | N/A | 55 | No specific source for milk concentration but 1 $\mu$g/kg is based on RNI. Need 100 mL colostral milk/d (0.2–0.3 $\mu$g vitamin K) during first week for normal hemostasis; 5 $\mu$g/d needed to prevent vitamin K deficiency bleeding. | 5 $\mu$g/d cannot be met if exclusively breastfed. Milk content is 100 times less than amount in formula |
| Iron, mg | 0.24 (from assumed intake of 0.3) | 0.3 | — | 6.2 | −9.6 | N/A | 10 | Source of milk value not cited. Age 7–12 mo AI assumes 15% bioavailability. In lactation, 0.3 mg in milk + 0.8 mg basal losses. | Milk concentration here calculated from assumed intake of 0.3 mg/d. Requirements in lactation lower due to amenorrhea |
| Copper | — | — | — | — | — | — | — | — | No recommendations |
| Zinc, mg | 2–3 (1 mo), 0.9 (>3 mo) | 1.4 (1–3 mo) | 1.1 | 0.8 | +2.8, +2.3, +1.3 for 0–3, 3–6, 6–12 mo respectively. | N/A | 5.8, 5.3, 4.3 for ages 0–3, 3–6, 6–12 mo, respectively. | Milk value from WHO (55) based on Krebs et al. (58); $n = 48$. Assumes 80% bioavailability when exclusively breastfed. | United States: age 1–12 mo; no justification provided for high bioavailability assumption |
| Calcium, mg | 360 | ∼280 | 300 | 400 | 0 | N/A | 1000 | Milk data from Nordin (59). For lactation, evidence of no increase in calcium requirements. | Recommended intake assumes 50% absorption provides 120 mg Ca/d needed for normal growth |
| Magnesium, mg | 29 | 20–26 | 26 | 54 | +50 | N/A | 270 | Milk concentration from 2 studies (60, 61). Assumes absorption from milk of 80–90%. | Original references not available. Justification for high bioavailability assumption is that isotope studies show 50–80% absorption from human milk and formulas. |
| Iodine, $\mu$g/kg | — | — | 15 | 15 | +1.5 | N/A | 3.5 | No data on milk included. | — |

(*Continued*)

**TABLE 2** (*Continued*)

| | Milk | | Infant AI | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | Data | Comments |
| Selenium, μg | 18.5 | 6 | 6 | 10 | +9 (0–6 mo), +16 (7–12 mo) | N/A | 35 (0–6 mo), 42 (7–12 mo) | Milk value from WHO-IAEA international survey in developed and developing countries (62); $n = >500$. Assumes 80% absorption. Wide range of milk concentrations depending on maternal intake. | Six countries, 3 mo of lactation. No justification provided for high bioavailability assumption |

[1] Data were obtained from the WHO/FAO (63). AI, Adequate Intake; DFE, dietary folate equivalent; EAR, Estimated Average Requirement; IAEA, International Atomic Energy Agency; IOM, Institute of Medicine; N/A, not available/not estimated; NE, niacin equivalent; NPNL, nonpregnant, nonlactating; RAE, retinol activity equivalent; RNI, recommended nutrient intake; TE, tocopherol equivalent.

[2] Calculation of infant intake assumes 0.75 L milk/d consumed from ages 0–6 mo.

This is in large part due to intakes in the United States being elevated by the use of iodophores in the dairy industry at the time when their cited study was conducted.

Interestingly, the estimates of breast-milk volume (expressed in mL/d) vary both at 6 mo (780 for IOM, 750 for FAO/WHO, 850 in the United Kingdom, and 800 by EFSA) and at 7 mo (600, 650, 850, and 800 for the same authorities, respectively) (56, 63, 65, 101). Arguably, there is a need for better agreement among authorities on this issue because it makes a substantial difference in recommended intakes.

Neither the United Kingdom nor EFSA has set recommended intakes for infants aged 0–6 mo, taking the general position that breast milk provides adequate amount of nutrients at this stage of life (65, 101). However, the panel that provided advice in the EFSA Scientific Opinion on nutrient requirements for infants and young children in the European Union did publish recommendations for ages 0–6 mo (75). That group accepted the milk micronutrient concentrations published by the IOM for B-vitamins and iron (2, 53, 101). The only substantial differences from other agencies were for vitamin E (3.5 mg/L), copper (250 μg/L), and iodine (50–100 μg/L), mainly because the panel focused on studies from Europe (101).

## Milk nutrient concentrations linked to maternal or infant deficiency or both

Published reports provide some values for concentrations of nutrients in milk collected from women, infants, or both who were defined as having clinical or biochemical signs of deficiency. Table 6 presents the reports and values selected and compares these with concentrations assumed to be adequate (i.e., those accepted as the basis for setting the AI for infants by the IOM) (2, 52–54). The criteria used to define the low values in the table are inconsistent across nutrients. This is essentially an update of a process that we have used previously to show how low (as a percentage of value used to set the AIs) concentrations of each nutrient can fall as a result of maternal deficiency (109). Overall, it is clear that milk micronutrient concentrations, and especially those of vitamins, can be affected substantially by poor maternal nutritional status and can produce clinical signs of deficiency in the infant.

## Descriptions of the selected values

***Thiamin.*** Older studies on infantile beriberi showed that when breast-milk thiamin is <0.12 μg/L, growth is poor, and if <0.06 μg/L, growth is completely arrested, with symptoms of deficiency and death from heart disease (110). In 1964–2001, milk concentrations in The Gambia (0.16 mg/L), India (0.22 and 0.11 mg/L), and Thailand (0.12 mg/L) were ∼50% of those used to set the AI (111). Maternal deficiency was common in these 3 locations.

***Riboflavin.*** Selected values for riboflavin were based on the milk concentration associated with high Erythrocyte Glutathione Reductase Activity Coefficients in infants in The Gambia. Five studies between 1964 and 1982 reported milk concentrations of 0.16–0.22 mg/L (111). Supplementing the infants reduced the Erythrocyte Glutathione Reductase Activity Coefficient values (104).

***Vitamin B-6.*** Infants fed formula in the 1950s developed clinical (convulsions) and metabolic signs of vitamin B-6 deficiency; the formula was manufactured by using high temperatures at which pyridoxine is unstable. The infants convulsed with a vitamin B-6 intake of 0.085 mg/d, which would be equivalent to ∼0.10 mg/L, although this may overestimate requirements because pyridoxal-lysine formed in the milk had antivitamin activity (105). In 1990, Egyptian mothers with low milk vitamin B-6 (<0.10 mg/L) showed less response to their infants vocalizations, and their infants had several indications of abnormal behaviors (111, 112).

***Vitamin B-12.*** Infants who received <0.06 μg vitamin B-12/d from breast milk developed megaloblastic anemia, which was cured by a daily supplement of 1 μg/d (113). An intake of 0.3 μg/d normalized infant urinary

**TABLE 3** RNIs for infants and lactating women: United Kingdom[1]

| | Milk | | Infant RNI[3] | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | Data | Comments |
| Thiamin, mg | 0.16 | 0.14 | 0.3 | 0.3 | 0 | 0.3 | 0.4 | Milk concentration from 5-center study in United Kingdom (66). | Infant RNI also considers report of higher concentration by 6 wk (67); $n = 5$; United Kingdom |
| Riboflavin, mg | 0.31 | 0.26 | 0.4 | 0.4 | +0.5 | 1.4 | 1.6 | Milk concentration from 5-center study in United Kingdom (66). Infant RNI is amount that restored normal EGRAC values in Gambia. In lactation, covers amount in milk + metabolic cost of its secretion. | Details of samples and populations not cited or available |
| Niacin, mg | 2.7 | 2.3 | 3 | 4 (7–9 mo), 5 (10–12 mo) | +2.3 | 12.3 | 15 | No reference for milk value. | — |
| Vitamin B-6, $\mu$g/g protein | 8 | 6.8 | 8 | 10–13 | 0 | 13 | 15 | Milk value from Department of Health and Social Security (68). | Original source of data not available. Units differ from those of other organizations |
| Vitamin B-12, $\mu$g | 0.2–1.0 | 0.3 | 0.3 | 0.4 | +0.5 | 1.75 | 2.0 | For infants, intake to normalize MMA (9); $n = 19$, of whom 13 were strict vegetarians. For lactation, 0.5 $\mu$g/d should ensure adequate supply to milk. | Source is 1988 textbook (69) |
| Folate, $\mu$g | 40 | 34 | 50 | 50 | +60 | 210 | 260 | Milk concentration based on Swedish study (70); $n = 91$. In lactation, to replace 40 $\mu$g/d in milk + incomplete absorption and utilization from diet. | Swedish women with adequate folate status. Samples from <1 mo ($n = 47$), 1 to <6 mo ($n = 13$), and 6–12 mo ($n = 31$). |
| Vitamin C, mg | 30–80 | 25–68 | 25 | 25 | +30 | 55 | 70 | Source of milk concentration not stated; 25 mg/d RNI to prevent scurvy (would be equivalent to 32 mg/L). For lactation, to maintain maternal stores and circulating concentration in upper 50% of physiologic range. | — |
| Vitamin A, $\mu$g RAE | — | — | 350 | 350 | +350 | 750 | 950 | FAO/WHO recommendation for infants in 1988 (71) | — |
| Vitamin D, IU | — | — | 340 | 280 | 10 | 0 | 10 | Milk vitamin D concentrations not used. In lactation to prevent observed decline in maternal 25(OH)D. | No RNI for NPNL adults |
| Vitamin E, mg TE | 3.2 | 2.7 | ≥0.4 mg TE/g PUFAs | — | N/A | N/A | N/A | Infant data from single study (23); $n = 24$. No DRVs for adults because requirement depends on PUFA intake. | Mature milk (12 d to 5 mo); from milk bank in Sweden collected after infant fed |

(*Continued*)

**TABLE 3** (*Continued*)

| | Milk | | Infant RNI[3] | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | Data | Comments |
| Vitamin K, μg | 1–10 | <1 | 10 | 10 | N/A | N/A | N/A | RNI based on milk value of 10 μg/L to prevent hemorrhagic disease of newborn (26); $n = 9$. Data judged inadequate to set adult DRVs. | Well-nourished German mothers, at 1, 3, 5, 8, 15, 22, 29, and 36 d of lactation. Complete breast expression. Median: 1.2 μg/L at 8–36 d. Higher in colostrum (median: 2.7 μg/L) and hind- vs. foremilk; 10 μg/L likely only possible with maternal supplementation |
| Iron, mg | 0.4 (6–8 wk), 0.29 (17–22 wk) | 0.25–0.34 | 1.7 (0–3 mo), 2.3 (4–6 mo) | 4.2 | 0 | 11.4 | 14.8 | Milk concentration from Finnish study (72). For infants, assumes 10% bioavailability from formula. Needs for lactation offset by amenorrhea. | 1–3 mo of lactation. Original data not available |
| Copper, mg | 0.22 | 0.19 | 0.3 | 0.3 | +0.3 | N/A | 1.5 | Milk concentration is average of 1–6 mo; $n = 10$ (73). For lactation, to replace copper excreted in milk, assuming 50% absorption. | Middle class, apparently well-nourished women in United States sampled monthly to 18 mo or weaning. No EAR for adults |
| Zinc, mg | 2.5 (1–4 mo), 1.1 (5–12 mo) | 2.13 (1–4 mo), 0.94 (5–12 mo) | 4.0 | 5.0 | +6.0 (0–4 mo), +2.5 (>4 mo) | 11.5 (0.4 mo), 8 (>4 mo) | 13 (0–4 mo), 9.5 (>4 mo) | Source of milk concentration not stated. Infant RNI based on factorial method. Assumes 30% absorption from adult diet. | — |
| Calcium, mg | 350 | 298 | 525 | 525 | +550 | 1075 | 1250 | No source for milk concentration. For infants, 160 mg/d retention, 40% absorption from formula. In lactation, 300 mg/d secreted in milk, and 40% absorption from diet. | — |
| Magnesium, mg | 28 | 25 | 55 (0–3 mo), 60 (4–6 mo) | 75 (7–9 mo), 80 (9–12 mo) | +50 | 250 | 320 | Milk concentration from Department of Health and Social Security guidelines (68). For lactation, assumes absorption from diet is 50%. | Original source not available |
| Iodine, μg | ~60 (estimated from DRI) | 50 | 50 (0–3 mo), 60 (4–6 mo) | 60 | 0 | N/A | 140 | 30–40 μg/L in milk produces no signs of deficiency in infant (45); $n = 37$. No increment for lactation. | United States, during period of high iodide intake. No EAR for adults |

(*Continued*)

**TABLE 3** (*Continued*)

| | Milk | | Infant RNI[3] | | Lactation, units/d | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, unit/L | Intake from milk (0–6 mo),[2] units/d | 0–6 mo | 7–12 mo | Increment over NPNL | EAR | RNI | Data | Comments |
| Selenium, μg | 12 | 5–13 | 10 (0–3), 13 (4–6 mo) | 10 | +15 | N/A | 75 | Milk concentrations of 8–30 μg/L in United Kingdom (74). In lactation, assumes 60% absorption. | Original data from report on selenium in British food. Data unavailable. No EAR for adults |

[1] Data are from DRVs for food energy and nutrients for the United Kingdom (65). DRV, Dietary Reference Value; EAR, Estimated Average Requirement; EGRAC, Erythrocyte Glutathione Reductase Activity Coefficient; MMA, methylmalonic acid; NPNL, nonpregnant, nonlactating; RNI, recommended nutrient intake; TE, alpha-tocopherol equivalent; 25(OH)D, 25-hydroxyvitamin D.

[2] Calculated by assuming 850 mL milk/d consumed from ages 0–6 mo.

[3] DRVs are intended for formula-fed infants; panel saw no value in setting them for breastfed infants.

methylmalonic acid excretion in infants who were breastfed by vegan mothers in Boston (9). Milk concentrations <200 pmol/L (0.25 μg/L) were found in the vegan mothers with serum vitamin B-12 <100 pmol/L. The milk concentration in Indian mothers with poor vitamin B-12 status was 87 pmol/L (0.12 μg/L) at 6 wk postpartum (106). In Guatemalan women with serum vitamin B-12 <147 pmol/L at 12 mo postpartum, milk vitamin B-12 was 0.29 μg/L (range: 0–984 μg/L) and the infants had very low plasma vitamin B-12 (107).

***Vitamin A.*** Breastfed infants do not show signs of deficiency with an intake only a little above 100 μg/d (114, 115). However in the reference cited, the mean concentration in the milk of the Navajo Indian women was 320 μg/L (115). The United Kingdom's Department of Health and Social Security made the decision that an infant intake of 100 μg/d will probably not be enough to build liver stores. Vitamin A is one of the few micronutrients for which a cutoff has been recommended [i.e., <1.05 μmol/L (<300 μg/L) or <8 μg/g fat] (116). In a recent survey, these values were consistent with the prevalence of low plasma retinol-binding protein (<0.78 μmol/L) in Cameroonian lactating women (117). However, substantially lower mean concentrations of milk retinol (172–200 μg/L) have been reported in other populations, including Indonesia and Guatemala, prefortification of sugar with vitamin A (118).

***Vitamin D.*** Breast milk alone does not provide adequate quantities of vitamin D; adequate exposure of the infant to UV light and the provision of some supplement or fortified food are necessary (108). Vitamin D in breast milk is quite strongly related to maternal exposure to UV light, but to our knowledge, there are no comparative data on milk vitamin D from women with high and low UV exposure. In Saudi Arabia, the milk of 8 deficient mothers (serum 25-hydroxyvitamin D <50 nmol/L) was below the 20-IU/L detection limit of the assay (108).

***Iodine.*** A review of 57 studies confirmed that breast-milk iodine parallels maternal urinary iodine. Milk iodine was 13–18 μg/L in women with goiter, 9–32 μg/L where the prevalence of goiter is high, and >90 μg/L where there is effective salt iodization (119).

***Selenium.*** The relation between maternal plasma selenium and breast-milk selenium was investigated in Malawi (120). Approximately 50% of the mothers had low plasma selenium. For those in the lowest tertile of plasma selenium, at 6 mo postpartum, breast milk contained 6 μg/L compared with 14 μg/L in the highest tertile. Clinical signs of deficiency were not investigated in either the mothers or the infants.

## Conclusions

This review of the nutrient concentrations used to set the AIs for infants and RDAs for older infants and lactating women shows the lack of studies designed to collect systematic data on milk composition. For many nutrients, and especially for vitamins, the data were obtained from <10 women; and frequently, a wide range of milk values was reported in the original data. There is a lack of information on longitudinal changes in composition, although samples were often collected early and again later in lactation and differences in composition were reported. Times and methods of breast-milk collection were not at all systematic and seldom were reported. The original source of many of the values is not stated, and it was not readily possible to trace the source of information in order to check details such as the number of women and the time postpartum when measurements were made. The fact that the 4 agencies examined in this review used some of the same milk concentrations is to a substantial extent due to the FAO/WHO's acceptance of the IOM's recommendations for B-vitamins (2, 64).

Clearly, there is a need to collect new data that can be used to develop reference values for micronutrients in human milk. In addition to serving as a standard for human-milk micronutrient quality, rigorously developed reference values will improve estimates of nutrient requirements for infants, young children, and lactating women. Despite being based on inadequate and often flawed data, existing estimates are the main source of information used to estimate the current AIs for infants. Currently, cutoffs for biomarkers of infant nutrient status are also poorly defined, especially from ages 0

**TABLE 4** Milk micronutrient concentrations used by the EFSA to set recommended intakes for older infants and lactating women[1]

| | Milk | | | Lactation | | | |
|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, units/L | Intake from milk,[2] units/d | Infant AI (7–12 mo) | Increment over NPNL | PRI | Data | Description of subjects and samples |
| Thiamin, mg | 0.18 | 0.15 | 0.1/MJ[3] | +0.2 | 0.1/MJ | Among studies, mean values of 0.14–0.22 mg/L; $n \approx$ 200 (5, 16, 67, 76–78) | Healthy mothers of term infants in Western countries, all stages of lactation, mostly unsupplemented. For mothers and older infants, increase based on energy requirements only. |
| Riboflavin, mg | — | — | — | — | — | — | Not yet published. |
| Niacin, mg | — | — | 1.6/MJ[3] | 0 | 1.6/MJ | DRVs for older infants and lactating women based on AR of 5.5 mg NE/1000 kcal | PRI; milk concentrations not used to set DRVs. |
| Vitamin B-6, mg | 0.125 rounded to 0.130 | 0.1 | 0.3 | 0.1 | 1.7 | Milk values from (7) ($n = 30$) at 2 mo postpartum and (79) ($n = 7$) | US unsupplemented women. For 7–11 mo, extrapolated up from intake from milk at 0–6 mo and down from adults on body weight basis. |
| Vitamin B-12, μg | 0.5 | 0.4 | 1.5 | +1.0 | 5.0 | "Representative" milk values based on data from 11 studies in Western countries | Older infant AI extrapolated down from adults due to uncertainties in milk values. For lactation, value is AI, 1 mg/d added to cover 1 mg in milk and 40% absorption. |
| Folate, μg DFE | 80 | 64 | 80 | +170 | 500 | Based on 6 studies by using best extraction methods for folate; range: 54–99 μg/L; average: 80 μg/L (80–85); total $n = 226$, mostly 1–6 mo of lactation | United States, Canada, and Mexico. For 7–12 mo, AI extrapolated up from intake from milk at 0–6 mo; assuming 50% absorption, need +130 mg/d lactation. |
| Vitamin C, mg | 50 | 40 | 20 | +60 | 155 | Milk values from 2 studies; $n = 142$ and $n = 200$ (13, 86); 1–12 mo postpartum | Selected 2 studies out of 7 due to large sample size and small SD (Finland, Switzerland). For lactation, to cover loss in milk and absorption efficiency of 80%. |
| Vitamin A, μg RAE | 530 | 424 | 250 | +630 | 1300 | Midpoint of range of average values: 229–831 μg/L in 5 studies (87–91); total $n \approx 363$ | Mostly European, in first 6 mo of lactation. For infants aged 7–11 mo, extrapolated down from adults on the basis of body and liver weight. In lactation, increase covers milk and assumes 80% absorption efficiency. |
| Vitamin D, μg | — | — | 10 | 0 | 15 | — | Milk vitamin D concentrations not used; AI for lactation |
| Vitamin E, mg TE | 4.6 | 3.7 | 5 | 0 | 11 | 2 studies in Europe (88, 92); mean values of 3.5 and 5.7 mg/L; total $n = 85$ | Unsupplemented women from Germany and Greece. 3 wk to 6 mo postpartum. For ages 7–11 mo, extrapolated up from intake of breastfed infants 0–6 mo. AI for lactation. |

(Continued)

**TABLE 4** (*Continued*)

| | Milk | | | Lactation | | | |
|---|---|---|---|---|---|---|---|
| Nutrient | Concentration, units/L | Intake from milk,[2] units/d | Infant AI (7–12 mo) | Increment over NPNL | PRI | Data | Description of subjects and samples |
| Vitamin K, μg | — | — | — | — | — | — | Not yet published. |
| Iron, mg | 0.3 | 0.24 | 11[3] | 0 | 16 | Milk values from (93) ($n = 27$) and (94) ($n = 86$) | Finland and Sweden, through 9 mo of lactation. For ages 7–11 mo, PRI based on requirement for absorbed iron and 10% absorption. No increment in lactation due to amenorrhea. |
| Copper, mg | 0.35 | 0.28 | 0.4 (7–11 mo) | 0.2 | 1.5 | Source of specific milk concentration not specified but likely estimated from review of 9 studies ($n \approx 860$) (95) | Review cited showed wide range of values (study means of 120–970 for mature milk). Samples collected ≤12 mo postpartum. For lactation, AI. |
| Zinc, mg | 2.52 at 1 mo, 1.37 at 1–2 mo, 0.86 at 3 to <6 mo | — | 2.9[3] | 1.6 | 7.5 | Milk data not used for infants aged 7–11 mo. For lactation, milk zinc from combined analysis of data from 33 studies (96). | For lactation, low-phytate diet. Milk data used only as one component of estimates for lactation. |
| Calcium, mg | 200–300 | — | 280 | 0 | 1000 (18–24 y), 950 (≥25 y) | 200–300 mg/L from review (97) citing data from (33) and (98) | United States, United Kingdom, and The Gambia (data not available). |
| Magnesium, mg | 31 | 25 | 80 | 0 | 300 | Milk value from a review (99) | Mean magnesium in 16 studies of term infants was 23–35, agreeing with review value (99). For lactation, AI. |
| Iodine, μg | 90 | 72 | 70 | 50 | 200 | Reviewed milk iodine data from Europe; noted median of 112 μg/L in one study with low maternal urinary iodine (100) ($n = 127$) so set value at 90 μg/L | Key study in Denmark, 31 d. For lactation, AI. |
| Selenium, μg | 15 | 12 | 15 | 15 | 85 | Milk values estimated from 15 reports of milk for term infants in Europe | Reported concentrations in milk varied widely, primarily due to iodine status of the mothers. For lactation, AI. |

[1] Data are from reference 101. AI, Adequate Intake; AR, Average Requirement; DFE, dietary folate equivalent; DRV, Dietary Reference Value; EFSA, European Food Safety Authority; NE, niacin equivalent; NPNL, nonpregnant, nonlactating; PRI, Population Reference Intake; TE, alpha-tocopherol equivalents.

[2] Calculated by assuming 800 mL milk/d consumed from ages 0–6 mo.

[3] Infant value represents the PRI.

to 6 mo, because there is sparse information on the distribution of normal values in well-nourished infants. For most nutrients, existing estimates of milk micronutrient concentrations are used to set the additional maternal nutrient requirements for lactation. They are also typically extrapolated to derive RNIs for young children, which, in turn, are the basis for estimating nutrient gaps and requirements for complementary feeding.

## Recommendations

To be scientifically rigorous, reference values should be developed and expressed as percentiles in several well-nourished, but nonsupplemented, populations during the period when breast milk contributes all or most of the nutrients to the infant. Developing human-milk micronutrient reference values would be conceptually similar to the process used in the development of fetal and newborn (121) and child (122)

**TABLE 5** Comparative values for milk micronutrient concentrations used to set recommended intakes for infants, lactating women, or both[1]

| Nutrient | United States/Canada, units/L | FAO/WHO, units/L | United Kingdom, units/L | EFSA, units/L |
|---|---|---|---|---|
| Thiamin, mg | 0.21 | 0.21 | 0.16 | 0.18 |
| Riboflavin, mg | 0.35 | 0.35 | 0.31 | Not yet published |
| Niacin, mg | 1.8 | 1.5 + 210 mg tryptophan/L = 5 mg NE | 2.7 | Milk data not used |
| Vitamin B-6, mg | 0.13 | 0.13 | 8 μg/g protein | 0.13 |
| Vitamin B-12, μg | 0.42 | 0.40 | 0.30 | 0.50 |
| Folate, μg DFE | 85 | 85 | 32 | 80 |
| Vitamin C, mg | 50 | 40 | 30–80[2] | 50 |
| Vitamin A, μg RAE | 485 | 485 | 485 | 530 |
| Vitamin D, μg | — | — | — | — |
| Vitamin E, mg | 4.9 | 3.2 | 3.2 | 4.6 |
| Vitamin K, μg | 2.5 | 2 | 10 | Not yet published |
| Iron, mg | 0.35 | 0.4 | 0.4 (6–8 wk), 0.29 (17–22 wk) | 0.3 |
| Copper, μg | 250 | — | 220 | 350 |
| Zinc, mg | 2.5 | 2–3 (1 mo), 0.9 (2–3 mo) | 2.5 (1–4 mo), 1.1 (>4 mo) | 2.52 (1 mo), 1.37 (1–2 mo), 0.86 (3 to <6 mo) |
| Calcium, mg | 259 | 360 | 350 | 200–300 |
| Magnesium, mg | 34 | 29 | 28 | 31 |
| Iodine, μg | 146 | — | 60 | 90 |
| Selenium, mg | 18 | 18.5 | 12 | 15 |

[1] DFE, dietary folate equivalent; EFSA, European Food Safety Authority; NE, niacin equivalent; RAE, retinol activity equivalent; TE, alpha-tocopherol equivalent.

[2] Range in the literature; the recommended nutrient intake was selected to prevent scurvy and equivalent to ∼21 mg/L (see Table 3).

growth standards. Exclusive breastfeeding in the study population would need to continue through ages 4–6 mo. Milk samples must be collected by using a standardized collection protocol taking into consideration time of day, time since last feeding, and method of milk extraction. Women engaging in optimal health behaviors should be included, whereas those who smoke or use other substances should be excluded. Information should be collected on maternal usual diet and recent intake, supplementation, parity, age, anthropometric measurements, and morbidity. Infant nutrient status, morbidity, development, and growth outcomes should be monitored. At some study visits, blood should be collected from both mother and infant to assess the adequacy of their micronutrient status, and to inform normative values for micronutrient status indicators during infancy. Laboratory analyses of micronutrients should be conducted as described by Hampel et al. (123) in this series. The study would also provide an opportunity to conduct metabolomic analyses of human milk, which are an efficient way to measure amino acids, choline, human-milk oligosaccharides, and other important constituents.

A tangential benefit of systematically designed research to develop reference values would be determining the influence of milk volume on milk micronutrient concentrations. Milk volume differs between women (124) and decreases with complementary feeding, but whether this is accompanied by any increase or decrease in the concentrations of micronutrients has not been studied systematically to our knowledge. This information is critical for determining the usual distribution of nutrient intakes by infants, and data will also help to validate the WHO's estimates of milk volume intake during lactation and after the introduction of complementary foods

**TABLE 6** Concentrations of micronutrients reported in milk where the lactating mother, the breastfeeding infant, or both were identified as deficient and percentages of the AI value[1]

| Nutrient | Deficient milk concentration reported (reference), units/L | Concentration used to set AI, units/L | Percentage of AI |
|---|---|---|---|
| Thiamin, mg | 0.16 (103) | 0.21 | 76 |
| Riboflavin, mg | 0.21 (104) | 0.35 | 60 |
| Vitamin B-6, mg | 0.06 (105) | 0.13 | 46 |
| Vitamin B-12, μg | 0.12 (106), 0.25 (9), 0.29 (107) | 0.42 | 29, 60, 69 |
| Vitamin A, μg | <300 (WHO cutoff) <200 | 485 | 62<br>41 |
| Vitamin D, IU | <20 (108) | — | — |
| Vitamin C, mg | 25 | 50 | 50 |
| Iodine, μg | 13–18 (mothers with goiter), 9–32 (high prevalence of goiter) | 146 | 6–22 |
| Selenium, μg | ∼6 | 18 | 33 |

[1] Values are reported concentration and percentage of the AI as recommended by Institute of Medicine. The criteria used to select the deficient milk values were inconsistent among studies but are described in the text. AI, Adequate Intake.

(55). Currently, the 4 organizations examined here assume substantially different volumes of milk intake when setting recommended intakes for infants and lactating women.

A multisite study following the above guidelines is in the initial stages of implementation, based at the USDA, Agricultural Research Service, Western Human Nutrition Research Center, and funded by the Bill & Melinda Gates Foundation. When reference values are available, it will be possible to describe milk micronutrient (and other nutrient) concentrations in various population groups relative to normative values and to identify lactating women and infants who would benefit directly from interventions on the basis of the distribution of specific milk constituents. Reference values for human-milk micronutrients can also be used as a tool to evaluate the efficacy or effectiveness of interventions by quantifying the effect of maternal supplementation or food fortification on breast-milk quality.

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