SUPPLEMENT

Vitamin B-12 in Human Milk: A Systematic Review

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ABSTRACT

Despite the critical role of vitamin B-12 in infant development, existing recommendations for infant and maternal intake during lactation are based on milk vitamin B-12 concentrations analyzed with outdated methods in a sample of 9 Brazilian women. Accurate quantification of vitamin B-12 in the milk matrix requires effective hydrolysis of the vitamin from haptocorrin, its binding protein. The objective of the present systematic review is to consider and critique evidence of associations between milk vitamin B-12 concentration and time postpartum, maternal vitamin B-12 consumption, maternal vitamin B-12 status, and sample collection methodology. A systematic search of published literature was undertaken using the US National Library of Medicine's MEDLINE/PubMed bibliographic search engine. Observational and intervention studies were included if research was original and vitamin B-12 concentration in human milk was measured using an appropriate method during the first 12 mo of lactation. Eleven studies met inclusion criteria. Vitamin B-12 concentration was highest in colostrum and decreased in a poorly delineated trajectory over the first 3–4 mo of lactation. There was some evidence of a positive association between habitual maternal vitamin B-12 intake and milk vitamin B-12 concentration in marginally nourished women. Supplementation with 50–250 μg vitamin B-12/d during pregnancy and lactation raised human milk vitamin B-12 concentrations while intervention was ongoing, whereas supplementation with 2.6–8.6 μg/d was effective in a population with poor baseline vitamin B-12 status but not in other populations. Whether milk vitamin B-12 concentration varies with maternal circulating vitamin B-12 concentrations or sampling methodology requires further research as existing data are conflicting. Additional research is needed to bridge knowledge gaps in the understanding of human milk vitamin B-12 concentrations. Reference values for vitamin B-12 in human milk and recommended intakes during infancy and lactation should be reevaluated using modern methods of analysis. *Adv Nutr* 2018;9:358S–366S.

Keywords: human milk, lactation, infant, vitamin B-12, cobalamin, deficiency, supplementation

Introduction

Vitamin B-12, an essential nutrient synthesized exclusively by prokaryotes, acts as a cofactor in 2 key enzymatic reactions essential for folate metabolism and DNA synthesis (1) and is critical for normal fetal and childhood growth and development (2). Livers of infants born to vitamin B-12 replete mothers contain ∼25–30 μg of vitamin B-12, whereas newborn endogenous stores may be considerably lower (2–5 μg) if the mother's intake or absorption of the vitamin during pregnancy is inadequate (3, 4). It is estimated that a growing infant requires ≥0.1 μg vitamin B-12/d to support tissue synthesis and neurologic development (3, 4), leaving the exclusively breastfed infant reliant upon human milk to avert depletion.

Infants born with limited hepatic vitamin B-12 reserves who are predominantly breastfed can develop symptoms of deficiency within several months of birth. The cluster of symptoms, well-documented in case studies and most commonly diagnosed between 4 and 10 mo of age, includes anemia, irritability, hypotonia, microcephaly, failure to thrive, apathy, anorexia, movement disorders, and gross developmental delay or regression (5, 6). Treatment with high-dose intramuscular and oral vitamin B-12 results in clinical improvement, but cognitive and developmental delay persists in 40–50% of cases (5).

Despite the critical role of vitamin B-12 in infant development, the mechanisms of vitamin B-12 uptake and secretion by the mammary glands remain a topic of

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.

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investigation. Newly ingested vitamin B-12 binds to intrinsic factor in the proximal ileum, and this complex enters mucosal cells in the distal ileum by cubam complex receptor-mediated endocytosis (7). A number of receptors and transporters are involved in the extracellular and transmembrane transport of vitamin B-12 in the gastrointestinal system and its subsequent distribution (7). The mammary gland likely acquires vitamin B-12 from maternal circulation via receptor-mediated endocytosis. Preliminary results from ex vivo studies suggest that the surfaces of mammary epithelial cells have a high affinity for the transcobalamin II–B-12 complex (8). Transport of vitamin B-12 from the lactating mammary gland into milk has not yet been delineated (7).

Lactating mothers acquire vitamin B-12 primarily via intake of animal source, fermented, and vitamin B-12–fortified foods. Following de novo biosynthesis of vitamin B-12 by bacteria inhabiting the gastrointestinal tract of ruminants and other animals, the vitamin is stored in muscle and liver and released in milk and eggs (1). The US Recommended Dietary Allowance (RDA) of 2.8 μg/d in lactation assumes that 50% of dietary vitamin B-12 is absorbed by healthy adults, but bioavailability measured by radioactive label varies from 9–36% from egg products, 30–42% from fish, and 52–89% from meat and is inversely related to the dose consumed (9–11). Foods fermented with vitamin B-12–producing bacteria can also contribute to dietary intake (9). Although bacteria in the human colon produce vitamin B-12, the site of synthesis is distant from the small intestine, where the vitamin is absorbed. Furthermore, it is postulated that the majority of microbial species in the human intestine require exogenous corrinoids; individuals with high bacterial loads in their small intestine have lower cobalamin status (12).

Human milk contains ∼100-fold more of the vitamin B-12 binding protein, haptocorrin, than serum, mostly in the unsaturated form of apo-haptocorrin (13). Accurate measurement of vitamin B-12 in milk requires treatment of the sample in order to release cobalamin from haptocorrin (13). Various methods have been employed to accomplish this, including digestion with papain; acid treatment; boiling, steaming, or autoclaving; and use of a cobinamide-sepharose column that physically removes the binding proteins (13, 14). The efficiency of papain digestion, acidity, and heat alone in denaturing protein is unknown but is likely to depend on specific experimental conditions. The cobinamide-sepharose column method has been validated in human milk and is considered effective at protein removal (13). Recently, protein deactivation by heat in the presence of dithiothreitol and potassium cyanide was found to be as effective as, and less labor-intensive than, the cobinamide-sepharose column (15).

Once isolated, vitamin B-12 has been quantified by microbiological assay or competitive protein binding assay with radioisotope or chemiluminescence detection. Both methods are susceptible to residual binding protein in the sample of human milk (13, 16). In foods as well as in serum, competitive protein binding with chemiluminescence detection yielded similar results to microbiological assay but had a lower coefficient of variation (17). The 2 methods have not been compared directly in the human milk matrix. Competitive protein binding with chemiluminescence detection has been validated for linearity, accuracy, precision, and stability and is now considered the gold-standard for vitamin B-12 measurement in human milk (15).

The US adequate intake for vitamin B-12 in infancy (0.4 μg/d) and the incremental RDA for vitamin B-12 in lactation are derived from a mean milk vitamin B-12 concentration of 310 pmol/L in a single longitudinal study of 9 Brazilian women (3, 18). In this study, samples did not undergo treatment and were analyzed using a radioisotope dilution assay kit validated for serum (18). Despite significant improvements in analytical methods and more accurate data from recent studies utilizing these methods, associations with milk vitamin B-12 concentration have not been summarized in the literature. The objective of the present systematic review is to consider and critique evidence of associations between human milk vitamin B-12 concentration and time postpartum, maternal vitamin B-12 consumption, maternal vitamin B-12 status, and sample collection methodology.

Methods

Search Strategy

A systematic search of published literature from 1 January 1966 through 15 July 2017 was undertaken using the US National Library of Medicine's MEDLINE/PubMed bibliographic search engine. Multiple PubMed searches were conducted using various combinations of Medical Subject Heading (MeSH) and Title/Abstract keywords. MeSH keywords included "Milk, Human," "Lactation," "Breast Feeding," and "Colostrum" as well as "vitamin B-12." Title/Abstract keywords included "lactation," "breastfeeding," "breast feeding," "breast-feeding," "breastmilk," "milk," "breast-milk," "human milk," "colostrum," "vitamin B-12," "vitamin B-12," "cobalamin," and "cyanocobalamin." Filters limited search results to human studies published in English, French, German, Portuguese, Spanish, Hebrew, Danish, Norwegian, or Swedish. Two individuals independently screened unique article titles and abstracts to identify a list of relevant studies for full-text review. When opinions differed, a third individual reviewed the abstracts to resolve the discrepancy by two-thirds majority. Review and original research articles were examined for references to other relevant studies not identified by the initial search. Studies were included if their research was original and vitamin B-12 concentration in human milk was measured at ≥1 time points during the first 12 mo of lactation. Studies were excluded if they were conducted in animals, measured vitamin B-12 in serum but not in milk, were case studies, or utilized an analytical protocol that did not include validated sample treatment to hydrolyze vitamin B-12 from its binding protein (**Supplemental Table 1**).

Using the search strategy, 255 unique titles were returned. Three additional studies were identified through cross-referencing (**Figure 1**). After abstract screening and

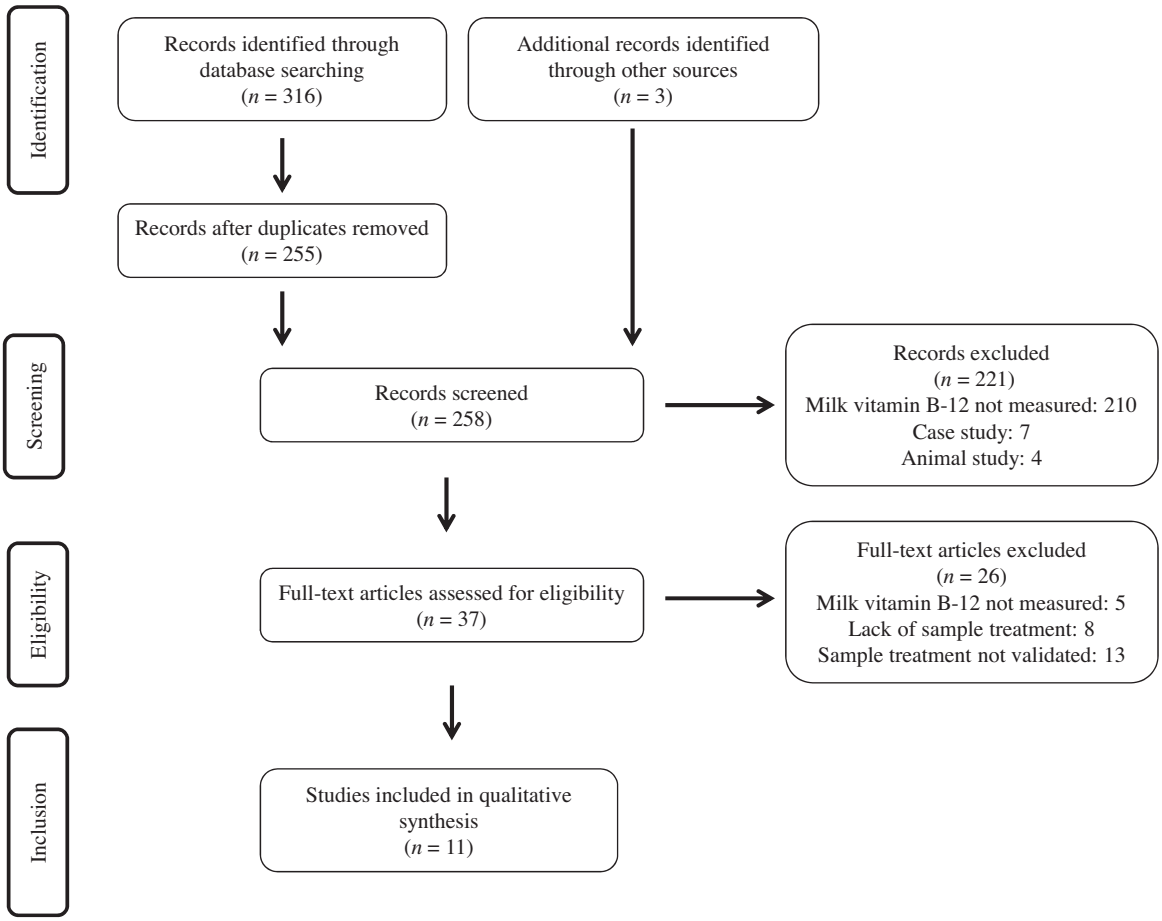

FIGURE 1 Flow diagram.

full-text review, 11 independent studies were included in the review. Risk of bias was assessed at the individual study level through review of methods, data analysis, and reporting. The principal summary measure was mean or median human milk vitamin B-12 concentration.

Results

Description of Included Studies

Of the 11 unique studies included in the systematic review (**Table 1**), 9 were conducted in low- or middle-income countries, typically in populations of low socioeconomic status (19, 21–24, 26–29). The remaining studies were conducted in lactating women without dietary restrictions living in developed countries (20, 25), including a subset of participants in the study by Chebaya et al. (21).

Human milk vitamin B-12 concentration was measured cross-sectionally in 5 studies (21, 22, 24, 27, 29), 3 at a defined time postpartum and 2 with a wide range of lactational stages (24, 29). One study was a short-term supplementation trial in which milk was collected at each feed over a 3-d period during which mothers received no intervention, 2.8 μg vitamin B-12, or 5.6 μg vitamin B-12 on consecutive days (26). In the 5 longitudinal studies, 3 involving interventions, human milk vitamin B-12 concentrations were measured between 2 and 6 times from colostrum to 3–9 mo lactation (19, 20, 23, 25, 28). All but 1 longitudinal study (20) included a control group. In 1 of the intervention studies both treatment and control mothers were HIV-infected (19).

Human milk samples were treated using either a cobinamide-sepharose column (14) or by boiling in dithiothreitol and potassium cyanide for 20 min (15). Solid-phase competitive protein binding immunoassay with chemiluminescence detection was the analytical method used for all included studies. Potential sources of bias in individual studies are described in Table 1.

Time Postpartum

A significant decrease in human milk vitamin B-12 concentration between earlier and later stages of lactation was shown amongst the control group in 3 of the 4 longitudinal studies that included controls. Siddiqua et al. (28) found a decrease in milk vitamin B-12 concentration from <72 h to 3 mo ($n = 33$, median 320 compared with 170 pmol/L, $P = 0.011$), Greibe et al. (25) from 2 wk to 4 mo ($n = 25$, median [range]: 760 [210–1880] compared with 290 [140–690] pmol/L, $P < 0.0001$), and Allen et al. (19) from 2 or

TABLE 1 Description of included studies[1]

Authors, year, and country (ref)	Study design, *n*, whether well-nourished, % of mothers taking B-12 supplements if reported	Sample collection and storage, time postpartum, and analytical methods	Major and additional results[2,3]	Bias/limitations	Important aspects/critical comments
Validated vitamin B-12 assay in human milk with adequate protein removal					
Allen et al., 2015, Malawi (19)	RCT. From delivery, daily supplementation with *1*) LNS (2.6 μg vitamin B-12/d [$n = 185$]), *2*) control [$n = 177$]. PN.	ME of casual sample at study visits, immediately frozen, shipped on dry ice, and stored at −80°C. 2 or 6 wk, 24 wk. Samples boiled in dithiothreitol and KCN, analyzed by solid-phase competitive chemiluminescent protein binding immunoassay (Siemens Immulite 1000) [method of Hampel et al. (15)].	2 or 6 wk: 302.6 [192, 598] (LNS) vs. 243.5 [162, 438] (control); 24 wk: 236.2 [170, 362] vs. 177.1 [140, 258] ($P = 0.0018$ combined time points). At 24 wk, 34% of LNS samples and 16% of control samples met infant vitamin B-12 AI for 0–6 mo.	Milk collected only after supplementation. All women HIV+.	Unblinded. Two other arms (ARV and ARV/LNS) not included.
Bae et al., 2015, United States (20)	Prospective cohort controlled feeding study, from 5–15 wk lactation daily 8.6 μg vitamin B-12 (6 μg food + 2.6 μg MVI supplement). $n = 28$. WN. None taking B-12 supplements.	Mothers fasted (10 h), full expression of 1 breast 2 h after first feed of the day, stored at −80°C. 15 wk. Analyzed using method of Hampel et al. (15).	Geometric means, baseline: 318, 95% CI [227, 447], study-end: 298 [213, 419] ($P = 0.46$). Positive correlation between HM and serum B-12 at baseline ($r = 0.48$, $P = 0.01$) but not at study-end ($r = 0.18$, $P = 0.36$).	No control group, convenience sample from separate study.	—
Chebaya et al., 2017, Canada and Cambodia (21)	Cross-sectional. Total $n = 168$; Canada [$n = 109$], Cambodia [$n = 59$]. Nutritional status not described. Canadian mothers consuming MVI containing 12 μg vitamin B-12/d from 13–22 wk gestation through 8 wk PP. Cambodian mothers unsupplemented.	Full expression of 1 breast using electric pump >2 h since previous feed, mainly in morning. Stored at −80°C. Canada: 8 wk, Cambodia: 3–27 wk. Analyzed using method of Hampel et al. (15).	Geometric means [95% CI]. Canada: 452 [400, 504], Cambodia 317 [256, 378] ($P < 0.001$). HM correlated with maternal and infant serum vitamin B-12 in Canada ($P < 0.05$) but not in Cambodia. Infant age borderline inversely associated with HM vitamin B-12 in Cambodian samples ($P = 0.05$).	Samples in 2 populations collected at different times PP.	In Canadian mothers, MVI supplementation continued until point of sampling.
Deegan et al., 2012, Guatemala (22)	Cross-sectional. Total $n = 183$; deficient (serum B-12 <150 pmol/L) [$n = 64$], marginal $n = 64$, adequate (serum B-12 >220 pmol/L) [$n = 55$]. PN.	ME of ∼50 mL casual sample into freezer-safe bag, storage conditions not reported. 11.5–12.5 mo. Milk pretreated with cobinamide-coated, epoxy-activated, hydrophilic sepharose and analyzed by solid-phase competitive chemiluminescent protein binding immunoassay (Advia Centaur Analyzer).	Deficient: 22 [0, 738], Marginal: 25 [0, 644], Adequate: 63 [50, 1300] ($P < 0.05$ in adequate vs. other groups). HM B-12 concentration associated with maternal intake ($r = 0.26$) and serum B-12 ($r = 0.30$), $P < 0.05$ for both.	—	High intra-individual variability in HM B-12. 65% of samples fell below analyzer detection limit of 50 pmol/L.
Duggan et al., 2014, India (23)	RCT. Daily oral supplementation from <14 wk gestation through 6 wk postpartum with Fe + folic acid and *1*) vitamin B-12 (50 μg) [$n = 68$], *2*) placebo [$n = 73$]. PN.	ME of 10 mL from 1 breast without regard to time since last feeding, stored at −80°C. 6 wk, 3 mo, 6 mo. Analyzed using method of Hampel et al. (15).	6 wk: 136 [93, 203] (B-12) vs. 87 [44, 127] (placebo), 3 mo: 97 [63, 146] vs. 68 [37, 102], 6 mo: 106 [65, 160] vs. 80 [51, 113] ($P < 0.0005$ at 6 wk). Difference between groups NS at 3 mo or 6 mo.	—	High loss to follow-up, $n = 37$ (B-12) and $n = 44$ (control) at 6 mo.

(*Continued*)

TABLE 1 (*Continued*)

Authors, year, and country (ref)	Study design, *n*, whether well-nourished, % of mothers taking B-12 supplements if reported	Sample collection and storage, time postpartum, and analytical methods	Major and additional results[2,3]	Bias/limitations	Important aspects/critical comments
Engle-Stone et al., 2017, Cameroon (24)	Cross-sectional at 2 time points, before and after introduction of wheat flour fortification. 2009 [$n = 23$] and 2012 [$n = 133$]. Urban population, nutritional status not described.	ME of 5–10 mL from fuller breast 30 s after infant started feeding. Stored at −20°C. ≥1 mo. Analyzed using method of Hampel et al. (15).	2009: 333 ± 46, 2012: 685 ± 31 ($P = 0.004$ after controlling for covariates).	Foremilk.	—
Greibe et al., 2013, Denmark (25)	Prospective cohort. $n = 25$. WN. At 2 wk, 4 mo, and 9 mo: 79%, 67%, and 50%, respectively, taking MVI containing 1.0–4.5 μg cobalamin/d.	ME of fore- and hindmilk before and after feed. Stored at 4°C up to 18 h, then −80°C. 2 wk, 4 mo, 9 mo. Analyzed using method of Hampel et al. (15).	2 wk: 760 [210, 1880], 4 mo: 290 [140, 690], 9 mo: 440 [160, 1940] (NS). Hindmilk contained more B-12 than foremilk ($P = 0.003$–0.005), but absolute difference small. Correlation between HM and plasma B-12 at 4 mo only ($r = 0.58$, $P = 0.002$).	—	—
Hampel et al., 2017, Bangladesh (26)	3-d supplementation study; Day 1: no supplement, Day 2: MVI containing 2.8 μg vitamin B-12, Day 3: MVI containing 5.6 μg vitamin B-12. $n = 18$. Nutritional status not described.	At every feed, 1 breast emptied by pump. Aliquots collected separately during first 2 min and remainder of feed, and third aliquot by mixing other 2. Stored at −20°C. 2–4 mo. Analyzed using method of Hampel et al. (15).	Medians. Day 1: 107, Day 2: 108, Day 3: 107. No difference between aliquots I, II, and III. No effect of supplementation.	—	—
Shahab-Ferdows et al., 2015, Cameroon (27)	Cross-sectional. Total $n = 116$; South [$n = 39$], North [$n = 55$], Yaoundé/Douala [$n = 22$]. PN/WN.	ME of 5–10 mL from fuller breast 30 s after infant started feeding. Storage conditions not reported. Nonspecific time PP. Analyzed using method of Hampel et al. (15).	National: 180 [55, 291]; South: 236 [159, 386], North: 47 [20, 201], Yaoundé/Douala: 287 [208, 422] (lower in North, $P = 0.02$).	Foremilk.	Unknown whether stage of lactation differed between regions.
Siddiqua et al., 2016, Bangladesh (28)	RCT. Daily during pregnancy through 3 mo PP: *1*) 250 μg B-12 + 60 mg Fe + 400 μg folate [$n = 35$], *2*) placebo [$n = 33$]. PN.	ME of unreported volume at the end of a feed. Stored at −80°C. <72 h and 3 mo. Analyzed using method of Hampel et al. (15).	Medians. <72 h: 778 (B-12) vs. 320 (placebo) ($P < 0.001$), 3 mo: 235 (B-12) vs. 170 (placebo) ($P < 0.03$).	Hindmilk.	Daily supplementation continued until point of sampling.
Williams et al., 2016, Kenya (29)	Cross-sectional. $n = 286$. PN/WN.	ME of 5 mL HM from right breast 1 min after initiation of feed and ≥90 min since previous feed, between 0900 and 1200. Stored at −4°C for ≤3 wk, then at −80°C. 1–6 mo. Heat treatment of whey fraction in presence of dithiothreitol and KCN followed by solid-phase competitive chemiluminescent protein binding immunoassay (Immulite 1000 Siemens).	*113 [61, 199]. Maternal age inversely associated with HM vitamin B-12 (*P* value not reported). No association between HM vitamin B-12 and maternal vitamin B-12 intake in last 24 h or BMI.	Potential recall and reporting bias in dietary data. Convenience sample from larger study.	

[1] AI, adequate intake; ARV, antiretroviral; HM, human milk; KCN, potassium cyanide; LNS, lipid-based nutrient supplement; ME, manual expression; MVI, multivitamin; PN, poorly nourished; PP, postpartum; RCT, randomized controlled trial; ref, reference; WN, well nourished.
[2] Values are means ± SD [range] or median [IQR].
[3] Significance defined as $P < 0.05$.

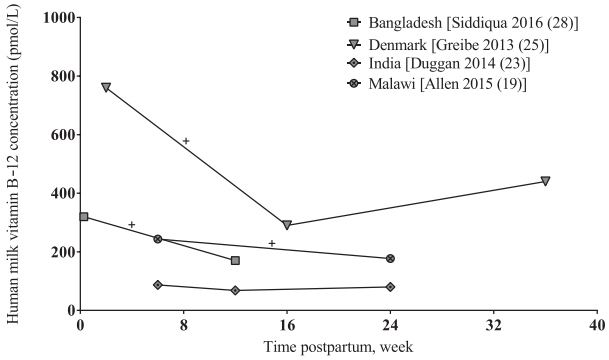

FIGURE 2 Human milk vitamin B-12 concentration in longitudinal studies of unsupplemented women. Significant changes are indicated by a plus sign (+).

6 wk to 24 wk ($n = 177$, median [IQR]: 244 [162, 438] compared with 177 [140, 258] pmol/L, $P < 0.0001$) (Figure 2). In a cross-sectional study of lactating Cambodian women between 3 and 27 wk postpartum, the association between milk vitamin B-12 concentration and time postpartum was of borderline significance ($P = 0.05$) (21). Two longitudinal studies suggested a nadir of human milk vitamin B-12 concentration at 3–4 mo compared with earlier and later time points (23, 25).

Maternal Vitamin B-12 Consumption

Three studies have evaluated the association between habitual maternal intake and milk vitamin B-12 concentration, all in marginally nourished women (22, 24, 29). In lactating women 11.5–12.5 mo postpartum in a peri-urban area near Guatemala City, maternal vitamin B-12 intake based on a validated semi-quantitative FFQ was positively associated with milk vitamin B-12 concentration ($n = 183$, $r = 0.26$, $P < 0.05$) (22). In Cameroon, mean milk vitamin B-12 concentration more than doubled a year after introduction of wheat flour fortification with vitamin B-12 (333 ± 46 compared with 685 ± 31 pmol/L, $n = 23$ and $n = 133$, respectively, $P = 0.004$) (24). Neither animal source food nor vitamin B-12 intake from a single 24-h food recall was related to human milk vitamin B-12 concentration in a Kenyan population with low overall milk vitamin B-12 (median 113 pmol/L); however, the authors suggest that recall bias and misclassification of serving sizes may have masked an association (29).

Five studies have investigated the effect of maternal supplementation on milk vitamin B-12 concentration (19, 20, 23, 26, 28). In Bangladesh, daily supplementation with 250 µg oral vitamin B-12/d during pregnancy and through 3 mo postpartum resulted in higher median milk vitamin B-12 concentrations in the intervention group compared with the control group both in colostrum (<72 h) and at 3 mo ($n = 68$, median 778 compared with 320 pmol/L at <72 h, $P < 0.001$ and 235 compared with 170 pmol/L at 3 mo, $P < 0.03$) (28). Daily supplementation of Indian women with 50 µg oral vitamin B-12/d from <14 wk gestation through 6 wk postpartum led to higher median milk vitamin B-12 concentration in the intervention compared with the control group at 6 wk ($n = 141$, median [IQR]: 136 [93, 203] compared with 87 [44, 127] pmol/L, $P < 0.0005$), but not at 3 mo or 6 mo postpartum (23).

In the remaining intervention studies, women in the treatment group received daily oral doses of 2.6–8.6 µg vitamin B-12/d (19, 20, 26), similar in magnitude to the US RDA for lactating women (3). A positive effect of supplementation on milk vitamin B-12 concentration was found in only 1 of the 3 studies. Supplementation of HIV-positive women in Malawi with 2.6 µg vitamin B-12/d as part of a lipid-based nutrient supplement from delivery through 6 mo raised milk vitamin B-12 concentration compared with controls at 2, 6, and 24 wk postpartum in a combined analysis ($n = 362$, median [IQR]: 303 [192, 598] compared with 244 [162, 438] pmol/L at 2 or 6 wk, 236 [170, 362] compared with 177 [140, 258] pmol/L at 24 wk, $P = 0.0018$ for combined analysis of both time points) (19). In contrast, supplementation of 18 Bangladeshi women with a single dose of 2.8 or 5.6 µg vitamin B-12 in the morning had no effect on measured milk vitamin B-12 concentrations over the course of the next 24 h (26), nor did daily supplementation with 8.6 µg oral vitamin B-12/d from 5–15 wk postpartum change milk vitamin B-12 concentration in a feeding study of 28 US women with no control group (20).

Maternal Vitamin B-12 Status

A positive association between milk and circulating vitamin B-12 concentration was found in 1 study and at some but not all time points in 2 additional studies. Milk vitamin B-12 concentration was positively correlated with serum vitamin B-12 in a cross-sectional study in a low-income population of Guatemalan women 11.5–12.5 mo postpartum ($n = 183$, $r = 0.30$, $P < 0.05$) (22). Positive correlations between milk and circulating vitamin B-12 concentrations were found at 4 mo ($n = 25$, $r = 0.58$, $P = 0.002$) but not at 2 mo or 9 mo postpartum in a longitudinal study in Denmark (25) and at baseline (5 wk lactation, $n = 28$, $r = 0.48$, $P < 0.01$) but not following a 10-wk controlled feeding intervention with 8.6 µg vitamin B-12/d in the United States (20).

Sample Collection

Separately collecting milk from the first 2 min and the remainder of the feed in full expressions of a single breast over a 24-h period, Hampel et al. (26) found no relation between time of day, time since last maternal food intake, or time during a feed and human milk vitamin B-12 concentration. However, Greibe et al. (25) consistently found higher vitamin B-12 and haptocorrin concentrations in foremilk collected before a feed than in hindmilk collected after a feed ($P = 0.005$).

Discussion

Despite a data pool limited by shortcomings of methods used in earlier analyses, the systematic review found

evidence of associations between human milk vitamin B-12 concentration and both time postpartum and maternal vitamin B-12 consumption. The relation of human milk vitamin B-12 concentration with maternal vitamin B-12 status and sample collection methodology was inconsistent. There is an urgent need for additional research to bridge knowledge gaps and improve the understanding of factors influencing human milk vitamin B-12 concentration.

Based on data collected using scientifically rigorous methods, human milk vitamin B-12 concentration is maximal in colostrum and decreases over the first several months of lactation to a potential nadir at 3–4 mo postpartum. The trajectory of the decrease in concentration is not clearly delineated given wide intervals between milk sample collections in the included studies. To enable a more thorough understanding of the relation between stage of lactation and human milk vitamin B-12 concentration, samples should be collected frequently, especially during early lactation when the concentration changes more rapidly.

Available evidence suggests a positive association between maternal vitamin B-12 intake and milk concentration in marginally nourished women. Relevant data were collected only in marginally nourished populations, precluding generalization to well-nourished populations. In order to understand the interaction between habitual vitamin B-12 intake and milk concentration, future studies must collect samples longitudinally from women with a variety of vitamin B-12 intake levels.

Supplementation with daily doses of vitamin B-12 in the range of 50–250 μg was effective at raising human milk vitamin B-12 concentration while intervention was ongoing (23, 28), but the effect was not sustained in an Indian study in which samples were collected following cessation of the intervention (23). This result raises questions about vitamin B-12's biological compartmentalization, storage, and transfer into human milk. Supplementation with daily doses closer to the current US RDA for lactating women (2.6–8.6 μg) effectively raised milk vitamin B-12 concentration in a population of HIV-positive Malawian mothers with poor baseline vitamin B-12 status (19), but not in well-nourished US women (20) or in Bangladeshi women given a single morning dose of vitamin B-12 (26). There is a need for randomized controlled intervention trials providing titrated vitamin B-12 supplementation protocols to evaluate changes in milk vitamin B-12 concentration both during and following intervention.

The relation between human milk and circulating vitamin B-12 concentration was inconsistent in the literature; however, serum vitamin B-12 concentration may not be the ideal indicator of maternal vitamin B-12 status. Vitamin B-12 deficiency is characterized initially by an increase in serum holotranscobalamin, followed by an increase in methymalonic acid and total homocysteine (30). A reduction in serum vitamin B-12 concentration is indicative of prolonged deficiency, and may not manifest for several years.

Whether human milk vitamin B-12 concentration is affected by sampling methodology requires more research, as existing data are inconsistent. Hampel et al. (26) found no association between time of day, time since last maternal intake, or time during a feed and human milk vitamin B-12 concentration amongst Bangladeshi women. Greibe et al. (25) found higher vitamin B-12 concentration in hindmilk collected after a feed compared with foremilk collected before a feed, though absolute differences were small. Higher haptocorrin in hindmilk than in foremilk corroborated the differing vitamin B-12 concentrations. The authors suggested that production and/or secretion of milk haptocorrin may be stimulated during the feed (25). Differences between sample storage methods were not evaluated, but in the majority of included studies samples were frozen (–20 to –80°C) and protected from light to prevent degradation of vitamin B-12. Available data suggest that human milk vitamin B-12 concentration is stable with freezing (31).

An emerging issue deserving further investigation is whether HIV infection or other chronic conditions influence human milk vitamin B-12 concentration. Preliminary data from the study of Allen et al. (19) in Malawi in which all women were HIV-positive suggest that HIV does not impact milk vitamin B-12 concentration. Although internal comparison with HIV-negative mothers was not possible, measured concentrations of human milk vitamin B-12 were within the range of other included studies conducted in HIV-negative women (23, 27, 28). Other chronic maternal conditions have not been evaluated for associations with human milk vitamin B-12 concentration.

Several additional knowledge gaps have been identified regarding the understanding of human milk vitamin B-12 concentration. To date no studies have monitored the relation between 24-h milk volume and vitamin B-12 concentration. One longitudinal study found a significant increase in vitamin B-12 concentration between 4 and 9 mo postpartum, corresponding to a probable decline in milk volume (25). Milk production decreases after 6 mo of lactation even if weaning is not deliberate (32), with decreasing concentrations of glucose, citrate, phosphate, and calcium and increasing concentrations of fat, lactose, protein, and sodium (33). How milk volume and vitamin B-12 concentration are related during late lactation requires additional research.

Another knowledge gap is the source and transport of vitamin B-12 in human milk. Milk hosts a diverse microbiome (34), including some bacteria that are known producers of vitamin B-12. Furthermore, mature human milk contains cobalt, which is essential for bacterial vitamin B-12 synthesis (35). Whether bacterial production contributes to the vitamin B-12 content of human milk is unknown, but deserving of investigation. A more thorough understanding of mammary gland uptake and secretion of vitamin B-12 would enable better nutritional management of both mother and infant.

With validated and highly automated analytical methods available for measuring vitamin B-12 in human milk, there is an urgent need for reevaluation of reference values for milk vitamin B-12 concentration in representative samples throughout lactation. To better evaluate how human

milk vitamin B-12 concentration changes over time, frequent sample collection is required in future studies, especially in early lactation. Prudent investigation should include assessment of more sensitive hematologic markers of maternal and infant vitamin B-12 status, including holotranscobalamin, methylmalonic acid, and homocysteine, as well as evaluation of infant developmental and neurologic outcomes in populations considered vitamin B-12 replete. In order to titrate supplementation, it would be informative to investigate how birth complications and maternal health conditions influence human milk vitamin B-12 concentration.

Conclusions

Human milk is a critical source of vitamin B-12 to the exclusively breastfed infant. Infants born to vitamin B-12 deficient mothers have compromised liver stores at birth and consume milk with a lower vitamin B-12 concentration, placing them at increased risk for vitamin B-12 deficiency and its neurologic and developmental consequences. Because there is some evidence that human milk vitamin B-12 concentrations can be modified via maternal intake and supplementation during lactation, intervention may be indicated in populations in which low maternal vitamin B-12 intake or poor status is common. Additional research is needed to define reference values for vitamin B-12 in human milk over the course of lactation and to establish revised recommendations for vitamin B-12 intake during infancy and lactation.

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