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Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: a 9-mo longitudinal study[1–3]

Eva Greibe, Dorte L Lildballe, Súsanna Streym, Peter Vestergaard, Lars Rejnmark, Leif Mosekilde, and Ebba Nexo

ABSTRACT

Background: Measurement of milk cobalamin is hampered by the high content of the cobalamin-binding protein haptocorrin, and limited data are available relating trustworthy measures of milk cobalamin to cobalamin status in healthy mothers and their children.

Objectives: The objectives were to explore the concentration of cobalamin and haptocorrin in foremilk and hindmilk during the first 9 mo of lactation and to relate these results to biomarkers of an impaired cobalamin status of mother and child.

Design: Milk samples from 25 mothers were collected at 2 wk, 4 mo, and 9 mo postpartum for the measurement of cobalamin and haptocorrin. Plasma samples from a larger cohort of lactating mothers (n = 107) and their infants (n = 108) were collected at the same time points for the measurement of cobalamin, holotranscobalamin, total transcobalamin, total haptocorrin, and methylmalonic acid.

Results: Median (range) concentrations of cobalamin in hindmilk were 760 (210–1880), 290 (140–690), and 440 (160–1940) pmol/L at 2 wk, 4 mo, and 9 mo, respectively; the respective haptocorrin concentrations were 25 (9–102), 22 (4–100), and 180 (30–460) nmol/L. We found slightly lower values in foremilk. A decrease in milk cobalamin at 4 mo was associated with decreases in plasma cobalamin ($P < 0.0001$) and holotranscobalamin ($P < 0.0001$) in the infants. Strong positive associations in paired maternal-infant cobalamin concentrations were found at all time points.

Conclusions: Foremilk and hindmilk contained comparable amounts of cobalamin and haptocorrin, but marked changes were observed during 9 mo of lactation. At 4 mo, low concentrations of milk cobalamin mirrored biochemical changes in infants, which suggests an impaired cobalamin status and indicates that nutrition from only mother's milk may not be sufficient for the supply of cobalamin from this age. This trial was registered by the Danish Data Protection Agency at www.datatilsynet.dk/english as 2008-41-2185. *Am J Clin Nutr* doi: 10.3945/ajcn.113.058479.

INTRODUCTION

Several studies have focused on the importance of cobalamin (vitamin B-12) during early life (1–3), when the lactating infant relies on the mother's milk for supply of the vitamin. Scandinavian mothers display a normal stable cobalamin status during lactation (2); however, studies have shown that their exclusively breastfed children at 6 mo of age have low plasma concentrations of cobalamin and holotranscobalamin (holoTC)[4] (or "biological active cobalamin") accompanied by high concentrations of methylmalonic acid (MMA) (3–5). Conversely, children nourished solely by infant formula based on cow milk and supplemented with vitamins have a sufficient cobalamin status (4), as do children receiving an intramuscular cobalamin injection given at 6 wk of age (6).

In human milk, virtually all cobalamin is bound to the cobalamin-binding protein haptocorrin (HC) (7)—a protein of unknown function. The high concentration of HC was recently shown to interfere with measures of cobalamin in assays using sample pretreatment at a high pH (8). We showed that this problem could be circumvented by removal of unsaturated HC before analysis of the content of cobalamin. This new method allows for reliable measurements of milk cobalamin (8).

In the current study, we explored the concentration of both cobalamin and HC in foremilk and hindmilk from healthy Danish mothers over a period of 9 mo and studied biomarkers of cobalamin status of both the mothers and children.

SUBJECTS AND METHODS

Participants and study design

This longitudinal cohort study was designed to investigate the content of cobalamin in human milk over a lactation period of 9 mo. For this purpose, milk was collected at 2 wk (15 ± 7 d), 4 mo (129 ± 12 d), and 9 mo (280 ± 15 d) postpartum from 25 healthy, white, Danish mothers. At the day of each visit (or the night before), foremilk (milk before feeding the child) and hindmilk (milk after feeding the child) were manually collected by the mothers following directions in a handed-out instruction. The mothers stored the milk at 4°C (for a maximum of 18 h)

[1] From the Departments of Clinical Biochemistry (EG, DLL, and EN) and Endocrinology and Internal Medicine (SS, PV, LR, and LM), Aarhus University Hospital, Aarhus, Denmark; the Faculty of Medicine, Aalborg University, Aalborg, Denmark (PV); and the Department of Endocrinology, Aalborg Hospital, Aalborg, Denmark (PV).

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[3] Address correspondence and reprint requests to E Greibe, Department of Clinical Biochemistry, Aarhus University Hospital, Norrebrogade 44, Building 9, 8000 Aarhus C, Denmark. E-mail: evagreibe@gmail.com.

[4] Abbreviations used: Cbi-Seph, cobinamide-coated sepharose; HC, haptocorrin; MMA, methylmalonic acid; TC, transcobalamin.

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before it was frozen and stored at −80°C at Aarhus University Hospital, Aarhus, Denmark, until further processed.

Mothers (n = 107) and children (n = 108) were recruited in relation to a project on vitamin D (S Streym, L Rejnmark, P Vestergaard, L Mosekilde, unpublished observations, 2008–2010). From this cohort, plasma samples at all time points were available for 60 mother-child matched pairs (including the 25 women who donated milk samples). The project on vitamin D was approved by the Central Denmark Region Ethics Committee (project no. 20040186).

Women between the ages of 24 and 41 y were included if they had a normal uncomplicated pregnancy, gave birth to healthy children, and had the intention of breastfeeding for 9 mo. Women taking medication regularly, with a chronic disease, or with an excessive consumption of alcohol were excluded from the study. The number of mothers and children included in the vitamin D study was based on power calculations by using multiple linear regression, showing a statistical power of 98% (α = 0.05). Data were collected at Aarhus University Hospital, Aarhus, Denmark, from 2008 to 2010. The study was performed within the confines of the Helsinki Declaration II, and all mothers gave their informed consent before inclusion in the study. The Central Denmark Region Ethics Committee (project no. 20090149) approved that the samples could be used also for measurement of cobalamin-related variables in the current study.

Nonfasting blood samples were drawn from the mothers and children (between 0800 and 1400) at 2 wk, 4 mo, and 9 mo postpartum; however, umbilical cord blood collected at birth was used as a substitute for collecting infant blood samples at 2 wk postpartum. Collected samples were analyzed for cobalamin and related variables (*see* Biochemical measurements).

Pretreatment of human milk samples

Milk was pretreated according to our previously described method (8). In brief, milk was thawed and centrifuged for 10 min at 15,000 × g at room temperature. The fat-reduced whey fraction was measured for totalHC before the addition of cobinamide sepharose (Cbi-Seph), prepared as previously described (8). Added Cbi-Seph binds HC to ensure an HC concentration that does not interfere with measures of cobalamin (HC <10 nmol/L) (8). Milk (200 μL whey) and Cbi-Seph were mixed with 0.9% NaCl to a final dilution of 2.5 and incubated under rotation for 1 h at room temperature before centrifugation for 2 min at 500 × g. The supernatant fluid was collected, and totalHC was measured again to ensure that the concentration was below the threshold of 10 nmol/L.

Biochemical measurements

For each variable, all samples from each mother and child were measured in one run. Total cobalamin in plasma and pretreated milk samples were assayed with an automatic Cobas 6000 system (Roche Diagnostics) that uses alkaline hydrolysis (sodium hydroxide and dithiothreitol) for cobalamin extraction and sodium cyanide for conversion into cyanocobalamin. The assay has a total imprecision of 5% and an intraassay imprecision of 2%. Plasma MMA was measured with a liquid chromatography–tandem mass spectrometer (model 6490; Agilent Technologies) with a total imprecision of 10.1% and an intraassay imprecision of 10%. TotalTC in plasma was measured by an in-house sandwich ELISA with a total imprecision of 4% to 6% and an intraassay imprecision of 3% (9). HoloTC in plasma was measured by the TC-ELISA after removal of the apoTC with cobalamin-coupled beads (10). The total imprecision was 8% (10), and the intraassay imprecision was 4% (11). TotalHC in milk (before and after pretreatment with Cbi-Seph) and plasma was measured with an in-house sandwich ELISA with a total imprecision of 5% and an intraassay imprecision of 2% (12). From the cohort of 107 mothers and 108 children, 60 mother-child matched pairs had complete sample materials at all time points; however, because of limited volume in some of the 60 infant plasma samples, measurements of totalTC and MMA could only be performed on a subgroup of these children (totalTC, n = 57; MMA, n = 28). For the same reason, plasma totalHC was not measured in the children.

Because protein measurements with bicinchoninic acid assays are susceptible to influence by lactose (13), total protein in milk was measured by using a Coomassie Brilliant Blue assay (Bio-Rad Protein Assay; Bio-Rad Laboratories). Milk samples were diluted 1:20 in 1.5 mmol NaH_2PO_4/L, 8.5 mmol Na_2HPO_4/L, and 145 mmol NaCl/L, pH 7.4, before analysis.

Statistical analysis

Results are presented as medians with ranges. For paired data, changes in the medians of the measured biochemical markers in plasma over time were tested by the nonparametric Friedmaṅs test for multivariate analyses with Dunn's multiple comparisons posttest (0.05/3 = 0.02; levels of significance = 0.02). A paired t test or Wilcoxon's signed-rank test was used as appropriate for comparisons between 2 time points or between 2 variables at the same time point. A 2-factor repeated-measures ANOVA was used to compare foremilk and hindmilk at all 3 time points. In this regard, Bonferroni correction (level of significance = 0.02) was used to allow for multiple comparisons. For unpaired data, the Mann-Whitney U test was used for pairwise comparisons, for comparisons between one variable at 2 time points or between exclusively breastfed children and children consuming a mixed diet at a given time point. Bivariate correlations were examined by using a Spearman's rank-order correlation test, and linear regression analysis was used to estimate the relative influence of various factors on the cobalamin status in the mothers and children. The data analysis was performed by using the statistical software available in GraphPad Prism version 5.

RESULTS

Characteristics of the study population

The median (range) age of the 107 mothers (including the 25 mothers who donated milk samples) was 30 (24–41) y. All mothers claimed to have an omnivorous diet, although 14 mothers reported that they rarely consumed dairy products (n = 11) or fish (n = 3). For a majority of the women (79% at 2 wk, 67% at 4 mo, and 50% at 9 mo), the diet was supplemented with a daily multivitamin pill containing 1.0–4.5 μg cobalamin. The subgroup consisting of the 25 mothers who donated milk samples was representative of the large cohort in all aspects. Most of the 108 children were exclusively breastfed at 2 wk (90%) and 4 mo (76%) of age. At 9 mo, all 108 children consumed a mixed diet of regular omnivorous food and infants' formula in addition to breast milk.

Cobalamin and HC in human milk

We measured the content of cobalamin and HC in milk collected at 2 wk, 4 mo, and 9 mo postpartum from 25 healthy lactating women (**Figure 1**). The median (range) cobalamin content of hindmilk was 760 (210–1880) pmol/L at 2 wk, 290 (140–690) pmol/L at 4 mo, and 440 (160–1940) pmol/L at 9 mo postpartum. At all time points, hindmilk contained more cobalamin than foremilk (Figure 1A). For milk HC, no difference in median (range) concentrations was found between 2 wk [25 (9–102) nmol/L] and 4 mo [22 (4–100) nmol/L] postpartum (Figure 1B). However, remarkably higher concentrations of milk HC [180 (30–460) nmol/L] were found at 9 mo ($P < 0.0001$). Hindmilk contained a mean of 14%, 9%, and 9% more HC than did the foremilk at 2 wk, 4 mo, and 9 mo, respectively (Figure 1B).

The median (range) total protein concentration in milk was higher at 2 wk [10 (7–13) g/L] than at 4 mo [7 (4–10) g/L] and 9 mo [7 (4–13) g/L] ($P < 0.0001$) after birth. No difference in protein concentration between foremilk and hindmilk was observed during the first 4 mo of lactation, which agrees with previous findings (14). At 9 mo, hindmilk had a slightly higher protein content than did foremilk (mean of difference: 0.5 g/L; $P = 0.002$). Correction for the protein content did not change the differences seen for HC in milk (data not shown).

Markers of cobalamin status in mothers and children

We explored biomarkers of the cobalamin status of lactating mothers (n = 107) and their newborn children (n = 108) by measuring plasma cobalamin and related variables at 2 wk, 4 mo, and 9 mo postpartum. The results of the 60 mother-child matched pairs from whom a complete data set at all time points could be obtained are shown in **Table 1**. The results of all measured samples from the 107 mothers and 108 children are presented elsewhere (*see* Supplementary data 1 under "Supplemental data" in the online issue).

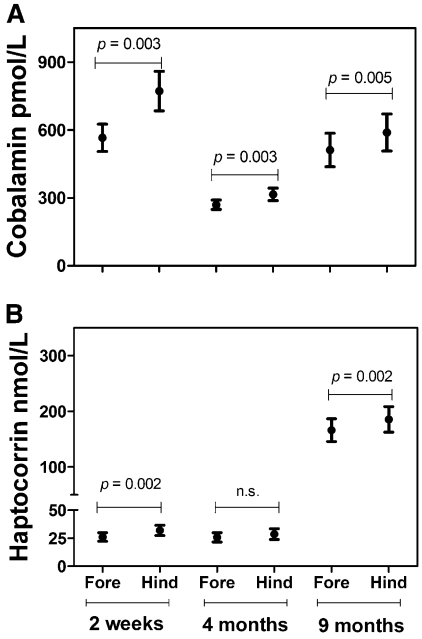

FIGURE 1. Mean (±SEM) cobalamin (A) and haptocorrin (B) concentrations in human milk. Foremilk and hindmilk from 25 healthy women were collected after 2 wk, 4 mo, and 9 mo of lactation. Differences between foremilk and hindmilk were estimated by using a paired t test or Wilcoxon's signed-rank test as appropriate. Differences in cobalamin and haptocorrin over time (2 wk, 4 mo, and 9 mo) were estimated by using a 2-factor repeated-measures ANOVA with Bonferroni corrections, $P < 0.02$. All multiple comparisons over time were found to be statistically significant (P values not shown).

In the mothers, no overall change in cobalamin was observed during the 9-mo lactation period. The concentrations of holoTC and totalTC gradually declined over time, whereas the concentrations of totalHC was unchanged. A small decrease in MMA concentrations between 2 wk and 9 mo was found (Table 1).

In the children, a remarkable decrease in plasma cobalamin was observed at 4 mo postpartum. This was accompanied by a decrease in holoTC and an increase in MMA concentrations. The totalTC concentration in the children increased gradually over time (Table 1).

No difference in any variable was found when data were analyzed as paired (60 mother-child matched pairs) (Table 1) compared with unpaired (107 mother and 108 children; *see* Supplementary data 1 under "Supplemental data" in the online issue), except for the increase in MMA in infants at 4 mo postpartum that was only significant when data were analyzed as paired.

When the mothers and children were analyzed as paired measurements (n = 60), strong positive associations in maternal and infant cobalamin concentration were found at all 3 time points: 2 wk ($r = 0.52$, $P = 0.0001$), 4 mo ($r = 0.47$, $P = 0.0001$), and 9 mo ($r = 0.29$, $P = 0.03$) postpartum (data not shown).

Separation of the 60 4-mo-old children according to diet, either being exclusively breastfed or consuming a mixed diet, showed that children consuming a mixed diet had higher concentrations of cobalamin and holoTC at 4 mo of age than did children nourished solely by breast milk (**Figure 2**). The same results were found when data from all 108 children were divided according to diet (*see* Supplementary data 2 under "Supplemental data" in the online issue).

Dividing the 4-mo-old children according to their mothers' intake of vitamin supplements containing cobalamin (1–4.5 μg/d), we found no difference in plasma cobalamin, holoTC, and MMA concentrations between children of mothers that took daily supplements and children of mothers who did not (*see* Supplementary data 3 under "Supplemental data" in the online issue).

Milk cobalamin and HC and cobalamin-related variables in mothers and children

The cobalamin concentrations of both mothers ($r = 0.58$, $P = 0.002$) and children ($r = 0.58$, $P = 0.005$) (n = 25) were positively correlated with milk cobalamin after 4 mo of lactation, when all 25 children were exclusively breastfed (**Figure 3**). As expected, no association was found at the other time points: at birth, when breastfeeding had not begun yet, and at 9 mo, when all children were consuming a mixed diet (data not shown). A correlation between maternal holoTC concentrations and milk cobalamin was found at 2 wk ($r = 0.45$, $P = 0.023$) and 4 mo ($r = 0.57$, $P = 0.003$), and an inverse correlation was found between maternal MMA concentrations and milk cobalamin at 2 wk postpartum ($r = -0.43$, $P = 0.03$). No correlation between holoTC and MMA and milk cobalamin was found in the children at any time point. We explored whether the high concentration of HC in milk correlated with the concentration observed in mother's blood, but found no correlation at any time point. At 9 mo,

TABLE 1
Plasma markers of cobalamin status in mothers and children during lactation[1]

Marker and reference interval for healthy adults	Visit 1 (birth/2 wk pp)	Visit 2 (4 mo pp)	Visit 3 (9 mo pp)	*P* value (visits 1–3)
Cobalamin (pmol/L)				
200–600 pmol/L[2]				
Mothers (n = 60)	400 (170–790)	390 (190–750)	420 (160–750)	0.85
Children (n = 60)	440[3] (130–1130)	240 (110–700)	320[3,4] (110–860)	<0.0001[5]
HoloTC (pmol/L)				
40–150 pmol/L[6]				
Mothers (n = 60)	140[3] (50–360)	130 (60–290)	110[3,4] (50–240)	<0.0001[5]
Children (n = 60)	210[3] (100–870)	60 (30–220)	100[3,4] (30–220)	<0.0001[5]
TotalTC (pmol/L)				
560–1550 pmol/L[6]				
Mothers (n = 60)	1200[3] (820–1640)	1000 (720–1780)	1040[4] (570–1840)	<0.0001[5]
Children (n = 57)	860[3] (350–1370)	1140 (750–1650)	1400[3,4] (980–2470)	<0.0001[5]
TotalHC (pmol/L)				
240–680 pmol/L[7]				
Mothers (n = 25)	420 (250–950)	460 (300–890)	480[3,4] (266–1000)	0.03
MMA (μmol/L)				
0.1–0.3 μmol/L[8]				
Mothers (n = 60)	0.18 (0.10–0.38)	0.17 (0.10–0.30)	0.16[3,4] (0.09–0.30)	<0.0001[5]
Children (n = 28)	0.27[3] (0.16–0.53)	0.44 (0.16–2.8)	0.24[3] (0.13–1.0)	0.009[5]

[1] All values are medians; ranges in parentheses. To convert the values for cobalamin to pg/mL, multiply by 1.35. Cobalamin, holoTC, totalTC, and MMA were measured in plasma samples from 60 healthy lactating mothers and their 60 newborn children at 2 wk, 4 mo, and 9 mo pp. TotalHC was also measured in the mothers (n = 25) who donated milk samples. Because of limited volume in some of the infants' plasma samples, totalTC and MMA were measured only for a subgroup of the infant population. For the first measurement (visit 1), umbilical cord blood was used for the children (birth), whereas blood from the mothers was drawn at 2 wk postpartum. Significant differences between 2 time points were estimated with Wilcoxon's signed-rank test, $P < 0.05$. HC, haptocorrin; MMA, methylmalonic acid; pp, postpartum; TC, transcobalamin.

[2] Nexo (15).

[3] Significantly different from visit 2.

[4] Significantly different from visit 1.

[5] Significant differences between all 3 visits were estimated by Friedman's test with Dunn corrections, $P < 0.02$.

[6] Nexo et al (10).

[7] Morkbak et al (12).

[8] Rasmussen et al (16).

a correlation between the concentration of milk HC and milk cobalamin was found (r = 0.41, P = 0.04).

DISCUSSION

We have reported data on biomarkers of cobalamin status of lactating mothers (n = 107) and their infants (n = 108) followed for 9 mo. Inclusion of 25 mothers donating milk samples allowed for paired data relating mother-child plasma measures to the cobalamin content of breast milk and a comparison of values obtained on foremilk and hindmilk.

The procedure for breast milk collection provided some uncertainties about the study outcome. Although the mothers were provided instructions on how to collect the foremilk and hindmilk samples, intraindividual differences in the collection procedure were difficult to assess. Also, because the mothers were allowed to collect the milk either on the day of the visit or on the night before, diurnal variation may have influenced the content of cobalamin and HC in the milk. Other milk components, such as fat and iron, have been shown to vary during the day-night cycle (17, 18); however, to our knowledge, this has never been investigated for cobalamin. We collected samples only 3 times during the 9-mo study period and thus cannot give a precise time limit for the changes observed. We did not plan to include measurements of hematologic variables and serum creatinine; however, because all of the women were healthy, it is unlikely that any of the women had any hematologic conditions or had impaired kidney function. Data on the relation between MMA and creatinine in infants are sparse, but Hogeveen et al (19) found that infants had low creatinine concentrations with no correlation to MMA concentrations. To spare the 2-wk-old infants from blood collection, umbilical cord blood was used. Some uncertainties are associated with this procedure, and great caution should be taken when comparing milk cobalamin contents collected at 2 wk postpartum and infant plasma measurements at birth. Despite these weaknesses, several interesting results were obtained in our study concerning the content of cobalamin and its binding protein HC in milk and the markers of cobalamin status in the mothers and children.

Few studies that used validated measures of milk cobalamin are available, and, to our knowledge, only the results of one study can be directly compared with our data (20). That study found a mean cobalamin content of 565 pmol/L in milk collected between 1 and 3 mo postpartum from 28 healthy Californian women supplemented with 6 μg cobalamin/d. Our data on 25 healthy Danish women adds to these findings in the following ways. First, we

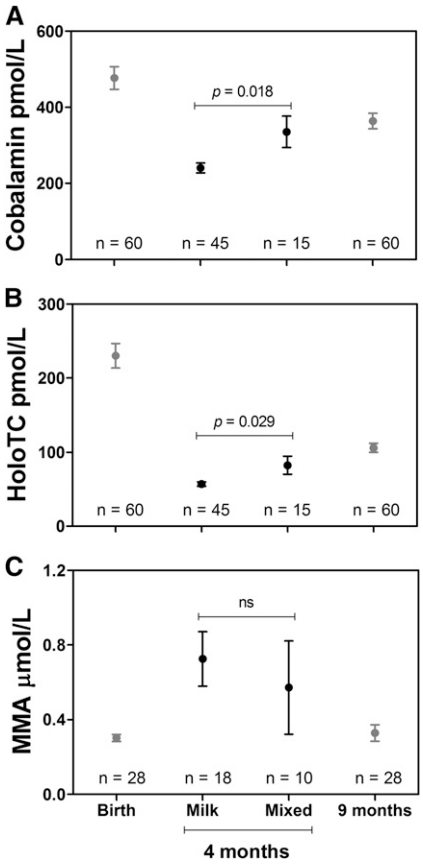

FIGURE 2. Markers of cobalamin status in 4-mo-old children according to diet. Mean (±SEM) plasma concentrations of cobalamin (A), holoTC (B), and MMA (C) in 60 children 4-mo of age divided according to nutritional source. "Milk" refers to children who were exclusively breastfed, and "mixed" refers to children who had a mixed diet (breast milk plus infant formula/regular food). For comparison, values for children at birth and at 9 mo of age are indicated (gray shading). At 9 mo of age, all children were nourished with a mixed diet. Differences between exclusively breastfed children and children consuming a mixed diet were assessed by using the Mann-Whitney *U* test. Differences related to diet were found for cobalamin and holoTC concentrations at 4 mo of age. holoTC, holotranscobalamin; MMA, methylmalonic acid.

showed a substantial decline in milk cobalamin between 2 wk (mean: 772 pmol/L) and 4 mo postpartum (mean: 316 pmol/L), which suggests that pooled data on milk samples removed during the first mo postpartum may give inconclusive results. Second, we found the content of milk cobalamin in hindmilk to be significantly higher than that in foremilk at all 3 time points after birth. However, the absolute difference was small and barely of importance.

At 2 wk and 4 mo postpartum, most of the children were exclusively breastfed. With the use of the mean cobalamin concentration in hindmilk and previously published data on daily intake of milk during lactation (21), we estimated the daily amount of cobalamin ingested through breast milk as follows: 0.7 μg/d at 2 wk of age (770 pmol/L, ~700 mL milk/d) and 0.3 μg/d at 4 mo of age (316 pmol/L, ~800 mL milk/d). In comparison, the Recommended Dietary Allowance for children aged 0–6 mo is 0.4 μg (22).

Human milk has long been known to contain ~100-fold more HC than plasma (7, 8). Here we showed for the first time that the milk HC content varies during lactation and reaches levels of up to 400-fold higher than in plasma at 9 mo postpartum (up to 450 nmol/L). The finding of higher concentrations of HC in hindmilk than in foremilk suggests that the production and/or secretion of milk HC are stimulated during feeding of the child.

The function of HC in milk is unsolved. Two hypotheses have been offered. A few studies point to an antimicrobial effect of HC in the intestine of infants, where HC, by withholding cobalamin, potentially protects the infant against pathogenic bacteria (23, 24). However, a recent systematic study of 34 strains of microorganisms present in the intestine of infants could not support this hypothesis (25). Another hypothesis is that HC mediates cobalamin absorption by being a "stand-in" for an immature intrinsic factor–mediated absorption in early infancy (26). This hypothesis is challenged by our finding that milk HC concentrations reach the highest concentrations in late lactation and also by the findings in the current study and by others (4) that children exclusively consuming mother's milk have lower concentrations of circulating cobalamin than do children consuming a mixed diet and thus depending on an intrinsic factor-mediate uptake.

In agreement with an earlier study (2), we found that the plasma cobalamin concentration of the lactating mothers was within the reference interval of healthy nonlactating adults. Even though some of the mothers had cobalamin values outside the normal range (either below or above), none had more than one measurement outside the reference interval. Given this and the fact that most of the women took cobalamin supplements, we judged that these women were cobalamin replete and that the measurements of cobalamin in their milk, therefore, can be considered normal and used for reference values.

In 4-mo-old children, we found low plasma concentrations of cobalamin and holoTC and high concentrations of MMA, which

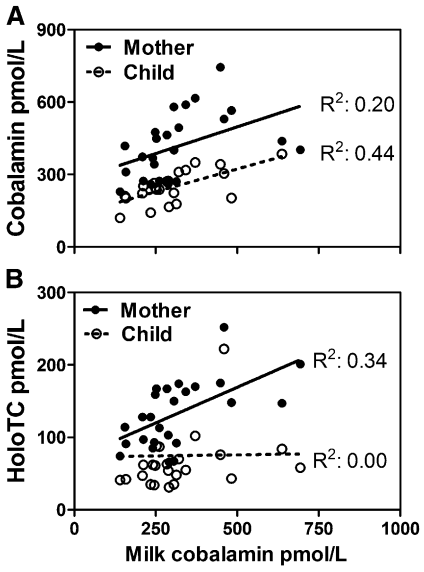

FIGURE 3. Relation (linear regression) between milk cobalamin and plasma cobalamin (A) and holoTC (B) in 25 mothers and their children at 4 mo postpartum. A positive association between milk cobalamin and plasma cobalamin was observed in both the mothers and their children and between milk cobalamin and holoTC in the mothers. No correlation was found for milk cobalamin and holoTC in children at 4 mo postpartum. holoTC, holotranscobalamin.

supports earlier data in 6-mo-old children (3, 4). The alterations in markers indicating a decline in cobalamin status correlated positively with the decline in milk cobalamin concentration observed at 4 mo postpartum (n = 25). Because the exclusively breastfed children were being nourished by healthy Scandinavian mothers, we speculated whether these findings in plasma cobalamin, holoTC, and MMA were physiologic and reflect normal cobalamin status in infants or were a consequence of an insufficient cobalamin supply, which suggests that at this age breast milk cannot provide enough cobalamin to meet the needs of the growing child. In support of the latter hypothesis was our finding—and those of others (4)—that children consuming a mixed diet had a better cobalamin status than did those solely fed on breast milk. Furthermore, the fact that the cobalamin status of infants getting cobalamin injections resembles that of older children and adults (6) suggests that the organ system of infants this age is matured and that the metabolic profile in our 4-mo-old children was attributable to a low cobalamin status and not to an immature organ system.

Concentrations of other micronutrients such as iron, zinc, copper, sodium, and potassium (21, 27) in human milk also decline during the first 6 mo of lactation, which makes the daily intake of some micronutrients lower than the recommended allowance for children (21). These data, and the data from our study, question the current WHO recommendations that children should be exclusively breastfed for ≥6 mo (28). Until it has been established whether the biochemical signs of low cobalamin status in 4-mo-old children are physiologic or a sign of insufficient cobalamin intake, we propose that a mixed diet of breast milk, infant formula, and regular omnivorous food be fed to infants beginning at least from the age of 4 mo to ensure a sufficient intake of cobalamin.

Reference values for cobalamin and related variables in early childhood are lacking. The 95% reference values of our 9-mo-old children (n = 108) were 140–800 pmol/L for cobalamin, 40–220 pmol/L for holoTC, and 0.14–1.2 μmol/L for MMA. The reference values for cobalamin and holoTC are comparable with the values of adults, but the reference values for MMA are distinctly higher. In comparison, vitamin-optimized IQR values measured in 4-mo-old children treated with one intramuscular cobalamin injection at 6 wk of age were 291–497 pmol/L for cobalamin and 0.15–0.43 μmol/L for MMA (6).

In conclusion, our data obtained in well-nourished lactating mothers provide reference values for cobalamin and its binding protein in human milk. Milk cobalamin shows the highest values early after birth and the lowest values 4 mo later, whereas the concentrations of HC increase dramatically with duration of lactation. We showed that the cobalamin concentration in milk was influenced by markers of cobalamin status in the mothers and was related to markers of cobalamin status in the children. Importantly, our data show that children fed solely on mother's milk had markers of cobalamin status (cobalamin, holoTC, and MMA) suggesting low cobalamin status at 4 mo of age. It remains to be determined whether this finding indicates that cobalamin intake through breast milk is insufficient to sustain a child's needs at this age.

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