Assessment of vitamin B-12, folate, and vitamin B-6 status and relation to sulfur amino acid metabolism in neonates[1–3]

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ABSTRACT

Background: Total serum homocysteine (tHcy) has been used as an indicator of intracellular vitamin B-12, vitamin B-6, and folate status in adults, but data for neonates and infants are lacking. Vitamin B-12 deficiency may have fatal effects on neurologic development in infants; therefore, early diagnosis is crucial.

Objective: Our aim was to provide a reference range for tHcy in neonates and to explore the relation of tHcy to *1*) serum vitamin concentrations, *2*) the product of the transsulfuration pathway (cysteine), and *3*) nutritional factors.

Design: tHcy, cysteine, folate, vitamin B-12, and vitamin B-6 were measured in 123 healthy, breast-fed neonates. The influence of nutrition (formula or human milk) on these variables was investigated in 60 infants.

Results: The mean (±SD) tHcy concentration was 7.8 ± 3.1 μmol/L. tHcy showed a linear association with log vitamin B-12 ($r = -0.64$, $P < 0.001$), red blood cell folate ($r = -0.33$, $P < 0.001$), and cysteine ($r = 0.36$, $P < 0.001$). The strongest linear association was found between tHcy and the ratio of log cysteine to log vitamin B-12 ($r = 0.71$, $P < 0.0001$). We found more neonates with probable tissue deficiencies of vitamin B-12 and folate on the basis of tHcy measurements than was expected from the analysis of serum vitamin concentrations alone (15.4% compared with 9.7%). Breast-fed infants had significantly lower vitamin B-12 concentrations and significantly higher serum tHcy and cysteine concentrations and ratios of log cysteine to log vitamin B-12 than did formula-fed infants ($P < 0.001$).

Conclusions: tHcy can be used as a functional indicator of vitamin B-12 and folate status in neonates. The ratio of cysteine to vitamin B-12 can be used as an additional index of impaired intracellular Hcy metabolism. tHcy and cysteine concentrations in infants are affected by nutritional factors. *Am J Clin Nutr* 2000;72:751–7.

KEY WORDS Homocysteine, cysteine, vitamin B-12, folate, vitamin B-6, neonates, infants, infant nutrition

INTRODUCTION

Fetuses, neonates, and pregnant women are in a state of rapid cell turnover that requires a high rate DNA synthesis (1). This high rate of DNA synthesis is associated with a great need for vitamin B-12, folate, and vitamin B-6. However, in the past, the assessment of functional and cellular vitamin status in these groups of subjects has been possible to only a limited extent. Early detection of deficiencies is important, however, because the neurologic changes that take place after pronounced vitamin B-12 deficiency in infants may be irreversible.

During the past decade it was established that subtle changes suggestive of a functional intracellular deficiency of these vitamins occur in many adult patients in the presence of minimal biochemical changes (2–4). In addition, neuropsychiatric disorders resulting from vitamin B-12 deficiency are not necessarily associated with typical clinical signs of anemia and macrocytosis (5). Indeed, megaloblastic anemia and neurologic disorders, such as hypotonia and delay in psychomotor development in infants, occur at a later stage of deficiency and are evident only in severe cases of folate and vitamin B-12 deficiency. As a result, the early signs of neurologic disturbance due to vitamin B-12 deficiency may be misinterpreted by physicians when they refer to classical signs of advanced vitamin B-12 deficiency. In this respect, serum vitamin concentrations have relatively poor sensitivity and specificity in detecting subjects with subtle changes suggestive of vitamin deficiency (6).

On the other hand, metabolites [methylmalonic acid, homocysteine (Hcy), cystathionine, and 2-methylcitric acid] involved in enzymatic reactions dependent on vitamin B-12, folate, and vitamin B-6 have been found to be sensitive estimates of both functional and intracellular deficiencies of these vitamins. Hcy especially is widely regarded as a reliable indicator for this purpose (7). Although this has been established in adults, there are few or no data indicating the use of serum Hcy for monitoring vitamins in neonates and young infants (8). Moreover, the reported reference ranges of vitamin concentrations in neonates and infants are broad (9).

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Hcy is a sulfur amino acid derived from methionine during transmethylation. The metabolism of Hcy depends on 2 pathways: the methionine cycle and transsulfuration. Hcy can be either remethylated to methionine or condensed with serine to form cysteine. The remethylation pathway (catalyzed by 5-methyltetrahydrofolate–homocysteine *S*-methyltransferase, or methionine synthase) requires vitamin B-12 and folate (methyltetrahydrofolate) as coenzyme and as cosubstrate (methyl donor), respectively, whereas the condensation to cysteine is dependent on vitamin B-6 (and is catalyzed by cystathionine β-synthase). In neonates, particularly in premature neonates, cysteine is a conditionally essential amino acid because the metabolic conversion of methionine to cysteine is impaired as a result of low activity of the hepatic enzyme cystathionine β-synthase (10).

In our study, we attempted to establish a relation between total serum Hcy (tHcy), cysteine, and subtle changes in functional vitamin status in neonates. We also investigated the relation between tHcy and neonatal and obstetric history. The influence of nutrition (human milk or infant formula) on tHcy, the transsulfuration pathway, and vitamin status was also examined in young infants.

SUBJECTS AND METHODS

Subjects

This was a 12-mo, cross-sectional study of consecutive samples of a representative, healthy infant population (129 neonates) attending our pediatric and obstetric departments (Baselbieter Kinderspital Bruderholz, Switzerland). All neonates underwent a complete physical examination and only apparently healthy neonates were investigated. In accord with the guidelines of the ethical committee, newborns with malformations, cerebral dysfunctions, renal diseases, or severe respiratory distress syndrome and premature newborns with birth weights <1501 g were not included in the study. Furthermore, newborns with laboratory evidence of hyperbilirubinemia (n = 3), suspected neonatal infection (n = 2), or hemolytic anemia (n = 1) were excluded from the statistical analyses. Thus, the complete study population consisted of 123 healthy neonates. To avoid possible nutritional bias, exclusively breast-fed neonates were examined. None of the infants had previously received vitamin B-12, vitamin B-6, or folate. Maternal history of complicated gestation and delivery and daily vitamin intake during gestation was also investigated.

We examined the influence of nutrition (human milk or infant formula) on serum tHcy, cysteine, and vitamin concentrations in an unselected sample of 60 healthy infants with a median age of 12 wk (range: 4–20 wk). The infant formula used was a hypoallergenic (hydrolyzed whey protein concentrate), standard (modified cow milk based) formula for neonates and young infants. In general, neonates receive breast milk directly after birth and not infant formula when attending our hospital. In the first 3–4 d of life, they do not consume much and most lose weight and are not completely enterally fed. By day 10, neonates commonly drink the full amount of milk necessary for adequate growth. For this reason, we studied young infants and not neonates for a better comparison with respect to intake volume and total daily protein intake.

The protocol was approved by the appropriate hospital ethical committee (Kantonsspital Bruderholz, Basel, Switzerland). Informed consent was obtained from all parents.

Methods

Blood sampling

We analyzed venous cord blood samples for 49 neonates and peripheral venous blood samples obtained during routine sampling for the remaining neonates (n = 74) and the 60 infants. Serum concentrations of tHcy, cysteine, vitamin B-12, vitamin B-6, folate, and red blood cell (RBC) folate were measured. Blood was drawn in anticoagulant-free tubes for the measurement of vitamins, tHcy, and cysteine. The tubes were cooled immediately at 4°C and centrifuged (at 3000 × g for 10 min at 4°C) 20 min after blood collection. Blood samples for the measurement of RBC folate were collected in tubes containing EDTA. Erythrocytes were washed 3 times with 0.9% saline to measure RBC folate concentrations. All samples were stored at −70°C until the day of the assay; the assay was performed within 4 wk.

Biochemical measurements

Hematologic and biochemical data were collected with use of standard methods. Concentrations of tHcy and cysteine were measured by reversed-phase HPLC with fluorometric detection after derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate according to the method of Ubbink et al (11), which was adapted to our working conditions as described previously (12).

Serum folate, vitamin B-12, and RBC folate concentrations were measured with a new competitive receptor binding immunoassay (Access Immunoassay; Beckman Coulter, Krefeld, Germany). Intraassay CVs for serum folate, serum vitamin B-12, and RBC folate were 3.8–6.5%, 5–7%, and 2.4–3.1%, respectively. Interassay CVs for serum folate, serum vitamin B-12, and RBC folate were 7.4–11%, 6–11%, and 5.8–7.8%, respectively. Vitamin B-6 concentrations were assayed as pyridoxal-*P* (PLP) by HPLC (13). In the deproteinized serum sample, PLP was transformed to pyridoxic acid-5′-phosphate. The separation was performed by reversed-phase HPLC followed by fluorometric detection with an excitation wavelength of 318 nm and an emission wavelength of 418 nm (13).

Statistical analysis

Vitamin data were log transformed before analysis when the Kolmogorov-Smirnov test did not indicate a normal distribution of the variables. Median values and 95th percentile ranges were calculated for these variables instead of mean values and SDs. Data were further analyzed by using parametric or nonparametric methods as appropriate. Unpaired comparisons were carried out with the two-sided Student's t test or Kolmogorov-Smirnov test. The chi-square test was used to compare frequency distributions between groups. Pearson's correlation coefficient was used only when the least-squares method supported a linear relation. A stepwise multiple regression analysis was used to assess the dependency of tHcy on other measured variables. P values <0.05 were considered to be statistically significant.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were drawn from 2 × 2 tables. For this purpose, the sensitivity and specificity (100 − specificity) of tHcy as a diagnostic index of vitamin B-12 and folate deficiency and of high cysteine concentrations were plotted at different tHcy cutoffs in the form of receiver operator characteristic (ROC) curves. The areas under the ROC curves (AUC^{ROC}) were calculated (means and 95% CIs) to compare the

TABLE 1
Serum vitamin concentrations of 123 neonates

Vitamins	Median	Log transformed
Vitamin B-12 (pmol/L)	264 (86–939)[1]	2.45 ± 0.29[2]
Serum folate (nmol/L)	35.6 (17.4–111)	1.57 ± 0.24
Vitamin B-6 (nmol/L)[3]	42.1 (8.1–202.7)	1.62 ± 0.38

[1] 95th percentile range in parentheses.
[2] $\bar{x}$ ± SD.
[3] Assayed as pyridoxal-*P*.

diagnostic performance of tHcy in identifying neonates with hypovitaminemia (12).

We applied the two-sided Student's *t* test and nonparametric test for the unpaired comparison of mean serum concentrations, birth weight, gestational age, and age of formula-fed and breast-fed infants. Data analyses were performed with the statistical program SPSS (version 6.1.3; SPSS Inc, Chicago). To calculate AUC^{ROC}, we used MEDCALC software (version 4.20.021 for WINDOWS 95/NT; F Schoonjans, Belgium). Values in the text are means ± SDs unless noted otherwise.

RESULTS

The investigated population consisted of 123 apparently healthy neonates (69 boys and 54 girls) aged between 1 and 28 d (median: 5 d) with a birth weight and gestational age of 3159 ± 685 g and 39 ± 2.4 wk, respectively. Of the neonates, 13% were premature (mean gestational age: 34 wk) and 10% were small for gestational age.

Serum vitamin values were not normally distributed as assessed by the Kolmogorov-Smirnov test but were positively skewed. We therefore calculated the median, the 95th percentile range, and the log-transformed values as shown in **Table 1**. In contrast, RBC folate concentrations were normally distributed (Kolmogorov-Smirnov = 0.84, *P* = NS) with a mean value of 1332 ± 213 nmol/L. The lowest serum folate value of 12.0 nmol/L was measured in one neonate who had a normal RBC folate concentration (553 nmol/L). Eleven neonates (8.9%) had serum vitamin B-12 concentrations below the lower reference range value of 129 pmol/L. In 13 neonates (10.6%) the PLP concentration was <12.1 nmol/L. Only one neonate had an RBC folate concentration <340 nmol/L, a value suggesting a possible deficiency of this vitamin (0.8%).

Concentrations of tHcy and cysteine were normally distributed. The mean serum concentration of tHcy was 7.8 ± 3.1 μmol/L; that of cysteine was 260 ± 58 μmol/L. Five neonates (4%) had a tHcy concentration >15.0 μmol/L. We used different cutoffs to test the hypothesis of a possible association between concentrations of tHcy and vitamins. Neonates with vitamin B-12 deficiency (vitamin B-12 < 129 pmol/L; *n* = 11) had significantly higher mean tHcy concentrations than did neonates with normal serum vitamin B-12 concentrations (13.6 ± 2.9 compared with 7.3 ± 2.5 μmol/L, respectively; *P* < 0.001). Neonates with low, subnormal serum folate concentrations (serum folate < 21.5 nmol/L; *n* = 23) had significantly higher mean tHcy concentrations than did neonates with normal and upper-normal folate concentrations (9.4 ± 3.1 compared with 7.6 ± 3.1 μmol/L, respectively; *P* < 0.05). Accordingly, neonates with low RBC folate concentrations (RBC folate < 680 nmol/L; *n* = 10) had significantly higher mean serum tHcy values than did neonates with normal RBC folate concentrations (11.75 ± 3.3 compared with 7.6 ± 2.9 μmol/L, respectively; *P* < 0.001). Low PLP concentrations were not associated with higher mean tHcy values.

As shown in **Table 2**, apparently healthy neonates with high tHcy concentrations (>11.0 μmol/L) had significantly lower mean log serum vitamin B-12, log serum folate, and RBC folate concentrations than did neonates with tHcy concentrations ≤11.0 μmol/L. In addition, after applying the nonparametric Kolmogorov-Smirnov test, we found a significant association between serum tHcy and vitamin B-12 values and between serum tHcy and serum folate values (not log transformed). PLP concentrations were not significantly associated with tHcy at the different cutoffs and therefore PLP was rejected for further linear analysis.

By increasing the cutoff to higher tHcy values, the association of tHcy with vitamin B-12 and folate was strengthened, suggesting a possible linear relation. Shown in **Figure 1** is the hyperbolic relation between tHcy and vitamin B-12. This indicates that with decreasing vitamin B-12, tHcy concentration increased exponentially. A logarithmic transformation of vitamin B-12 values showed a significant, inverse linear relation between tHcy and vitamin B-12 concentrations (**Figure 2**). In comparison, the linear association between tHcy and folate concentrations was

TABLE 2
Serum vitamin concentrations in neonates according to serum total homocysteine (tHcy) concentration[1]

	tHcy ≤ 11.0 μmol/L (*n* = 104)	tHcy > 11.0 μmol/L (*n* = 19)	*P*
Cysteine (μmol/L)	255 ± 54[1]	288 ± 70	0.02[2]
Vitamin B-12 (pmol/L)	323 (91–939)[3]	136.5 (62–279)	<0.01[2]
Log vitamin B-12	2.51 ± 0.26	2.14 ± 0.19	<0.001[4]
Serum folate (nmol/L)	36.8 (17.9–123)	23.8 (12.0–99.7)	0.03[2]
Log serum folate	1.59 ± 0.23	1.45 ± 0.25	0.02[4]
RBC folate (nmol/L)	1376.8 ± 488.0	1121.38 ± 408.6	0.03[2]
Log RBC folate	3.14 ± 2.69	3.0 ± 2.6	0.02[4]
Vitamin B-6 (nmol/L)[5]	45.5 (10.1–202)	26.7 (8.1–113)	NS
Log vitamin B-6	1.66 ± 0.38	1.47 ± 0.36	NS

[1] $\bar{x}$ ± SD.
[2] Kolmogorov-Smirnov test.
[3] Median; 95th percentile range in parentheses.
[4] Student's *t* test.
[5] Assayed as pyridoxal-*P*.

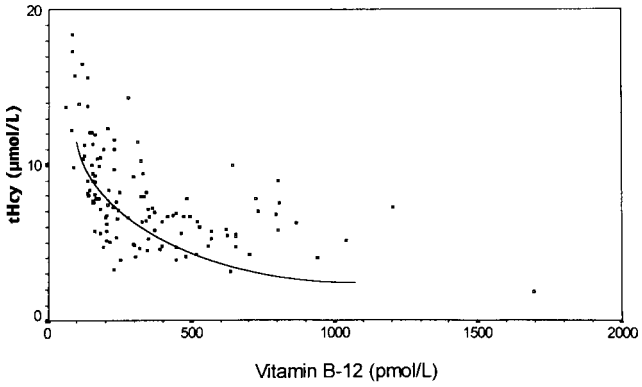

FIGURE 1. Regression plot between serum vitamin B-12 and serum total homocysteine (tHcy) concentrations. The line drawn in the plot shows a hyperbolic (exponential) relation ($r = -0.50$, $P < 0.001$).

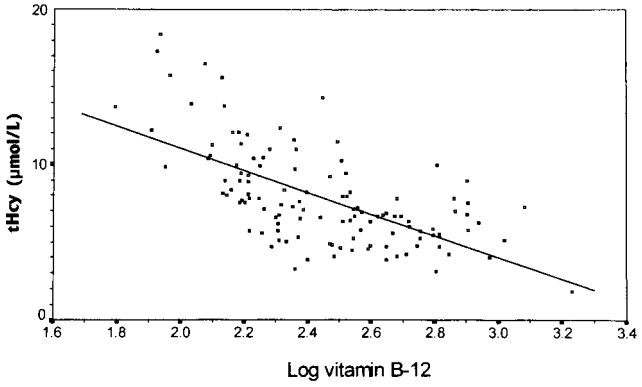

FIGURE 2. Relation between serum total homocysteine (tHcy) concentrations and log vitamin B-12 ($r = -0.64$, $P < 0.001$). Regression line: $y = 25.5 - 7.08x$.

weak for both serum folate ($r = -0.30$, $P < 0.001$) and RBC folate ($r = -0.33$, $P < 0.001$).

No significant association was found between the serum cysteine concentration and the examined vitamins. In contrast, high serum cysteine concentrations were associated with significantly higher mean tHcy concentrations ($r = 0.36$, $P < 0.001$).

Parity and age of the mother were not significantly related to tHcy, cysteine, or vitamin concentrations. Additionally, we found no significant relation between the tHcy concentrations of the neonates and complications during gestation or delivery. Neither birth weight nor gestational or postgestational age of the neonates showed a significant interaction with tHcy, cysteine, or vitamin status. Serum vitamin values, as well as cysteine and tHcy concentrations, did not differ significantly between boys and girls (tHcy: 7.74 ± 3.2 and 7.98 ± 3.1 μmol/L, respectively).

In the stepwise multiple regression analysis, vitamin B-12 was the most powerful independent predictor of tHcy, followed by cysteine and RBC folate (**Table 3**). These factors together explained 57% of the variation in tHcy.

As shown in **Figure 3**, a strong linear association existed between the log of the ratio of cysteine to vitamin B-12 (log Cys:vitamin B-12) and tHcy concentrations. This indicates that log Cys:vitamin B-12 constitutes an effective determinant of Hcy status. The mean value of the log ratio was -0.04 ± 0.31.

On the basis of the tHcy measurements (tHcy > 11.0 μmol/L), we found significantly more neonates [15.4% ($n = 19$) compared with 9.7% ($n = 12$); $P < 0.01$] with a possible tissue deficiency of vitamin B-12 and folate than was expected from the analysis of serum vitamin B-12 (<129 pmol/L; $n = 11$) and RBC folate (<340 nmol/L; $n = 1$) concentrations alone. At a cutoff of 11.0 μmol/L, tHcy showed a sensitivity of 90% and a specificity of 86% in identifying neonates with serum vitamin B-12 deficiency (PPV: 42%, NPV: 97%). Sensitivity was highest (100%) at a tHcy cutoff value of 10.0 μmol/L (specificity: 84%, NPV: 100%, PPV: 38%), whereas specificity was highest (99%) at tHcy concentrations ≥15.0 μmol/L (PPV: 80%, NPV: 94%).

ROC curves for tHcy as an indicator of pathologic vitamin B-12 concentrations (<129 pmol/L), low RBC folate concentrations (<680 nmol/L), and high log Cys:vitamin B-12 (>0.41) are combined in **Figure 4**. The AUC^{ROC} values provided by tHcy in identifying vitamin B-12–deficient neonates and neonates with low RBC folate concentrations were 0.948 (95% CI: 0.89, 0.98) and 0.846 (95% CI: 0.77, 0.91), respectively. In comparison, tHcy provided an AUC^{ROC} value for high log Cys:vitamin B-12 of 0.970 (95% CI: 0.92, 0.99).

At a cutoff of 11.0 μmol/L for tHcy, the sensitivity and specificity of detecting vitamin B-12 deficiency were 90% and 86%, respectively. Sensitivity and specificity were 100% and 89% for log Cys:vitamin B-12 (PPV: 30%, NPV: 100%) and 50% and 87% for RBC folate (PPV: 26%, NPV: 95%). tHcy is therefore a highly sensitive and specific index of cysteine-dependent functional vitamin B-12 deficiency in neonates. Cys:vitamin B-12 (cutoff > 0.18) was an accurate index of impaired tHcy metabolism (tHcy > 15.0 μmol/L), with a sensitivity and a specificity of 100% and 80%, respectively (PPV: 20%, NPV: 100%).

Breast-fed infants had significantly higher serum cysteine and significantly lower serum vitamin B-12 concentrations than did formula-fed infants (**Table 4**). Accordingly, tHcy concentrations and log Cys:vitamin B-12 were significantly higher in breast-fed than in formula-fed infants.

DISCUSSION

Two troublesome issues in clinical practice are *1*) defining subclinical vitamin deficiencies and *2*) deciding whether a latent vitamin deficit should be treated before the onset of overt clinical signs. In addition, reported vitamin concentrations in neonates differ considerably from those in adults and therefore there are no clearly defined reference ranges for neonates. However, our data are consistent with those published by Davis et al (14), Dostálová (15), and Baker et al (16).

TABLE 3
Multiple linear regression analysis to show the proportion of variation in serum total homocysteine (tHcy) explained by vitamin and serum cysteine concentrations[1]

	R	R^2	P
		%	
Vitamin B-12	0.64	41	<0.001
Cysteine	0.73	54	<0.001
RBC folate	0.76	57	<0.05

[1] R, multiple correlation coefficient, after stepwise regression analysis; R^2, percentage of variation in tHcy explained by the biochemical indexes; RBC, red blood cell.

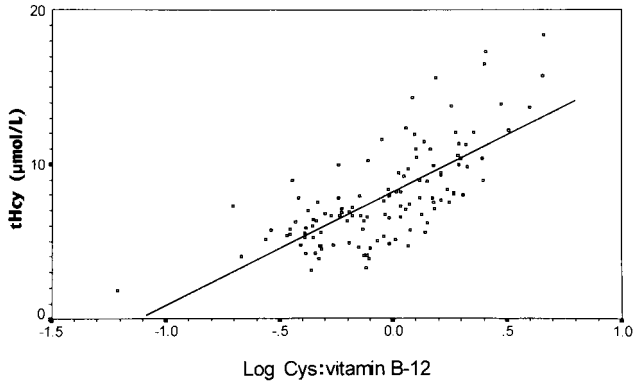

FIGURE 3. Relation between serum total homocysteine (tHcy) concentrations and the calculated log ratio of cysteine (Cys) to vitamin B-12 ($r = 0.71$, $P < 0.0001$). Regression line: $y = 8.2 + 7.34x$.

Assessing vitamin nutritional status in adults by measuring tHcy is more sensitive and specific than using serum vitamin concentrations to predict functional vitamin deficiency (4, 6, 7) because serum vitamin B-12 and folate assays do not completely differentiate deficient patients from healthy persons. About 5–10% of patients with clinically confirmed vitamin B-12 deficiency have normal serum vitamin B-12 values (5, 17). Furthermore, ≈25% of patients with low serum vitamin B-12 concentrations do not appear to be deficient (6, 18). These findings were reported for adults but not for neonates.

Our data show that the tHcy concentration in neonates is determined by serum vitamin B-12, serum cysteine, and RBC folate concentrations. The strongest association was found between vitamin B-12 (log vitamin B-12) and tHcy. The tHcy concentration rose exponentially with decreasing vitamin B-12 values, even when these values were within the lower end of the reference range (≤220 nmol/L). This indicates that the intracellular need for this vitamin is sensitive to minor changes in the serum vitamin B-12 concentration. Neonates with subnormal serum vitamin B-12 or folate concentrations (or both) had significantly higher tHcy concentrations. Even after exclusion of patients with manifest serum vitamin deficiency, there was a strong association between high tHcy and low vitamin B-12 or folate values (results not shown). From the present data, it can be statistically estimated that a cutoff of 11.0 μmol tHcy/L provides evidence of a possible functional, intracellular deficiency of vitamin B-12 and folate in neonates with normal serum concentrations. This cutoff value is in line with data reported in healthy adults (19) and children (8).

Previously reported data are conflicting and do not support the role of vitamin B-12 as a determinant of the tHcy concentration in adults. Pietrzik and Brönstrup (20) found folate to be the most important determinant of the tHcy concentration in healthy subjects aged ≤60 y but found no significant association between tHcy and serum vitamin B-12 concentrations. It was also found that age-related differences in tHcy concentrations are significantly and inversely correlated with serum vitamin B-12 and blood folate in both men and women (21). Nevertheless, we found a strong, significant association between tHcy and vitamin B-12 with a correlation coefficient of 0.64, which is 3-fold higher than coefficients reported in adults (12). This finding supports the assumption that the intracellular needs of vitamins are higher in neonates than in adults. In a randomized, placebo-controlled trial we showed that, irrespective of pretreatment serum vitamin B-12 values, initially elevated tHcy concentrations returned to normal when breast-fed neonates received a vitamin B-12 supplement (J-C Minet, E Bissé, A Beil, H Wieland, J Lütschg, G Schubiger, unpublished observations, 1999). Moreover, vitamin B-12–supplemented neonates never had tHcy concentrations exceeding 15.0 μmol/L (data not shown), a cutoff defined as pathologic in adults with a risk of cardiovascular disease (22). Furthermore, we confirmed that the upper limit of the reference range for tHcy was 11.0 μmol/L, as derived from the reference range of the vitamin B-12–supplemented neonates.

A suitable approach might be to analyze serum concentrations of methylmalonic acid to confirm functional vitamin B-12 deficiency in neonates with high tHcy serum concentrations. Methylmalonic acid is a slightly more sensitive index than tHcy for detecting patients with vitamin B-12 deficiency (98.4% compared with 95.9%). However, even methylmalonic acid may be elevated in concomitant renal failure, hypovolemia, and folate deficiency (6).

The significant association found between tHcy, cysteine, and log Cys:vitamin B-12 is the most important and newest finding of this study. This association suggests that cysteine is an independent factor in determining vitamin B-12–dependent tHcy metabolism in neonates and confirms our own observations in adults (data not shown). Moreover, it was shown by others that the cystathionine concentration is significantly associated with both vitamin B-12 and tHcy concentrations (23). The positive correlation between tHcy and cysteine in our study proves the direct relation between these compounds in vivo. When the association between tHcy and log Cys:vitamin B-12 is taken into account, it seems that the intracellular vitamin B-12 need increases in proportion to low-normal serum vitamin B-12 concentrations and high serum cysteine concentrations. Accordingly, it might be more relevant to consider this relation when assessing the functional

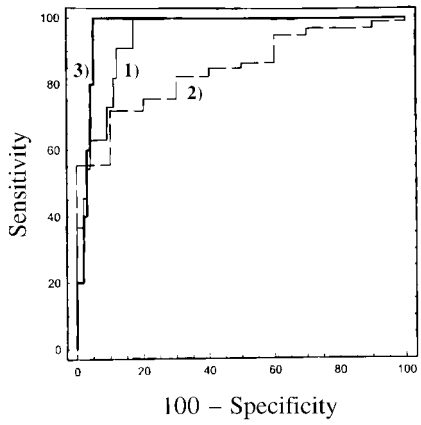

FIGURE 4. Receiver operating characteristic (ROC) curves for serum total homocysteine (tHcy) as an indicator of *1*) vitamin B-12 deficiency and *2*) red blood cell (RBC) folate deficiency in neonates ($n = 123$). *3*) The log cysteine–vitamin B-12 ratio was used to diagnose a cysteine-dependent functional vitamin B-12 deficiency in neonates. This test was achieved by using different cutoffs of tHcy. It was assumed that patients with serum vitamin B-12 <129 pmol/L ($n = 11$) and a log cysteine–vitamin B-12 ratio >0.41 ($n = 6$) had a vitamin B-12 deficiency. The cutoff selected for RBC folate was <680 nmol/L ($n = 10$). The resulting ROC curves are combined into one graph.

TABLE 4
Comparison of biochemical indexes and clinical data between breast-fed and formula-fed infants (median age: 12 wk)[1]

	Breast-fed (n = 30)	Formula-fed (n = 30)	P
tHcy (μmol/L)	10.4 ± 3.4[2]	7.0 ± 1.7	< 0.001[3]
Cys (μmol/L)	275 ± 48	244 ± 43	< 0.05[3]
Vitamin B-12 (pmol/L)	263 (62–703)[4]	456 (121–886)	< 0.005[5]
Log vitamin B-12	2.22 ± 0.24	2.74 ± 0.15	< 0.001[3]
Log Cys:vitamin B-12	0.22 ± 0.25	−0.36 ± 0.2	< 0.001[3]
Gestational age (wk)	37 ± 4	38 ± 3	NS
Birth weight (g)	2780 ± 985	2992 ± 833	NS
Weight (g)	4850 ± 860	5040 ± 820	NS

[1] tHcy, serum total homocysteine; Cys, cysteine.
[2] $\bar{x}$ ± SD.
[3] Student's t test.
[4] Median; 95th percentile range in parentheses.
[5] Kolmogorov-Smirnov test.

vitamin B-12 need. Furthermore, log Cys:vitamin B-12 may be useful in predicting vitamin B-12 deficiency.

The pronounced differences found between breast-fed and formula-fed infants with respect to tHcy and cysteine concentrations provide convincing evidence that concentrations of these metabolites are considerably affected by nutritional factors and lifestyle. Indeed, most infant formulas are enriched with vitamin B-12 but only to some extent with cysteine, whereas human milk contains a high amount of bioavailable cysteine but a comparably low vitamin B-12 concentration (24, 25). This could explain the marked difference in serum tHcy concentration between breast-fed and formula-fed infants. In addition, the methionine-sparing effects of cysteine observed in infants (10) and animals (26, 27) might explain the elevated tHcy concentrations in the serum of breast-fed infants. The methionine-sparing effect was explained by a possible inhibitory feedback loop between cysteine and methionine (10, 27). It was shown in young rats that a cysteine-enriched diet results in a product inhibition of cystathionine β-synthase, an enzyme that metabolizes Hcy to cysteine (26). High amounts of nutritional cysteine may therefore enhance intracellular vitamin B-12 demand and consecutively increase the tHcy concentration in serum. This finding underlines the need to assess the vitamin nutritional status of mothers and suggests vitamin supplementation during the breast-feeding period.

Interestingly, we found no significant association between vitamin B-6 (measured as PLP) and cysteine, despite the role of PLP as a cofactor for cystathionine β-synthase, or between PLP and tHcy. Our observation that vitamin B-6 is not relevant as a determinant of tHcy status in neonates was reported previously in adults; however, no explanation for this finding was provided (20, 28). We suppose that this finding may be due to a limited tissue distribution of vitamin B-6–dependent cystathionine β-synthase (29).

In conclusion, tHcy is a sensitive index for detecting serum vitamin B-12 deficiency in neonates and for determining the individual, intracellular need for this vitamin. The intracellular need may be higher than expected from measurements of the serum vitamin concentration alone. Our observations suggest that nutritional factors play a considerable role in young infants' metabolism of homocysteine. Cysteine and vitamin B-12 are independent factors of tHcy metabolism in neonates and taken together explain 54% of the variation in tHcy concentration. The intracellular, functional balance between cysteine and vitamin B-12 is very delicate in neonates and young infants. The ratio of cysteine to vitamin B-12 therefore provides an additional index of impaired intracellular Hcy metabolism. Validation of the use of this ratio as such an index is currently in progress.

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