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Cobalamin Status and Its Biochemical Markers Methylmalonic Acid and Homocysteine in Different Age Groups from 4 Days to 19 Years

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Background: **Recent data indicate that cobalamin and folate status, including the metabolic markers methylmalonic acid (MMA) and total homocysteine (tHcy), undergo marked changes during childhood, particularly during the first year.**

Methods: **Serum cobalamin, serum and whole-blood folate, and plasma MMA and tHcy were determined in a cross-sectional study of 700 children, ages 4 days to 19 years.**

Results: **During the first 6 months, serum cobalamin was lower than and plasma MMA, tHcy, and serum folate were higher than the concentrations detected in the other age groups. In infants 6 weeks to 6 months of age, median MMA and tHcy concentrations were >0.78 and >75 μmol/L, respectively. In older children (>6 months), serum cobalamin peaked at 3–7 years and then decreased, median plasma MMA remained low (<0.26 μmol/L), median plasma tHcy was low (<6 μmol/L) and increased from the age of 7 years on, and serum folate gradually decreased. Plasma MMA was inversely associated with cobalamin ($r = -0.4$) in both age groups, but across the whole range of cobalamin concentrations, MMA was markedly higher in infants (≤6 months) than in older children. Plasma tHcy showed a strong negative correlation to cobalamin ($r = -0.52$) but not to serum folate in infants ≤6 months. In older children, tHcy showed the expected association with both cobalamin ($r = -0.48$) and folate ($r = -0.51$).**

Conclusions: **In infants 6 weeks to 6 months, concentrations of the metabolic markers MMA and tHcy were higher than in the other age groups and strongly correlated to cobalamin, whereas in older children, both makers showed correlations to cobalamin and folate concentrations documented in adults. Whether this metabolic profile in infants is explained by impaired cobalamin status, which in turn may have long-term effects on psychomotor development, remains to be addressed in intervention studies.**

During the first year of life, cobalamin uptake may be limited because of a low cobalamin content in breast milk *(1)* and an immature intrinsic factor system *(2, 3)*, but the estimated cobalamin stores in the neonatal liver are assumed to be sufficient for normal growth *(1)*. In older children, an omnivorous diet is thought to ensure the daily dietary requirement for cobalamin. Consequently, nutritional cobalamin deficiency in childhood is considered rare and limited to infants born to cobalamin-deficient mothers or children adhering to a strict vegetarian diet low in cobalamin *(1, 4)*.

Several case reports, mainly from developing countries *(1, 5, 6)*, demonstrate the importance of maintaining adequate cobalamin concentrations during periods of rapid growth and development. In infancy, cobalamin deficiency may present as failure to thrive, developmental delay and regression, progressive neurologic disorders, or hematologic changes. The symptoms may be evident as early as 3–4 months of age *(7)*, but are often nonspecific and difficult to detect.

Cobalamin is a coenzyme in a methyl transfer reaction that converts homocysteine to methionine and in a separate reaction converts L-methylmalonyl-CoA to succinyl-CoA *(8)*. This explains why increased total homocysteine (tHcy) and/or methylmalonic acid (MMA) in the blood are measures of impaired cobalamin status, which may

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occur even in the presence of normal serum cobalamin concentrations and the absence of the classic signs myelopathy or megaloblastosis *(8)*.

Recent data indicate that cobalamin and folate status, including the metabolic markers MMA and tHcy, undergoes marked changes during the first year of life *(9)*. In newborns and infants, tHcy shows a strong relationship with serum cobalamin, but not with serum or erythrocyte folate *(10, 11)*. In older children, the associations are similar to those documented in adults: plasma tHcy correlates strongly with serum folate and less with serum cobalamin *(12–15)*. tHcy and MMA as markers of cobalamin and folate status in infancy and childhood have been the subject of a recent review article *(9)*.

MMA concentrations are frequently high in the blood or urine in infants. Specker et al. *(16)* noted more than 10 years ago that the reference interval for urinary MMA in infants was wider and higher than that for adults and older children. Reports based on newborn-screening programs indicate the common occurrence of a benign or less severe form of methylmalonic aciduria not related to inborn errors of the MMA-CoA mutase reaction *(17, 18)*. We recently reported the occurrence of low serum cobalamin and increased plasma MMA in a significant portion of infants born to healthy, nonvegetarian mothers *(11)*. Taken together, these data may indicate that impaired cobalamin status in newborns may be more prevalent than formerly recognized.

In the present work we investigated cobalamin concentrations and the association to folate and the metabolic markers in 700 healthy children 4 days to 19 years of age, who consumed a typical Western diet.

Participants and Methods

PARTICIPANTS

The study population included 700 children 4 days to 19 years of age, recruited in the period from 1996 to 1998. Newborns (n = 173) and mothers were recruited at the Department of Obstetrics and Gynaecology at Haukeland University Hospital (Bergen, Norway) and invited for a second investigation at 6 weeks (n = 46). Data on cobalamin status in these newborns have been published previously *(11)*. Mothers and infants 6 weeks (n = 10), 4 months (n = 28), 6 months (n = 34), and 12 months (n = 24) of age were recruited during well-baby visits at a local health service, and children 10–19 years (n = 213) of age were recruited from two different schools. Children 1.5–15 years (n = 172) attending an outpatient clinic for minor ear, nose, and throat surgery at Haukeland University Hospital were also enrolled, and all of these children had normal hematologic indices.

For the age group 4 days to 1 year, blood samples were collected from the mothers (n = 308), who also completed a questionnaire on maternal indices, including data on current and former pregnancies. This information was checked against the Medical Birth Register of Norway.

Newborns born to mothers on regular drug treatment, with diabetes, or who experienced Rh incompatibility during pregnancy were excluded. In addition, children with malformations or serious, chronic diseases were excluded.

Ethics approval of the protocol was granted by the local Committee on Medical Research Ethics, and one parent of each child gave written, informed consent.

DATA COLLECTION

Data on each child's diet and intake of vitamin supplements and drugs were obtained through a questionnaire filled out by a parent for the younger age groups or by self-reporting in the older age groups (>13 years).

In the age group 4 days to 1 year, additional data on breastfeeding, formula feeding, use of solid foods, and maternal diet, vitamin supplement intake, and smoking habits were collected. For this age group, information on current and former pregnancies was obtained from the Medical Birth Registry of Norway.

Intake of vitamin supplements containing cobalamin and/or folate was categorized as daily and never/sporadic; the latter category was used for those taking no supplement or supplements containing neither folate nor cobalamin. Nutrition during the first year of life was classified as nonexclusive (additional formula and/or solid food) and exclusive breastfeeding (breast milk with no additional formula or food). Smoking habits were recorded as either nonsmoker or daily smoker.

BLOOD SAMPLING AND STORAGE

Fasting blood samples were obtained by antecubital venipuncture. For infants up to 1 year, the prandial state was not registered, but all samples were collected in the morning. The samples used for tHcy determinations were placed in ice water, and plasma was separated from the blood cells within 2 h. Plasma and serum samples were stored at −20 °C until analysis.

BIOCHEMICAL ANALYSES

Serum cobalamin was determined by a *Lactobacillus leichmannii* microbiological assay *(19)* and serum and whole-blood folate by a *L. casei* microbiological assay *(20)*. Both cobalamin and folate assays were adapted to a microtiter plate format *(21)* and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG). The microbiological assays correlated with immunoassays (Immuno 1; Bayer) for estimation of serum (n = 373; $r = 0.91$) and whole-blood folate (n = 253; $r = 0.61$) and serum cobalamin (n = 380; $r = 0.96$).

Plasma MMA concentrations were assayed using a gas chromatography–mass spectrometry method based on ethylchloroformate derivatization *(22)*, and plasma tHcy was assayed by a liquid chromatography–tandem mass spectrometry method *(23)*.

Table 1. Demographic characteristics of the total study population.

	Age groups				
	4 days	**6 weeks–6 months**	**1–10 years**	**10.5–15 years**	**15.5–19 years**
n	173	118	172	109	128
Boys, n (%)	98 (57)	68 (58)	106 (62)	55 (51)	68 (53)
Intake of vitamin supplements[a]					
Never or sporadic, n (%)		98 (83)	105 (72)	100 (98)	104 (85)
Daily, n (%)		20 (17)	41 (28)	2 (2)	19 (15)

[a] Data on vitamin intake are not complete for age groups 1–10 years [data for 26 children (15%) missing], 10.5–15 years [data for 7 children (6%) missing], and 15.5–19 years [data for 5 children (4%) missing].

STATISTICAL ANALYSES

Results are presented as medians and interquartile ranges. Medians were compared by the Mann–Whitney test. Differences in vitamin and metabolite concentrations by age groups were assessed by the Kruskal–Wallis test. Correlation was assessed by the Spearman correlation.

Serum folate and serum cobalamin concentrations as determinants of high tHcy (upper quartile for the total group, >7.85 μmol/L) at the ages below and above 6 months were assessed by ROC analysis. Two-sided *P* values <0.05 were considered statistically significant.

The SPSS statistical package (Ver. 10) was used for all statistical analyses.

Results

CHARACTERISTICS OF STUDY POPULATION

The characteristics of the study population are listed in Table 1. A total of 700 children 4 days to 19 years of age were studied; 56% were boys. All children >1 year consumed an omnivorous diet. Only 17% of the total population were daily users of vitamin supplements that contained cobalamin and/or folate. Fifty-six percent of the supplements used contained both vitamins, and this percentage was equally distributed among the age groups.

Children 4 days to 1 year of age (n = 315) had a median gestational age at birth of 40 weeks (range, 39–41 weeks), and the median birth weight was 3690 g (range, 3340–4000 g). Daily use of multivitamin supplements increased during the first year and was 11% at 6 weeks and 33% at 12 months. Exclusive breastfeeding decreased from 73% at 6 weeks to 35% at 6 months, and at 12 months, all infants received additional solid foods.

Fifty-nine percent of the infants were born to multiparous women who had a mean of 1.5 other children (range, 1–10 children). Eighteen percent of the mothers took multivitamin supplements daily, and 17% were daily smokers.

VITAMINS AND METABOLIC MARKERS

The median (interquartile range) concentrations of cobalamin, MMA, tHcy, and folate for each age group are listed in Table 2 and presented visually in Fig. 1. Apart from a significantly higher median plasma MMA (0.32 vs 0.28 μmol/L; $P = 0.012$) in girls compared with boys at day 4

Table 2. Vitamins and metabolites in children according to age.

	Age groups				
	4 days	**6 weeks–6 months**	**1–10 years**	**10.5–15 years**	**15.5–19 years**
n	173	118	172	109	128
Serum cobalamin, pmol/L					
Median	314	217	551	436	369
Interquartile range	238–468	147–290	456–683	295–529	294–452
Serum folate, nmol/L					
Median	27.0	31.6	14.9	11.9	9.7
Interquartile range	20.4–36.3	21.3–43.3	12.0–21.1	9.0–15.1	7.7–12.6
Whole-blood folate, nmol/L					
Median	532	299	271	213	245
Interquartile range	438–723	239–384	203–361	175–247	204–313
Plasma MMA, μmol/L					
Median	0.29	0.78	0.13	0.17	0.14
Interquartile range	0.24–0.39	0.36–1.51	0.11–0.17	0.13–0.22	0.12–0.18
Plasma tHcy, μmol/L					
Median	6.22	7.47	5.24	6.52	7.75
Interquartile range	5.00–7.48	6.10–9.22	4.68–5.97	5.70–7.75	6.61–9.12

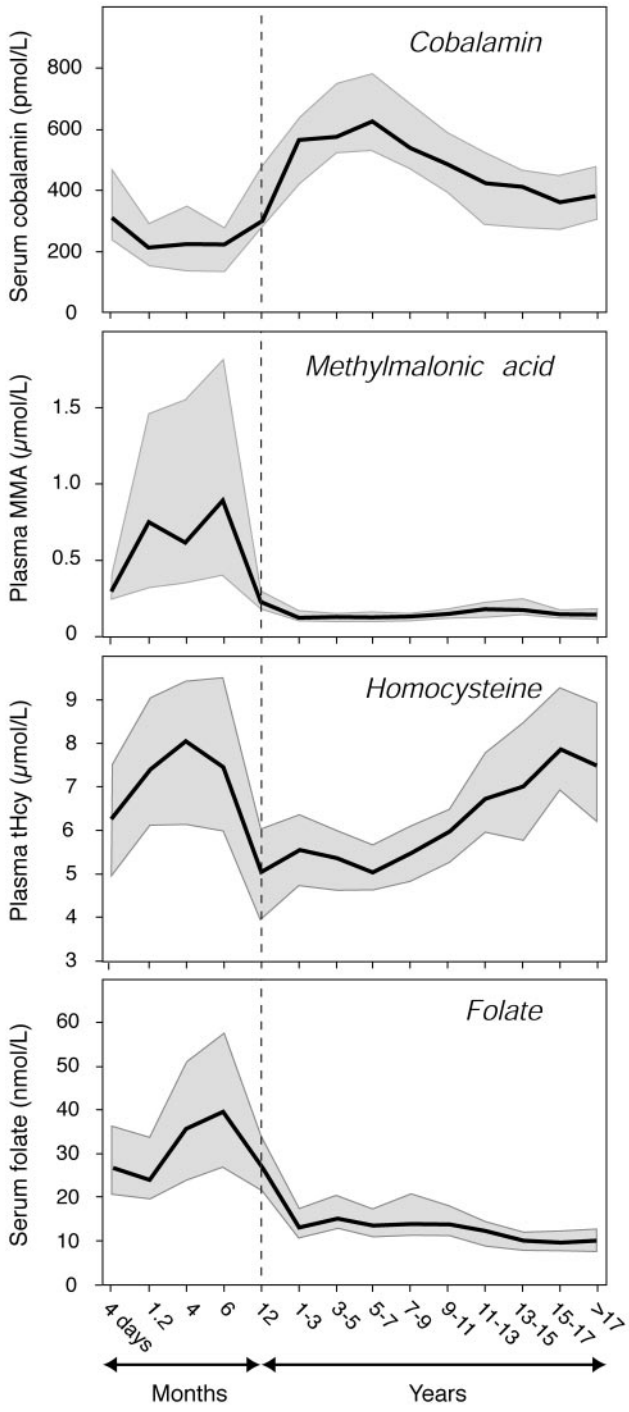

Fig. 1. Changes in serum cobalamin, serum folate, plasma tHcy, and plasma MMA in children from day 4 throughout adolescence.

The *solid lines* indicate the median values; *shaded areas* indicate the 25th and 75th percentiles.

after birth, there were no sex-related differences, and the data for both genders were analyzed together.

The most notable findings were low median cobalamin (<350 pmol/L) and high median plasma MMA (>0.26 μmol/L) during the first 6 months (Fig. 1). Median serum cobalamin decreased markedly after birth (median, 314 pmol/L) to reach a nadir (median, 217 pmol/L) between 6 weeks and 6 months (P <0.001). Thereafter, serum cobalamin increased to a maximum concentration at ~3–7 years, after which the median concentration gradually decreased toward the concentrations observed in adults *(24)*. The period of low serum cobalamin was associated with a marked increase in plasma MMA (from a median of 0.29 to 0.78 μmol/L; P <0.001) and a slight increase in plasma tHcy (from a median of 6.22 to 7.47 μmol/L; P <0.001) and serum folate (from a median of 27.0 to 31.6 nmol/L; P = 0.02; Table 2). At the age of ~1 year, all three indices had decreased to the median concentrations observed in early childhood. Thereafter, plasma MMA and tHcy were stable, and after age 7 years, plasma tHcy increased toward adult concentrations, whereas serum folate gradually decreased up to the age of 15 years (Table 2 and Fig. 1). Whole-blood folate was high (median, 532 nmol/L) in newborns, remained moderately high (median, 299 nmol/L) during the first 6 months, and reached stable median concentrations of ~220 nmol/L from 3 years on (Table 2).

SIMPLE CORRELATIONS

Spearman rank correlation coefficients demonstrated that the relationships between vitamins and the metabolites MMA and tHcy were dependent on age (Table 3). Notably, during the first 6 months of life, tHcy was inversely and strongly related to serum cobalamin, but not related to serum folate. Another unique trait in this age group was serum folate, which was positively related to MMA.

In children older than 6 months, tHcy showed an equally strong relationship with both cobalamin and folate (Table 3). We observed a strong correlation between serum cobalamin and plasma MMA (r = −0.4) and a significant correlation between tHcy and MMA in both age groups (Table 3).

We plotted the plasma tHcy concentrations according to serum folate quintiles (for the whole study group) in

Table 3. Correlations between concentrations of vitamins and metabolites in children.

	Spearman correlation coefficients			
	Serum cobalamin	**Serum folate**	**Whole-blood folate**	**Plasma MMA**
Infants (4 days–6 months)				
Serum folate	−0.05			
Whole-blood folate	0.34[a]	0.26[a]		
Plasma MMA	−0.38[a]	0.12[b]	−0.30[a]	
Plasma tHcy	−0.52[a]	−0.02	−0.27[a]	0.39[a]
Older children (>6 months)				
Serum folate	0.18[a]			
Whole-blood folate	0.05	0.48[a]		
Plasma MMA	−0.44[a]	0.01	0.02	
Plasma tHcy	−0.48[a]	−0.51[a]	−0.19[b]	0.17[a]

[a] P <0.001.
[b] P <0.05.

children younger and older than 6 months of age (Fig. 2, top left panel). The inverse dose–response relationship between tHcy and folate was similar in the age groups, but serum folate in the younger children was distributed to higher concentrations and included few individuals with low folate (Fig. 2, bottom left panel). tHcy concentrations were significantly higher in infants ≤6 months than in older children across each quintile in folate quintiles 2–4 ($P < 0.01$), but not in quintile 1.

A similar graphic presentation of plasma MMA concentrations according to serum cobalamin quintiles in the two age groups is shown in the top right panel of Fig. 2. Cobalamin and MMA were inversely associated up to a serum cobalamin concentration of 572 pmol/L in both age groups. Notably, MMA concentrations in the infants ≤6 months were markedly higher than in the older children across each quintile in cobalamin quintiles 1–4 ($P < 0.001$). The difference was largest (0.53 vs 0.19 μmol/L) in individuals with low cobalamin (quintile 1; Fig. 2, top right panel).

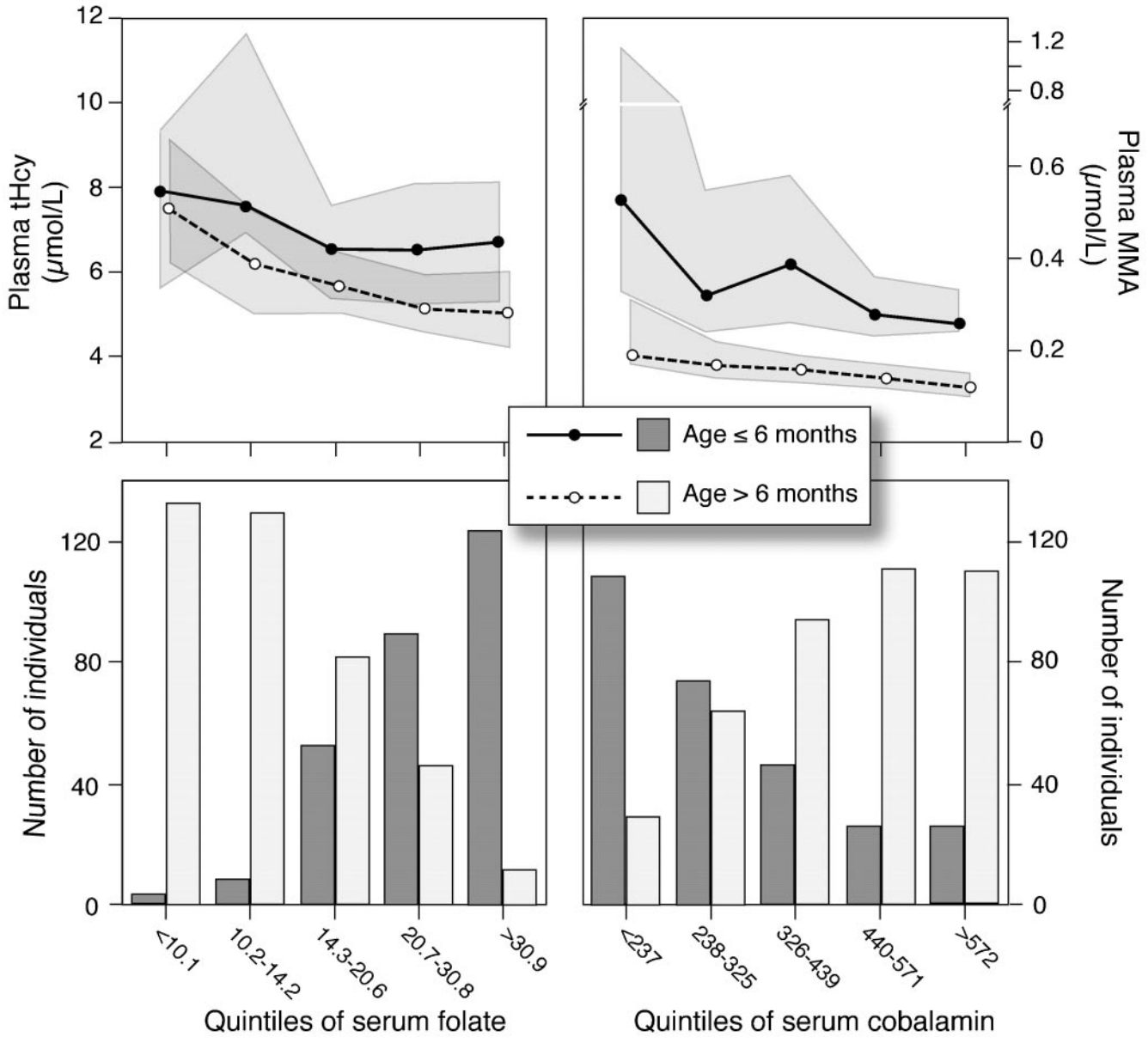

Fig. 2. Vitamin concentrations vs metabolic markers according to age.

(*Top panels*), plasma tHcy and plasma MMA plotted against quintiles of serum folate (nmol/L) and serum cobalamin (pmol/L), respectively, for 291 infants ≤6 months of age (●) and for 409 children >6 months of age (○). Categorization represents quintiles for the whole study group (n = 700). The data are given as median values with the 25th and 75th percentiles (*shaded areas*). (*Bottom panels*), frequency distributions according to quintiles of folate or cobalamin for the two age groups.

INCREASED PLASMA tHcy ACCORDING TO VITAMIN CONCENTRATION

The age-related association between high plasma tHcy (>75 percentile, 7.85 μmol/L) and the vitamins was confirmed and visualized by ROC analyses (Fig. 3). Below the age of 6 months, the curve for folate was close to the 45-degree diagonal, showing that serum folate was not related to tHcy, whereas the curve for cobalamin was located in the upper triangle throughout the whole range of sensitivity-specificity pairs, demonstrating a significant association ($P < 0.001$) between tHcy and cobalamin. In children older than 6 months, folate and cobalamin both showed a significant ($P < 0.001$) and equal discriminatory power, and both were related to increased tHcy.

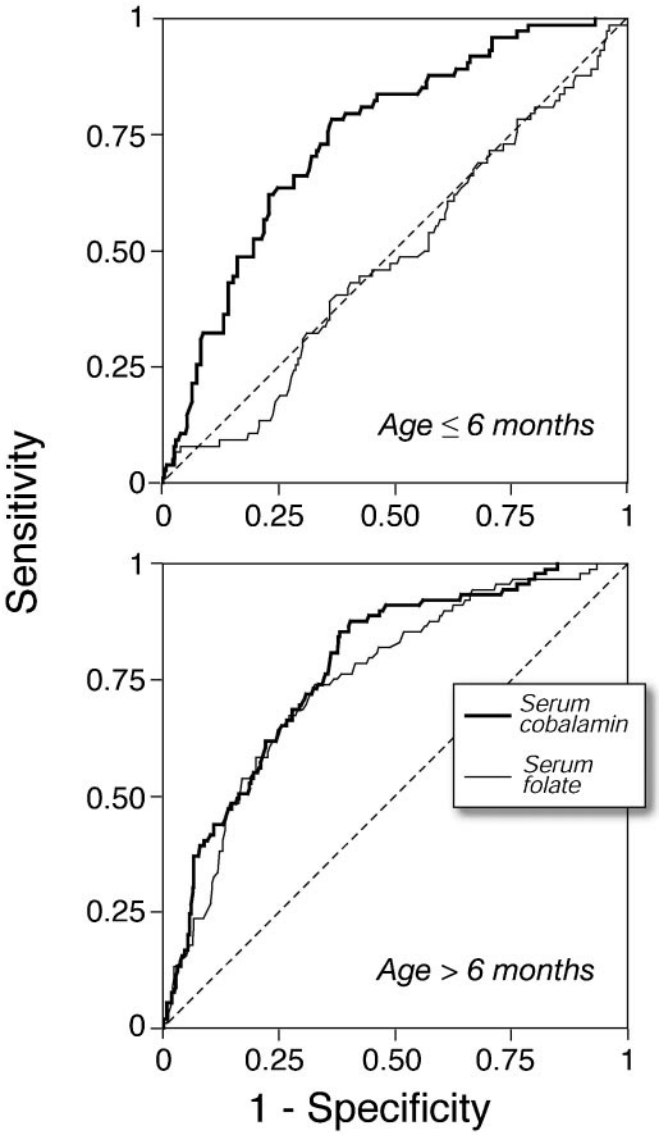

Fig. 3. Nonparametric ROC plot for serum cobalamin and serum folate in distinguishing tHcy concentrations above the 75th percentile (>7.85 μmol/L).

(*Top*), ROC graph for 291 infants ≤6 months of age. The areas under the curves are 0.76 ($P < 0.001$) and 0.48 ($P = 0.6$) for cobalamin and folate, respectively. (*Bottom*), ROC graph for 409 children >6 months of age. The areas under the curves are 0.77 ($P < 0.001$) and 0.75 ($P < 0.001$) for cobalamin and folate, respectively.

Discussion

In the present study, serum cobalamin and folate, and the metabolic markers plasma MMA and tHcy, varied markedly in the span of 4 days to 19 years of age. Between birth and 6 months, median serum cobalamin was lower than in the other age groups, and tHcy and, particularly, MMA were relatively high, suggesting impaired cobalamin function.

STUDY DESIGN AND LIMITATIONS

We investigated a large number of children (n = 700) recruited to obtain an even age distribution from 4 days to 19 years. The study was mainly cross-sectional, and only 46 children were followed from birth to 6 weeks *(15)*. The study population included presumably healthy infants and children, and the subgroup recruited from the outpatient clinic for minor ear, nose, and throat surgery had normal hematologic indices.

In older children, the blood samples were taken in the fasting state. This may decrease the intraindividual variability in tHcy, because tHcy may vary according to prandial status in children, as has been demonstrated previously in adults *(25)*. For the infant group, we could not obtain fasting samples. Whether the time since last meal influences tHcy in infants is unknown.

VITAMIN AND METABOLITE CONCENTRATIONS IN CHILDREN

We found relatively high median serum folate concentrations (>25 nmol/L) during the first 6 months of life. Similar observations have been made previously by others *(26, 27)*. The concurrent low median serum cobalamin (<350 pmol/L) described by us has been demonstrated in some *(10, 28)*, but not all *(27)* published studies. The cobalamin and folate concentrations in children older than 6 months reported here (Fig. 1 and Table 2) are in accordance with data published by others *(27, 29–31)*.

In children older than 6 months, we measured plasma tHcy concentrations (Fig. 1 and Table 2) that agreed with published data, which consistently demonstrated a plasma tHcy of ~4–8 μmol/L at age <12 years and an increase in tHcy as a function of age *(9)*. We observed no sex differences. Some *(32–34)*, but not all *(14, 35–37)* published studies have demonstrated a slightly higher plasma tHcy concentration in boys than in girls, and this gender effect is enhanced during and after puberty.

Published data on plasma MMA in infants are scarce *(4, 11, 38)*. In infants 6 weeks to 6 months of age, we observed median plasma MMA concentrations 3 times higher (Fig. 1 and Table 2) than the upper reference limits established in adults *(39)*. Similarly, Specker and coworkers reported markedly higher urinary MMA excretion in infants than in adults, in particular in infants fed human milk *(16)* and breast-fed infants of vegetarian mothers

(40). In children older than 6 months, we observed plasma MMA concentrations (Table 2) comparable to those established in adults.

homocysteine and vitamin status by age

In infants <6 months of age, plasma tHcy was strongly correlated with serum cobalamin, but not with serum folate, whereas in older children, serum concentrations of both vitamins were associated with tHcy (Table 3 and Figs. 2 and 3). A similar observation of serum cobalamin as the main tHcy determinant in neonates has been reported by Minet et al. *(10)*, and there are consistent reports showing that serum folate is strongly associated with tHcy in older children *(9, 15, 32)*.

The lack of correlation between tHcy and serum folate coincided with a period (first 6 months of life) of high serum folate (Fig. 1 and Table 2). During this period, whole-blood folate was essentially normal (Table 2) and showed a significant inverse association with tHcy (Table 3).

possible mechanisms and implications of increased metabolites in infants

The increased metabolite concentrations in infants may be a harmless phenomenon related to developmental, physiologic, or nutritional factors or may reflect the common occurrence of impaired cobalamin function in infants.

High concentrations of plasma tHcy during the first 6 months of life correlated to serum cobalamin but not serum folate. The lack of a relationship between tHcy and folate may reflect that the high serum folate in infants (Fig. 2 and Table 2) exceeds the subnormal or deficient folate concentrations associated with increased tHcy in adults *(8)*. Alternatively, low cobalamin and high MMA may reflect impaired cobalamin status *(8)*, which would lead to inhibition of methionine synthase and thus to methylfolate trapping and increased 5-methyltetrahydrofolate in serum/plasma *(41)*. The observations that serum folate in infants (but not in older children) tended to be inversely associated with serum cobalamin and positively related to plasma MMA (Table 3) support this contention. However, long-term methylfolate trapping is expected to cause folate depletion *(42)*, whereas these infants had high-normal whole-blood folate (Table 2).

The possible effects of constituents in human milk on plasma tHcy should be considered. The methionine content in human milk is actually lower than in milk-based formulas, and breastfeeding has been associated with low plasma methionine *(43)*. In adults, ingestion of large amounts of methionine during methionine loading increases tHcy *(44)*, whereas dietary methionine does not seem to affect plasma tHcy *(45)*. Thus, dietary methionine may not explain the variations in plasma tHcy in infants.

In infants ≤6 months, MMA was inversely related to cobalamin, but the MMA concentrations were higher than in older children at each cobalamin quintile and therefore through the range of cobalamin concentrations (Fig. 2). The higher plasma MMA concentrations in infants than in older children (Fig. 1 and Table 2) and adults *(39)* may be related to processes independent of cobalamin function, such as propionate production by intestinal bacteria *(46)* and liver *(47)* and kidney immaturity *(48)*. The tubular system responsible for secretion of organic acids is not fully developed at birth *(48)*. Impaired secretion of MMA or its precursor(s) in infants may explain high plasma MMA concentrations, but certainly not the increased urinary MMA excretion, which is reported to be inversely related to maternal cobalamin status *(16, 40)*.

Because urinary MMA excretion in infants is related to breastfeeding *(16)*, dietary causes of increased MMA should be considered. Cholesterol *(49)* and odd-chain fatty acids *(50)* are constituents of human milk and, together with some amino acids (methionine, isoleucine, threonine, and valine), are precursors of propionic acid and MMA *(8)*. Total blood cholesterol is higher in breast-fed than formula-fed infants *(51, 52)*, but there are indications that the high cholesterol may be related to enhanced synthesis rather than intake *(53)*. Fecal concentrations of propionic acid have been determined to be lower rather than higher in breast-fed infants compared with infants given milk formula *(54)*. Finally, protein intake on a weight basis may be high in infants, but the plasma concentrations of methionine, isoleucine, and threonine are lower in breast-fed than in formula-fed infants *(43)*. Thus, the data cited above do not provide any firm evidence that high plasma MMA in infants is related to intake of compounds degraded to propionic acid and MMA.

Alternatively, high plasma MMA reflects impaired cobalamin function. Several lines of evidence support the latter contention. The relationships between serum folate, serum cobalamin, and metabolites suggest a methylfolate trap, as outlined above. Furthermore, the increase in MMA concurs with increased tHcy and low plasma cobalamin (Figs. 1 and 2) and is predicted by maternal cobalamin status and factors expected to put a strain on maternal cobalamin status, such as multiparity *(11)*. There are consistent reports demonstrating higher urinary MMA *(16)* or tHcy *(10, 55)* in breast-fed infants than in infants given formula, which is usually enriched with cobalamin *(56)*. Likewise, initially increased tHcy in breast-fed neonates has been corrected by cobalamin supplementation *(10)*. Thus, the increased MMA and its relationship with other blood indices suggest the common occurrence of impaired cobalamin status in infants *(57)*.

In conclusion, the combinations of lower serum cobalamin and increased MMA, tHcy, and folate in the first 6 months of life compared with other age groups may reflect normal, transient physiologic processes. However, this metabolic profile is related to factors expected to affect maternal cobalamin balance and seems to be normalized by cobalamin supplementation. These observations point to the possibility that impaired cobalamin

status may be common in infants born to nonvegetarian mothers on a typical Western diet, as suggested previously by others *(1, 57)*. Because impaired cobalamin function may have long-term effects related to psychomotor development *(58)*, our findings may motivate intervention studies with cobalamin supplementation in pregnancy and infancy.

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References

1. Allen LH. Vitamin B_{12} metabolism and status during pregnancy, lactation and infancy. Adv Exp Med Biol 1994;352:173–86.
2. Kelly EJ, Brownlee KG, Newell SJ. Gastric secretory function in the developing human stomach. Early Hum Dev 1992;31:163–6.
3. Adkins Y, Lonnerdal B. Mechanisms of vitamin B_{12} absorption in breast-fed infants. J Pediatr Gastroenterol Nutr 2002;35:192–8.
4. Schneede J, Dagnelie PC, van Staveren WA, Vollset SE, Refsum H, Ueland PM. Methylmalonic acid and homocysteine in plasma as indicators of functional cobalamin deficiency in infants on macrobiotic diets. Pediatr Res 1994;36:194–201.
5. Higginbottom MC, Sweetman L, Nyhan WL. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B_{12}-deficient breast-fed infant of a strict vegetarian. N Engl J Med 1978;299:317–23.
6. Stollhoff K, Schulte FJ. Vitamin B_{12} and brain development. Eur J Pediatr 1987;146:201–5.
7. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B_{12} deficiency in infants. J Pediatr 1992;121:710–4.
8. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin-B12) and folate deficiency. FASEB J 1993;7:1344–53.
9. Bjorke Monsen AL, Ueland PM. Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. Am J Clin Nutr 2003;78:7–21.
10. Minet JC, Bisse E, Aebischer CP, Beil A, Wieland H, Lutschg J. Assessment of vitamin B-12, folate, and vitamin B-6 status and relation to sulfur amino acid metabolism in neonates. Am J Clin Nutr 2000;72:751–7.
11. Bjorke-Monsen AL, Ueland PM, Vollset SE, Guttormsen AB, Markestad T, Solheim E, et al. Determinants of cobalamin status in newborns. Pediatrics 2001;108:624–30.
12. Tonstad S, Refsum H, Sivertsen M, Christophersen B, Ose L, Ueland PM. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. Pediatr Res 1996;40:47–52.
13. Tonstad S, Refsum H, Ueland PM. Association between plasma total homocysteine and parental history of cardiovascular disease in children with familial hypercholesterolemia. Circulation 1997; 96:1803–8.
14. De Laet C, Wautrecht JC, Brasseur D, Dramaix M, Boeynaems JM, Decuyper J, et al. Plasma homocysteine concentration in a Belgian school-age population. Am J Clin Nutr 1999;69:968–72.
15. Delvin EE, Rozen R, Merouani A, Genest J Jr, Lambert M. Influence of methylenetetrahydrofolate reductase genotype, age, vitamin B-12, and folate status on plasma homocysteine in children. Am J Clin Nutr 2000;72:1469–73.
16. Specker BL, Brazerol W, Ho ML, Norman EJ. Urinary methylmalonic acid excretion in infants fed formula or human milk. Am J Clin Nutr 1990;51:209–11.
17. Shih VE, Coulombe JT, Maties M, Levy HL. Methylmalonic aciduria in the newborn. N Engl J Med 1976;295:1320–1.
18. Ledley FD, Levy HL, Shih VE, Benjamin R, Mahoney MJ. Benign methylmalonic aciduria. N Engl J Med 1984;311:1015–8.
19. Kelleher BP, Broin SD. Microbiological assay for vitamin B_{12} performed in 96-well microtitre plates. J Clin Pathol 1991;44: 592–5.
20. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol 1992;45:344–7.
21. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
22. Husek P. Simultaneous profile analysis of plasma amino and organic acids by capillary gas chromatography. J Chromatogr B Biomed Appl 1995;669:352–7.
23. Nexo E, Engbaek F, Ueland PM, Westby C, O'Gorman P, Johnston C, et al. Evaluation of novel assays in clinical chemistry: quantification of plasma total homocysteine. Clin Chem 2000;46: 1150–6.
24. Wahlin A, Backman L, Hultdin J, Adolfsson R, Nilsson LG. Reference values for serum levels of vitamin B_{12} and folic acid in a population-based sample of adults between 35 and 80 years of age. Public Health Nutr 2002;5:505–11.
25. Nurk E, Tell GS, Nygard O, Refsum H, Ueland PM, Vollset SE. Plasma total homocysteine is influenced by prandial status in humans: The Hordaland Homocysteine Study. J Nutr 2001;131: 1214–6.
26. Salmenpera L, Perheentupa J, Siimes MA. Folate nutrition is optimal in exclusively breast-fed infants but inadequate in some of their mothers and in formula-fed infants. J Pediatr Gastroenterol Nutr 1986;5:283–9.
27. Hicks JM, Cook J, Godwin ID, Soldin SJ. Vitamin B_{12} and folate. Pediatric reference ranges. Arch Pathol Lab Med 1993;117: 704–6.
28. Casterline JE, Allen LH, Ruel MT. Vitamin B-12 deficiency is very prevalent in lactating Guatemalan women and their infants at three months postpartum. J Nutr 1997;127:1966–72.
29. Doyle W, Jenkins S, Crawford MA, Puvandendran K. Nutritional status of schoolchildren in an inner city area. Arch Dis Child 1994;70:376–81.
30. Requejo AM, Ortega RM, Navia B, Gaspar MJ, Quintas E, Lopez-Sobaler A. Folate and vitamin B_{12} status in a group of preschool children. Int J Vitam Nutr Res 1997;67:171–5.
31. Wright JD, Bialostosky K, Gunter EW, Carroll MD, Najjar MF, Bowman BA, et al. Blood folate and vitamin B_{12}: United States, 1988–94. Vital Health Stat 1998;11:1–78.
32. Osganian SK, Stampfer MJ, Spiegelman D, Rimm E, Cutler JA, Feldman HA, et al. Distribution of and factors associated with serum homocysteine levels in children: Child and Adolescent Trial for Cardiovascular Health. JAMA 1999;281:1189–96.
33. Minniti G, Cerone R, Piana A, Armani U, Lorini R. Plasma and serum total homocysteine concentrations in paediatric patients, evaluated by high-performance liquid chromatography with fluorescence. Clin Chem Lab Med 2000;38:675–6.
34. Jacques PF, Rosenberg IH, Rogers G, Selhub J, Bowman BA,

Gunter EW, et al. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999; 69:482–9.

35. Vilaseca MA, Moyano D, Ferrer I, Artuch R. Total homocysteine in pediatric patients. Clin Chem 1997;43:690–2.

36. Rauh M, Verwied S, Knerr I, Dorr HG, Sonnichsen A, Koletzko B. Homocysteine concentrations in a German cohort of 500 individuals: reference ranges and determinants of plasma levels in healthy children and their parents. Amino Acids 2001;20:409–18.

37. Bates CJ, Mansoor MA, Gregory J, Pentiev K, Prentice A. Correlates of plasma homocysteine, cysteine and cysteinyl-glycine in respondents in the British National Diet and Nutrition Survey of young people aged 4–18 years, and a comparison with the survey of people aged 65 years and over. Br J Nutr 2002;87:71–9.

38. Rogers LM, Boy E, Miller JW, Green R, Sabel JC, Allen LH. High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. Am J Clin Nutr 2003;77:433–40.

39. Bolann BJ, Solli JD, Schneede J, Grottum KA, Loraas A, Stokkeland M, et al. Evaluation of indicators of cobalamin deficiency defined as cobalamin-induced reduction in increased serum methylmalonic acid. Clin Chem 2000;46:1744–50.

40. Specker BL, Miller D, Norman EJ, Greene H, Hayes KC. Increased urinary methylmalonic acid excretion in breast-fed infants of vegetarian mothers and identification of an acceptable dietary source of vitamin B-12. Am J Clin Nutr 1988;47:89–92.

41. Das KC, Herbert V. Vitamin B_{12}–folate interrelations. Clin Haematol 1976;5:697–745.

42. Scott JM, Weir DG. The methyl folate trap. A physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid-induced exacerbation of subacute combined degeneration in pernicious anaemia. Lancet 1981;2:337–40.

43. Janas LM, Picciano MF, Hatch TF. Indices of protein metabolism in term infants fed human milk, whey-predominant formula, or cow's milk formula. Pediatrics 1985;75:775–84.

44. Ubbink JB, Becker PJ, Delport R, Bester M, Riezler R, Vermaak WJ. Variability of post-methionine load plasma homocysteine assays. Clin Chim Acta 2003;330:111–9.

45. Ward M, McNulty H, Pentieva K, McPartlin J, Strain JJ, Weir DG, et al. Fluctuations in dietary methionine intake do not alter plasma homocysteine concentration in healthy men. J Nutr 2000;130: 2653–7.

46. Thompson GN, Walter JH, Bresson JL, Ford GC, Lyonnet SL, Chalmers RA, et al. Sources of propionate in inborn errors of propionate metabolism. Metabolism 1990;39:1133–7.

47. Sniderman LC, Lambert M, Giguere R, Auray-Blais C, Lemieux B, Laframboise R, et al. Outcome of individuals with low-moderate methylmalonic aciduria detected through a neonatal screening program. J Pediatr 1999;134:675–80.

48. Jones DP, Chesney RW. Development of tubular function. Clin Perinatol 1992;19:33–57.

49. Giovannini M, Agostoni C, Riva E. Fat needs of term infants and fat content of milk formulae. Acta Paediatr Suppl 1994;402:59–62.

50. Koletzko B, Mrotzek M, Bremer HJ. Fatty acid composition of mature human milk in Germany. Am J Clin Nutr 1988;47:954–9.

51. Agostoni C, Riva E, Scaglioni S, Marangoni F, Radaelli G, Giovannini M. Dietary fats and cholesterol in Italian infants and children. Am J Clin Nutr 2000;72:1384S–91S.

52. Owen CG, Whincup PH, Odoki K, Gilg JA, Cook DG. Infant feeding and blood cholesterol: a study in adolescents and a systematic review. Pediatrics 2002;110:597–608.

53. Bayley TM, Alasmi M, Thorkelson T, Krug-Wispe S, Jones PJ, Bulani JL, et al. Influence of formula versus breast milk on cholesterol synthesis rates in four-month-old infants. Pediatr Res 1998;44:60–7.

54. Edwards CA, Parrett AM, Balmer SE, Wharton BA. Faecal short chain fatty acids in breast-fed and formula-fed babies. Acta Paediatr 1994;83:459–62.

55. Fokkema MR, Woltil HA, van Beusekom CM, Schaafsma A, Dijck-Brower J, Muskiet FAJ. Plasma total homocysteine increases from day 20 to 40 in breastfed but not formula-fed low-birthweight infants. Acta Paediatr 2002;91:507–11.

56. Ford C, Rendle M, Tracy M, Richardson V. Vitamin b-12 levels in human milk during the first nine months of lactation. Int J Vitam Nutr Res 1996;66:329–31.

57. Rosenblatt DS, Whitehead VM. Cobalamin and folate deficiency: acquired and hereditary disorders in children. Semin Hematol 1999;36:19–34.

58. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, Ueland PM, et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. Am J Clin Nutr 2000;72:762–9.