Folate and cobalamin status in relation to diet in healthy 2-y-old children

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ABSTRACT

Background: Limited data exist on sources of folate and cobalamin in the toddler diet.

Objective: We examined the influence of diet on folate and cobalamin status in healthy toddlers in an unfortified population.

Design: Dietary intake was assessed in 178 children, aged 24 mo, by using 7-d food records and related to serum folate and cobalamin status in 155 children.

Results: Median (25th–75th percentile) daily intakes of folate and cobalamin were 87 μg (74–104 μg) and 3.1 μg (2.4–3.8 μg), respectively. Thirty-five percent of subjects had a folate intake below the Norwegian recommendations (80 μg folate/d), but only 5.8% of subjects had low serum folate concentrations (<10 nmol/L). All children reached the recommended cobalamin intake (0.8 μg cobalamin/d). Median (25th–75th percentile) serum concentrations were as follows: folate, 19 nmol/L (14–24 nmol/L); cobalamin, 410 pmol/L (334–521 pmol/L); holotranscobalamin, 94 pmol/L (67–121 pmol/L); holohaptocorrin, 315 pmol/L (241–409 pmol/L); methylmalonic acid, 0.16 μmol/L (0.13–0.20 μmol/L); and total homocysteine, 5.0 μmol/L (4.2–5.7 μmol/L). Folate intake correlated with serum folate concentrations (ρ = 0.25, P < 0.01), and cobalamin intake correlated with serum holotranscobalamin concentrations (ρ = 0.21, P < 0.05). In multivariate models, serum folate concentrations were significantly positively associated with the consumption of fruit and berries and grain products; however, this was not the case with dairy products, which was the food group that contributed most to folate intake. Cobalamin status was associated with dairy products (cobalamin and holotranscobalamin), cobalamin supplements (cobalamin and holohaptocorrin), and liver pâté (holotranscobalamin).

Conclusions: In this unfortified toddler population, folate status was associated with intakes of fruit and berries and grain products. Cobalamin status was associated with intakes of dairy, liver pâté, and supplements. In the assessment of vitamin sources, vitamin availability must be considered.

INTRODUCTION

The B vitamins folate and cobalamin play vital roles in the development of the brain, nervous system, and blood-forming organs and thus are essential for optimal growth and development in children. Folate participates in numerous single-carbon transfer reactions in the metabolism of nucleic and amino acids, whereas cobalamin functions as a coenzyme for a critical methyl transfer reaction that converts homocysteine to methionine and for a separate reaction that converts L-methylmalonyl-coenzyme A to succinyl-coenzyme A (1). Deficiency of cobalamin or folate may cause megaloblastic, macrocytic anemia as a result of interference with the normal DNA synthesis (1). In addition, cobalamin deficiency may cause demyelination of the central nervous system and, hence, neurologic disorders (2). Clinical symptoms (anemia or neurologic deficits) usually only become apparent at a late stage of deficiency (3).

Limited data exist on the serum status of these vitamins in children in unfortified populations (4, 5), and the interpretation of serum results is complicated because age-specific reference values related to functional outcome variables are sparse (4). Furthermore, there is little data on the influence of the various components in the toddler diet on folate and cobalamin status (6–8).

Many countries do not have general fortification of folate or cobalamin and therefore depend on a diet with sufficient amounts of food items with a natural content of these vitamins in a bioavailable form. Thus, it is of great interest to identify the sources of folate and cobalamin in the diet and, furthermore, to examine the association between various food groups and food items and relevant serum vitamin indexes.

We previously reported on iron, folate, and cobalamin serum status from a longitudinal study in children aged 0–24 mo (4, 9) as well as diet (4, 10). In the current study, we examined the folate and cobalamin intake in relation to serum vitamin status in healthy 2-y-olds by using data from 7-d weighed food records (WRs).

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Received September 8, 2010. Accepted for publication December 23, 2010. First published online January 26, 2011. doi: 10.3945/ajcn.110.003426.
SUBJECTS AND METHODS

Subjects

We examined serum status and dietary intakes of folate and cobalamin in a group of healthy Norwegian toddlers at 24 mo of age who were followed longitudinally from birth. Invitations were sent to 471 pregnant women of Norwegian or other Nordic descent who were registered to deliver at Aker University Hospital, Oslo, Norway, between April and June 1997. The inclusion process and study design was previously described (4, 9, 11). Out of the 364 infants included at birth, 192 children completed dietary records at 24 mo of age. We previously showed that breastfeeding status significantly influenced cobalamin status, even at 24 mo (4). Furthermore, it is difficult to assess nutrient intake in breastfed children. Therefore, we excluded 14 children who were still breastfed and one child with an unknown breastfeeding status, which left a total of 178 children with dietary records. For 155 of these children (68 girls and 87 boys), serum samples were collected.

Written informed consent was obtained from each child’s parent or parents. The study was approved by the Regional Committee for Research Ethics and the Norwegian Data Directorate.

Data collected

The data collected from the 2-y-old children included characteristics, dietary data (7-d WR), and blood samples. Information on breastfeeding status was obtained by questionnaires. Weight and height data, which were available in part of a proportion of the population, were collected from health care centers. In 119 children, measurements were performed between 23 and 25 mo of age. For another 20 children, results were extrapolated from the growth chart by using measurements collected at ages 18–23 or 25–30 mo. Food records were completed shortly before blood samples were drawn. The average (±SD) recording started 2.4 ± 2.8 wk before the child’s second birthday, and the blood sample was drawn 1 ± 2.6 wk before the child’s second birthday.

Dietary intake

The WR covered 7 consecutive days. For 160 children, 7 d were recorded. For some children, fewer or more days were recorded (5 d, n = 2; 6 d, n = 7; 8 d, n = 8; 9 d, n = 1). The average intake per day was used in the calculations.

Details on the collection of dietary data, including instructions to participants, have been described elsewhere (10). In short, participants were given careful instructions by 3 trained nutritionists who also coded the forms after 7-d WRs were completed. Parents were instructed to carefully record all intakes of food and drink in the food records. The use of supplements was also recorded (amount, types, and frequency). About 80% of the children attended day care or had a childminder outside the home, and one of the employees in the day-care center or the childminder was trained by one of the nutritionists to record the child’s diet.

The daily intakes of energy, nutrients, and food items were computed by using a food database and software systems developed at the University of Oslo (KOSTBEREGNINGSSYSTEM software, version 3.2; Department of Nutrition, University of Oslo, Oslo, Norway). The food database is mainly based on the official Norwegian food-composition table (12). The folate and cobalamin content in foods were determined by using microbiological assays (cobalamin, Lactobacillus delbrueckii (American Type Culture Collection 4965, Manassas, VA); folate, Lactobacillus casei (American Type Culture Collection 8014). Intake was calculated without losses from preparation methods. Folate and cobalamin intakes from supplements were determined on the basis of the frequency of intake and listed product content. The total intake of folate and cobalamin included vitamin intake from foods and supplements. There was no fortification with cobalamin, and cobalamin was not added to infant cereals at the time of the study. There was also no folic acid fortification of flour or other food items except for baby foods. In baby cereals to be reconstituted with water, the content of folate, including extra added folic acid, was 40–60 μg/100 g powder. Few infants consumed baby cereals at 24 mo of age (n = 39), and baby cereals contributed to <5% of the total folate intake in cereal consumers.

The content of folate and cobalamin in canned baby dinners (potatoes, rice, or pasta, vegetables, and meat or fish) was obtained from the near-exclusive manufacturer of baby foods at the time (Nestlé Norway, Oslo, Norway). The content was calculated by the manufacturer by using Nordic food-composition tables with an estimated 10% loss of cobalamin because of preparation. Details on the categorization of food groups are given in Table 1.

Blood sampling and blood analyses

Serum was obtained from venous nonfasting blood samples. The procedures of the blood sampling and handling of the samples have been published previously (4, 11). After sampling, serum was kept cold until stored in aliquots at −70°C. Serum indexes of folate status included serum concentrations of folate and total homocysteine (tHcy), whereas serum cobalamin status was assessed by using serum concentrations of cobalamin, holotranscobalamin, holohaptocorrin, methylmalonic acid (MMA), and tHcy. Microbiological assays were used to determine serum cobalamin and folate concentrations by using a colistin sulfate-resistant strain of Lactobacillus leichmanii (13) and L. casei (14), respectively. Serum folate concentrations were determined in samples that were not previously thawed. Serum holotranscobalamin concentrations were measured with magnetic beads (microspheres) with immobilized monoclonal antibody specific for human transcobalamin to isolate transcobalamin, followed by a microbiological assay for cobalamin (13). Holohaptocorrin (ie, cobalamin bound to haptocorrin) was calculated by subtracting holotranscobalamin from cobalamin amounts. The CV for serum folate and cobalamin in the laboratory is 5%, whereas the CV for holotranscobalamin is 5–8% (13). Serum MMA and tHcy were analyzed by a modified gas chromatography–mass spectrometry method on the basis of ethylchloroformate derivatization (15). The CVs for these measurements are <5%.

Statistical analyses

Nutrient intake, food intake data, and serum indexes were not normally distributed, and hence, medians and interquartile ranges are presented. The Mann-Whitney U test was used to compare independent groups. Bivariate correlations were examined using Spearman’s rank-order correlation test. Independent associations
were explored by using linear regression analyses with log values of serum indexes as outcome variables. Dependent variables included intakes of relevant food items or food groups (in quartiles), cobalamin supplement use (3 categories: 0, < 0.8, and ≥0.8 μg cobalamin/d) and folate supplement use (2 categories: users and nonusers). Analyses were adjusted for energy intake (serum folate status) and sex (serum cobalamin status). All nonusers). Analyses were adjusted for energy intake (serum folate and serum indexes, which were adjusted for the same variables as in the linear regression analyses. We did not control for multiple comparisons. The possibility of multicollinearity was examined by using the variance inflation factor. In all cases, the variance was not a cause for concern (16).

All statistical analyses were performed with PASW Statistics software (version 17.0; SPSS Institute, Chicago, IL). P < 0.05 was considered significant.

RESULTS

Characteristics, nutrient intake, and vitamin status

Characteristics of the population (mothers and newborns) were reported previously (11). Characteristics of the 2-y-olds (all children, boys, and girls separately), including intakes of selected nutrients and serum vitamin indexes, are listed in Table 2.

The median (25th–75th percentile) age of the children was 23.7 mo (23.4–24.2) mo. Boys were significantly heavier and taller than were girls. The energy intake was 4147 kJ/d (3692–4492) kJ/d. Protein, carbohydrates, and fat yielded 14% (12–15%), 52% (48–55%), and 34% (31–36%) of total energy intake, respectively. Sugar contributed 15% (11–19%) of the energy. Boys had significantly higher intakes of energy, carbohydrates, sugar, and folate than did girls.

In this unfortified population, the median intake of folate was 87 μg folate/d, and 63 out of 178 children (35%) had folate intakes below the Norwegian recommendations of 80 μg/d. Only 4 out of 178 children had folate intake above the US Recommended Dietary Allowance (RDA) (150 μg folate/d) (17). Folate intake was higher in boys than in girls, which was related to the higher energy intake in boys (data not shown). The median cobalamin intake was 3.1 μg cobalamin/d, and none of the children had cobalamin intakes below the Norwegian recommendations (0.8 μg cobalamin/d) or the US RDA (0.9 μg/d) (17). The cobalamin intake did not differ between the sexes. Children staying at home (n = 32) had significantly lower folate intakes than did children minded outside the home (n = 146) (P < 0.01). However, this difference was explained by the higher energy intake in the latter group; the difference disappeared with adjustment. There was no difference in cobalamin intake according to day care.

There was no difference in serum folate status between the sexes; however, girls had significantly higher holotranscobalamin and lower MMA concentrations than did boys. Serum folate status was significantly correlated with tHcy concentrations (r = −0.18, P < 0.05). Holotranscobalamin was significantly correlated with MMA concentrations (r = −0.41, P < 0.001) but only borderline correlated with tHcy concentrations (r = −0.14, P = 0.09). Neither total cobalamin nor holohaptocorrin concentrations correlated significantly with either MMA or tHcy concentrations.
Food intake and dietary sources of cobalamin and folate

Boys had significantly higher intakes of grain products, fruit and berries, fats and oils, and sweet beverages than did girls (see supplemental Table 1 under “Supplemental data” in the online issue for intakes of various food groups and food items in the total group and in girls and boys separately).

Dietary sources of folate and cobalamin on the basis of contribution to total vitamin intake are listed in Table 1. When calculated as the percentage of total intake for each child, the 3 main dietary sources of folate were 1) dairy products, 2) grain products, and 3) fruit and berries. These 3 food groups accounted for 70.5% of total folate intake. The use of folic acid–containing supplements was reported for only 7 children (4%), with a median intake from supplements in users of 20 µg folic acid/d (7.5–71.4 µg folic acid/d). The 3 main dietary sources of cobalamin were 1) dairy products, 2) meat products, and 3) fish products, which together accounted for 80.3% of total intake.

Cobalamin supplements were used by 59 children (33%). The contribution from supplements was modest, even in users [median: 0.73 µg cobalamin/d (0.25–1.08 µg cobalamin/d)] significantly correlated with total folate intake and intakes of fruit and berries and cakes and pastries and was borderline significantly correlated with the intake of grain products. However, the intake of dairy products, which was the food group with the highest contribution to total folate intake, was negatively correlated with serum folate concentrations, although not significantly. Serum folate concentrations were significantly negatively correlated with the percentage of energy from sugar and sweet beverages.

Serum holotranscobalamin concentrations were positively and significantly correlated with total cobalamin intake and the percentage of energy from protein and fat as well as intake of dairy products and liver pâté. Serum cobalamin concentrations were only significantly correlated with the intake dairy products. However, intakes of meat and fish products, which were the second and third largest contributors to total cobalamin intake, were not significantly correlated with any of the cobalamin indexes. The intake of liver pâté, although low in total amounts, was significantly correlated with holotranscobalamin concentrations, which possibly reflected the high cobalamin content. The use of cobalamin supplements (3 categories) was significantly correlated with serum cobalamin (r = 0.20, P < 0.05) and holotranscobalamin (r = 0.23, P < 0.01) concentrations.

Folate and cobalamin status related to dietary intake

Univariate analyses

Spearman’s correlations between intakes of nutrients and food groups and serum indexes of folate and cobalamin status are shown in Table 3. Serum folate concentration was positively and significantly correlated with total folate intake and intakes of fruit and berries and cakes and pastries and was borderline significantly correlated with the intake of grain products. However, the intake of dairy products, which was the food group with the highest contribution to total folate intake, was negatively correlated with serum folate concentrations, although not significantly. Serum folate concentrations were significantly negatively correlated with the percentage of energy from sugar and sweet beverages.

In regression analyses, we investigated the association between log serum folate and cobalamin indexes as dependent variables and quartiles of intakes of relevant food items (P < 0.1

Multivariate models

In regression analyses, we investigated the association between...
For holotranscobalamin, significant results were obtained for food groups and items. Intakes of supplements (partial categories) were entered into the model. For cobalamin, significant associations were observed for fruit and berries (partial \( r = 0.15, P < 0.01 \)) and dairy products (partial \( r = 0.11, P < 0.05 \)). For holotranscobalamin, significant results were obtained for intakes liver pâte (partial \( r = 0.31, P < 0.001 \)) and dairy products (partial \( r = 0.25, P < 0.01 \)). With holohaptocorrin as the dependent variable, only supplement use contributed (partial \( r = 0.19, P < 0.05 \)). For MMA, a borderline significant association was shown for the intake of liver pâte (partial \( r = 0.15, P = 0.06 \)). With the use of either the folate or cobalamin model, no associations were observed for tHcy concentrations.

Finally, we repeated the analyses for intakes of folate, cobalamin, and the most important sources of these nutrients by using ANOVA. Quartiles of intake of relevant food items or other categories (2 or 3 categories for folate and cobalamin supplements, respectively) were used in the models. The adjusted geometric means (95% CIs) of the relevant serum indexes were in univariate analysis) as independent variables. The analyses were adjusted for energy intake (folate and cobalamin status) and sex (cobalamin status). For serum folate, intakes of dairy products, grain products, fruit and berries, and sweet beverages were simultaneously included in the model. Significant associations were observed for fruit and berries (partial \( r = 0.25, P < 0.01 \)) and grain products (partial \( r = 0.17, P < 0.05 \)). For cobalamin indexes, intakes of dairy products, liver pâte, grain products, sweet beverages, and cobalamin supplements (3 categories) were entered into the model. For cobalamin, significant associations were shown for intakes of supplements (partial \( r = 0.19, P < 0.05 \)) and dairy products (partial \( r = 0.17, P < 0.05 \)). For folate, intakes of dairy products, liver pâte (partial \( r = 0.31, P < 0.001 \)) and dairy products (partial \( r = 0.25, P < 0.01 \)). With holohaptocorrin as the dependent variable, only supplement use contributed (partial \( r = 0.19, P < 0.05 \)). For MMA, a borderline significant association was shown for the intake of liver pâte (partial \( r = 0.15, P = 0.06 \)). With the use of either the folate or cobalamin model, no associations were observed for tHcy concentrations.

**FIGURE 1.** Geometric means (95% CIs) of serum folate and cobalamin status by quartile of total vitamin intake. Data shown were adjusted for energy intake (all markers) and sex (serum cobalamin and holotranscobalamin). \( P \) values were obtained by using ANOVA. The total numbers for each serum index were as follows: folate, \( n = 155 \); cobalamin, \( n = 155 \); holotranscobalamin (holoTC), \( n = 152 \).
estimated and plotted against the respective median folate or cobalamin intake in each category. Results are depicted in Figure 1 (total folate intake compared with serum folate concentrations and total cobalamin intake compared with serum cobalamin and holotranscobalamin concentrations) and Figure 2 (folate and cobalamin intake from various food items and supplements compared with serum folate, cobalamin, and holotranscobalamin concentrations). Folate intake was associated with a near linear increase in serum folate concentrations ($P < 0.001$), and a similar pattern was observed for intakes of fruit and berries ($P = 0.001$), grain products ($P = 0.08$), and folic acid–containing supplements ($P < 0.05$). Although dairy products were a major source of folate, no effect on serum folate status was apparent. Vegetables did not contribute, possibly because the intake of vegetables was low. For THcy, a significant effect was shown only for folic acid–containing supplements (geometric mean (95% CI) 4.22 (3.50–5.08) compared...
with 5.11 (4.92–5.30) in users and nonusers, respectively; \( P < 0.05 \).

Serum cobalamin and holotranscobalamin concentrations increased significantly with increasing total cobalamin intake \( (P < 0.05 \) for both). However, a plateau in serum concentrations for these 2 cobalamin indexes seemed to be reached at an intake of 3.0–3.5 \( \mu g \) cobalamin/d. Interestingly, the only other factor that influenced serum cobalamin concentrations was supplement use \( (P = 0.06) \) and only when used in doses above 0.8 \( \mu g \) cobalamin/d. The effect of supplements was significant for holohaptocorrin \( (P < 0.05) \) (data not shown) but not for holotranscobalamin concentrations.

Another interesting observation was the marked increase in holotranscobalamin concentrations in response to the intake of liver pâté. A similar intake of cobalamin from meat products was associated with nonsignificant changes. The intake of fish products was low in this group and not related to cobalamin indexes.

**Findings in children with low serum folate or serum cobalamin status**

Serum folate concentrations are defined as low at concentrations <10 nmol/L (18). Despite the low folate intake in our study population, only 9 out of 155 (5.8%) subjects had low serum folate concentrations. In children with low serum folate concentrations, the median tHcy concentration was modestly elevated \( (5.8 \mu mol/L \ [5.0, 8.2 \mu mol/L]) \) compared with 5.0 \( \mu mol/L \ [4.2, 5.6 \mu mol/L] \); \( P < 0.05 \). These children also had a significantly lower median total folate intake than did children with serum folate concentrations \( \geq 10 \) nmol/L \( (70 \mu g \) folate/d \ [58, 82 \( \mu g \) folate/d] \) compared with \( 90 \mu g \) folate/d \ [74, 106 \( \mu g \) folate/d]; \( P < 0.01 \). Intakes of food items of children with a low serum folate status did not differ significantly from those of children with serum folate concentrations \( \geq 10 \) nmol/L, possibly because of the small group with low values.

Only 3 children had serum cobalamin concentrations \( <150 \) pmol/L (1.4%), and 7 children had serum cobalamin concentrations \( <200 \) pmol/L (3.3%). In the latter group, MMA and tHcy concentrations were not significantly elevated. There were too few subjects with serum cobalamin concentrations \( <200 \) pmol/L and also dietary data \( (n = 4) \) to assess associations. If the low cobalamin group was expanded to include children with serum cobalamin concentrations \( <250 \) pmol/L \( (n = 12) \), a significant difference was seen only in meat intake (median: 21 compared with \( 35 \) g meat/d; \( P < 0.05 \)). The intake of dairy products was also lower (277 compared with \( 338 \) g dairy products/d); however, this difference was not significant.

**DISCUSSION**

We investigated diet related to folate and cobalamin status in a group of toddlers in a population without folic acid fortification. Folate and cobalamin status in this group was determined not only by the total intake of the vitamins but also by dietary sources. The intake of fruit and berries was most significantly associated with serum folate concentrations, whereas dairy products was the food group that was best related with serum cobalamin status.

Thirty-five percent of the children did not meet the age-appropriate Norwegian recommendations for folate intake \( (80 \mu g \) folate/d), and 98% of the children did not meet the US RDA \( (17) \) or the World Health Organization (WHO) recommended nutrient intake (RNI) \( (150 \mu g \) folate/d) \ (19). The Norwegian recommendations are based on a small study showing that 3.5–5.0 \( \mu g \) folate/kg maintained growth, hemapoiesis, and clinical wellbeing in infants aged 2–11 mo. The values have been adjusted to older children \( (20) \). The US RDA is based on extrapolation from adult values because of lack of data in children. This probably explains the marked differences in recommendations. Both approaches have shortcomings; and more data on folate intake, serum status, and clinical outcomes in children are critical.

The folate intake in our study (mean: \( 87 \mu g \) folate/d) was lower than that shown in other nonfortified toddler populations \( (21–23) \). In US children of the same age, the folate intake was much higher \( (mean: 254–311 \mu g \) folate/d) \ (24, 25), probably because of the US fortification practice combined with the frequent use of folic acid–containing supplements \( (~30\% \) compared with \( 4\% \) in our group) \ (24, 25). A direct comparison of data on folate status in a nonfortified population such as ours with a population markedly influenced by folic acid intake is difficult, if not impossible.

Despite the overall low dietary intake of folate, the proportion of children with low serum folate concentrations \( (<10 \) nmol/L) in our group was only 5.7%. Furthermore, the marked change in serum folate concentrations according to folate intake was not accompanied by a change in tHcy concentrations, which suggested that the serum folate concentration, even in the lowest quartile intake category, was sufficient to maintain low tHcy concentrations. Only in those few subjects who had a low serum folate concentrations \( (<10 \) nmol/L) was there a modest rise in tHcy concentrations. Because tHcy is a sensitive marker of folate status in children \( \geq 24 \) mo of age \ (26), these results indicate that the recommendations for folate intake in this age group, in particular that of 150 \( \mu g \) folate/d, may be unnecessary high. In the United States, the combination of fortification and supplement use results in high intakes of folic acid in children, with many exceeding the tolerable upper intake \( (24, 27) \).

Among food groups, the intake of fruit and berries was best associated with serum folate concentration. The intake of grain products was also associated. These food groups were the second and third largest contributors to dietary folate intake. In contrast, milk products, which were apparently the best source of dietary folate, were not associated with folate status. Notably, milk folate has been quantified after pasteurization, and most of the milk in this study was consumed without further processing. Thus, heat loss is not a likely cause. All naturally occurring folates are chemically labile and poorly bioavailable because they exist as polyglutamates, and as much as three-quarters of the dietary folate may be unavailable for absorption \ (19). A similar lack of association between folate in dairy products and serum folate has also been observed in adults \ (28, 29); however, in these populations, the contribution of folate from dairy was modest. Therefore, the lack of association between dairy intake and serum folate status, even after adjustment for other sources, could be related to poor bioavailability, which is a concern that should be addressed in future studies. The negative effect of sugar and sweet beverages on serum folate status is likely to be caused by a diluting effect on diet quality, as has been shown by others, especially in girls \ (30).
The median intake of cobalamin (3.1 μg cobalamin/d) was high compared with the Norwegian recommendation of 0.8 μg cobalamin/d or the 0.9 μg cobalamin/d used by the US RDA and the WHO RNI (17, 19). None of the children had cobalamin intake below the cutoffs. Total serum cobalamin and holotranscobalamin concentrations increased significantly according to cobalamin intake. For holotranscobalamin, a plateau was reached at intakes of ~3 μg cobalamin/d. However, neither MMA nor tHcy concentrations decreased with increasing cobalamin intakes in these children, which could reflect that all children had quite good cobalamin status. Without considering the effect on serum indexes, dairy products were clearly the best source of cobalamin and accounted for about one-half of the intake (44%), whereas meat contributed one-fourth of intake. Fish products were the third most important contributor to cobalamin intake (11%).

In the multivariate models, the intake of dairy products was significantly associated with both cobalamin and holotranscobalamin as the dependent variable. Thus, our results are in agreement with previous findings (31) that cobalamin from dairy products appears to be highly available. Vogiatzoglou et al (31) showed that plasma cobalamin concentrations increased in adults with increasing intakes of total cobalamin (up to ~10 μg cobalamin/d) and with the intake of dairy products. A significant association was also shown for fish but not for meats or eggs. The lack of association with fish intake in our study may be due to the low fish intake in this age group. Apart from milk products, we showed positive associations for intakes of liver pâté (significant for holotranscobalamin) and cobalamin supplements (holohaptocorrin). Liver pâté has a high content of cobalamin, and this is a popular spread among Norwegian children. Microgram for microgram, the same amount of cobalamin in liver pâté appeared more bioavailable than did cobalamin in meat products (Figure 1). Liver was the source that was successfully used by Minot and Murphy in the initial treatment of pernicious anemia (32). The effect was explained by the high cobalamin content of liver. However, it is possible that it is not only the high content in liver but also that cobalamin in liver is more available for absorption than is cobalamin in meat.

The finding that the effect of cobalamin-containing supplements was stronger for holohaptocorrin and cobalamin than for holotranscobalamin is in agreement with our previous observations in infants aged 6 and 12 mo (4). Overall, our data suggested that holotranscobalamin was more significantly associated with cobalamin intake from foods than was serum cobalamin and holohaptocorrin concentrations. The serum holotranscobalamin concentration is believed to reflect recent changes in cobalamin intake (33, 34), and this probably explains why holotranscobalamin appears to be a better marker for the assessment of dietary cobalamin sources. Strengths of the study included the dietary survey method (7-d WR) combined with multiple folate and cobalamin markers. The WR has been shown to be the method that provides the best estimate for energy intake in children 0.5–4 y of age compared with the doubly labeled water method (35). Underreporting leads to the underestimation of nutrient intake. However, only 7 children in our study had an energy intake <3 MJ (10), which suggested overall good data on most children. Weaknesses with our study were that we did not have blood samples for the complete data set, data on weight and height were incomplete, and only children with Nordic heritage were included. Results from dietary studies should be interpreted with caution and always take ethnic and regional differences into account. For example, one of the best sources of cobalamin in our study, liver pâté, is popular among children in Norway but may not be consumed by children in many other countries. Furthermore, as with all cross-sectional studies, we could only report associations and not document causality.

In conclusion, we have shown that folate and cobalamin intake in toddlers were reflected by changes in serum vitamin concentrations. Concentrations of metabolic markers tHcy and MMA did not change according to intake, which suggests that functional vitamin status was sufficient, even if the folate intake in a large proportion of the children was below recommendations. Dairy products provided dietary folate and cobalamin but were only associated with serum cobalamin status. The food group best associated with serum folate status was fruit and berries. Liver pâté was both a good dietary source of cobalamin and was significantly associated with serum holotranscobalamin concentrations. In contrast, meat, although rich in cobalamin, had no effect on serum cobalamin status. Finally, our results raise the question about the Dietary Reference Intake for these vitamins in children, in particular for folate; this issue should be addressed in larger studies and preferably also in populations with a wider range of intake.

We are indebted to the families who participated in the study, the laboratory technicians at Aker University Hospital under the leadership of Britt Eieland, and several project workers and graduate students who contributed considerably to the study. We especially thank Marianne Hope Abel, Gunn Helene Arsky, Janne Liabø, Ellen Margrethe Holvand, Kirsti Kverndokk Bjerkan, and Kathrine C Haavardsholm for their invaluable contribution to data collection and Otto Andreas Bårholm and Cynthia Prendergast for help with analyses of cobalamin and folate indexes. We greatly appreciated Elfrid Blomdal’s help with the literature search.

The authors’ responsibilities were as follows—GH, KT, and AW: designed the research; GH: conducted the research; GH, KT, CJ, and HR: analyzed the data; GH and HR: performed statistical analyses and wrote the manuscript; HR: had primary responsibility for the final content; AW: critically reviewed the manuscript; and all authors: read and approved the final manuscript. The funding sources had no direct influence on the design, collection, and analyses of the data or the decision to submit this report for publication. None of the authors reported a conflict of interest.

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