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Dietary Intake and Biomarkers of Folate and Cobalamin Status in Norwegian Preschool Children: The FINS-KIDS Study

Beate S Solvik,[1,2] Tor A Strand,[1,2] Ingrid Kvestad,[3] Maria W Markhus,[4] Per M Ueland,[5] Adrian McCann,[5] and Jannike Øyen[4]

[1]Innlandet Hospital Trust, Lillehammer, Norway; [2]Centre for International Health, University of Bergen, Bergen, Norway; [3]Regional Center for Child and Youth Mental Health and Child Welfare, NORCE Norwegian Research Center, Bergen, Norway; [4]Institute of Marine Research, Bergen, Norway; and [5]Bevital AS, Bergen, Norway

ABSTRACT

Background: Folate and cobalamin (vitamin B-12) are essential for growth and development. However, few population-based studies have investigated B-vitamin status in children.

Objectives: This study aimed to assess biomarkers of folate and vitamin B-12 status and to explore their dietary determinants in healthy Norwegian children.

Methods: Using baseline data obtained from a randomized controlled trial on the effect of fish intake on neurodevelopment in children aged 4–6 y, we measured the plasma concentrations of folate, cobalamin, total plasma homocysteine (tHcy), and methylmalonic acid (MMA). Food-frequency questionnaires (FFQs) were used to assess dietary intake. We used unadjusted and multiple linear regression models to explore the determinants of biomarker concentrations.

Results: The median (IQR) of plasma folate ($n = 197$) and plasma cobalamin ($n = 195$) concentrations were 15.2 (12.2–21.1) nmol/L and 785 (632–905) pmol/L, respectively. Plasma folate concentrations of <10 nmol/L were observed in 13% of the children. No child had a cobalamin concentration <148 pmol/L. Two children were identified with elevated plasma MMA concentrations (>0.26 μmol/L) and 8 children had elevated tHcy concentrations (>6.5 μmol/L). Plasma folate concentration was inversely correlated with tHcy ($\rho = -0.24$, $P < 0.001$); we found no correlation between tHcy and cobalamin ($\rho = -0.075$, $P = 0.30$). Children who consumed vitamin supplements had 51% higher plasma folate concentrations ($P < 0.0001$) than those who did not. Consumption of red meat for dinner more than twice a week was associated with 23% lower plasma folate ($P < 0.01$). No other significant associations between dietary intake and the biomarkers were observed.

Conclusions: The Norwegian preschool children from this cohort had adequate vitamin B-12 status. Poor folate status was common and associated with elevated tHcy. The implications of poor folate status during childhood should be a prioritized research question. This trial was registered at ClinicalTrials.gov as NCT02331667. *J Nutr* 2020;150:1852–1858.

Keywords: children, folate, cobalamin, homocysteine, methylmalonic acid, vitamin B-12

Introduction

Deficiencies of folate and cobalamin (vitamin B-12) represent a common health issue affecting both high- and low-income countries (1). These B-vitamins are essential for optimal growth and development through critical physiological functions, such as DNA synthesis, cell division, RBC maturation, and myelination of the nervous system (2, 3).

Typically, folate and vitamin B-12 deficiencies are associated with hematologic changes, including megaloblastic anemia, but the impact of mild or subclinical deficiency is less understood (4). Subclinical deficiency may not lead to immediate clinical symptoms but has, over the long-term, been associated with poor growth and impaired neurodevelopment (5–10). Early detection of subclinical deficiency is important and dependent on circulating functional markers such as total plasma homocysteine (tHcy) and methylmalonic acid (MMA) (3, 11).

Both folate and cobalamin are essential for methionine synthase, an important enzyme for the synthesis of methionine from homocysteine (12). Deficiency of either of the aforementioned vitamins will lead to elevated tHcy, a functional marker and potential risk factor for cardiovascular disease and impaired cognition (3, 13, 14). In a separate reaction, cobalamin serves as a cofactor for methylmalonyl-CoA mutase,

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an essential enzyme in energy metabolism, that converts L-methylmalonyl-CoA to succinyl-CoA. Impaired cobalamin status will, consequently, lead to an accumulation of MMA—a functional marker of cobalamin status (11). Each of the biomarkers has its limitations, in addition to being influenced by factors such as age, lifestyle, and disease (15). Algorithms that combine these markers, such as the combined indicator of vitamin B-12 (cB12), have therefore been proposed (16). This algorithm was originally modeled to define vitamin B-12 status based on tHcy, MMA, cobalamin, and holotranscobalamin (holo-TC). Holo-TC is another established marker of cobalamin (16). Recently, the cB12 has been improved to adjust for age, folate concentrations, and the absence of 1 or 2 biomarkers (17).

Fruits and vegetables are excellent sources of folate. According to national dietary surveys, however, the consumption of these food items in Norwegian children is below dietary recommendations (18). Moreover, Norway does not have a folic acid fortification policy, which, in turn, increases the risk of folate deficiency. As the primary dietary source of cobalamin is animal-derived foods, deficiency is less likely among children consuming a typical Western omnivore diet. Most evidence on cobalamin deficiency in Western societies has been observed among children with inborn errors of cobalamin metabolism, malabsorption, and in high-risk groups such as vegans (19). In addition, Norwegian studies have suggested that breastfed infants may be prone to cobalamin deficiency (20, 21).

Little is known about vitamin status in preschoolers, and biochemical values in children are difficult to interpret due to limited age-specific reference values. Thus, it is of great interest to evaluate B-vitamin status in a healthy population of children. We have previously reported on iodine (22) and vitamin D (23) status among Norwegian preschool children participating in a randomized controlled trial (24). In the current study, we investigated folate and vitamin B-12 status and their associations with dietary intake.

Methods

Study procedure and participants

We examined baseline data from a randomized controlled trial; the Fish Intervention Studies–KIDS (FINS-KIDS). A total of 70 kindergartens from different districts in Bergen municipality, Norway, were invited to participate. Children were recruited from the 13 kindergartens that agreed. The study was undertaken from January to June 2015. The general trial design, enrollment, blinding, and randomization have been described elsewhere (24). The ethical procedures were in accordance with the Declaration of Helsinki. Prior to study start, the participants' caregivers provided signed, informed consent. The trial was approved by the Regional Committees for Medical and Health Research Ethics North (2014/1396) and registered in ClinicalTrials.gov (NCT02331667).

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Address correspondence to JO (e-mail: jannike.oyen@hi.no).
Abbreviations used: cB12, combined indicator of vitamin B-12; FFQ, food-frequency questionnaire; FINS-KIDS, Fish Intervention Studies–KIDS; holo-TC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total plasma homocysteine; 3cB12, 3 combined indicator of vitamin B-12 status.

Assessment of folate and cobalamin status

Blood sampling was performed in the kindergartens. Venous blood for plasma preparation was collected in BD Vacutainer® K2E 7.2-mg vials (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and centrifuged (1000 × *g*, 20°C, 10 min) within 30 min of sampling. In cases in which venipuncture was problematic, capillary blood was collected from the fingertip with an ACCU-CHEK® Safe-T-Pro Plus lancet (Roche Diagnostics, Rotkreuz, Switzerland) into BD Microtainer® blood collection tubes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). EDTA-plasma was transferred to cryotubes (Nunc, Roskilde, Denmark) and transported on dry ice to storage at −80°C until analyses. Plasma folate and cobalamin concentrations were determined by microbiological assays based on a chloramphenicol-resistant strain of *Lactobacillus casei* (25) and colistin sulfate-resistant strain of *Lactobacillus leichmannii* (26), respectively. The functional markers, tHcy and plasma MMA, were analyzed by GC–tandem MS (GC-MS/MS) based on methylchloroformate derivatization (27). The within-day CV was 4% for both folate and cobalamin and ranged from 1% to 5% for tHcy and MMA. The between-day CV was 5% for both folate and cobalamin and ranged from 1% to 3% for tHcy and MMA (27). All blood samples were analyzed at Bevital Laboratory, Bergen, Norway (www.bevital.no).

Definitions and cutoff values

To our knowledge, no age-specific reference values exist for plasma folate and cobalamin concentrations. Therefore, the following cutoffs were used: plasma folate concentrations of >45.3 nmol/L, <13.4 nmol/L, and <6.8 nmol/L were considered as "elevated," "possible deficiency," and "deficiency," respectively (28). The WHO also suggests a cutoff of <10 nmol/L, which we also used (28). Moreover, plasma cobalamin concentrations of <148 pmol/L, 148–221 pmol/L, and >221 pmol/L were considered as "B-12 deficiency," "low B-12," and "B-12 adequate," respectively (15). For adults, plasma tHcy and MMA concentrations >13 μmol/L and 0.26 μmol/L, respectively, are considered as elevated concentrations (15). For children, tHcy values >6.5 μmol/L have been used to indicate elevated concentrations in a Norwegian intervention study (20). We calculated the 3 combined indicator of vitamin B-12 status (3cB12) by using the log of cobalamin over the product of tHcy and MMA concentrations, minus an age factor. A score of < −0.5 is considered low vitamin B-12 status (16). The score is corrected for low folate status (<10 nmol/L) (17).

Dietary intake

A modified and validated version of a short food-frequency questionnaire (FFQ) was completed online by one of the participant's caregivers (29). The FFQ contained questions regarding the consumption of meat, fish, dairy products, eggs, fruits, and vegetables during the preceding 3 mo. The frequencies were categorized as follows: never, <1 times/mo, 1–3 times/mo, 1 or 2 times/wk, or ≥3 times/wk. In addition, information concerning the consumption of dietary supplements was collected, defined as never, 1–3 times/mo, 1–3 times/wk, 4–6 times/wk, or daily. To estimate weekly dietary intake (**Table 1**), we converted frequency data from the FFQ to numeric data by using methods based on a previously validated seafood index (30). The questionnaire also collected information on demographics, such as children's weight, height, parental education, and family income.

Statistical methods

Categorical variables were reported as frequencies and percentages (gender and family income). The dietary intake responses were given as dinner, portion or number per week, or as portions per day. Age, height, weight, and years of parents' education were reported as means and SDs. Other continuous variables were reported as medians and IQRs due to the skewed distribution of the data. The biomarkers were also enumerated (frequency and percentages) according to suggested cutoff values. Unadjusted linear regression analyses were used to explore the possible associations between biomarker concentrations (log-transformed concentrations of cobalamin, folate, tHcy, and MMA, and the 3cB12) and dietary variables (vitamin supplements, fish, meat,

TABLE 1 Baseline characteristics of Norwegian children aged 4–6 y[1]

	n	Values
Demographics		
Age, y	197	5.2 ± 0.6
Body height, cm	163	114 ± 6.3
Body weight, kg	161	20.2 ± 3.1
Parents' education, y	175	15.4 ± 1.7
Boys, %	96	48.7
Girls, %	101	51.3
Gross household income (NOK),[2] %		
<200,000–749,999	42	24.1
750,000–1,249,999	102	58.6
>1,250,000	30	17.2
Dietary intake from FFQ		
Seafood, dinners/wk	181	1.7 ± 0.9
Red meat, dinners/wk	181	2.5 ± 0.9
Chicken, dinners/wk	181	1.3 ± 0.9
Eggs, number/wk	181	1.6 ± 1.1
Dairy products, portions/d	181	1.8 ± 1.0
Fruits and vegetables, portions/d	181	2.9 ± 1.3
Vitamin supplements (1–7 times/wk), %	67	37.4
Vitamin supplements (<1 time/wk), %	112	62.6
Biomarkers		
Plasma folate, nmol/L	197	15.2 (12.2–21.1)
Plasma cobalamin, pmol/L	195	785 (632–904)
Plasma tHcy, μmol/L	197	4.71 (4.23–5.34)
Plasma MMA, μmol/L	197	0.15 (0.13–0.18)
3cB12	195	1.26 (1.01–1.43)

[1]Values are means ± SDs or medians (IQRs). FFQ, food-frequency questionnaire; MMA, methylmalonic acid; NOK, Norwegian Krone; tHcy, total plasma homocysteine; 3cB12, 3 combined indicator of vitamin B-12 status.
[2]Median gross household income in Norway, 2015: 628 000 NOK (100 NOK = ~10€ or 11 US$) [Statistisk Sentralbyrå. 06944: Inntekts- og formuesstatistikk for husholdninger 2005–2018 (Income and Wealth Statistics for Households 2005–2018). Oslo (Norway): Statistics Norway; 2018. Available from: https://www.ssb.no/en/statbank/table/06944/].

dairy, egg, fruits, and vegetables). The variable "Vitamin supplements" was categorized as yes (1–7 times/wk) or no (1–3 times/mo or never). For bread, the variable was categorized as white (0–50% whole-wheat flour or whole grains) or whole-grain bread (50–100% whole-wheat flour or whole grains). The remaining dietary variables were categorized as more or less than 2 times/wk. The analyses were also undertaken adjusted for gender and parental education. Spearman's rank-order correlation coefficient was used to describe the monotonic correlations between the different biomarkers. To explore nonlinear relations between the biomarkers, fractional-polynomial prediction plots were used. Outliers defined as values >97.5th percentile were not included in the plot due to few observations in the tails, causing a high uncertainty in this range. Statistical analyses were performed using the statistical software Stata, version 15 (StataCorp.). *P* values <0.05 were considered statistically significant.

Results

General characteristics

From the participating kindergartens, a total of 232 out of 314 eligible children agreed to participate. Baseline concentrations of folate, tHcy, and MMA were available for 197 children, while cobalamin concentrations were available for 195 children. Data from the online questionnaire were available for 181 of these children. Children's demographic information and FFQ responses are summarized in Table 1. Both intakes of seafood (1.7 portion/wk) and vegetables plus fruits (2.9 portions/d) were below the Nordic dietary recommendations (31). No children were vegetarian or vegan.

TABLE 2 Suggested cutoff values and corresponding prevalence of deficiency among Norwegian children aged 4–6 y[1]

Cutoff	Definition (ref)	*n* (%)
Plasma folate <13.4 nmol/L	Possible deficiency (28)	73 (37.1)
Plasma folate <10 nmol/L	Deficiency (28)	26 (13.2)
Plasma folate <6.8 nmol/L	Deficiency (28)	6 (3.0)
Plasma folate >45.7 nmol/L	Elevated folate (28)	7 (3.6)
Plasma cobalamin <148 pmol/L	Deficiency (15)	0 (0.0)
Plasma cobalamin 148–221 pmol/L	Low vitamin B-12 (15)	1 (0.5)
tHcy >13 μmol/L	Elevated (15)	0 (0.0)
tHcy >6.5 μmol/L	Elevated (20)	8 (4.1)
Plasma MMA >0.26 μmol/L	Elevated (15)	2 (1.0)
3 Combined indicator of vitamin B-12 < −0.5	Low status (17)	0 (0.0)

[1]Values are counts and percentages. References for cutoff values and definitions are included in parentheses.
n = 197 for folate, tHcy, and MMA; *n* = 195 for plasma cobalamin and 3 combined indictor of vitamin B-12. MMA, methylmalonic acid; tHcy, total plasma homocysteine.

Folate and cobalamin status

The plasma concentrations of both direct and functional biomarkers as well as the 3cB12 are given in Table 1. Suggested cutoff values and respective prevalences are given in Table 2. Correlations between the plasma biomarkers are shown in Table 3. Plasma folate was inversely correlated with plasma tHcy ($P < 0.001$), and the correlation was also observed in a nonlinear correlation plot (Figure 1).

Dietary intake and its association with folate and cobalamin status

Results from the unadjusted regression analysis are given in Table 4. Children who consumed solid or liquid vitamin supplements had 51% higher plasma folate concentrations ($P < 0.0001$), whereas consumption of red meat for dinner >2 times/wk was associated with 23% lower plasma folate ($P < 0.01$) and 9% higher tHcy ($P < 0.05$). These associations remained after adjustments for gender and parental years of education. No other significant associations between dietary intake and biomarkers were observed. We did not find any correlations between red meat and fruit ($\rho = 0.007$, $P = 0.92$) or vegetable ($\rho = 0.03$, $P = 0.67$) intake.

Discussion

In this cross-sectional analysis, the status of folate and cobalamin and their relation to dietary intake were examined

TABLE 3 Correlation coefficients between direct and functional plasma biomarkers of folate and cobalamin status among Norwegian children aged 4–6 y[1]

	Folate	Cobalamin	tHcy	MMA
Folate	1.00			
Cobalamin	0.064	1.00		
tHcy	−0.25*	−0.09	1.00	
MMA	−0.073	−0.135	0.114	1.00

[1]Values are Spearman's rank correlation coefficient. *n* = 195. *$P < 0.001$. MMA, methylmalonic acid; tHcy, total plasma homocysteine.

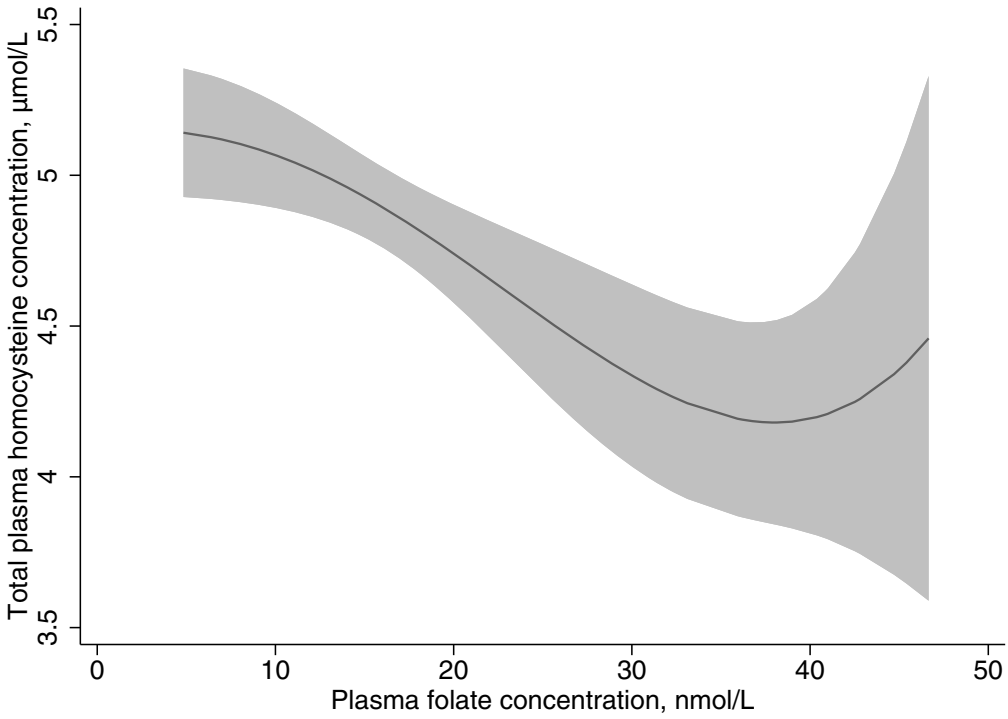

FIGURE 1 Correlation between plasma total homocysteine and plasma folate among Norwegian children aged 4–6 y. The solid line represents a 2-way fractional-polynomial prediction plot; the gray area represents the 95% CI.

in 197 healthy Norwegian preschoolers. Among the children in this cohort, 13% had a folate concentration <10 nmol/L, whereas all children had an adequate cobalamin concentration. Daily intake of vitamin supplements was directly associated with plasma folate concentration, while a high intake of red meat was associated with lower plasma folate and tHcy. As expected, plasma folate concentration was inversely correlated with plasma tHcy. We observed that 1 out of 8 children had a folate concentration <10 nmol/L, and the median folate concentration was 15.2 nmol/L. According to WHO's definitions of folate deficiency, 73 (37%) were possibly deficient and 6 (3%) were deficient.

The FINS-KIDS cohort had a lower median folate concentration compared with other European studies, where reported values ranged from ~19 to 25 nmol/L (32–34). In a British study, children aged 4–10 y who consumed fortified cereals and vitamin supplements had a higher folate concentration compared with consumers of nonfortified cereals and non–vitamin-supplement users (32). We did not explore the relation between the B-vitamins and fortified cereals in the current study, as these items are not commonly available in Norway. We found, however, a direct association between vitamin supplement use and plasma folate concentrations. The effect of regular supplement consumption on plasma folate concentrations has previously been observed in other studies (35). This is expected, as supplements typically contain folic acid, a synthetic form of folate, which is more bioavailable than folate in its natural form (36).

We identified an inverse association between meat intake and folate status. A reasonable explanation is that children who eat more meat might eat fewer fruits and vegetables. We did not find such a relation between meat intake and fruit and vegetable intake. The crudeness of the dietary assessment method, however, might have limited the ability to identify such an association.

A study in Norwegian 2-y-old children found a direct association with fruit and berry intake and circulating folate concentrations (34). We did not identify such associations in the present study. The implemented semiquantitative FFQ used in our study was designed and validated to assess intake of seafood and omega-3 fatty acids (29). Hence, our FFQ may not be optimal in assessing the intake of other food items and their associations with non–seafood-related biomarkers.

In the present study, no children were identified with vitamin B-12 deficiency (plasma cobalamin <148 pmol/L) and the median cobalamin concentration was 785 pmol/L. The absence of vitamin B-12 deficiency is in accordance with the aforementioned study among Norwegian children (34). Among these 2-y-old children, Hay and colleagues (34) found that only 3% had cobalamin concentrations <150 pmol/L and the median was 407 pmol/L. The lower concentrations observed by Hay and colleagues are comparable to other European studies that included preschool children (32, 33). Among Dutch children aged 2–5 y, the geometric mean plasma cobalamin concentration was 497 pmol/L (33). Similarly, in the British study discussed above among children aged 4–10 y, the median serum cobalamin concentrations were 471 and 436 pmol/L in boys and girls, respectively (32). A common feature of the latter studies is a decline in cobalamin status from early childhood towards adolescence (32, 33). In another Norwegian cross-sectional study, which included children aged 4 d to 19 y, the concentration of cobalamin increased from 6 mo of age to a maximum at 3–7 y of age before declining (37). If the children in the FINS-KIDS population follow the same trend, we would anticipate that they are currently at their peak in terms of vitamin B-12 status. This trend, and the fact that all

TABLE 4 Generalized linear models for the associations of dietary variables with log-transformed plasma concentrations of folate, cobalamin, tHcy, MMA, and 3cB12[1]

Variables	*n*	Plasma folate[2]	Plasma cobalamin[2]	Plasma tHcy[2]	Plasma MMA[2]	3cB12[3]
Supplements						
<1 time/wk	112	Ref	Ref	Ref	Ref	Ref
≥1 time/wk	67	1.51 (1.32, 1.73)*	1.00 (0.90, 1.11)	0.97 (0.92, 1.03)	0.97 (0.91, 1.05)	0.03 (−0.70, 0.12)
Egg						
<2 times/wk	106	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	77	1.01 (0.89, 1.16)	0.96 (0.86, 1.06)	1.00 (0.95, 1.06)	1.02 (0.95, 1.10)	− 0.04 (−0.13, 0.05)
Bread						
White bread	33	Ref	Ref	Ref	Ref	Ref
Whole grain	148	0.97 (0.82, 1.16)	0.93 (0.81, 1.07)	1.02 (0.95, 1.10)	1.07 (0.98, 1.18)	− 0.10 (−0.22, 0.03)
Dairy						
<2 times/d	91	Ref	Ref	Ref	Ref	Ref
≥2 times/d	90	1.05 (0.92, 1.19)	1.08 (0.97, 1.19)	1.00 (0.95, 1.06)	0.96 (0.90, 1.03)	0.07 (−0.02, 0.16)
Poultry						
<2 times/wk	131	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	50	0.99 (0.86, 1.15)	1.04 (0.93, 1.17)	1.04 (0.98, 1.11)	1.01 (0.93, 1.09)	− 0.01 (−0.11, 0.10)
Red meat						
<2 times/wk	26	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	155	0.77 (0.64, 0.92)**	0.93 (0.80, 1.08)	1.09 (1.00, 1.18)***	1.04 (0.94, 1.15)	− 0.11 (−0.24, 0.02)
Fish						
<2 times/wk	93	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	88	0.92 (0.81, 1.05)	1.07 (0.97, 1.19)	1.00 (0.94, 1.06)	0.94 (0.88, 1.01)	0.08 (0.01, 0.18)
Fruit						
<2 times/wk	58	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	123	1.03 (0.88, 1.21)	0.88 (0.78, 1.00)	0.93 (0.87, 1.00)	0.99 (0.91, 1.08)	− 0.04 (−0.15, 0.08)
Vegetables						
<2 times/wk	34	Ref	Ref	Ref	Ref	Ref
≥2 times/wk	147	0.90 (0.75, 1.09)	1.12 (0.96, 1.30)	1.01 (0.93, 1.09)	0.98 (0.88, 1.08)	0.10 (−0.04, 0.23)

[1] * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$. MMA, methylmalonic acid; Ref, reference; tHcy, total homocysteine; 3cB12, combined indicator of vitamin B-12 status including 3 biomarkers (cobalamin, MMA, and tHcy).
[2] Data are exponentiated regression coefficients (95% CIs) of unadjusted linear regression.
[3] Data are regression coefficients (95% CIs) of the unadjusted linear regression generalized.

of the children had an omnivore diet, may, in part, explain the high cobalamin concentration observed in the present study. We observed elevated MMA in 2 children. It has been suggested that propionate, produced by intestinal bacteria or by catabolism of odd-chain fatty acids, acts as a precursor to MMA (38). Consequently, an increase in propionate may result in elevated MMA. The plasma concentration of odd-chain fatty acids has been associated with dietary fiber and dairy fat intake (39, 40). This indicates that elevated MMA could be due to alterations in the microbiota or to other dietary factors not captured by our assessments. Moreover, we did not identify any associations between dietary intake and the markers of vitamin B-12 status. The absorption of vitamin B-12 varies by food items and the absorption is not proportional with dietary intake due to a proposed saturation of the active absorption mechanism (41). Given that all participants had an omnivore diet, it is reasonable to believe that most of the children had a dietary intake of vitamin B-12 sufficient to saturate the absorption mechanisms. This, in turn, will dilute the potential associations between dietary intake and plasma biomarkers of vitamin B-12 status.

Although we did not identify any correlations between plasma concentrations of tHcy or MMA with cobalamin, we found an inverse correlation between plasma concentrations of tHcy and folate. In the study in Dutch children, elevation of tHcy was mainly observed when plasma cobalamin concentrations were <200 pmol/L (33). The lack of a correlation between tHcy and cobalamin in the current study may be explained by the adequate vitamin B-12 status, in which only 1 child had a concentration <200 pmol/L.

Our observed correlation between plasma tHcy and folate is consistent with findings from other studies, suggesting that tHcy is a sensitive biomarker for folate status among children (37). Given that 13% of the children in this study sample were categorized as folate deficient (<10 pmol/L), the correlation with tHcy is of significance and could indicate that low folate status in children has metabolic consequences (Figure 1). Although elevated tHcy has been associated with cardiovascular disease (13) also in children (3), the high tHcy is probably caused by disruption of the methionine cycle due to low availability of folate. The clinical consequences of tHcy in this range are uncertain but may reflect an increased risk of cardiovascular illness. Strategies to reduce plasma tHcy concentrations, such as increased intake of folate or folic acid, may therefore be an important public health consideration for all ages. In addition, our data suggest that children have a lower fruit and vegetable intake than recommended (31), and although we did not find an association between fruit and vegetable intake and folate status, an increased consumption of these foods could also be a feasible approach to improve folate status and consequently decrease plasma tHcy concentration. This also further emphasizes the importance of developing healthy dietary patterns in early childhood for the prevention of disease later in life.

It should also be mentioned that neither vitamin D nor iodine deficiency were common in this population. The median spot

urinary iodine concentration was 132 μg/L (22), and ~80% of the children had vitamin D concentrations 50 nmol/L during wintertime (23).

The current study has some limitations. It was not designed to estimate the prevalence of folate or vitamin B-12 deficiencies, nor designed to measure dietary intake in relation to B-vitamin status. In addition, the cutoff values for defining deficiencies are not clear, particularly for children. The applied FFQ was validated for dietary intake of fish and seafood and did not accurately estimate the portion size (29). Consequently, there were few questions with regard to certain food groups important for the B-vitamins that are the focus of the current study (e.g., meat, fruits, and vegetables). Given these limitations, and due to the inadequacy of portion size for children, we were not able to estimate the nutrient intake of folate and vitamin B-12. The participants were instead ranked according to the level of dietary intake (> or <2 times/wk) for each food group (Table 4). This is a crude method, which limits the ability to identify associations between diet and biomarkers. Moreover, the FFQ was not validated for this population. We did not assess holo-TC in the present study. The complete combined indicator of vitamin B-12 status was originally designed for 4 biomarkers: cobalamin, tHcy, MMA, and holo-TC. Using only 3 biomarkers, as we did in our analyses, could limit the validity of this indicator (17). Nevertheless, the datasets that were used for modeling the algorithms of cB12 did not include children, which limits the interpretation of this indicator regardless of whether holo-TC was included or not (17).

To conclude, in this study population of Norwegian preschool children representing an unfortified population, 1 out of 8 children had poor folate status while all had adequate vitamin B-12 status. Plasma folate was inversely correlated with tHcy, and vitamin supplement consumption was the main dietary determinant for plasma folate concentration. The implications of poor folate status during childhood should be a prioritized research question.

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