

Dietary Intakes of Folic Acid and Methionine in Early Childhood Are Associated with Body Composition at School Age^{1–3}

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Abstract

Background: Deficiency of vitamin B-6, vitamin B-12, folate, folic acid, or methionine may lead to dysregulation of DNA methylation, which might lead to disturbed energy and lipid metabolism.

Objective: We aimed to explore whether intakes of vitamin B-6, vitamin B-12, folate, folic acid, and methionine at 1 y are associated with measures of growth and body composition at the age of 6 y.

Methods: This study was performed in 2922 children participating in The Generation R Study, a population-based prospective cohort study. Dietary intakes of vitamins B-6 and B-12, folate, folic acid, and methionine were assessed at a median age of 12.9 mo by using a validated food-frequency questionnaire. At the age of 6 y, height and weight were measured, and body mass index (BMI; in kg/m²) was calculated. Body fat was measured with dual-energy X-ray absorptiometry, and body fat percentage and the ratio of android fat mass to gynoid fat mass (android:gynoid) were calculated.

Results: In models adjusted for maternal and child characteristics, children with folic acid intakes in the highest tertile had a 0.16 SD score (SDS) lower weight (95% CI: −0.31, −0.02 SDS) and a 0.14 SDS lower BMI (95% CI: −0.26, −0.01 SDS) than children in the lowest tertile. Children with vitamin B-12 intakes in the highest tertile had a 0.13 SDS higher android:gynoid (95% CI: 0.00, 0.25 SDS) than children in the lowest tertile. In addition, children with intakes in the highest tertile of methionine had a 0.09 SDS higher BMI (95% CI: 0.01, 0.17) and a 0.12 SDS higher android:gynoid (95% CI: 0.02, 0.22) than children in the lowest tertile. Vitamin B-6 and folate intakes were not associated with any of the body composition outcomes measured.

Conclusions: In this population of children, early high folic acid intakes were associated with a lower body weight and BMI at the age of 6 y. In contrast, early higher methionine intakes were associated with unfavorable body composition at the age of 6 y. Future studies should investigate long-term consequences of these outcomes on health. *J Nutr* 2015;145:2123–9.

Keywords: vitamin B-12, folic acid, methionine, body composition, children

Introduction

The prevalence of overweight is rising among children, increasing the risk of metabolic syndrome, type 2 diabetes, and obesity

in adulthood (1, 2). For adequate prevention, it is important to identify factors that influence the development of overweight in early life. One of the mechanisms that might be involved in the early development of overweight is DNA methylation. DNA methylation is a mechanism in which gene expression is altered in response to environmental influences, such as nutrition and physical activity (3). For example, several nutrients, including vitamin B-6, vitamin B-12, methionine, and folate, can directly

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³ Supplemental Tables 1–7 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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or indirectly act as methyl donors in DNA methylation (4–6). Deficiency of these methyl donor nutrients could lead to dysregulation of DNA methylation and might generate metabolic disturbances, including disturbed energy and lipid metabolism, and a higher future risk of cardiovascular disease (7–9).

Considering that DNA methylation might influence the development of overweight, this might be particularly important during early life, when epigenetic changes are actively occurring (10–12). Therefore, it is possible that methyl donor nutrient intake during critical periods of growth, for instance during early childhood, could have an influence on the development of overweight in children. For instance, Perng et al. (13) observed that a lower DNA methylation was associated with the development of adiposity in 5- to 12-y-old boys, but not in girls.

Several animal studies have examined the effects of methyl donor nutrient supplementation on body composition. Results of those studies suggest that high intakes of methyl donor nutrients could have a protective effect on liver fat accumulation (14) and that methyl donor nutrient supplementation during pregnancy has a protective effect on the development of obesity in the offspring (15, 16). A recent study by Gunanti et al. (17) reported that low serum concentrations of folate and vitamin B-12 were associated with higher BMI and fat mass in Mexican American children aged 8–15 y. Furthermore, a few other cross-sectional studies investigated the associations between folate and vitamin B-12 and body composition in children and adolescents. Results of those studies show that lower concentrations or intakes of vitamin B-12 or folate were associated with unfavorable body composition outcomes, such as a higher BMI and waist circumference (18–21). In addition, a few studies suggest that a higher homocysteine concentration, which could be the result of a vitamin B-12 and/or folate deficiency, is associated with unfavorable body composition in children (22–24). The aim of this study was to investigate the associations between dietary intakes of vitamin B-6, vitamin B-12, folate, folic acid, and methionine in early childhood and growth and body composition outcomes at school age in 2922 children participating in a prospective cohort study.

Methods

Design and subjects. This study was embedded in The Generation R Study, a population-based prospective cohort study from early fetal life onward in Rotterdam, Netherlands (25). The study was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participating mothers. All children were born between 2002 and 2006, and data on follow-up in early childhood were available for 7893 children (Figure 1). A FFQ to assess infant diet was sent to 5088 mothers who provided informed consent for postnatal follow-up and who mastered the Dutch language sufficiently (26). This FFQ was returned by 3650 mothers (72%). After exclusion of subjects with invalid dietary data and withdrawn consent, information on nutrition was available for 3629 children (26). Of these children, growth and body composition data at the age of 6 y were available for 2922 singleton born children (Figure 1).

Dietary assessment. Dietary intake of the children was assessed at a median age of 12.9 mo (95% range: 12.2–18.9 mo) by using a semiquantitative FFQ filled in by 1 of the parents, covering the previous month (26). This FFQ was developed for our study population and included foods that are frequently consumed by children aged 9–18 mo according to a Dutch national food consumption survey in 2002 (27). The final FFQ consisted of 211 food items and included questions on frequency, quantity, type, and preparation methods. This FFQ was validated against 3 24-h recalls, obtained by trained nutritionists, in a representative sample of 32 Dutch children aged 14 mo (26). Intraclass

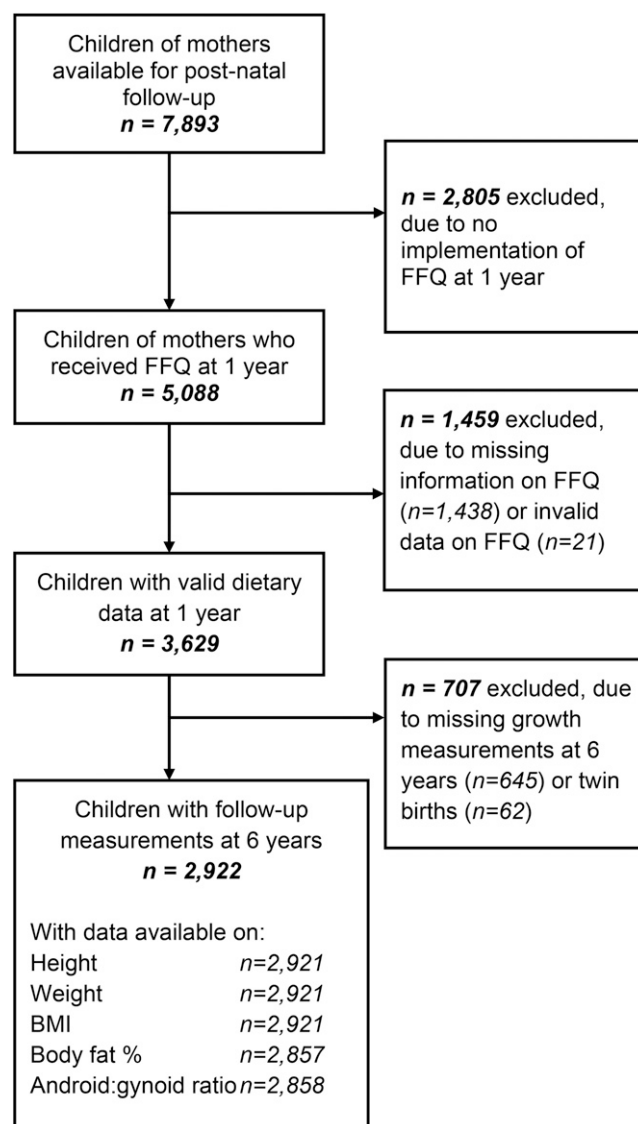


FIGURE 1 Population for analysis.

correlation coefficients for nutrient intakes ranged from 0.36 to 0.74 (28). Food frequencies and quantities were converted into grams per day by using standardized portion sizes. Vitamin B-6, vitamin B-12, folate, and methionine intakes were calculated with the Dutch Food Composition Table 2006 (29). For folate intakes we calculated both naturally occurring dietary folate and synthetically produced folic acid, which is added to fortified foods. Total dietary folate equivalents (DFEs)⁹ were calculated, taking into account the differences in bioavailability of dietary folate and synthetic folic acid (1 DFE = 1 µg dietary folate or 0.6 µg folic acid added to food), according to a commonly used formula for DFE (30).

Growth and body composition outcomes. At a median age of 5.9 y (95% range: 5.7–6.6 y) children visited our research center in the Erasmus Medical Center for a detailed physical examination. Height was determined in the standing position to the nearest millimeter by a Harpenden stadiometer (Holtain Limited). Weight was measured with a mechanical personal scale (SECA). Height and weight were measured without shoes and heavy clothing, and BMI (kg/m²) was calculated. Age- and sex-specific SD scores (SDSs) for height, weight, and BMI were

⁹ Abbreviations used: android:gynoid, ratio of android fat mass to gynoid fat mass; DFE, dietary folate equivalent; *MTHFR*, methylenetetrahydrofolate reductase gene; SDS, SD score.

obtained from Dutch reference growth charts (31). Total body, android, and gynoid fat mass were measured with DXA (iDXA; General Electric) by using enCORE software version 13.6, as described in detail previously (32). Body fat percentage was calculated, and android fat mass was divided by gynoid fat mass to obtain the ratio (android:gynoid). Age- and sex-specific SDSs for body fat percentage and android:gynoid were calculated on the basis of data from our study population.

Covariates. Maternal height and weight were measured at enrollment, and BMI was calculated. Data on maternal age, educational level, household income, parity, and folic acid supplement use were available from self-administered questionnaires at enrollment. Maternal educational level was categorized as no/primary; secondary, or higher education. Net monthly household income was categorized as <€1400; €1400–€2200; or >€2200 (equivalent to <\$1600; \$1600–\$2500; or >\$2500 US\$ in 2015). Parity was categorized as nulliparous or multiparous. Smoking and alcohol consumption during pregnancy were assessed with questionnaires in each trimester and categorized as never, until pregnancy was known, or continued during pregnancy. Folic acid supplement use during pregnancy was categorized as none during early pregnancy, during the first 10 wk of pregnancy, or periconceptual. Child's methylenetetrahydrofolate reductase (*MTHFR*) variants C677T (rs1801133) and A1298C (rs1801131) were genotyped in cord blood (33). Information on child's sex, ethnicity, gestational age at birth, and birth weight were obtained from hospital medical records. Ethnicity was defined as Dutch or non-Dutch according to Statistics Netherlands (34). Information on breastfeeding during infancy was obtained with postnatal questionnaires and categorized as exclusively for ≥ 4 mo, partially in the first 4 mo, or never. Infant formula intake at 1 y was obtained from the previously mentioned FFQ. A previously defined child diet score was used to quantify overall diet quality at the age of 1 y (28). The diet score was calculated for each child by using data from the FFQ. Information on child's screen time (television watching and computer use, h/d) and sports participation (yes/no) at the age of 6 y was obtained with a questionnaire.

Statistical analysis. To control for confounding by total energy intake, we used the residual method to adjust the intakes of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine for total energy intake (35). In short, linear regression analyses were used to calculate energy adjusted intake of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine for each subject, with energy intake as independent variable and intake of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine as dependent variables. Because methionine is an amino acid and therefore part of dietary protein, methionine intake was additionally adjusted for total protein intake with the residual method. After visual inspection, all outcome variables were considered to be normally distributed. Multivariable linear regression models were used to assess associations between intake of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine with age- and sex-adjusted SDSs for growth and body composition outcomes (height, weight, BMI, body fat percentage, and android:gynoid). As recommended for dietary intake variables, nutrient intakes were analyzed both as continuous variables and categorized into tertiles, with the lowest tertile as the reference category (35). Potential covariates were selected according to the literature or a $\geq 10\%$ change in effect estimates (36). The adjusted models included maternal age, BMI, education, parity, folic acid supplement use, smoking, and alcohol consumption during pregnancy; household income; child's ethnicity, birth weight *z* score, breastfeeding during infancy, intake of formula, total energy intake, diet quality score, screen time, and participation in sports. Considering that vitamin B-12 only occurs in animal foods, models that included vitamin B-12 were additionally adjusted for animal protein intake. To reduce potential bias due to attrition, missing values of covariates were multiple imputed, based on the correlation between each variable with missing values and other subject characteristics (Supplemental Table 1). Five independent data sets were generated. Because characteristics were similar for imputed and unimputed data (Supplemental Table 2) and we found similar results, we present results that are based on the imputed data sets (37). Because the FFQ was developed and validated for Dutch children,

we performed sensitivity analyses in Dutch children only. Previous studies suggest that the influence of several nutrients in early life might differ between boys and girls (38, 39). Furthermore, it is suggested that nutrients involved in the 1-carbon metabolism, such as folate, might interact with *MTHFR* polymorphisms (40). To assess if associations between intakes of vitamin B-6, vitamin B-12, folate, folic acid, and methionine and body composition outcomes differed by sex or by *MTHFR* polymorphisms, we analyzed interaction terms. A statistical interaction was analyzed by adding a product of each of the nutrients (e.g., folic acid) and the potential effect modifier (e.g., sex) as an independent variable to the linear regression model. The linear regression model in which the interaction was tested thus included 1 of the nutrients, the effect modifier, and the interaction term of these 2 variables as independent variables, and 1 of the outcomes as dependent variables. Stratified analyses were performed in case the interaction term was significant ($P < 0.05$). All statistical analyses were conducted with SPSS version 21.0 (SPSS Inc.). Results were considered statistically significant with a $P < 0.05$. Values are presented as mean \pm SD, median (95% range), *n* (%), and regression coefficients (95% CI: lower limit, upper limit).

Results

Subject characteristics. Characteristics of the children and their mothers are presented in Table 1. Most of the children had a Dutch ethnicity (68.7%) and 49.1% were boys. Mean total energy intake at the median age of 12.9 mo (95% range: 12.2–18.9 mo) was 1310 ± 393 kcal/d. Median intakes of vitamin B-6 and vitamin B-12 were comparable with the median intakes among Dutch children aged 2–3 y from the general population (41), but intake of DFEs were considerably higher (i.e., 182 μ g/d vs. 106 μ g/d) (41). This high DFE intake in our population was primarily due to added folic acid in infant formula. Main sources of vitamin B-12 and methionine in our population were meat, fish, and eggs. Various food groups contributed to vitamin B-6 and naturally present folate intake. In nonresponse analyses, we observed that children without FFQ data had a higher BMI and body fat percentage at the age of 6 y than children with FFQ data (Supplemental Table 3).

Intake of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine and growth and body composition outcomes. The covariate-adjusted associations between intake of vitamin B-6, vitamin B-12, folate, folic acid, DFEs, and methionine and growth and body composition outcomes are shown in Table 2. Children with intake of vitamin B-12 in the highest tertile had a 0.13 SDS higher android:gynoid (95% CI: 0.00, 0.25 SDS) than children in the lowest tertile. In addition, for the association between vitamin B-12 and android:gynoid *P*-trend was significant ($P = 0.03$). Additional adjustment for saturated fat intake did not change this result (data not shown). In contrast, children with intakes of folic acid in the highest tertile had a 0.16 SDS lower weight (95% CI: -0.31 , -0.02 SDS) and a 0.14 SDS lower BMI (95% CI: -0.26 , -0.01 SDS) than children with intakes in the lowest tertile. In line with this, children with intakes of DFEs in the second and highest tertile had a lower weight, -0.10 SDS (95% CI: -0.19 , -0.01 SDS) and -0.11 SDS (95% CI: -0.22 , -0.00 SDS), respectively, than children with intakes in the lowest tertile. However, intake of DFEs in the third tertile was also associated with a lower height (-0.12 SDS; 95% CI: -0.23 , -0.01 SDS) than intake in the lowest tertile. Children with intakes in the highest tertile of methionine had a 0.09 SDS higher BMI (95% CI: 0.01, 0.17 SDS) and a 0.12 SDS higher android:gynoid (95% CI: 0.02, 0.22 SDS) compared with children in the lowest tertile. In addition, *P*-trend was significant for body fat percentage ($P = 0.03$) and

TABLE 1 Population and measurement characteristics ($n = 2922$)¹

Characteristics	Values
Child characteristics	
Sex, male	1434 (49.1)
Ethnicity	
Dutch	2007 (68.7)
Non-Dutch	915 (31.3)
Gestational age at birth, wk	40.1 [36.0–42.3]
Birth weight, g	3470 \pm 552
Breastfeeding	
Exclusively ≥ 4 mo	864 (29.6)
Partially ≥ 4 mo	1708 (58.4)
Never	350 (12.0)
Dietary measurement characteristics at 1 y	
Age filling out FFQ, mo	12.9 [12.2–18.9]
Total energy intake, kcal/d	1310 \pm 393
Vitamin B-6 intake, mg/d	1.2 [0.5–2.2]
Vitamin B-12 intake, μ g/d	2.5 [0.7–5.6]
Folate intake	
Dietary folate, μ g/d	91 [32–209]
Added folic acid, μ g/d	430 [0–1420]
Folate equivalents, μ g/d	182 [67–783]
Methionine intake, mg/d	642 [197–1390]
Growth measurement characteristics at 6 y	
Age, y	6.0 \pm 0.2
Height, cm	118 \pm 5
Weight, kg	22.4 \pm 3.4
BMI, kg/m ²	16.0 \pm 1.6
Body fat percentage, %	24.2 \pm 5.1
Android:gynoid fat ratio	0.24 \pm 0.06
Sports participation (yes), %	1291 (44.2)
Screen time, h/d	1.6 [0.3–4.4]
Maternal characteristics	
Age at enrollment, y	31.5 \pm 4.5
BMI at enrollment, kg/m ²	24.4 [18.8–35.6]
Educational level	
No or primary education	302 (10.3)
Secondary	911 (31.2)
Higher	1709 (58.5)
Household income per month ²	
<€1400	414 (14.2)
€1400–€2200	556 (19.0)
>€2200	1952 (66.8)
Parity	
0	1761 (60.3)
1	842 (28.8)
≥ 2	319 (10.9)
Folic acid supplement use	
None	469 (16.0)
First 10 wk of pregnancy	890 (30.5)
Periconceptional	1563 (53.5)
Smoking during pregnancy	
Never	2277 (78.0)
Until pregnancy was known	296 (10.1)
Continued	349 (11.9)
Alcohol consumption during pregnancy	
Never	1119 (38.3)
Until pregnancy was known	443 (15.2)
Continued	1359 (46.5)

¹ Values are mean \pm SD, median [95% range], or n (%).² Equivalent to <\$1600; \$1600–\$2500; or >\$2500 US\$ in 2015.

android:gynoid ($P = 0.01$). In the covariate-adjusted models, vitamin B-6 and folate intakes were not associated with body composition outcomes.

Results of the crude analyses are presented in **Supplemental Table 4**, showing associations in similar directions to the adjusted models. However, in the crude models higher folate and vitamin B-12 intakes were associated with higher weight and BMI. These results were no longer significant in the adjusted model.

Additional analyses. Analyses restricted to Dutch children ($n = 2002$) were similar to analyses in the whole study population (**Supplemental Table 5**). Only 2 of 30 interaction terms for *MTHFR* polymorphisms were statistically significant (folate and folic acid on height); however, stratification on *MTHFR* for these 2 models revealed no clear differences in associations (data not shown). Interactions of sex with vitamin B-12, folic acid, and methionine intake were significant for height and weight ($P < 0.05$) but not for BMI, body fat percentage, or android:gynoid. We stratified all analyses for sex (**Supplemental Tables 6 and 7**), but analysis stratified for sex showed effect estimates in the same direction as the whole group. Only for vitamin B-6 intake did results differ between girls and boys. Girls with higher vitamin B-6 intake had a lower BMI, whereas boys had a higher body fat percentage.

Discussion

In this population-based prospective cohort study, we investigated associations between dietary intake of vitamin B-6, vitamin B-12, folate, folic acid, and methionine in early childhood and growth and body composition outcomes at the age of 6 y. We hypothesized that higher intake of vitamin B-6, vitamin B-12, folate, folic acid, and methionine were associated with favorable body composition outcomes, because these nutrients play a role in DNA methylation, which may be involved in obesity risk (13). We observed that folic acid intake in the highest tertile was associated with a lower body weight and BMI, which is in line with our hypothesis. However, in contrast to our hypothesis, a higher methionine intake was associated with a higher BMI, a higher body fat percentage, and a higher android:gynoid, and a higher vitamin B-12 intake was associated with a higher android:gynoid. Finally, no consistent associations were found between vitamin B-6 intake and any of the body composition outcomes in the total group. However, when analyses were stratified for sex, a higher vitamin B-6 intake was associated with a higher body fat percentage in boys but with a lower BMI in girls.

Our findings for vitamin B-6 are in line with findings of the study by Gunanti et al. (17) in Mexican American children, which also did not find an association between vitamin B-6 intake and body composition. However, 2 cross-sectional studies in adults reported that vitamin B-6 concentrations were lower in obese subjects or patients with metabolic syndrome than in control subjects (42, 43). These findings are in concurrence with our finding that a higher vitamin B-6 intake was associated with a lower BMI in girls. However, in boys a higher intake was associated with a higher body fat percentage. No difference was found in vitamin B-6 intake between boys and girls, suggesting that divergent results might be due to sex-specific differences.

In our study, we observed that compared with the lowest tertile a folic acid intake in the highest tertile was associated with a lower weight and a lower BMI but not with body fat percentage or android:gynoid. Similar patterns of associations

TABLE 2 Covariate-adjusted associations between intakes of vitamin B-6, vitamin B-12, folate, folic acid, and methionine with growth and body composition outcomes in children ($n = 2922$)¹

	Height, SDS	Weight, SDS	BMI, SDS	Body fat percentage, SDS	Android-to-gynoid ratio, SDS
Vitamin B-6, mg/d					
Tertile 1 (<1.1)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (1.1–1.3)	–0.01 (–0.09, 0.09)	0.04 (–0.05, 0.12)	0.05 (–0.03, 0.12)	0.09 (–0.00, 0.18)	–0.00 (–0.10, 0.09)
Tertile 3 (>1.3)	–0.04 (–0.14, 0.06)	–0.05 (–0.15, 0.04)	–0.04 (–0.12, 0.05)	0.02 (–0.07, 0.12)	–0.01 (–0.11, 0.09)
<i>P</i> -trend	0.63	0.20	0.15	0.19	0.36
Vitamin B-12, ² µg/d					
Tertile 1 (<2.2)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (2.2–2.9)	–0.01 (–0.11, 0.08)	–0.01 (–0.10, 0.08)	–0.00 (–0.08, 0.08)	–0.06 (–0.15, 0.04)	0.00 (–0.10, 0.10)
Tertile 3 (>2.9)	–0.01 (–0.12, 0.11)	0.06 (–0.06, 0.17)	0.08 (–0.02, 0.18)	0.04 (–0.08, 0.16)	0.13 (0.00, 0.25)*
<i>P</i> -trend	0.35	0.51	0.66	0.15	0.03*
Folate, µg/d					
Tertile 1 (<80.9)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (80.9–110.3)	0.03 (–0.07, 0.13)	0.08 (–0.02, 0.18)	0.08 (–0.01, 0.16)	0.05 (–0.05, 0.15)	–0.01 (–0.15, 0.13)
Tertile 3 (>110.3)	–0.02 (–0.15, 0.10)	0.06 (–0.06, 0.18)	0.09 (–0.01, 0.20)	0.05 (–0.08, 0.17)	0.03 (–0.15, 0.20)
<i>P</i> -trend	0.12	0.58	0.59	0.74	0.63
Folic acid, µg/d					
Tertile 1 (<304.3)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (304.3–669.5)	–0.03 (–0.13, 0.07)	–0.06 (–0.16, 0.03)	–0.06 (–0.15, 0.03)	–0.04 (–0.14, 0.06)	–0.09 (–0.19, 0.02)
Tertile 3 (>669.5)	–0.10 (–0.25, 0.05)	–0.16 (–0.31, –0.02)*	–0.14 (–0.26, –0.01)*	–0.06 (–0.21, 0.09)	–0.09 (–0.24, 0.07)
<i>P</i> -trend	0.60	0.13	0.06	0.12	0.93
Folate equivalent, µg/d					
Tertile 1 (<167.8)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (167.8–214.4)	–0.07 (–0.17, 0.02)	–0.10 (–0.19, –0.01)*	–0.08 (–0.16, 0.00)	0.03 (–0.07, 0.12)	–0.02 (–0.12, 0.08)
Tertile 3 (>214.5)	–0.12 (–0.23, –0.01)*	–0.11 (–0.22, –0.00)*	–0.06 (–0.15, 0.03)	0.08 (–0.03, 0.19)	–0.03 (–0.15, 0.09)
<i>P</i> -trend	0.75	0.14	0.05	0.67	0.61
Methionine, ³ mg/d					
Tertile 1 (<681.5)	Reference	Reference	Reference	Reference	Reference
Tertile 2 (681.5–682.2)	0.01 (–0.08, 0.09)	0.00 (–0.09, 0.09)	0.00 (–0.08, 0.08)	–0.04 (–0.13, 0.05)	–0.00 (–0.10, 0.09)
Tertile 3 (>682.2)	0.03 (–0.06, 0.12)	0.08 (–0.00, 0.17)	0.09 (0.01, 0.17)*	0.08 (–0.02, 0.17)	0.12 (0.02, 0.22)*
<i>P</i> -trend	0.87	0.70	0.65	0.03*	0.01*

¹ Values are regression coefficients (95% CI) from linear regression models and reflect the difference in body composition for tertiles of vitamin B-6, vitamin B-12, folate, folic acid, and methionine intake, compared with the lowest tertile. Tests for trend were conducted with nutrient intake as a continuous variable in the regression model. All outcomes are age- and sex-specific SDSs. Nutrient intakes are adjusted for total energy intake by using the residual method. Models are adjusted for child ethnicity, birth weight z score, breastfeeding during infancy, infant formula intake (energy adjusted), total energy intake, diet quality score, participation in sports, screen time, and for maternal age, maternal BMI, maternal education, household income, parity, folic acid supplement use during pregnancy, smoking during pregnancy, and alcohol use during pregnancy. * $P < 0.05$. SDS, SD score.

² Additionally adjusted for animal protein intake.

³ Additionally adjusted for total protein intake by using the residual method.

were observed for intake of DFEs, probably because folic acid intake was the main contributor to the intake of DFEs (30). In line with this, a previous cross-sectional study in Brazilian children aged 10–19 y reported that a higher total folate intake was associated with a lower waist circumference (19). However, in this latter study no distinction was made between natural folate and added folate (folic acid). Moreover, Gunanti et al. (17), Huemer et al. (20), and Gallistl et al. (21) observed unfavorable body composition outcomes in relation to lower folate concentrations in children and adolescents aged 8–15, 2–17, and 4–17 y, respectively. However, 2 cross-sectional studies did not observe associations between folate concentrations and overweight in children and adolescents aged 6–17 y (44, 45).

In contrast to our results for folic acid, intakes of folate were not associated with any of the body composition outcomes. In sensitivity analyses that included Dutch children only, a higher folate intake was even associated with a higher BMI (P -trend = 0.03). Only 1 study reported similar results. In that cross-sectional study concentrations of folate were significantly higher in overweight and obese boys than in boys with a normal weight

(18). Potential mechanisms behind these associations remain unclear, but they may involve DNA synthesis induced by folate. Intakes of folate and vitamin B-12 have been associated with increased DNA synthesis (46), which stimulates growth and might also increase BMI.

In line with this latter mechanism, vitamin B-12 intakes were associated with a higher android:gynoid. However, these findings are not in agreement with findings from 3 previous studies, which reported higher vitamin B-12 concentrations among children and adolescents aged 8–15, 2–17, and 10–19 y with a lower BMI than children with higher BMI (17, 18, 20). These conflicting results might be explained because previous studies used blood concentrations of vitamin B-12, rather than dietary vitamin B-12 intakes. Another reason for our unexpected findings might be residual confounding. Vitamin B-12 only occurs in animal products, which are usually also high in energy, protein, and fat. However, we adjusted our models for total energy intake, animal protein intake, diet quality, and saturated fat. Other possible explanations for this unexpected finding might be other mechanisms involved, such as DNA synthesis. In

addition to folate, vitamin B-12 is also associated with an increased DNA synthesis, and this could lead to a stimulated growth, which might explain our higher android:gynoid among children with a higher vitamin B-12 intake. Potential mechanisms underlying these associations should be further investigated in future studies.

We observed that a higher methionine intake, independent of total protein intake, was associated with a higher BMI, body fat percentage, and android:gynoid. Although this is in contrast with our hypothesis that focused on DNA methylation, it is in line with a number of other studies. Results from a randomized controlled trial in adults with metabolic syndrome conducted by Plaisance et al. (47) suggested that a methionine-restricted diet led to an increase in fat oxidation and a decrease in intrahepatic lipid content. Previous animal studies performed by Hasek et al. (48) and Malloy et al. (49) showed that rats fed methionine-restricted diets had limited fat deposition and reduced visceral fat mass. These results suggest that a potential beneficial effect on body composition of higher DNA methylation because of a higher methionine intake might be overruled by other mechanisms, such as decreased energy expenditure (48). For methionine and other nutrients we observed larger effect estimates in girls than in boys. A possible explanation for these different findings may be differences in adiposity rebound between boys and girls. This onset of rapid growth in body fat occurs around the age of 6 y (50) and seems to occur earlier in girls than in boys (51).

The present study has several strengths and limitations. An important strength is the size of the population included and the prospective, population-based design. There was a large amount of information available on potentially confounding sociodemographic and lifestyle factors of the children and their parents. However, residual confounding may still be an issue, as in any observational study. For instance, detailed information on physical activity levels of children was not available. However, we adjusted for proxies of physical activity, including participation in sports and screen time. In addition, a diet score was used to control for overall healthy diet. Detailed body fat measurements were available by using DXA, which is a more objective measure than several anthropometric measures, such as skinfold thickness. We aimed to reduce attrition bias as much as possible. Therefore, we used a multiple imputation procedure, which is an appropriate method to deal with missing data (37).

A limitation of our study was that the FFQ was only validated for Dutch children, whereas we had a multiethnic study population. Furthermore, the FFQ was validated for several nutrients but not for the nutrients used in the present study (26). This might have led to measurement error in the assessment of vitamin B-6, vitamin B-12, folate, folic acid, and methionine intakes, which may have led to random misclassification and, consequently, attenuation of effect estimates (52). However, we adjusted all the nutrients for energy intake by using the nutrient residual method, which was described by Kipnis et al. (53) as an accurate approach for reducing attenuated effect estimates in nutritional epidemiology. In addition, we analyzed the nutrient intakes in tertiles to avoid misspecification and to decrease the influence of outliers (35). Unfortunately, as the intake within the tertiles show, the variation of vitamin B-6 and vitamin B-12 intakes in our population is quite low, which made it less likely to find an association with body composition outcomes. Furthermore, there might be some distortion in calculating the concentrations of DFEs in human milk. Folate in human milk is considered naturally present, but it might have the same bioavailability as added folic acid. However, the number of children still being breastfed at the age of 1 y was small in our

population ($n = 267$); therefore, the impact this error might have on our results is minor. The FFQ was only sent to mothers of participating children who mastered the Dutch language sufficiently, of whom 72% returned the questionnaire, which may have led to selection bias. We observed that children without FFQ data were taller and heavier than children with FFQ data (Supplemental Table 3). However, this would only result in selection bias if the associations between intake of vitamin B-6, vitamin B-12, folate, folic acid, and methionine and body composition outcomes were different between included and excluded participants in the final analysis. Unfortunately, blood concentrations of nutrients were not available, which would be a more objective measurement of nutritional status than dietary intake. In addition, we did not have information on choline intake, which is another important nutrient involved in DNA methylation. Finally, an important limitation of our study is that we had no information available on DNA methylation. We could therefore not examine whether intake of vitamin B-6, vitamin B-12, folate, folic acid, and methionine were associated with child body composition via changes in DNA methylation, as suggested by animal studies. Future studies in population-based samples of children are needed to investigate this effect.

In conclusion, results of this prospective cohort study suggest that high folic acid intake during early childhood may be associated with a lower weight and BMI at the age of 6 y. In contrast, a higher methionine intake was associated with a higher BMI, body fat percentage, and android:gynoid. Future studies are needed to confirm these findings, investigate the underlying mechanisms, and examine long-term consequences of these outcomes on health.

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