Molecular Nutrition & Food Research
www.mnf-journal.com

Imbalanced Folate and Vitamin B12 in the Third Trimester of Pregnancy and its Association with Birthweight and Child Growth up to 2 Years

Rima Obeid, Simone J.P.M. Eussen, Monique Mommers, Luc Smits, and Carel Thijs*

Scope: Folic acid supplementation during pregnancy may lead to an imbalance when vitamin B12 intake is low (folate trap) and may affect child's growth.
Methods: The authors study the association between third trimester maternal intakes of folate and B12 and birthweight and postnatal growth of 2632 infants from the KOALA Birth Cohort Study. Plasma vitamin biomarkers are measured in 1219 women.
Results: Imbalanced total intakes (folate > 430 µg day^{-1} combined with B12 < 5.5 µg day^{-1}) are not associated with birthweight [β adj (95% CI) = –14.87 (–68.87, 39.13)] compared with high intakes of both. Imbalanced intake is associated with a lower z score of weight at 1–2 years [β adj = –0.14 (–0.25, –0.03)]. Having red blood cell folate > 745 nmol L^{-1} and plasma B12 < 172 pmol L^{-1} is not associated with birthweight [β adj = –7.10 (–97.90, 83.71) g]. Maternal dietary B12 intake [β adj = –9.5 (–15.6, –3.3)] and plasma methylmalonic acid [β adj = 234 (43, 426)] are associated with birthweight.
Conclusion: Low maternal dietary B12 intake and elevated methylmalonic acid rather than imbalanced vitamins are associated with higher birthweight, suggesting that low maternal B12 can predispose the infants for later obesity.

1. Introduction

Folate and vitamin B12 are cofactors in one-carbon metabolism that participates in synthesis of nucleotides and methyl groups.[1] During pregnancy, sufficient folate supply in the mother is associated with a lower risk of neural tube defects.[2] There are strong recommendations to supplement folic acid in the preconceptional period until the end of the first trimester of pregnancy when the neural tube is closed.[2] No specific recommendations exist regarding vitamin B12 supplementation during pregnancy. Antenatal multivitamins are commonly used until the end of pregnancy, thus resulting in high blood folate and low plasma total homocysteine (tHcy).[3,4] There is inconclusive evidence regarding the association between maternal folate and vitamin B12 supply during pregnancy and birthweight[5] and infant's growth.[6–8]

Recently, excess folate intake (or status) during pregnancy has raised concerns,[9,10] especially in women with low vitamin B12.[11–14] High maternal folate combined with low vitamin B12 (i.e., also known as imbalanced folate to B12) could cause folate trap and affect child growth[9] by mechanisms related to DNA methylation[15] or impaired DNA synthesis. Imbalanced maternal folate to vitamin B12 intakes[13] or status markers[14,16,17] showed associations with low birthweight and insulin resistance in the children in studies among Asians who were predominantly vegetarians. In contrast, concentrations of blood folate did not show interaction with those of vitamin B12 in the association with birthweight in Spanish pregnant women.[3] Depletion of maternal vitamin B12 stores during late pregnancy as evident by lowered concentrations of vitamin B12 and elevated methylmalonic acid (MMA)[18] could increase the likelihood of imbalanced folate to vitamin B12. It is debatable whether imbalanced folate to vitamin B12 in 3rd trimester pregnant women is associated with birthweight and postnatal growth trajectories in a population without common vitamin B12 deficiency.

Green leafy vegetables are the main source of folate in the diet, whereas animal-foods are the main source of vitamin B12. Vegetarian lifestyle is gaining popularity due to a global trend to reduce meat consumption.[19] Thus, many women could have

R. Obeid
Department of Clinical Chemistry and Laboratory Medicine
Saarland University Hospital
Kirrberg street, Building 57, Homburg, Saarland D-66421, Germany
E-mail: rima.obeid@uks.eu
S. J. Eussen, M. Mommers, L. Smits, C. Thijs
Department of Epidemiology
Maastricht University Medical Centre+
Care and Public Health Research Institute (CAPHRI)
Maastricht, The Netherlands
S. J. Eussen
CARIM School for Cardiovascular Diseases
Maastricht University
Maastricht, The Netherlands

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202100662

DOI: 10.1002/mnfr.202100662

low vitamin B12 status during pregnancy, which predisposes them to develop imbalanced folate to vitamin B12, especially when using supplemental folic acid.

The present study investigated whether imbalanced maternal third trimester folate to vitamin B12 intake and imbalanced folate to B12 biomarkers are inversely related to birthweight among term neonates. Moreover, we studied the longitudinal associations between imbalanced maternal folate to vitamin B12 and child *z*-scores of weight, height, and body mass index (BMI) at age of 1–2 years. The study was conducted in 2632 pregnant women and their children participating in the KOALA Birth Cohort Study from the Netherlands.

2. Experimental Section

2.1. Study Design

The present study was conducted within the prospective KOALA Birth Cohort Study (in Dutch, the Child, Parent and Health: Lifestyle and, Genetic Constitution study) in the Netherlands. The study design has been described elsewhere.[20] Briefly, starting in October 2001, pregnant women (10–14 weeks of pregnancy) were recruited from an ongoing cohort study on pregnancy-related pelvic girdle pain (the conventional recruitment group). Additionally, pregnant women (10–14 weeks of pregnancy) were recruited via other recruitment channels such as posters in organic food shops, anthroposophical physician offices, and midwives. The inclusion criteria were delivery at ≥37 weeks of gestation and willingness to respond to the follow-up questionnaires. The exclusion criteria were perinatal death, congenital abnormalities (i.e., chromosomal defects, inborn errors of metabolism), and no response to any of the questionnaires in the first year of life. We further excluded women with incomplete data from the food frequency questionnaires (FFQ) and the questionnaires on multivitamin supplement use, women using vitamin B12 injections, women with multiple pregnancies and anomalies that affect child growth.

The study was conducted according to the ethical principles for medical research on human subjects set out in the Declaration of Helsinki. The medical ethics committee of the Maastricht University/University Hospital of Maastricht has reviewed and approved the study (approval numbers: MEC 01–139.3 2001-10-09 and MEC 00-182-13 2003-11-14). All participants provided written informed consent to the study.

2.2. The Cohort Included in the Present Study

The KOALA study included 2834 pregnant women who gave birth between 2001 and 2003. We excluded 32 women due to incomplete data from FFQ; 5 who received B12 injections; 78 with preterm birth; 32 with twin pregnancy; 49 with birth anomalies; two because the parents did not respond to any of the follow-up questionnaires in the first year of life; and three because the infants died in the first 2 weeks after birth. The present study included 2633 women. Gestational age was missing in one case, thus leaving 2632 women for the data analyses (**Figure 1**).

2.3. Maternal Information

We used a questionnaire around the 34th week of gestation to collect information on maternal age, height and pre-pregnancy weight, number of previous pregnancies, lifestyle (conventional or alternative), maternal education, cigarette smoking (number of cigarettes per day), and alcohol consumption (number of glasses per week) during the third trimester, supplement use, and ethnic origin of the mother and the father (information on either of the grandparents born outside the Netherlands).

2.4. Dosage Information / Dosage Regimen

Information on dietary intakes of several nutrients was collected at 34 weeks of gestation using a semi-quantitative FFQ. The FFQ included approximately 200 food items[21] and asked about the frequency and amounts of each of the items consumed in the last month. The Dutch Food Composition Database (NEVO) version 2011[22] was used to calculate the daily intake of micronutrients as reported before.[23]

The questionnaire that was distributed at 34th week specifically asked about the use of single or multivitamin supplements containing folic acid, the brand name, and the timing of folic acid use (before or during pregnancy). The nutrient contents per portion were documented. Total folate intake (from the diet and multivitamin supplements) is expressed in μg dietary folate equivalent (DFE) day^{-1}, assuming that the supplements were taken regularly on a daily basis. Likewise, dietary vitamin B12 intake[23] and total vitamin B12 intake (from the diet and multivitamin supplements) were calculated and expressed in μg day^{-1}. Scatter plots of dietary versus supplemental folate and B12 are shown in Figures S1 and S2, respectively (Supporting Information).

Due to the higher bioavailability of folic acid compared to dietary folate, folic acid intake from supplements and fortified foods was converted to DFE as recommended by the Food and Nutrition Board of the US National Academy of Sciences.[24]

Folate intake (μgDFE) = μg naturally occurring folate + (1.7 × μg folic acid)

Folate intake from food (μgDFE) is the sum of natural food folate and folic acid from fortified foods (μgDFE). There is no mandatory fortification with folic acid in the Netherlands and voluntary fortification was uncommon before 2004. The mean and standard deviation (SD) of folic acid intake from fortified foods in the present study were 1.6 (8.0) μg day^{-1}.

2.5. Information on Birthweight and Child Anthropometric Markers

Birthweight was reported by the mothers at 2 weeks postpartum and was verified against the obstetric record. After birth, mothers filled out a questionnaire including questions on child weight and height, breast feeding habits, and introduction of complementary foods. At child's age 1 and 2 years, the mothers filled out questionnaires where they provided information on the most recent weight and height measured at the child health clinic and the age of the child at the time of measurement. *Z*-scores of weight,

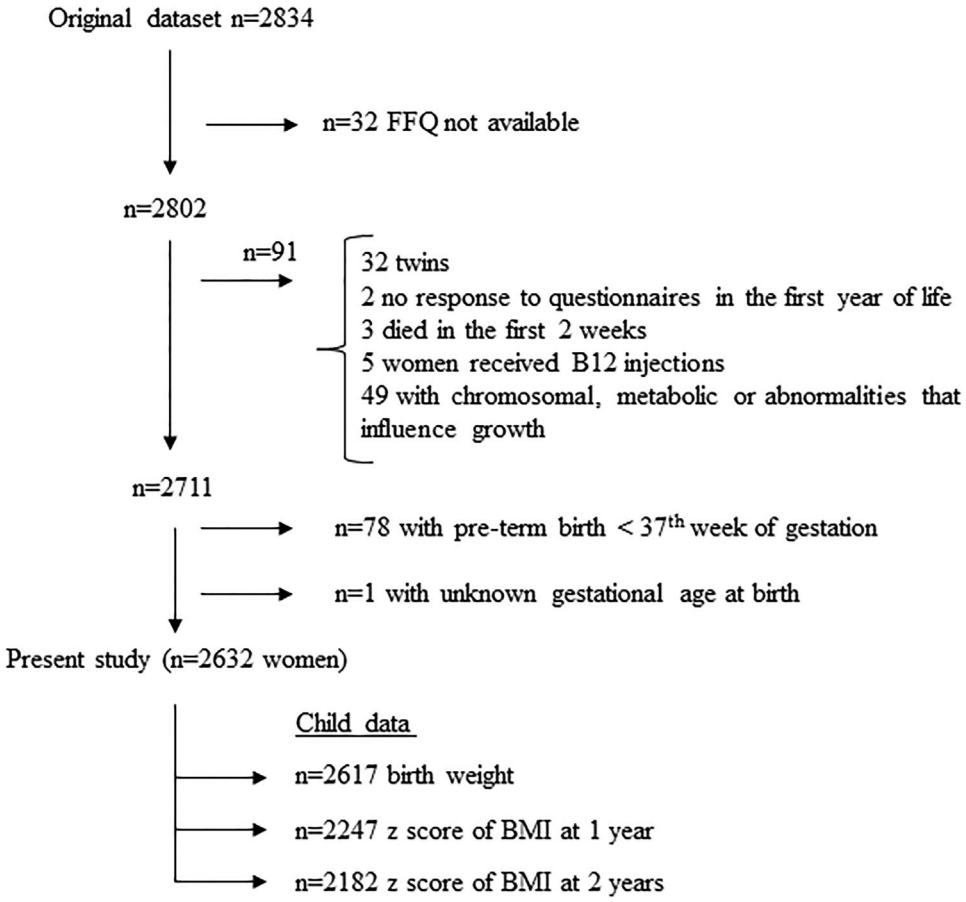

Figure 1. Study flow diagram. FFQ, food frequency questionnaire; BMI, body mass index.

height, and BMI in kg m^{-2} were calculated and standardized for sex and age against the 4th Dutch National Growth Study.[25]

2.6. Blood Sampling and Biochemical Measurements

Trained study nurses collected blood samples from a subgroup of 1355 women between gestational weeks 34th and 36th. Blood was collected in the morning hours into tubes containing EDTA-K^+. The samples were centrifuged on site within 30 min at 3000 rpm for 10 min at room temperature. The plasma was separated, transported at 4°C to the study biobank, and stored at −80°C until analysis.

Concentrations of folate and vitamin B12 markers were measured at the Department of Clinical Chemistry, Erasmus MC Medical Center, Rotterdam, Netherlands. Plasma total vitamin B12 concentrations were measured using electrochemiluminescence immunoassay "ECLIA" on Cobas 401 autoanalyzer (Roche Diagnostics). Plasma holotranscobalamin (holoTC) concentrations were measured using Abbott ARCHITECT Active-B12 immunoassay (Abbott Diagnostics). Plasma MMA levels were measured using liquid chromatography tandem mass spectrometry.[26] Plasma tHcy concentrations were measured using LX20 Pro autoanalyzer (Beckman Coulter). The concentrations of folate in lysed-blood erythrocytes were measured using fluorescence immunoassay (Perkin-Elmer). The concentration of red blood cell (RBC)-folate was calculated as; level of folate in the erythrocyte lysate (nmol L^{-1}) × 23 × (100/hematocrit %), where 23 is the assay dilution factor. The between-days coefficient of variations were; < 7% for plasma vitamin B12, < 15% for holoTC, < 8% for MMA, and < 7% for tHcy.[27,28]

The concentrations of tHcy, vitamin B12, MMA, and holoTC were available from 1219 women. The concentrations of RBC-folate, tHcy, vitamin B12, MMA, and holoTC were available from 766 women.

2.7. Statistical Analyses

The statistical analyses were conducted using version 27 of IBM SPSS Statistics package (SPSS Inc., Chicago, IL, USA). p values < 0.05 were considered statistically significant.

2.8. General Statistical Procedures

The descriptive data are shown as mean (SD) or median [interquartile range, IQR] for continuous variables and absolute (n) and relative frequencies (%) for categorical variables. One-sample Kolmogorov-Smirnov Test and Q-Q plots were used to

study the distribution of the continuous variables. Birthweight and *z*-scores of height were not normally distributed, but log-transformation did not improve the distribution. Thus, we used the variables without log-transformation in all data analyses.

The Curve Estimation procedure was used to explore whether the associations between birthweight and intake values or plasma levels (all continuous variables) fit into linear, quadratic, or cubic models. The curves and the regression statistics (p values for different regressions models) indicated that linear associations can be assumed.

2.9. Defining Imbalanced Folate to B12

We defined categories of imbalanced folate to B12 intakes by dichotomizing the data using median values in the study population (Figures S3 through S7, Supporting Information). Categories of RBC-folate concentrations below and above the median were combined with categories of a single vitamin B12 marker each time (plasma B12, holoTC, or MMA) to evaluate the consistency of the results. This strategy was chosen because of the low variability of supplemental folic acid and vitamin B12 intakes, the low prevalence of overt deficiency conditions, the controversy about using cutoff values for the biomarkers during pregnancy due to trimester-related variations,[18] and to avoid imbalanced sample size in the subgroups.

The following groups were defined as having imbalanced nutrients: dietary folate above the median and dietary B12 intakes below the median (folate intake > 268 μgDFE day^{-1} and vitamin B12 intake < 4.9 µg day^{-1}); total folate above the median and total B12 intakes below the corresponding medians (folate > 430 μgDFE day^{-1} and B12 < 5.5 µg day^{-1}); RBC-folate above the median and plasma B12 below the median (RBC-folate > 745 nmol L^{-1} and plasma B12 < 172 pmol L^{-1}); RBC-folate above the median and holoTC below the median (RBC-folate > 745 nmol L^{-1} and plasma holoTC < 68 pmol L^{-1}); both RBC-folate and MMA above the corresponding medians (RBC-folate > 745 nmol L^{-1} and plasma MMA > 0.220 µmol L^{-1}) (Table 3 and Table S2, Supporting Information). The corresponding reference groups consisted of women who had high folate and B12 intakes or statuses (above the corresponding medians).

2.10. Statistical Analyses of Birthweight

The Generalized Linear Models (GENLIN) were used to study the associations between maternal vitamin intakes or statuses (as continuous variables) and birthweight (Table 3 and Table S2, Supporting Information). The beta coefficients (β) and 95% Confidence Intervals (95% CI) show the changes of birthweight per 1 unit changes of maternal exposures. In addition, the associations of imbalanced intake or biomarker category with birthweight were studied compared to the corresponding reference category. The β (95% CI) estimate the change of birthweight in the imbalance category compared to the reference category.

Statistical interactions between categories of folate and vitamin B12 intakes or biomarkers were studied to evaluate whether the results of the associations with birthweight might differ by the levels of the exposures.

2.11. Statistical Analyses Of Child Anthropometric Measured at 1 and 2 Years

The associations between maternal folate and vitamin B12 intakes or biomarkers and *z*-scores of child's weight, height, and BMI at 1 and 2 years (secondary outcomes) were studied using Generalized Estimating Equation (GEE) methods (Table 3 and Tables S2 and S3, Supporting Information). A separate GEE model was constructed for longitudinal measurements of each growth measurement. The models used unstructured correlation matrix and the unique child identification number as a cluster variable. The β (95% CI) estimate the change in the outcome variable across the population for one unit change in the exposure (Table S3, Supporting Information) or in the imbalance category versus the reference group (Table 3 and Table S2, Supporting Information) after accounting for within-subject correlation.

The interactions between categories of folate and vitamin B12 intakes or their biomarkers were studied in separate GEE models. For the purpose of this study, the GEE models did not include time-interactions (i.e., to judge whether the associations differed by age of the child at 1 and 2 years).

2.12. Covariates

The regression models were adjusted for factors known to be associated with the exposure and the outcome in the same time or factors that have been shown to be associated with the outcome only. The covariates were determined a priori and include maternal gravidity, dietary intakes of vitamin D, zinc, and iron, maternal age, pre-pregnancy BMI, height, education, gestational diabetes, smoking and alcohol consumption in third trimester, ethnic origin of the mother and the father, recruitment group (conventional/alternative), season of completing the FFQ, and child sex.

The associations between maternal variables and birthweight were not adjusted for gestational age at birth and maternal weight gain during pregnancy because these variables are potential colliders.[29] For the postnatal longitudinal growth outcomes, we did not adjust for birthweight, because this could be a mediator in the association between maternal exposures and child growth.

Missing information on the covariates was limited to some cases, those cases were not excluded from the study and the information was not imputed, implying that cases with missing information on one variable were automatically excluded in multivariate analyses.

2.13. Sensitivity Analysis

The regression models of *z*-scores of child outcomes were further adjusted for lactation patterns that could influence child growth. The regression models including maternal dietary intakes as exposure variables were further adjusted for caloric intake (kcal).

Table 1. Patterns of supplements use and intakes and blood biomarkers of folate and vitamin B12 among third trimester pregnant women from the KOALA Birth Cohort Study.

Patterns of supplements use in third trimester ($n = 2632$)	n(%) or median [IQR]
Folic acid-containing supplements: did not use supplements	1352 (51.4)
used supplements containing folic acid	1280 (48.6)
up to 200 µg	65 (2.5)
> 200 µg up to 400 µg	1158 (44.0)
> 400 µg	57 (2.2)
% contribution of supplements to total folate intake	71.6 [7.4]
Vitamin B12-containing supplements: did not use supplements	1362 (51.7)
used supplements containing vitamin B12	1270 (48.3)
0.5-2.5 µg	1077 (40.9)
3–6 µg	123 (4.7)
≥ 10 µg	70 (2.7)
% contribution of supplements to total B12 intake	17.9 [10.6]
Vitamin intakes ($n = 2632$)	**Mean (SD); median** [IQR]
Dietary folate intake, µgDFE/d[1,2]	274 (73); 268 [88]
Folic acid in brands of multivitamin supplements, µg	196 (213); 0 [680]
Total folate intake (diet and supplements)[3,4], µgDFE day^{-1}	607 (370); 430 [680]
Dietary vitamin B12 intake, µg/day[1]	5.6 (3.4); 4.9 [2.6]
Vitamin B12 in brands of multivitamin supplements, µg	2.1 (16.2); 0 [1.0]
Total vitamin B12 intake (diet and supplements)[3], µg day^{-1}	7.7 (16.4); 5.5 [3.2]
Vitamin biomarkers[5]	
RBC-folate, nmol L^{-1}	830 (413)
Plasma vitamin B12, pmol L^{-1}	184 (72)
Plasma tHcy, µmol L^{-1}	9.1 (3.8)
Plasma MMA, µmol L^{-1}	0.250 (0.131)
Plasma holoTC, pmol L^{-1}	71 (33)

[1] Information on dietary intake in 3rd trimester was collected using semi-quantitative FFQ. [2] Intake of folate from the diet including fortified foods. [3] Total intakes from diet plus supplements assuming that women took 1 portion of the reported brand daily. [4] Total intake of folate from diet and multivitamin supplements (µg DFE) was calculated as: µg naturally occurring folate + (1.7 × µg folic acid). [5] RBC-folate was measured in 766 women and vitamin B12, tHcy, MMA, and holoTC were measured in 1219 women between gestational weeks 34th and 36th. DFE, dietary folate equivalent; FFQ, food frequency questionnaire; holoTC, holotranscobalamin; IQR, interquartile range; MMA, methylmalonic acid; RBC-folate, red blood cell folate; tHcy, total homocysteine.

3. Results

3.1. Population Characteristics

Among the 2632 women, 1280 (48.6%) reported using multivitamin supplements containing folic acid during the third trimester. The majority of the supplements contained 200–400 µg folic acid and 0.5-2.5 µg B12 (**Table 1**). Medians [IQR] of dietary and total folate intake were 268 [88] µgDFE day^{-1} and 430 [680] µgDFE day^{-1}, respectively. Dietary and total vitamin B12 intakes were 4.9 [2.6] µg day^{-1} and 5.5 [3.2] µg day^{-1}, respectively (Table 1). The contribution of folic acid to total folate intake was 71.6 [7.4] % in women who took multivitamin supplements. Supplemental vitamin B12 contributed to 17.9 [10.6] % of total B12 intake. Additional child and mother characteristics and maternal nutrient intakes are shown in Table S1 (Supporting Information). The newborns had a median [IQR] birthweight of 3540 [600] g (Table S1, Supporting Information). Birthweight was not available in 0.7% of the infants and growth measurements were not available in 14.6% of the children at 1 year, and in 17.1% at 2 years (lost to follow up) (**Table 2**).

3.2. Maternal Imbalanced Folate to Vitamin B12 and Birthweight

Compared to women with high total intakes of folate and B12 ($n = 846$), imbalanced intakes of the vitamins [in 459 of 2603 women (17.6%)] was not associated with birthweight [adjusted β (95% CI) = –14.87 (–68.87, 39.13)] (**Table 3**). Similarly, birthweight was not associated with other categories of total folate and B12 intakes and there was no interaction between categories of the intakes in their association with birthweight (p for interaction = 0.188).

Imbalanced maternal RBC-folate to plasma vitamin B12 levels was not associated with birthweight [–7.10 (–97.90, 83.71); p for interaction = 0.545] (Table 3). Similarly, imbalanced maternal RBC-folate to MMA levels [36.10 (–52.47, 124.67); p for interaction = 0.552] (Table 3); imbalanced dietary folate to B12 intakes [6.46 (–48.99, 61.92); p for interaction = 0.954]; and imbalanced RBC-folate to holoTC [17.32 (–72.86, 107.49); p for interaction = 0.524] were not associated with birthweight (Table S2, Supporting Information).

In a regression model with dietary vitamin B12 intake as a sole exposure variable, higher maternal dietary vitamin B12 intake was associated with lower birthweight [–9.5 (–15.5, –3.5)]. This association remained significant after further adjustment for caloric intake [–9.5 (–15.6, –3.3)]. Also, higher maternal MMA levels were associated with higher birthweight [234 (43, 426)] (Table S3, Supporting Information). Low maternal dietary vitamin B12 intake (< vs > 4.9 µg day^{-1}) was not associated with having a birthweight above 4000 g [adjusted odds ratio (95% CI) = 0.86 (0.67, 1.14)] or with having a birth below 2500 g [adjusted odds ratio (95% CI) = 0.64 (0.29, 1.45)].

Maternal dietary and total folate intakes, total B12 intake, RBC-folate, plasma vitamin B12, tHcy, and holoTC showed no association with birthweight in fully adjusted regression models that included one maternal exposure variable per model (Table S3, Supporting Information).

3.3. Maternal Imbalanced Folate to Vitamin B12 and Postnatal Child Growth

Imbalanced maternal total intakes of folate and B12 and imbalanced biomarkers were not associated with child's z scores of weight, height, and that of BMI at 1–2 years (Table 3 and Table S2, Supporting Information).

Imbalanced maternal dietary intakes of folate to B12 was associated with lower z scores of weight [adjusted β (95% CI) = –0.14

Table 2. Child growth measurements at age of 1 year and 2 years in children from the KOALA birth cohort study.

	At age 1 year		At age 2 years	
	Number	Mean (SD)	Number	Mean (SD)
Age at growth measurements [months]	2274	11.5 (0.9)	2209	22.1 (3.8)
Weight[1] [kg]	2330	9.7 (1.1)	2290	12.2 (1.6)
Weight z-score[2]	2268	−0.09 (0.92)	2198	−0.12 (0.95)
Height[1] [cm]	2284	75.0 (2.9)	2245	86.0 (4.9)
Height z-score[2]	2250	−0.05 (0.99)	2187	−0.09 (1.06)
BMI[3] [kg/m^2]	2279	17.2 (1.40)	2237	16.4 (1.4)
BMI z-score[2]	2247	−0.01 (1.01)	2182	−0.03 (1.03)

[1] Parent's reported weight and height. [2] z scores for weight, height, and BMI were age- and sex-standardized. [3] Body mass index (BMI) was calculated as (weight in kg/height in m^2).

(–0.25, –0.03); p for interaction = 0.116] and tended to be associated with a lower z scores of height [–0.12 (–0.23, 0.002), p = 0.054; p for interaction = 0.128] at age of 1–2 years compared to when both intakes were above the corresponding medians (Table S2, Supporting Information). Moreover, low maternal RBC-folate combined with low plasma B12 was associated with lower child z scores of BMI at 1–2 years [–0.20 (–0.37, –0.03); p for interaction = 0.321] (Table 3).

Maternal nutrient intakes and biomarkers were not associated with child's anthropometric measures in adjusted regression models that included one maternal exposure variable per model (**Table S3**, Supporting Information). Further adjustment for breastfeeding patterns did not alter the association (data not shown).

4. Discussion

We investigated whether maternal third trimester imbalanced folate to vitamin B12 is related to birthweight and child postnatal growth. Imbalanced intakes and markers of folate to B12 were not associated with birthweight. Higher maternal dietary B12 intake was associated with lower birthweight [–9.5 (–15.5, –3.5) g lower weight for each 1 μg day^{-1} higher intake] in models that did not take folate intake into account. Maternal B12 intake was not associated with high birthweight (> 4000 g) or low birthweight (< 2500 g). Compared to when both dietary folate and B12 intakes were above the corresponding medians, imbalanced folate to B12 dietary intakes (folate > 268 μgDFE day^{-1} and B12 < 4.9 μg day^{-1}) was associated with lower child z scores of weight between 1 and 2 years of age [ß = –0.14 (–0.25, –0.03)].

The lack of association between imbalanced folate to B12 and birthweight in our study is in line with an earlier study in Spanish women.[30] Similarly, imbalanced vitamin markers in Canadian first- and second trimester-pregnant women were not associated with birthweight.[31] In contrast, imbalanced folate to B12 in Indian women was associated with higher birthweight.[32] The women in the Indian study received 5 mg day^{-1} folic acid during the pregnancy and 35% of them had plasma vitamin B12 below 150 pg mL^{-1}.[32] It is possible that the association could be different at very low B12 or very high folic acid intakes or could be due to other accompanying nutritional deficiencies. Imbalanced folate to B12 implies accumulation of folate as 5-methyltetrahydrofolat that cannot be used for synthesis of purines and pyrimidines (folate trap) to support cell growth. However, when high dose of folic acid is provided for subjects with low B12, the DNA synthesis and cell division can be partly maintained.[33] Beside the total intake of folate, it is possible that the source of folate (diet or supplemental folic acid) could be differentially related to child weight.

The association between lower maternal vitamin B12 intake (or higher MMA) and birthweight is in line with earlier studies showing inverse associations between cord plasma B12 and birth size.[34,35] Due to the strong association between maternal and cord plasma vitamin B12 levels,[36] mothers of the heaviest babies are expected to have the lowest B12 levels in their plasma. Maternal folate levels and folic acid use were not associated with child insulin resistance.[37] In contrast, vitamin B12 deficiency has been related to overweight, obesity, and insulin resistance.[37–39] Lowered serum vitamin B12 during early pregnancy was associated with higher HOMA-IR (indicating insulin resistance) in Nepalese children at age of 6–8 years.[37] The prevalence of vitamin B12 deficiency in Indian adolescents was proportional to the degree of overweight and obesity.[39] In adults' young women, low vitamin B12 showed associations with higher BMI[40–42] and plasma lipids.[43] Restriction of vitamin B12 in pregnant rats caused lower birthweight compared to the controls.[44,45] However, the offspring of the deficient rats showed higher percentage of body fats, disturbed lipid metabolism, and intracellular lipid accumulation.[44,45] Thus, lower maternal vitamin B12 could be associated with alternations in body composition that are not reflected by birthweight.

Our results do not generally support an association between imbalanced folate to B12 and child anthropometric measures in the first 2 years. Previous studies showed mixed results.[16,46,47] Maternal vitamin markers and child anthropometrics were not associated in Guatemalan mothers and their 3-month-old breastfed infants[46] or in mothers and their 12-months old infants despite prevalent vitamin B12 deficiency.[47] Both vitamin B12 and RBC-folate measured during pregnancy, but not their imbalance, were associated with child insulin resistance at age of 6 years.[16] In a 6 months randomized, double-blind trial of folic acid and/or vitamin B12, or placebo in 6–35 months old Indian children, improvements in anthropometric measures after B12

Table 3. The associations between imbalanced total intakes of folate and B12 and their biomarkers in third trimester pregnant women and birthweight and child *z* scores of weight, height, and BMI at age 1 and 2 years in the KOALA Birth Cohort Study.

Group definitions	Reference	Imbalanced vitamins	Only folate is low	Both vitamins are low	
Total folate and B12 intakes[1,5]	**High folate–high B12**	**High folate–low B12**	**Low folate–high B12**	**Low folate–low B12**	***p* (inter-actions[6])**
Birthweight, n (total = 2603)	846	459	458	840	
Crude β (95% CI)[2]	0 (reference)	–33.81 (–87.51, 19.88)	–31.97 (–85.70, 21.77)	–16.15 (–61.27, 28.97)	
Fully adjusted β (95% CI)[2,4]	0 (reference)	–14.87 (–68.87, 39.13)	–27.96 (–78.06, 22.13)	4.62 (–40.77, 50.00)	0.188
Weight *z* scores, *n* (total = 4441)	1438	774	763	1466	
Crude β (95% CI)[3]	0 (reference)	–0.04 (–0.14, 0.07)	0.01 (–0.09, 0.12)	–0.07 (–0.16, 0.01)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.03 (–0.14, 0.08)	0.01 (–0.09, 0.11)	–0.03 (–0.12, 0.06)	0.869
Height z scores, n (total = 4412)	1432	768	755	1457	
Crude β (95% CI)[3]	0 (reference)	–0.11 (–0.22, –0.003)	–0.06 (–0.18, 0.05)	–0.10 (–0.19, –0.004)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.07 (–0.19, 0.04)	–0.06 (–0.16, 0.04)	–0.02 (–0.16, 0.08)	0.131
BMI z scores, n (total = 4404)	1429	767	754	1454	
Crude β (95% CI)[3]	0 (reference)	0.04 (–0.07, 0.15)	0.08 (–0.04, 0.19)	–0.02 (–0.11, 0.07)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	0.02 (–0.09, 0.14)	0.07 (–0.04, 0.18)	–0.02 (–0.12, 0.08)	0.148
RBC-folate and plasma B12 levels[5]	**High RBC-folate–high B12**	**High RBC-folate–low B12**	**Low RBC-folate–high B12**	**Low RBC-folate–low B12**	**P (inter-actions[6])**
Birthweight, n (total = 762)	226	155	168	213	
Crude β (95% CI)[2]	0 (reference)	–34.84 (–132.62, 62.94)	3.84 (–91.67, 99.35)	–21.40 (–110.93, 68.14)	
Fully adjusted β (95% CI)[2,4]	0 (reference)	–7.10 (–97.90, 83.71)	19.20 (–69.22, 107.63)	6.14 (–78.78, 91.07)	0.545
Weight *z* scores, *n* (total = 1384)	408	288	301	387	
Crude β (95% CI)[3]	0 (reference)	–0.15 (–0.34, 0.04)	0.02 (–0.16, 0.20)	–0.12 (–0.30, 0.06)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.10 (–0.28, 0.08)	0.04 (–0.13, 0.21)	–0.13 (–0.30, 0.04)	0.624
Height *z* scores, *n* (total = 1378)	407	285	301	385	
Crude β (95% CI)[3]	0 (reference)	–0.20 (–0.40, –0.002)	–0.06 (–0.26, 0.15)	–0.04 (–0.23, 0.15)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.07 (–0.26, 0.12)	0.02 (–0.17, 0.21)	0.02 (–0.15, 0.20)	0.597
BMI *z* scores, *n* (total = 1378)	406	285	299	385	
Crude β (95% CI)[3]	0 (reference)	–0.06 (–0.26, 0.14)	0.08 (–0.11, 0.26)	–0.13 (–0.30, 0.04)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.10 (–0.30, 0.09)	0.04 (–0.14, 0.22)	**–0.20 (–0.37, –0.03)**	0.321
RBC-folate and MMA levels[5]	**High RBC-folate – low MMA**	**High RBC-folate–high MMA**	**Low RBC-folate–low MMA**	**Low RBC-folate–high MMA**	**P (inter-actions[6])**
Birthweight, *n* (total = 762)	198	183	182	199	
Crude β (95% CI)[3]	0 (reference)	11.86 (–84.30, 108.01)	–7.86 (–104.15, 88.44)	25.56 (–68.56, 119.69)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	36.10 (–52.47, 124.67)	–7.09 (–95.79, 81.60)	66.93 (–19.42, 153.28)	0.552
Weight *z* scores, *n* (total = 1384)	369	327	324	364	
Crude β (95% CI)[3]	0 (reference)	0.01 (–0.18, 0.19)	0.02 (–0.17, 0.21)	–0.01 (–0.19, 0.18)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	0.01 (–0.17, 0.19)	–0.03 (–0.20, 0.15)	0.02 (–0.19, 0.05)	0.767
Height *z* scores, *n* (total = 1378)	368	324	323	363	
Crude β (95% CI)[3]	0 (reference)	0.05 (–0.14, 0.25)	0.03 (–0.18, 0.23)	0.09 (–0.10, 0.28)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	0.07 (–0.11, 0.25)	0.03 (–0.16, 0.21)	0.13 (–0.05, 0.31)	0.794
BMI z scores, n (total = 1375)	367	324	321	363	
Crude β (95% CI)[3]	0 (reference)	–0.06 (–0.25, 0.14)	0.006 (–0.18, 0.19)	–0.08 (–0.27, 0.10)	
Fully adjusted β (95% CI)[3,4]	0 (reference)	–0.06 (–0.25, 0.13)	–0.06 (–0.25, 0.12)	–0.09 (–0.26, 0.10)	0.765

[1] Total folate and B12 intakes are the sum of dietary intakes from FFQ and the content of the vitamins in the reported brands of the multivitamin supplements, assuming that women took one tablet of the supplements per day. [2] Generalized Linear Models (GLM) were used to study the association between categories of maternal folate and B12 intakes and biomarkers and birthweight. [3] Generalized Estimating Equations (GEE) were used to study the association between categories of maternal folate and B12 intakes and biomarkers and z scores of weight, height, and BMI at age of 1 year to 2 years. The β (95% CI) show the changes of birthweight or z scores of the child measurements compared to the reference group. [4] The models are adjusted for; maternal intakes of vitamin D, zinc, and iron, maternal age, pre-pregnancy BMI, maternal height (as continuous variables); sex of the child (two categories), mother gravidity (1,2,≥3), maternal education (four categories: low, middle, high/academic, and others), ethnic origin of the mother and the father (each as two categories: both grandparents born in Europe vs all other possibilities), recruitment group (alternative or conventional), season of FFQ (4 categories), maternal smoking during third trimester (yes or no), maternal alcohol consumption during third trimester (yes or no), and gestational diabetes (yes or no). [5] All exposure variables were dichotomized by the corresponding medians in the whole population. The median values are as follow: total folate intake (diet plus supplements) = 430 μgDFE day^{-1}; total vitamin B12 intake (diet plus supplements) = 5.5 µg day^{-1}; RBC-folate = 745 nmol L^{-1}; plasma vitamin B12 = 172 pmol L^{-1}; and plasma MMA = 0.220 µmol L^{-1}. [6] The interactions were studied in the GLM (for birthweight) or GEE (anthropometrics at 1–2 years) models between categories of folate intake (or markers) * vitamin B12 intake (or markers). CI, confidence intervals; DFE, dietary folate equivalent; FFQ, food frequency questionnaire; MMA, methylmalonic acid; RBC-folate, red blood cell folate.

supplementation were mostly confined to wasted, underweight, and stunted children.[6] Thus, there appears to be a threshold for deficiency to affect child growth.

The present study included rather well-nourished pregnant women who gave birth to term babies and were not exposed to mandatory fortification with folic acid. The study included 457 women with alternative lifestyle. Thus, the sample was enriched with women who were likely to have imbalanced vitamins. However, the prevalence of overt vitamin deficiency or supplement overdose was low. The women were generally educated and following alternative lifestyle is likely to be associated with better education and socioeconomic status that play a role in food selection, healthy lifestyle, and supplement use. Another factor is that vitamin B12 deficiency takes years to develop, while following alternative lifestyles is rather a new trend. Thus, the deficiency was not precipitated over generations like in India. These factors may explain heterogeneous results from European, Canadian, and Indian populations.

The strengths of the present study include the prospective design that made it possible to investigate child anthropometrics beyond birthweight. Moreover, the study included a well characterized group, had a large sample size and had collected information on maternal intake and biomarkers at the same time. The study has also some limitations. First, detailed information on exact dose and frequency of supplements was not available. However, the associations between maternal exposures and child anthropometric markers were generally consistent for maternal intakes and biomarkers.

5. Conclusion

Imbalanced maternal total folate to B12 intakes was not associated with birthweight in this study among generally well-nourished women from The Netherlands. Except for child *z* scores for weight at 1–2 years, the associations of imbalanced vitamins with child anthropometric measures after birth were not significant. Low maternal dietary vitamin B12 intake and high plasma MMA levels (but not those of folate) were associated with higher birthweight. Further studies should investigate whether children from women with low vitamin B12 and elevated MMA may have different body composition or higher risk of future obesity and insulin resistance.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors thank the families who participated to the KOALA Birth Cohort Study.

The collection of the data for this study was supported by Royal Friesland Foods (Leeuwarden, now FrieslandCampina); Triodos Foundation (Zeist); Phoenix Foundation; Raphaël Foundation; Iona Foundation; Foundation for the Advancement of Heilpedagogie; Ministry of Public Health, Welfare, and Sport; Hersenstichting (Dutch Brain foundation) and the Dutch Dairy Association (all in the Netherlands). The co-funders had no influence on the design, analysis, and reporting of the present study.

Conflict of Interest

The authors declare no conflict of interest. RO received consulting and speaker honoraria from P&G Health, HIPP, and Merck &Cie.

Author Contributions

R.O., planning and conceptualization of the present research question, conducted the data analysis, and wrote the manuscript. S.J.P.M.E., planning and conceptualization, general supervision of the statistical analysis and reporting, and critical revision of the manuscript. M.M., planning and conceptualization, data curation, contributed to data analyses, and critical review of the manuscript. L.S., RBC-folate measurements and critical review of the manuscript. C.T., planning and conceptualization, data curation, funding and execution of the KOALA Birth Cohort Study, general supervision of the statistical analysis, and reporting and critical review of the manuscript.

Data Availability Statement

Data subject to third-party restrictions. Data described in the manuscript, code book, and analytic code will be made available upon request.

Keywords

birthweight, child growth, folate trap, imbalanced folate and vitamin B12, pregnancy

Received: July 11, 2021
Revised: November 12, 2021
Published online: December 11, 2021

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