Associations of Early Pregnancy and Neonatal Circulating Folate, Vitamin B-12, and Homocysteine Concentrations with Cardiometabolic Risk Factors in Children at 10 y of Age

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ABSTRACT

Background: Higher circulating folate and vitamin B-12 concentrations and lower circulating homocysteine concentrations during pregnancy seem to be associated with fetal development. These micronutrients may also be associated with cardiometabolic health.

Objective: We examined the associations of circulating folate, vitamin B-12, and homocysteine concentrations during pregnancy and in neonates with childhood cardiometabolic outcomes.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward. We sampled blood in early pregnancy and cord blood. We measured cardiometabolic outcomes in the children at school age. Among 4449 children aged 10 y (median: 9.7; 95% range: 9.3, 10.7), we examined associations of plasma folate, serum vitamin B-12, and plasma homocysteine concentrations in early pregnancy and at birth with BMI, body fat distribution, heart rate, blood pressure, and insulin, glucose, and lipid concentrations, using linear regression models. Using logistic models, we examined the associations of these micronutrients with risks of overweight/obesity and clustering of cardiovascular risk factors.

Results: One standard deviation score (SDS) higher maternal plasma folate concentration was associated with lower BMI (−0.04 SDS; 95% CI: −0.08, −0.01), android-to-gynoid fat ratio (−0.04 SDS; 95% CI: −0.07, −0.01), systolic blood pressure (−0.06 SDS; 95% CI: −0.10, −0.03), risk of overweight (OR: 0.87; 95% CI: 0.78, 0.96), and clustering of cardiovascular risk factors (OR: 0.79; 95% CI: 0.68, 0.91). One SDS higher maternal serum total B-12 concentration was associated with lower glucose (−0.06 SDS; 95% CI: −0.10, −0.02) and higher HDL cholesterol concentrations (0.04 SDS; 95% CI: 0.00, 0.08). Cord blood folate, vitamin B-12, and homocysteine concentrations were not consistently associated with cardiometabolic outcomes.

Conclusions: Subtle differences in circulating folate and vitamin B-12 concentrations in early pregnancy may be associated with child cardiometabolic health at age 10 y. The causality and mechanisms underlying these associations need further study. *J Nutr* 2021;151:1628–1636.

Keywords: folate, vitamin B-12, holotranscobalamin, homocysteine, cardiovascular health, cohort, childhood

Introduction

An adverse maternal nutritional status during pregnancy may have long-term consequences for offspring cardiometabolic health (1). Folate, vitamin B-12, and homocysteine interact in 1-carbon metabolism and are crucial for cellular growth and differentiation, nucleic acid synthesis, and DNA methylation (2–5). Without supplementation, circulating folate, vitamin B-12, and homocysteine concentrations decline during pregnancy due to increased demand for fetal growth, hormonal changes, and hemodilution (6–9). Periconceptional folic acid supplementation is advised as standard prenatal care (10). Circulating folate or vitamin B-12 deficiency may result in

elevated circulating homocysteine concentrations (5, 11, 12). In adults, hyperhomocysteinemia is associated with endothelial dysfunction and increased cardiovascular risk (13–15). Previous prospective birth cohorts, including the cohort in which the current study was embedded, have suggested associations of not taking folic acid supplements and of suboptimal maternal folate, vitamin B-12, and homocysteine blood concentrations in pregnancy with lower uteroplacental vascular resistance, lower offspring birth weight, higher BMI, higher heart rate, and lower kidney function in offspring at school age (4, 16–21). No previous studies have assessed associations of multiple 1-carbon metabolism markers, measured at 2 time points in pregnancy, with a wide range of detailed cardiometabolic outcomes in childhood. Also, an imbalance between circulating maternal high folate and low vitamin B-12 blood concentrations may be associated with more adverse health outcomes in the children, but research on this topic is limited (12, 22). Active B-12, or holotranscobalamin, is the biologically active fraction of vitamin B-12 and possibly is a more reliable marker of an impaired vitamin B-12 status in pregnancy (5). Associations of active B-12 concentrations in pregnancy with cardiometabolic health in childhood were not studied previously.

Based on the findings of previous studies, we hypothesized that higher circulating folate and vitamin B-12 concentrations, as well as lower circulating homocysteine concentrations, are associated with better fetal development and subsequently cardiometabolic outcomes in childhood. In a population-based study among 4449 mother–child pairs, we examined associations of plasma folate, serum total and active B-12, and plasma homocysteine concentrations in early pregnancy and in cord blood with BMI, body fat distribution, heart rate, blood pressure, and insulin, glucose, and lipid concentrations in the children at age 10 y. We explored whether imbalanced maternal circulating folate and vitamin B-12 status was associated with these outcomes. Also, we assessed associations of circulating 1-carbon metabolism markers with risks of overweight and clustering of cardiovascular risk factors in childhood.

Methods

Participants

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onward in Rotterdam, the Netherlands (23). It was designed to identify early environmental and genetic causes of normal and abnormal growth, development, and health (23). Pregnant women with an expected delivery date between April 2002 and January 2006 living in Rotterdam were eligible to participate. Enrollment was aimed at early pregnancy but was allowed until the birth of the child. The Medical Ethical Committee of the Erasmus University Medical Center in Rotterdam approved the study (MEC 198.782/2001/31). Written informed consent was obtained from all participants. Data collection included blood sampling in pregnancy and in cord blood (23). Also, we measured cardiometabolic outcomes in the children at 10 y. In the current study, we included 4449 singleton multiethnic children aged 10 (median: 9.7; 95% range: 9.3, 10.7) years with at least 1 measurement of circulating folate, total or active B-12, or homocysteine concentrations in either pregnancy or in cord blood and 1 or more cardiometabolic risk factors measured. A flowchart of the study population is shown in **Supplemental Figure 1**.

Folate, vitamin B-12, and homocysteine concentrations

As described previously, maternal venous blood samples were drawn in early pregnancy (median weeks of gestation, 13.2; IQR: 12.2–14.8 wk), and cord blood samples were taken immediately after delivery by midwives (40.1; IQR: 39.3–41.0 wk of gestation) (18). Blood samples were stored at room temperature for a maximum of 3 h before being transported to the regional laboratory for processing and storage at $-80°C$. After thawing, EDTA plasma folate and homocysteine concentrations and serum total and active B-12 concentrations were analyzed in the Department of Clinical Chemistry at the Erasmus University Medical Centre, Rotterdam, using an immune-electrochemiluminescence assay on the Architect System (18). Active B-12 was analyzed in stored serum samples only and therefore available in a smaller subgroup. We also dichotomized maternal and cord blood folate (≥ 8 and <8 nmol/L, respectively), total B-12 (≥ 145 and <145 pmol/L, respectively), active B-12 (≥ 21 and <21 pmol/L, respectively), and homocysteine (<19 and ≥ 19 μmol/L, respectively) concentrations into normal and low according to the 95% reference interval for healthy adults (4, 6, 24, 25). To explore whether an imbalance of circulating maternal high folate status and low vitamin B-12 status is associated with detrimental cardiometabolic outcomes in childhood (12, 22), we categorized mothers based on their combined circulating folate/total B-12 status and similarly based on their combined circulating folate/active B-12 status. Plasma folate concentrations in the first/second tertile (<22.6) were defined as "low/normal"; concentrations in the third tertile (≥ 22.6 nmol/L) were defined as "high." Serum total B-12 was again dichotomized based on the 95% reference interval, as values <145 pmol/L corresponded to the first tertile. Serum active B-12 concentrations in the first tertile (<36 pmol/L) were defined as "low" and concentrations in the second/third tertile as "normal/high." Groups to analyze any circulating folate/total B-12 imbalance were low/normal folate + normal/high total B-12 (reference), high folate + normal/high total B-12, low/normal folate + low total B-12, and high folate + low total B-12. Similarly, we grouped mothers into circulating low/normal folate + normal/high active B-12 (reference), high folate + normal/high active B-12, low/normal folate + low active B-12, and high folate + low active B-12. Periconceptional folic acid supplementation was assessed by questionnaires. We categorized mothers into no supplementation, started before 10 wk gestational age, and started preconception (reference).

Childhood fat and cardiometabolic measurements

When children visited the research facility at age 10 y, we calculated BMI (in kg/m^2) from weight and height and subsequently sex- and age-adjusted standard deviation scores (SDSs), based on Dutch reference growth charts (Growth Analyzer 4.0; Dutch Growth Research Foundation) (26). We categorized BMI into underweight, normal weight, and overweight/obesity, based on the International Obesity Task Force cutoffs (27). We measured children's body composition with a DXA scanner (iDXA; Ge-Lunar 2008, Madison, WI, USA) using enCORE software version 12.6 (28). From these measurements, of which details have been described previously, we calculated fat mass index [FMI, fat mass (kg)/height (m^4)] and android fat mass as a

percentage of total fat mass (29). We calculated the ratio of android and gynoid fat mass, which reflects the relation between fat mass in the abdominal (android) and hip (gynoid) regions. We assessed heart rate and blood pressure at the right brachial artery 4 times with 1-min intervals with the validated automatic sphygmomanometer Datascope Accutorr Plus (Paramus, NJ, USA) (30). Mean heart rate and systolic and diastolic blood pressure were calculated from the last 3 measurements. We obtained venous blood samples after 30 min of fasting and measured insulin, glucose, total cholesterol, HDL cholesterol, and triglyceride concentrations (23). We measured insulin concentrations with the electro-chemiluminescence immunoassay on the E411 module (Roche) and glucose, total and HDL cholesterol, and triglyceride concentrations with the c702 module on the Cobas 8000 analyzer. We calculated LDL cholesterol concentrations using the Friedewald formula (31). As previously, we defined clustering of cardiovascular risk factors as having at least 3 of the following 4 risk factors: android fat mass percentage >75th percentile, systolic or diastolic blood pressure or both >75th percentile, HDL cholesterol <25th percentile or triglycerides >75th percentile or both, and insulin concentration >75th percentile of our study population (32, 33).

Covariates
We selected potential covariates based on previous literature and constructed a directed acyclic diagram (**Supplemental Figure 2**). From questionnaires sent out in each trimester of pregnancy, we obtained information on maternal age, highest completed education according to the classification of Statistics Netherlands as a proxy for socioeconomic status, prepregnancy BMI, parity, and smoking and alcohol consumption during pregnancy (23, 34). From questionnaires and midwife and hospital records, we obtained information on gestational age at blood sampling, sex, child ethnicity according to the classification of Statistics Netherlands, and birth weight, for which we created sex- and gestational age–adjusted SDSs based on a North European reference chart (35, 36).

Statistical analysis
First, we performed a nonresponse analysis by comparing child characteristics with and without at least 1 outcome measurement using Student t tests, Mann–Whitney tests, and χ^2 tests. Second, we examined the associations of circulating folate, total and active B-12, and homocysteine concentrations with all outcomes using multivariable linear regression models. Basic models were adjusted for sex, child age, and gestational age at blood sampling. Main models were also adjusted for maternal age, education, prepregnancy BMI, smoking and alcohol consumption, parity, and child ethnicity. To compare effect estimates, exposures and outcomes were analyzed in SDSs after natural log transformation of FMI, android fat mass percentage, android-to-gynoid fat ratio, and insulin and triglyceride concentrations, which all had skewed distributions (4, 33). As a sensitivity analysis, we assessed whether effect estimates of the associations were similar in an ethnic homogeneous Dutch subgroup. Also, we explored associations of folic acid supplementation and of dichotomized maternal circulating early pregnancy folate and total B-12 concentrations with all outcomes. Similar analyses were performed to explore associations of potentially imbalanced maternal circulating folate/vitamin B-12 status. In case of associations with $P \leq 0.05$ in the main models, we ran 3 mediator models to explore whether these associations were explained by child BMI (BMI model), birth weight (birth weight model), or plasma homocysteine (homocysteine model). We used multivariable logistic models to analyze associations of circulating folate, vitamin B-12, and homocysteine concentrations with the odds of overweight and clustering of cardiovascular risk factors. We did not perform sex-stratified analyses because none of the exposures showed interactions with sex ($P > 0.05$). We used multiple imputations for covariates with missing values, using the Markov chain Monte Carlo method. We created 5 data sets and analyzed these together (37). We performed all statistical analyses using the Statistical Package of Social Sciences version 25.0 for Windows (SPSS IBM).

Results

Participant characteristics
Table 1 and **Supplemental Table 1** show participant characteristics before and after imputation for covariates, respectively. **Table 2** shows the distribution of circulating folate, vitamin B-12, and homocysteine concentrations in early pregnancy and cord blood. Correlations between circulating folate and vitamin B-12 concentrations in early pregnancy and cord blood were low ($r < 0.2$, **Supplemental Table 2**). The nonresponse analysis suggested that, compared with children included in the analyses, not included children were less frequently European and had lower-educated, multiparous mothers who used less often alcohol and folic acid supplements and had lower plasma folate concentrations (**Supplemental Table 3**).

Childhood fat measurements
Table 3 shows that 1 SDS (9.1 nmol/L) higher maternal plasma folate concentrations were associated with lower childhood BMI (−0.04 SDS; 95% CI: −0.08, −0.01) and FMI (−0.04 SDS; 95% CI: −0.07, −0.01). The associations of dichotomized early pregnancy folate were similar (**Table 3**). Among Dutch children only, these effect estimates were similar but not significant because of lower numbers (**Supplemental Table 4**). Compared with taking no supplementation, starting folic acid preconception was associated with lower childhood BMI (−0.17 SDS; 95% CI: −0.28, −0.06), FMI (−0.27 SDS; 95% CI: −0.37, −0.17), fat mass percentage (−0.23 SDS; 95% CI: −0.33, −0.12), and android-to-gynoid fat ratio (−0.24 SDS; 95% CI: −0.35, −0.14). Active B-12, total B-12, and homocysteine blood concentrations were not associated with childhood fat measurements. The results from basic models were largely similar (data not shown). Mediator models are shown in **Supplemental Table 5**. We observed no evidence for associations of maternal imbalanced circulating folate/vitamin B-12 status with childhood fat measurements (**Supplemental Table 6**).

Childhood cardiometabolic measurements
Table 4 shows that 1 SDS higher maternal plasma folate concentrations were associated with lower childhood systolic blood pressure (−0.06 SDS; 95% CI: −0.10, −0.03). Also, compared with taking no supplementation, starting folic acid preconception was associated with lower childhood systolic blood pressure (−0.19 SDS; 95% CI: −0.30, −0.08). One SDS (93 pmol/L) higher serum total B-12 concentrations in early pregnancy were associated with lower glucose concentrations (−0.06 SDS; 95% CI: −0.10, −0.02) and higher HDL cholesterol concentrations (0.04 SDS; 95% CI: 0.00, 0.08) in childhood, whereas 1 SDS (20 pmol/L) higher serum active B-12 concentrations were associated with lower childhood diastolic blood pressure (−0.05 SDS; 95% CI: −0.09, −0.01). Models with dichotomized circulating early pregnancy folate and total B-12 concentrations showed consistent results (**Table 4**). In neonates, 1 SDS (29 pmol/L) higher serum active B-12 concentrations were associated with lower childhood heart rates (−0.04 SDS; 95% CI: −0.08, −0.01) and glucose concentrations (−0.05 SDS; 95% CI: −0.10, −0.01) but higher HDL cholesterol concentrations (0.05 SDS; 95% CI: 0.00, 0.09). For heart rate and glucose, these associations had similar effect estimates but attenuated into nonsignificance after adjustment for plasma homocysteine (**Supplemental Table 7**). One SDS (2.9 μmol/L) higher cord blood homocysteine concentrations were associated with higher childhood

TABLE 1 Characteristics of participating mother–child pairs ($n = 4449$)[1]

Characteristic	Value
Maternal characteristics	
Age,[2] y	30.7 (4.8)
Educational level	
No or primary	310 (7.3)
Secondary	1777 (41.9)
Higher	2158 (50.8)
Parity	
Nulliparous	2610 (59.0)
Multiparous	1817 (41.0)
Prepregnancy BMI,[2] kg/m^2	22.6 (18.1, 34.3)
Smoking	
Nonsmoker or smoked until pregnancy was known	3377 (84.3)
Smoked throughout pregnancy	629 (15.7)
Alcohol consumption	
Nonuser or consumption until pregnancy was known	2234 (56.5)
Sustained consumption	1722 (43.5)
Gestational age at blood sampling,[2] wk	13.2 (9.8, 17.4)
Newborn characteristics	
Gestational age,[2] wk	40.1 (36.0, 42.3)
Birth weight,[2] kg	3.47 (2.28, 4.46)
Sex	
Boy	2196 (49.4)
Girl	2253 (50.6)
Ethnicity	
European	3005 (68.5)
Non-European	1380 (31.5)
Childhood characteristics	
Child age at visit,[2] y	9.7 (9.3, 10.7)
Childhood BMI,[2] kg/m^2	17.6 (2.8)
Underweight	315 (7.1)
Normal weight	3333 (75.1)
Overweight	632 (14.2)
Obese	158 (3.6)
Fat mass index,[2,3] kg/m^4	2.2 (1.2, 5.0)
Android fat mass,[2,3] %	4.0 (2.4, 7.7)
Android-to-gynoid fat ratio[2,3]	0.24 (0.15, 0.49)
Heart rate,[2] beats per minute	73.4 (9.8)
Blood pressure,[2] mmHg	
Systolic	103 (7.9)
Diastolic	59 (6.4)
Insulin,[2,3] pmol/L	175 (216, 636)
Glucose,[2] mmol/L	5.2 (0.9)
Total cholesterol,[2] mmol/L	4.3 (0.7)
HDL cholesterol,[2] mmol/L	1.5 (0.3)
LDL cholesterol,[2] mmol/L	2.3 (0.6)
Triglycerides,[2,3] mmol/L	1.0 (0.4, 2.6)
Prevalence cardiovascular clustering,[5] n (%)	391 (13.3)

[1] Values are based on nonimputed data and are mean (SD) or median (95% range) for continuous variables and numbers (%) for categorical variables.
[2] Standard deviation scores were calculated for these variables.
[3] Indicate values before natural log transformation.
[4] Underweight, normal weight, overweight, and obesity were defined based on the International Obesity Task Force cutoffs (sex and age specific), defined to pass through a BMI of 25 and 30 kg/m^2 at age 18 y (27).
[5] Cardiovascular clustering was defined as having 3 or more risk factors (android fat mass >75th percentile, systolic or diastolic blood pressure >75th percentile or both, HDL cholesterol <25th percentile or triglycerides >75th percentile or both, insulin concentration >75th percentile of our study population). The prevalence of cardiovascular clustering was calculated in the subgroup of $n = 4401$ children with complete cardiovascular outcomes.

insulin (0.06 SDS; 95% CI: 0.02, 0.11; **Supplemental Table 8**) and glucose (0.05 SDS; 95% CI: 0.01, 0.10) concentrations. Only among Dutch children, 1 SDS higher maternal plasma homocysteine concentrations were associated with lower childhood LDL cholesterol concentrations (−0.05 SDS; 95% CI: −0.10, −0.01; **Supplemental Tables 9–10**). An imbalance between circulating maternal high folate and low total B-12, compared with low/normal folate and normal vitamin B-12, was only associated with higher childhood glucose concentrations (0.18 SDS; 95% CI: 0.03, 0.33; **Supplemental Tables 11–12**). The interaction term between maternal total B-12 and folate blood concentrations was significant for this association ($P = 0.045$).

Risks of childhood overweight and clustering of cardiovascular outcomes

Table 5 shows that only 1 SDS higher maternal plasma folate concentrations were associated with lower odds of childhood overweight/obesity (OR: 0.87; 95% CI: 0.78, 0.96) and clustering of cardiovascular risk factors (OR: 0.79; 95% CI: 0.68, 0.91).

Discussion

Our results suggest that higher maternal plasma folate concentrations in early pregnancy are associated with lower BMI and FMI in children at age 10 y and with lower risk of overweight and clustering of cardiometabolic outcomes. We found some evidence that optimal circulating folate, vitamin B-12, and homocysteine concentrations during fetal development are associated with individual favorable cardiometabolic outcomes in childhood. An imbalance between circulating maternal high folate and low total B-12 was associated with higher childhood glucose concentrations.

An adverse nutritional status during fetal life may contribute to development of disease in later life (1). Folate and vitamin B-12 are essential for cellular growth and differentiation and regulate homocysteine metabolism (6, 12). Higher circulating folate and vitamin B-12 concentrations and lower homocysteine concentrations during pregnancy may be associated with more favorable cardiometabolic outcomes in childhood.

We observed that higher maternal plasma folate concentrations in early pregnancy were associated with lower BMI and FMI, as well as lower risk of overweight in the children at age 10 y. Periconceptional folic acid supplementation was associated with a favorable body fat distribution in childhood. Five smaller previous studies, embedded in prospective birth cohort studies, have examined associations of any 1-carbon metabolism markers with body fat measurements but are less comprehensive compared with our study (16, 17, 22, 38, 39). Of these, 3 Western studies assessed BMI only in younger children (16, 17, 38). The first, among 1950 multiethnic children from Amsterdam, reported associations of higher early pregnancy folate concentrations with lower BMI at age 5 y. This association was similar among Dutch children only. The second study included 1517 six-year-old children from low-income families in Boston and suggested a higher risk of overweight among children of mothers with postpartum circulating folate concentrations in the lowest quartile compared with higher quartiles. In our study population, high folic acid intakes as toddlers were associated with lower body weight and BMI

TABLE 2 Characteristics of participants' plasma folate, serum total and active B-12, and plasma homocysteine concentrations in early pregnancy and in cord blood[1]

Characteristic	Maternal early pregnancy (*n* = 3701)	Cord blood (*n* = 3112)
Plasma folate concentration,[2] nmol/L	17.4 (6.0, 38.0)	20.8 (10.6, 38.5)
1 SDS, nmol/L	9.1	7.6
≥8 nmol/L	3254 (89.6)	3014 (99.8)
<8 nmol/L	378 (10.4)	7 (0.2)
Serum total B-12 concentration,[2] pmol/L	172 (76, 414)	303 (120, 903)
1 SDS, pmol/L	93	202
≥145 pmol/L	2301 (66.3)	2877 (94.2)
<145 pmol/L	1170 (33.7)	178 (5.8)
Serum active B-12 concentration,[2] pmol/L	42 (18, 98)	87 (36, 128)
1 SDS, pmol/L	20	29
≥21 pmol/L[2]	2478 (95.5)	2909 (99.8)
<21 pmol/L	118 (4.5)	7 (0.2)
Plasma homocysteine concentration,[2] μmol/L	6.9 (4.6, 11.6)	9.0 (7.4, 16.2)
1 SDS, μmol/L	2.0	2.9
<19 μmol/L	3583 (99.6)	2905 (99.1)
≥19 μmol/L	13 (0.4)	25 (0.9)
Folic acid supplement use[3]		
No	550 (19.0)	—
From early pregnancy	937 (32.4)	—
Yes, from preconception	1406 (48.6)	—

[1]Values are based on nonimputed data and are median (95% range) for continuous variables and numbers (%) for categorical variables. SDS, standard deviation score.
[2]Folate, vitamin B-12, and homocysteine were dichotomized based on the 95% reference interval for healthy adults.
[3]Information on folic acid supplement use was available in 2893 mothers with information on maternal folate concentration in early pregnancy.

(*n* = 2922) at age 6 y ([38]). Only 2 small Indian studies examined associations of 1-carbon metabolism markers with body fat distribution ([22], [39]). One study among 674 children aged 6 y observed positive associations between folate concentrations in pregnancy and fat mass and fat mass percentage but not lean mass ([22]). These associations of higher folate concentrations with unfavorable body fat distribution may be explained by differences in socioeconomic status and diet ([16], [22], [39]). The second study, among 478 children, found no associations of folate concentrations in pregnancy with skinfold or fat mass percentage at 3 ages ([39]). In our relatively large study population, we were able to confirm absence of associations between circulating vitamin B-12 concentrations in pregnancy and childhood BMI or body fat distribution, which were investigated previously by the study from Amsterdam and both Indian studies ([16], [22], [39]). To our knowledge, we were the first to study associations of active B-12 and homocysteine concentrations with childhood fat outcomes and thus cannot compare our null findings with others. If cord blood is a proxy for late pregnancy, our results suggest that only in early pregnancy are plasma folate concentrations critical for offspring body fat development. Alternatively, we lacked power to detect associations of cord blood folate due to lower numbers. Taken together, our findings suggest that higher early pregnancy plasma folate concentrations are associated with more beneficial body fat measurements in children aged 10 y. These observations seem similar to associations in younger children but need confirmation in larger non-Western studies.

Higher plasma folate concentrations in early pregnancy and periconceptional folic acid supplementation were associated with lower childhood systolic blood pressure. Higher early pregnancy serum active B-12 concentrations were associated with lower childhood diastolic blood pressure. Also, higher early pregnancy serum total B-12 concentrations were associated with lower childhood glucose and higher HDL cholesterol concentrations. Only early pregnancy folate concentrations were associated with a lower risk of clustering of cardiovascular risk factors in childhood. Multiple studies have reported on any associations of 1-carbon metabolism markers in pregnancy with offspring blood pressure, glucose, insulin, and lipid concentrations ([16], [17], [22], [39], [40]). Most studies focused on folate and vitamin B-12 concentrations and on younger children, and moreover, results were inconsistent. The study from Amsterdam reported an inverse association of maternal folate but not vitamin B-12 concentrations in pregnancy with diastolic blood pressure at age 6 y, which was no longer significant in the full model. No information on homocysteine concentrations was available ([16]). A second study, embedded in the Boston cohort, examined maternal postpartum folate blood concentrations in 492 mothers with a cardiometabolic risk factor and found that concentrations above the median were associated with a lower risk of elevated systolic blood pressure in their children aged 6 y ([41]). A third study among 2863 children, embedded in our cohort, reported that higher maternal early pregnancy total B-12 but not folate and homocysteine concentrations were associated with higher diastolic blood pressure at age 6 y ([21]). A similar unexpected inverse association has been reported between vitamin B-12 and birth weight and could be explained by reverse causation, as smaller fetuses require less vitamin B-12 for development ([18]). To our knowledge, we are the first reporting on associations of higher cord blood active B-12 concentrations with lower childhood heart rates and glucose concentrations but higher HDL cholesterol concentrations. Also, higher cord blood homocysteine concentrations were positively associated with childhood insulin and glucose concentrations. One of the studies from India observed positive associations between homocysteine concentrations in late pregnancy and glucose concentrations in children aged 5 and 10 y ([39]). The differences

TABLE 3 Associations of circulating folate, vitamin B-12, and homocysteine concentrations in early pregnancy and in cord blood with body fat measurements in children aged 10 y[1]

Characteristic	Difference (95% CI) in SDS			
	Body mass index ($n = 4438$)	Fat mass index ($n = 4401$)	Android fat mass, % ($n = 4401$)	Android-to-gynoid fat ratio ($n = 4401$)
Maternal early pregnancy[2]				
Folate,[3] SDS	− 0.04 (−0.08, −0.01)*	− 0.04 (−0.07, −0.01)*	− 0.02 (−0.05, 0.01)	− 0.03 (−0.06, 0.01)
≥8 nmol/L	Reference	Reference	Reference	Reference
<8 nmol/L	0.15 (0.05, 0.26)*	0.16 (0.07, 0.26)**	0.14 (0.03, 0.24)**	0.16 (0.00, 0.03)*
Total B-12,[4] SDS	0.02 (−0.02, 0.05)	− 0.00 (−0.03, 0.03)	− 0.02 (−0.05, 0.01)	− 0.02 (−0.05, 0.01)
≥145 pmol/L	Reference	Reference	Reference	Reference
<145 pmol/L	0.00 (−0.07, 0.07)	0.02 (−0.04, 0.08)	0.05 (−0.02, 0.12)	0.04 (−0.03, 0.11)
Active B-12, SDS	0.03 (−0.01, 0.07)	0.00 (−0.03, 0.04)	− 0.03 (−0.07, 0.01)	− 0.02 (−0.05, 0.02)
Homocysteine, SDS	− 0.00 (−0.03, 0.03)	− 0.01 (−0.04, 0.02)	0.02 (−0.01, 0.05)	0.02 (−0.01, 0.05)
Folic acid supplement use				
No	Reference	Reference	Reference	Reference
From early pregnancy	− 0.05 (−0.16, 0.06)	− 0.11 (−0.21, −0.01)*	− 0.10 (−0.20, 0.01)	− 0.12 (−0.23, −0.02)*
From preconception	− 0.17 (−0.28, −0.06)**	− 0.27 (−0.37, −0.17)**	− 0.23 (−0.33, −0.12)**	− 0.24 (−0.35, −0.14)**
Cord blood[2]				
Folate, SDS	− 0.01 (−0.04, 0.03)	− 0.00 (−0.04, 0.03)	0.01 (−0.03, 0.04)	0.01 (−0.02, 0.05)
Total B-12, SDS	0.01 (−0.03, 0.04)	0.01 (−0.02, 0.05)	− 0.01 (−0.04, 0.02)	− 0.01 (−0.04, 0.03)
Active B-12, SDS	0.02 (−0.01, 0.06)	0.02 (−0.02, 0.05)	− 0.00 (−0.04, 0.03)	0.00 (−0.03, 0.04)
Homocysteine, SDS	− 0.01 (−0.04, 0.03)	− 0.02 (−0.05, 0.02)	− 0.01 (−0.04, 0.03)	− 0.01 (−0.04, 0.03)

[1]The main models were adjusted for child sex, gestational age at blood sampling, child age at outcome, maternal confounders (parity, age, education, prepregnancy BMI, smoking, alcohol consumption), and child ethnicity. Folate and homocysteine were measured in plasma and total and active B-12 were measured in serum. *$P < 0.05$. **$P < 0.01$. SDS, standard deviation score.

[2]For early pregnancy active B-12 and homocysteine, and cord blood folate, total and active B-12 and homocysteine, the distribution of participants after dichotomization was deemed too uneven for meaningful analyses.

[3]Plasma folate ≥8 nmol/L: $n = 3254$; plasma folate <8 nmol/L: $n = 378$.

[4]Serum total B-12 ≥145 pmol/L: $n = 2301$; serum total B-12 <145 pmol/L: $n = 1170$.

in our study for early pregnancy and cord blood may possibly be explained by changes in maternal metabolism during pregnancy (42).

In the most thorough analysis so far, we observed an association of imbalanced maternal circulating high folate and low vitamin B-12 status with higher offspring glucose concentrations. One Indian study reported that offspring of mothers with such an imbalance were more insulin resistant (22). An underlying mechanism for these associations could be that high folic acid intake (e.g., via supplements) prevents the acceptation of the methyl group from 5-methyl tetrahydrofolate and dysregulates 1-carbon metabolism by oxidizing the cobalt of vitamin B-12 (12). This association needs further replication.

The mechanisms underlying the observed associations may include epigenetic fetal reprogramming in response to an adverse environment in utero (1). The 1-carbon metabolism is essential for DNA methylation (6). Folate and vitamin B-12 are important for fetal growth and development. Accelerated postnatal growth to compensate for fetal growth restriction is associated with cardiometabolic disease in later life (18). Suboptimal vitamin B-12 status may affect fetal neurodevelopment and autonomic regulation, potentially influencing blood pressure and heart rate (16). High homocysteine concentrations have been associated with endothelial dysfunction and increased cardiovascular risk in adults but also with preeclampsia, prematurity, and low birth weight (3, 4, 13, 18). In pregnancy, active B-12 seems critical as greater amounts of total B-12 are partitioned toward active B-12 (6). This could explain why we observed more associations for active compared with total B-12.

Although effect estimates were small, our findings emphasize that even minor fluctuations in circulating folate, vitamin

B-12, and homocysteine concentrations during fetal development may be associated with childhood cardiometabolic health. This is relevant, as childhood health may contribute to health in adult life. Strengths of this study are its implementation in an observational cohort study, large sample size, active B-12 measurements, and availability of detailed cardiometabolic outcomes. After sampling, blood was stored at room temperature for up to 3 h. Time-dependent increases in plasma homocysteine concentrations, due to continued production and release of homocysteine from blood cells, may have limited our capacity to detect associations between plasma homocysteine concentrations and all outcomes (43). Although we excluded few children, the nonresponse analysis suggested that they were from a lower socioeconomic background, and selection bias could have led to underestimation of our results. We measured plasma glucose and insulin in nonfasting samples. This may have led to misclassification, although this seems no major issue for cardiovascular risk prediction in adults (44). We performed many statistical tests but did not adjust for multiple testing, as exposures and outcomes were correlated. We adjusted for potential confounders but cannot exclude residual confounding due to unmeasured factors. After dichotomization based on the 95% reference ranges, only maternal circulating folate and total B-12 concentrations were evenly distributed for meaningful analyses. Using lower cutoffs (e.g, 12–15 μmol/L as used in previous studies for elevated blood homocysteine concentrations) was not possible due to low numbers (45–47). Similarly, our analyses on the associations of imbalanced maternal circulating folate and vitamin B-12 status were limited by the distribution of plasma folate concentrations in our cohort. Few mothers had extreme plasma folate concentrations, corresponding to the upper limit of the 95% reference range.

TABLE 4 Associations of circulating folate, vitamin B-12, and homocysteine concentrations in early pregnancy and in cord blood with cardiometabolic measurements in children aged 10 y[1]

Characteristic	Difference (95% CI) in SDS					
	Heart rate (n = 4237)	Systolic blood pressure (n = 4295)	Diastolic blood pressure (n = 4295)	Glucose (n = 3092)	Total cholesterol (n = 3092)	HDL cholesterol (n = 3092)
Maternal early pregnancy[2]						
Folate,[3] SDS	0.02 (−0.02, 0.05)	−0.06 (−0.10, −0.03)**	−0.03 (−0.06, 0.01)	0.00 (−0.04, 0.05)	0.02 (−0.02, 0.06)	0.01 (−0.03, 0.06)
≥8 nmol/L	Reference	Reference	Reference	Reference	Reference	Reference
<8 nmol/L	0.02 (−0.09, 0.13)	0.17 (0.06, 0.28)**	0.01 (−0.10, 0.12)	0.00 (−0.13, 0.13)	−0.07 (−0.20, 0.07)	−0.09 (−0.22, 0.04)
Total B-12,[4] SDS	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	0.01 (−0.02, 0.05)	−0.06 (−0.10, −0.02)**	0.03 (−0.01, 0.08)	0.04 (0.00, 0.08)*
≥145 pmol/L	Reference	Reference	Reference	Reference	Reference	Reference
<145 pmol/L	0.09 (0.02, 0.16)*	0.03 (−0.04, 0.10)	0.01 (−0.06, 0.09)	0.10 (0.01, 0.18)*	−0.05 (−0.13, 0.04)	−0.03 (−0.11, 0.06)
Active B-12, SDS	−0.03 (−0.07, 0.01)	−0.03 (−0.07, 0.01)	−0.05 (−0.09, −0.01)*	−0.01 (−0.06, 0.04)	−0.01 (−0.06, 0.04)	0.02 (−0.03, 0.06)
Homocysteine, SDS	−0.02 (−0.05, 0.01)	0.03 (−0.01, 0.06)	−0.01 (−0.04, 0.03)	−0.01 (−0.04, 0.03)	−0.03 (−0.07, 0.01)	0.01 (−0.03, 0.05)
Folic acid supplement use						
No	Reference	Reference	Reference	Reference	Reference	Reference
From early pregnancy	−0.05 (−0.17, 0.06)	−0.11 (−0.23, 0.00)	−0.01 (−0.12, 0.11)	0.03 (−0.11, 0.17)	0.05 (−0.08, 0.19)	0.10 (−0.03, 0.23)
From preconception	0.02 (−0.09, 0.13)	−0.19 (−0.30, −0.08)**	−0.05 (−0.17, 0.07)	0.01 (−0.13, 0.14)	0.04 (−0.09, 0.18)	0.18 (0.04, 0.31)**
Cord blood[2]						
Folate, SDS	−0.02 (−0.05, 0.02)	−0.04 (−0.07, 0.00)	−0.01 (−0.05, 0.03)	0.01 (−0.04, 0.05)	0.01 (−0.04, 0.06)	−0.01 (−0.05, 0.04)
Total B-12, SDS	−0.01 (−0.05, 0.02)	0.00 (−0.03, 0.04)	0.03 (−0.01, 0.06)	−0.04 (−0.08, 0.01)	0.04 (−0.01, 0.08)	0.04 (−0.00, 0.08)
Active B-12, SDS	−0.04 (−0.08, −0.01)*	0.01 (−0.03, 0.04)	0.03 (−0.004, 0.07)	−0.05 (−0.1, −0.01)*	0.02 (−0.02, 0.07)	0.05 (0.00, 0.09)*
Homocysteine, SDS	0.03 (−0.01, 0.06)	0.02 (−0.02, 0.06)	−0.02 (−0.06, 0.01)	0.05 (0.01, 0.10)*	−0.03 (−0.07, 0.02)	−0.03 (−0.08, 0.01)

[1]The main models were adjusted for child sex, gestational age at blood sampling, child age at outcome, maternal confounders (parity, age, education, prepregnancy BMI, smoking, alcohol consumption), and child ethnicity. Folate and homocysteine were measured in plasma and total and active B-12 were measured in serum. *P < 0.05. **P < 0.01. SDS, standard deviation score.

[2]For early pregnancy, active B-12 and homocysteine, and cord blood folate, total and active B-12 and homocysteine, the distribution of participants after dichotomization was deemed too uneven for meaningful analyses.

[3]Plasma folate ≥8 nmol/L: n = 3254; plasma folate <8 nmol/L: n = 378.

[4]Serum total B-12 ≥ 45 pmol/L: n = 2301; serum total B-12 <145 pmol/L: n = 1170.

TABLE 5 Associations of circulating folate, vitamin B-12, and homocysteine concentrations in early pregnancy and in cord blood with the risk of overweight/obesity and cardiovascular clustering at age 10 y[1]

Characteristic	OR (95% CI) for overweight/obesity[2] (n = 4123)	OR (95% CI) for clustering cardiovascular risk factors[3] (n = 4401)
Maternal early pregnancy		
Folate, SDS	0.87 (0.78, 0.96)**	0.79 (0.68, 0,91)**
Total B-12, SDS	0.99 (0.89, 1.10)	0.92 (0.80, 1.05)
Active B-12, SDS	0.99 (0.88, 1.10)	0.92 (0.79, 1.06)
Homocysteine, SDS	1.02 (0.93, 1.12)	1.04 (0.93, 1.16)
Cord blood		
Folate, SDS	0.98 (0.87, 1.09)	1.00 (0.86, 1.15)
Total B-12, SDS	0.99 (0.89, 1.10)	0.92 (0.80, 1.07)
Active B-12, SDS	1.10 (0.91, 1.12)	0.90 (0.78, 1.04)
Homocysteine, SDS	1.00 (0.90, 1.11)	1.10 (0.97, 1.25)

[1] Associations are based on the main models with adjustment for child sex, gestational age at blood sampling, child age at outcome, maternal confounders (parity, age, education, prepregnancy BMI, smoking, alcohol consumption), and child ethnicity. Sensitivity analysis among mother–child pairs from Dutch ethnicity resulted in similar results (data not shown). Folate and homocysteine were measured in plasma and total and active B-12 were measured in serum. **$P < 0.01$. SDS, standard deviation score.
[2] Reference group: children with normal weight. Overweight and obesity were defined based on the International Obesity Task Force cutoffs and were present in 790 (17.8%) of n = 4438 children with information on childhood BMI; 315 children with underweight were excluded.
[3] Reference group: children without cardiovascular clustering. Cardiovascular clustering was defined as having 3 or more risk factors (android fat mass >75th percentile, systolic or diastolic blood pressure >75th percentile or both, HDL cholesterol <25th percentile or triglycerides >75th percentile or both, insulin concentration >75th percentile of our study population). Cardiovascular clustering was calculated in the subgroup of 4401 children with complete cardiovascular outcomes and present in n = 391 children.

The associations of extremely imbalanced maternal circulating folate/vitamin B-12 status with childhood cardiometabolic outcomes need further study.

Conclusions

Our results suggest that higher plasma folate concentrations in early pregnancy are associated with a more favorable body fat distribution and lower risk of obesity and clustering of cardiovascular risk factors in children aged 10 y. We observed no consistent associations of cord blood folate, vitamin B-12, and homocysteine concentrations with cardiometabolic outcomes at age 10 y. Imbalanced maternal circulating high folate and low vitamin B-12 status may be associated with higher offspring glucose concentrations. The causality and underlying mechanisms for these associations need to be further studied.

Acknowledgments

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