Vitamin B$_{12}$ and folate status in early pregnancy and cardiometabolic risk factors in the offspring at age 5–6 years: findings from the ABCD multi-ethnic birth cohort

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Objective To explore whether maternal vitamin B$_{12}$ and folate status during early pregnancy are associated with cardiometabolic risk factors in the offspring at age 5–6.

Design Prospective multi-ethnic birth cohort, the Amsterdam Born Children and their Development study (ABCD).

Setting 12 373 pregnant women living in Amsterdam were approached between 2003 and 2004 for participation in the study.

Population Mother–child pairs for whom information on maternal vitamin B$_{12}$ or folate status in early gestation and health at age 5–6 years was available ($n = 1950$).

Methods Vitamin B$_{12}$ and folate concentrations were determined in maternal serum at intake in early pregnancy (median 13 weeks' gestation). Anthropometric measurements, blood pressure and fasting blood samples were collected during a health check of children aged 5–6 years. Multiple linear regression was performed to investigate the association between maternal serum concentrations and children's outcomes, corrected for confounders.

Main outcome measures Gestational age at birth, birthweight, body mass index (BMI), glucose levels, triglyceride levels, blood pressure and heart rate of the offspring at age 5–6.

Results Low maternal folate levels during early pregnancy were associated with slightly higher BMI in the offspring [decrease per 10 units: β 0.07 kg/m^2, 95% confidence interval (CI) 0.01, 0.13]. Low maternal vitamin B$_{12}$ concentrations were associated with higher heart rates (decrease per 100 units: β 0.49 beats/min, 95% CI 0.11, 0.87).

Conclusion This study provides further evidence that maternal nutrition in early pregnancy may possibly program cardiometabolic health of the offspring.

Keywords Body mass index, folate, heart rate, offspring, pregnancy, vitamin B$_{12}$.

Tweetable abstract Low folate and vitamin B$_{12}$ levels during pregnancy are associated with higher BMI and heart rate in offspring.

Introduction

Non-communicable diseases such as hypertension and type 2 diabetes mellitus are a major public health burden in the modern Western world. Adverse conditions during

pregnancy can contribute to the susceptibility of developing these diseases in later life, a concept known as developmental programming.[1,2] The foundation for this concept was laid when Barker et al.[3] showed that adult men who had been small at birth had the highest death rates from ischaemic heart disease. Later studies showed that maternal undernutrition during gestation is associated with many

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chronic diseases in the offspring in later life, including type 2 diabetes and coronary heart disease.[4–6] In general, when the developing fetus is faced with an adverse environment, i.e. macronutrient or micronutrient undernutrition, it may have to sacrifice growth of organs non-essential to fetal life in order to guarantee growth of organs essential for survival. Similarly, fetal endocrine and metabolic systems may adapt to best cope with scarce resources. Many of these adaptations have lasting effects and may lead to disease in later life.[4–6] It is thought that epigenetic programming is one of the mechanisms behind these lasting effects.[7–9]

DNA methylation is involved in the functioning of genes and forms the basis of epigenetic programming.[7–10] This methylation depends on the supply of methyl groups by methyl donors (i.e. methionine) in the diet, and on B vitamins (i.e. vitamin B$_{12}$ and folate) as cofactors and substrates of the methionine-homocysteine cycle.[8] Animal studies have shown that in ewes, a low dietary intake of vitamin B$_{12}$, folate and methionine around conception led to offspring with altered DNA methylation and phenotype.[8] They were heavier, fatter, more often insulin-resistant and had higher blood pressure. In India, mothers with high folate concentrations and low vitamin B$_{12}$ concentrations (shortage of only one micronutrient), gave birth to offspring with the highest levels of insulin resistance at age 6.[11] Furthermore, pregnant women and children of various ethnicities have marked differences in vitamin B$_{12}$ and folate concentrations and cardiometabolic risk profile.[12,13]

We hypothesized that a decrease in concentrations of vitamin B$_{12}$ and folate in early pregnancy would increase markers of cardiometabolic risk in the offspring at age 5–6. We furthermore hypothesized that ethnicity-related differences in maternal vitamin B$_{12}$ and folate levels may explain some of the ethnic differences in cardiometabolic risk in the offspring.

Methods

This study is part of a large prospective multi-ethnic birth cohort study: the Amsterdam Born Children and their Development (ABCD) study. The ABCD study was designed to investigate associations between maternal lifestyle and dietary habits during pregnancy and birth outcomes and future health of the child.

Study population and design

The cohort has been described in detail elsewhere.[14] In short, 12 373 pregnant women living in Amsterdam were invited between January 2003 and March 2004 to participate in the ABCD study at their first visit to an obstetric caregiver (Figure S1). Of these women, 8266 returned the

pregnancy questionnaire and 4389 provided a blood sample in which vitamin B$_{12}$ and folate concentrations were determined. The questionnaire covered socio-demographic characteristics, obstetric history, lifestyle and emotional wellbeing. Of these respondents, 7863 women gave birth to a live singleton infant. A total of 6735 women gave permission for follow up.

Approximately 2 weeks after their child's fifth birthday, 6161 mothers were sent a questionnaire (in Dutch, English or Turkish) asking for information about the child's health, development and behaviour (Figure S1). Attrition of this follow-up number was largely due to untraceable addresses or migration. In this questionnaire the mothers were also asked for consent regarding participation of their child in the ABCD health check. In all, 4488 mothers returned the questionnaire and gave informed consent for follow up. Anthropometric measurements (e.g. height, weight) and blood pressure measurements took place during a health check of 3321 children. In a subsample of 2108 children, whose parents gave additional permission, capillary blood was collected during the health check. We included only mother–child pairs for whom information on maternal vitamin B$_{12}$ or folate status and health at age 5–6 years was available ($n = 1950$). Twin pregnancies ($n = 135$) and major congenital anomalies ($n = 133$) were excluded at an earlier stage. At follow up, children with diseases that could interfere with outcome measures (severe cardiac, pulmonary or immune system-related diseases) ($n = 10$) were excluded. Information retrieved via the neonatal questionnaire and the 5-year questionnaire was used to complete data on severe diseases and congenital anomalies.

Maternal characteristics

Pregnancy questionnaire
Maternal age, pre-pregnancy body mass index (BMI), parity, ethnicity (based on self-reported country of birth of the pregnant woman's mother), socioeconomic status (SES; based on years of education after primary school), maternal smoking during pregnancy (yes or no) and alcohol use during pregnancy (yes or no) were available from the pregnancy questionnaire, hence were all self-reported. Country of birth included the following categories, based on the main ethnic populations in Amsterdam: the Netherlands, Netherlands Antilles, Surinam, Turkey, Morocco, Ghana, other Western countries and other non-Western countries. Children of Surinamese (Surinam-Creole), Antillean and Ghanaian descent were combined in the 'African descent' group because of the small sample size among these ethnicities and in accordance with other studies in this cohort.[13]

Maternal vitamin B_{12} and folate concentrations
Concentrations of vitamin B_{12} and folate of the mother were determined in blood samples collected at their first prenatal visit. The procedure has been described in detail elsewhere.[12] All samples were sent to the Regional Laboratory of Amsterdam for processing.[15] Plasma and serum were prepared in the laboratory by centrifugation and stored as 1-ml aliquots at $-80°C$ until analysis. The National Institute for Public Health and the Environment (Bilthoven, the Netherlands) performed the analysis of vitamin B_{12}. The concentration was determined by immunoassay with chemiluminescence detection on the Access Immunoassay System (Beckman Coulter, Bilthoven, the Netherlands), with an inter-assay coefficient of variation (CV) $\leq 7.8\%$ and an upper detection limit of 1500 pg/ml. Two measurements were above this limit and therefore excluded, leaving 4201 useable vitamin B_{12} measurements.

Folate analysis was performed at the Medical Laboratory (Maastricht, the Netherlands) by immunoassay with chemiluminescence detection on the ADVIA Centaur System (Bayer Group). Inter-assay CV was $\leq 6.1\%$. In all, 362 measurements were above upper detection limit (54.4 nmol/l) and were given a fictional value of 100 nmol/l; we included these cases with values above detection limit in our analysis by adding a variable identifying them as such.

This gave us 4243 useable folate measurements.

Child characteristics

Perinatal registration
Birthweight and gestational age at birth were available from the Dutch Perinatal Registry (PRN) and Youth Health Care Registration. Gestational age (days) was based on ultrasound by the obstetric care provider or, when unavailable (<10%), on the first day of the last menstrual period.

Health check
The health check was held at the child's primary school or at a central location (e.g. a museum in Amsterdam) during the weekend and holidays when no space was available for the health check at school or when the children and parents had moved outside of Amsterdam.[14]

Height was measured to the nearest millimetre using a Leicester portable height measure (Seca), and weight to the nearest 100 g using a Marsden weighing scale, model MS 4102. BMI was calculated by dividing weight in kilograms by height in metres squared. Systolic and diastolic blood pressure (SBP and DBP) was measured in the supine position. The device used was the Omron 705 IT (Omron Health Care Inc., Bannockburn, IL, USA) with a small cuff. Blood pressure was measured twice, after a test measurement, and was considered valid if the difference between the two measurements was less than 10 mmHg, otherwise a third measurement was taken. SBP and DBP were calculated by taking the mean value of these measurements. Heart rate (HR) was derived from the time between two adjacent R waves on a three-lead ECG during 4 min in the supine position by the VU-AMS.[16] A resting heart rate of more than 150 beats/min was considered abnormal. Therefore, we excluded the heart rate variable of one case.

Blood sampling and processing
Fasting plasma glucose and triglycerides were determined from capillary blood using a well validated collection kit developed for ambulatory purposes (Demecal, Haarlem, the Netherlands). Sixty-seven children did not fast and the glucose levels of these cases were therefore excluded.

Statistical analysis

Univariate linear regression, adjusted for blood sampling moment, was performed to check associations between maternal characteristics and vitamin B_{12} and folate levels. To examine the independent associations of maternal vitamin B_{12} or folate concentration with birthweight and cardiometabolic risk factors in offspring, univariate and multiple linear regression analysis was performed, adjusting for gestational age at blood sampling (because vitamin B_{12} and folate concentrations decline during pregnancy) (model 2) and the covariates birthweight, gestational age at birth, BMI, age and gender of the child (model 3). We also adjusted for maternal age, pre-pregnancy BMI, parity, ethnicity, SES, smoking and alcohol use during pregnancy (model 4). The variables parity, smoking, alcohol use, gender and ethnicity were entered as categorical variables in the analysis, and the other variables were included on a continuous scale. For those cardiometabolic risk factors in which significant ethnic differences were present after adjustment for all covariates (using Dutch as the reference group), as well as those displaying an association with vitamin B_{12} or folate in general, the explanatory role of vitamin B_{12} or folate was assessed in two separate models by adding vitamin B_{12} or folate to a fully adjusted model. Assumptions of linearity and normality were checked. To express effect size, Cohen's d was used. Data were analysed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). In all analyses a P-value <0.05 was considered significant.

Results

Maternal and child characteristics
Characteristics of the study population are displayed in Table 1. The women included in our analysis (with known vitamin B_{12} or folate level) compared with women not included because vitamin B_{12} or folate samples were missing, were significantly ($P < 0.001$) older (30.9 versus 30.5 years), taller (169.1 versus 167.4 cm), thinner (22.9

versus 23.4 kg/m^2), more often nulliparous (57.6 versus 52.8%), more often of Dutch ethnicity (68.2 versus 53.6%), had a higher SES (9.2 versus 8.0 years of education after primary school) and used more alcohol (24.4 versus 16.5%) and folic acid (75.9 versus 64.4%) during pregnancy (results not shown).

Maternal vitamin B$_{12}$ and folate concentrations according to maternal characteristics

In all, 13.8% of the mothers were vitamin B$_{12}$-deficient based on non-pregnant reference values (<200 pg/ml) (re-

Table 1. Maternal and child characteristics

Maternal characteristics	n*	%	Mean	Standard deviation
Age (year)	4219		30.94	4.86
Height (cm)	4106		169.11	7.08
BMI (kg/m^2) (before pregnancy)	3879		22.90	3.75
Multiparous				
No	2431	57.6		
Yes	1788	42.4		
Ethnicity				
Dutch	2877	68.2		
Surinamese	196	4.6		
Antillean	50	1.2		
Turkish	139	3.3		
Moroccan	213	5.0		
Ghanaian	61	1.4		
Other non western	305	7.2		
Other western	378	9.0		
Education after primary school (year)	4187		9.21	3.84
Smoking (during pregnancy)				
No	3811	90.4		
Yes	403	9.6		
Alcohol use (during pregnancy)				
No	3189	75.6		
Yes	1029	24.4		
Folic acid use (before/during pregnancy)				
No	1008	24.1		
Yes	3181	75.9		
Folate (nmol/l)	4155		29.69	24.11
Vitamin B$_{12}$ (pg/ml)	4116		317.34	126.96
Child characteristics at birth				
Sex of child				
Boy	2022	48.9		
Girl	2119	51.1		
Birthweight (g)	4114		3432	624
Gestational age at birth (days)	4114		276.94	20.93
Child characteristics at age 5–6				
BMI (kg/m^2)	1928		15.47	1.45
HR (bpm)	1724		85.36	9.66
SBP (mmHg)	1899		99.42	7.39
DBP (mmHg)	1892		57.13	6.05
Glucose (mmol/l)	1223		4.56	0.50
Triglyceride (mmol/l)	1263		0.65	0.30

*Mothers and children included with known maternal vitamin B$_{12}$ or folate level.

Table 2. Vitamin B$_{12}$ and folate concentrations in early pregnancy according to maternal characteristics

Maternal characteristics	Vitamin B12 (pg/ml) Median	Folate (nmol/l) Median
Age (years)		
<25	**261.92****,***	**13.80****,***
25–34	**292.67****,***	**24.20****,***
>34	**313.30****,***	**25.40****,***
Height (cm)		
<167	282.88	**18.60****,***
167–172	304.06	**24.50****,***
>172	295.61	**26.30****,***
BMI (kg/m^2) (before pregnancy)		
BMI <18.51	**303.29****,***	**23.90****,***
BMI 18.51–24.99	**299.96****,***	**25.30****,***
BMI 25–29.99	**281.66****,***	**19.70****,***
BMI>29.99	**252.99****,***	**16.30****,***
Multiparous		
No	**291.32****	**25.30****
Yes	**295.89****	**20.25****
Ethnicity****		
Dutch	295.72	25.80
Surinamese	276.89	**13.60****
Antillean	324.19	19.50
Turkish	**204.39****	**14.20****
Moroccan	285.09	**12.10****
Ghanaian	**600.27****	**11.80****
Other non-Western	289.54	**17.70****
Other Western	297.63	27.50
Education after primary school (years)		
<6	270.83	**13.80****,***
6–10	294.53	**22.90****,***
>10	301.57	**27.60****,***
Smoking (during pregnancy)		
No	**296.24****	**23.80****
Yes	**270.38****	**18.10****
Alcohol use (during pregnancy)		
No	**289.02***	**22.20****
Yes	**306.09***	**25.85****
Folic acid use (before/during pregnancy)		
No	**266.53***	**11.80****
Yes	**302.02***	**27.60****

Univariate linear regression.
*$P < 0.05$.
**$P < 0.001$.
***Age, height, BMI and education after primary school displayed in categories, but analysed as continuous variables adjusted for blood sampling moment.
****Dutch is reference group
Bold indicates significant values

sults not shown). Also, 11.9% were folate-deficient (<10 nmol/l). Table 2 shows the median maternal vitamin B_{12} and folate concentrations in early pregnancy according to maternal characteristics.

In our study population, younger women had significantly lower levels of vitamin B_{12} [per year: β 3.08 pg/ml, 95% confidence interval (CI) 2.28, 3.87] and folate (per year: β 0.47 nmol/l, 95% CI 0.4, 0.54) than older women. Also, folate concentrations dropped with a decrease in maternal height (per cm: β 0.23 nmol/l, 95% CI 0.1, 0.27). Furthermore, higher maternal BMI was associated with lower vitamin B_{12} (per kg/m^2: β −2.22 pg/ml, 95% CI −3.27, −1.17) and folate levels (per kg/m^2: β −0.43 nmol/l, 95% CI −0.53, −0.34). Multiparous women had significantly higher vitamin B_{12} concentrations (β 15.71 pg/ml, 95% CI 7.89, 23.52) compared with nulliparous women, but their folate levels were lower (β −2.69 nmol/l, 95% CI −3.38, −1.99).

A very low vitamin B_{12} status was found in mothers of Turkish ethnicity (mean 204.39 pg/ml) (normal range 200−700 pg/ml). In contrast, Ghanaian women had extremely high concentrations of vitamin B_{12} (mean 600.27 pg/ml). Folate concentrations were highest among Dutch, Antillean and other Western women (mean 25.8, 19.5 and 27.5 nmol/l, respectively). Women with lower socioeconomic status showed lower folate levels (per year of secondary education after primary school: β 0.80 nmol/l, 95% CI 0.71, 0.89). Low vitamin B_{12} (β −29.25 pg/ml, 95% CI −42.47, −16.04) and folate concentrations (β −3.02 nmol/l, 95% CI −4.18, −1.85) were found in women who smoked during pregnancy. Remarkably, alcohol use was associated with higher vitamin B_{12} (β 15.78 pg/ml, 95% CI 6.77, 24.79) and folate levels (β 2.58 nmol/l, 95% CI 1.78, 3.38).

Associations of maternal vitamin B_{12} and folate with birth outcomes and cardiometabolic outcomes

Table 3 shows mean birth outcomes and cardiometabolic outcomes at the age of 5–6 years according to maternal vitamin B_{12} and folate levels (displayed in quartiles, analysed as continuous variables) in early pregnancy. The results of multivariate linear regression, adjusted for blood sampling moment (model 2), the child covariates gender, age, gestational age at birth, birthweight and BMI (model 3), and the maternal covariates age, pre-pregnancy BMI, parity, ethnicity, SES, smoking and alcohol use (model 4), are shown in Table 4.

Lower maternal vitamin B_{12} levels during early pregnancy were associated with higher heart rates in offspring at age 5–6 years (per 100 units: β −0.49 beats/min, 95% CI −0.87, −0.11). This association remained significant after adjustment for maternal and child covariates. Also, when we compared the lowest maternal vitamin B_{12} level quartile

Table 3. Birth outcomes and cardio-metabolic outcomes in children at age 5–6 years according to maternal vitamin B_{12} and folate levels in early pregnancy

	Gestational age at birth (days)		Birthweight (g)		BMI (kg/m^2)		HR (beats/min)		SBP (mmHg)		DBP (mmHg)		Glucose (mmol/l)		Triglyceride (mmol/l)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vitamin B_{12} (pg/ml)																
Q1 < 233.11	278.86	12.42	3448	574	15.57	1.76	**85.84*,***	9.66	99.88	7.76	57.55	6.50	4.60	.54	.68	.37
Q2 233.11–294.10	278.97	12.31	3526	551	15.42	1.34	**86.48*,***	10.02	99.15	7.21	56.95	5.62	4.55	.45	.65	.29
Q3 294.11–378.66	279.52	10.63	3489	560	15.45	1.33	**84.78*,***	9.52	99.39	7.50	56.92	6.01	4.53	.47	.62	.28
Q4 > 378.66	279.94	11.72	3485	528	15.39	1.31	**84.57*,***	9.55	99.25	6.96	57.17	5.98	4.58	.52	.65	.26
Folate (nmol/l)																
Q1 < 15.8	279.01	12.13	3469	580	**15.74*,*,***	1.71	86.30	9.12	**100.06*,***	7.54	**58.30*,*,***	6.56	4.63	.51	.65	.37
Q2 15.8–25.1	279.19	12.18	3470	567	**15.50*,*,***	1.46	84.73	10.03	**99.71*,***	7.70	**56.97*,*,***	6.13	4.54	.46	.64	.30
Q3 25.2–36.7	279.57	11.17	3508	501	**15.31*,*,***	1.33	85.20	9.89	**98.72*,***	6.81	**56.74*,*,***	5.54	4.55	.50	.64	.25
Q4 > 36.7	279.36	11.85	3488	553	**15.31*,*,***	1.23	85.35	9.53	**99.32*,***	7.41	**56.61*,*,***	5.83	4.54	.50	.66	.29

*P < 0.05.
**P < 0.001.
***Univariate linear regression displayed in quartiles but analysed as continuous variables, adjusted for blood sampling moment.
Bold indicates significant values

Table 4. Multivariate linear regression of maternal vitamin B$_{12}$/folate level and cardiometabolic outcomes in children

	Model	SBP (mmHg)		DBP (mmHg)		HR (beats/min)		BMI (kg/m^2)***	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI
Vitamin B$_{12}$	1	−0.004	−0.287; 0.279	−0.016	−0.245; 0.213	**−0.539***	**−0.926; −0.152**	−0.048	−0.102; 0.007
(pg/ml) (×100)	2	−0.015	−0.300; 0.269	−0.011	−0.241; 0.219	**−0.526***	**−0.914; −0.138**	−0.047	−0.102; 0.008
	3	0.080	−0.189; 0.348	0.049	−0.171; 0.268	**−0.545***	**−0.921; −0.170**	−0.042	−0.096; 0.012
	4	0.115	−0.157; 0.386	0.099	−0.123; 0.320	**−0.490***	**−0.871; −0.109**	0.002	−0.051; 0.054
Folate	1	−0.248	−0.555; 0.060	**−0.494****	**−0.743; −0.246**	−0.269	−0.687; 0.150	**−0.140****	**−0.199; −0.081**
(nmol/l) (×10)	2	−0.280	−0.597; 0.036	**−0.507****	**−0.762; −0.251**	−0.228	−0.657; 0.201	**−0.145****	**−0.206; −0.085**
	3	0.061	−0.239; 0.361	**−0.280***	**−0.526; −0.034**	−0.385	−0.806; 0.035	**−0.135****	**−0.195; −0.075**
	4	0.112	−0.201; 0.424	−0.170	−0.426; 0.085	−0.307	−0.743; 0.129	**−0.066***	**−0.126; −0.006**

Model 1: Crude analysis.
Model 2: Adjusted for model 1, blood sampling moment.
Model 3: Adjusted for model 2, gender, gestational age at birth, birthweight, BMI of child, age.
Model 4: Adjusted for model 3, maternal age, pre-pregnancy BMI, parity, non-Western ethnicity, SES, smoking, alcohol use.
*$P < 0.05$.
**$P < 0.001$.
***Model 3 without BMI of child.
Bold indicates significant values

(vitamin B$_{12}$ <233.11 pg/ml, mean HR 85.84 beats/min, SD 9.66) with the highest vitamin B$_{12}$ level quartile (vitamin B$_{12}$ >378.66 pg/ml, mean HR 84.57 beats/min, SD 9.55), Cohen's effect size value was 0.13 (negligible effect). When we restricted the analysis to Dutch mothers only, there was no significant association between maternal vitamin B$_{12}$ levels and heart rate in offspring (see Tables S1 and S2).

A decrease in maternal folate concentration was associated with an increase of diastolic blood pressure in offspring when corrected for blood sampling moment and child covariates. However, after adjustment for maternal covariates, this association was no longer significant. Children born to mothers with lower folate levels had significant higher BMI after adjustment for all covariates (per 10 units: β −0.07 kg/m^2, 95% CI −0.13, −0.01). This association remained significant when we restricted the analysis to Dutch mothers and children (see Tables S1 and S2). Furthermore, when we compared the lowest maternal folate level quartile (folate <15.8 nmol/l, mean BMI 15.74 kg/m^2, SD 1.71) with the highest folate level quartile (folate >36.7 nmol/l, mean BMI 15.31 kg/m^2, SD 1.23), Cohen's effect size value was 0.29 (small effect). We did not find any associations between maternal folate or vitamin B$_{12}$ levels and the outcome measures glucose and triglyceride (Table 3).

The explanatory role of vitamin B$_{12}$ or folate concentration in cardiometabolic ethnic differences

Table S3 presents data on ethnic differences in heart rate and BMI and the explanatory role of maternal vitamin B$_{12}$ and folate concentration in this after adjustment for all covariates. African descent children had a significantly higher BMI (β 0.40 kg/m^2, 95% CI 0.16, 0.64), compared with ethnic Dutch children. After adjustment for folate, the difference in BMI of African descent children when compared with the reference group was unaltered. Turkish children showed higher heart rates (β 5.69 beats/min, 95% CI 3.25, 8.13) and BMI (β 0.73 kg/m^2, 95% CI 0.39, 1.06) compared with ethnic Dutch children. Moroccan children showed only higher BMI (β 0.62, 95% CI 0.34, 0.90) compared with ethnic Dutch children. However, these differences were unaltered after adjustment for vitamin B$_{12}$ and folate.

Discussion

Main findings
This study focused on the relationship between the vitamin B$_{12}$ and folate status of pregnant women during early pregnancy and cardiometabolic risk factors in their offspring at age 5–6 years. The results showed that, after correcting for multiple confounders, low maternal folate levels during pregnancy were associated with higher BMI in offspring, and low maternal vitamin B$_{12}$ concentrations during pregnancy were associated with an increased heart rate in children 5–6 years of age. Although effect sizes are small, these findings suggest that body composition and autonomic regulation in the offspring may be permanently altered in response to maternal micronutrient levels in early pregnancy and may contribute to the risk of metabolic and cardiovascular disease in later life. We were unable to confirm that maternal

micronutrient status contributes to ethnic differences in cardiometabolic risk of offspring.

Strengths and limitations

The prospective nature of the study, the multivariate analysis design and the large number of included women, children, blood samples and questionnaire data are major strengths of this study. The applicability of our findings could have been affected by selective participation. As often seen in follow-up studies, we found that, in comparison with non-participants, women included in our analysis were older, taller, thinner, more often nulliparous, more often of Dutch ethnicity, had a higher socioeconomic status and used more alcohol and had higher folic acid levels. Furthermore, only a subsample of children (63%) had blood samples taken. Selective participation will have only introduced bias if the associations between maternal micronutrient status and offspring's health is different among participants and non-participants, which seems unlikely. A limitation of the study is that it is observational, and therefore causality cannot be determined. Intervention studies in animal models have, however, suggested that the causal pathway is mediated by altered gene methylation.[8] Our study did not focus on changes in methylation or other epigenetic changes. Furthermore, this study only focused on maternal serum vitamin B_{12} and folate levels and not on (ethnic differences in) dietary intake of these or other micronutrients. Because we have no dietary intake information, we cannot rule out that low maternal micronutrient status is a marker of other dietary factors. Unfortunately, maternal serum levels of homocysteine, methionine and other methyl donors were not known. Further studies could focus on the associations of maternal intake of vitamin B_{12}, folate and methyl donor-rich foods before and during pregnancy and cardiometabolic risk factors in the offspring. Associations of cardiometabolic risk with maternal homocysteine, methionine and other methyl donor serum levels should also be explored. No adjustment for multiple testing was done, therefore results should be interpreted with caution. Because of small sample sizes of some ethnic groups (i.e. those of Ghanaian descent), separate analysis was not possible due to a lack of power. Finally, we have adjusted for several potential covariates. However, residual confounding may still be present due to the observational design of the study.

Interpretation

Many studies have linked maternal nutrition during gestation with cardiometabolic disease in the offspring in later life.[2,4–6] Some studies have associated fetal shortage of specific micronutrients, i.e. folate and vitamin B_{12}, with cardiometabolic disease in later life.[8,11] A possible mechanism explaining the permanent adaptations in children is epigenetic programming.[8,9,11] The heritable changes involved in epigenetic programming, in response to a micronutrient shortage during periconception, are a result of changes in DNA methylation.[10,17] DNA methylation changes gene expression and depends on the supply of methyl by methyl donors in the methionine-homocysteine cycle.[17] Vitamin B_{12} and folate are essential in this methionine–homocysteine cycle and therefore a shortage or subtle restriction within the normal physiological ranges of one of these micronutrients is associated with changes in epigenetic programming and phenotype.[8,17] This might explain the higher BMI in offspring of mothers with a lower folate status during pregnancy in this study. The methionine–homocysteine cycle is also involved in the myelination process of nerve tissue, and therefore the neurodevelopment of fetuses and children.[18–20] Disorders and changes in myelination can have important effects on the central nervous system functioning.[18–20] Several studies report severe neurological effects and classical demyelination lesions in infants of mothers on diets that are deficient in vitamin B_{12}.[21–23] Although this study did not focus on deficient versus non-deficient groups, lower levels of vitamin B_{12} could contribute to the changes in autonomic regulation such as an increased heart rate. Furthermore, low vitamin B_{12} and folate concentrations are linked to high homocysteine concentrations.[18] This, in turn, is associated with developmental disorders and vascular disease in later life [24,25] and might also contribute to higher heart rates and increased BMI.

Our findings are in line with experimental research.[8] However, we only found associations between maternal micronutrient status and heart rate and BMI of the children. Other studies in humans and animals also showed associations between low maternal vitamin B_{12} and folate concentrations and insulin resistance and high blood pressure in offspring.[8,11] However, these studies used insulin resistance as the primary outcome, whereas we only had fasting glucose levels available. Possibly, glucose levels and blood pressure were not yet significantly raised in our subjects due to the relative young age of the cohort. Also, we looked at vitamin B_{12} and folate observationally in an unrestricted diet, unlike animal models.[8] Indian mothers with low vitamin B_{12} and high folate concentrations had children with higher adiposity,[11] not higher weight or BMI. The higher adiposity possibly reflects the lower socioeconomic status and different diet of the Indian mothers and children. We used BMI as the outcome, not adiposity or fat free mass, and information on lifestyle and diet was lacking. Therefore, further research is needed to investigate possible effects of maternal micronutrients on offspring body composition.

Conclusion

In conclusion, this study shows that in a Western urban setting, subtle alterations in micronutrient status within the normal range might affect the body composition and markers of autonomic control of offspring, although effects are small. This provides further evidence for an early nutritional origin of cardiometabolic risk factors. Further research is necessary to support this initial evidence, including studies into the causal pathways and epigenetic effects of suboptimal micronutrient status and the potential beneficial effects of intervention studies. If confirmed, prenatal checks of pregnant women should be extended with diagnosis and treatment of women with a low vitamin B$_{12}$ or folate concentration.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

GGK performed the statistical analysis and drafted the manuscript together with IJG. The study was originally designed by RCP. TJR, RCP and IJG monitored the statistical analysis. TGV and ME rendered and provided the ABCD Study data. TJR, RCP, TGV, ME and IJG all made substantial contributions in revising the manuscript. The final manuscript was read and approved by all authors.

Details of ethical approval

Approval for the ABCD study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of Amsterdam.

Funding

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow diagram of the ABCD-study.

Table S1. Birth outcomes and cardio-metabolic outcomes in children at age 5–6 years according to maternal vitamin B12 and folate levels in early pregnancy of Dutch mothers.

Table S2. Multivariate linear regression maternal vitamin B12/folate level and gestational age at birth and cardiometabolic outcomes in ethnic Dutch children.

Table S3. Ethnic differences in heart rate and body mass index (ethnic Dutch = reference group) and the effect of additional adjustment for vitamin B12 or folate. ∎

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Folic acid in pregnancy

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BJOG PERSPECTIVES

BJOG SINCE 1902

In 1931, Lucy Wills, a British haematologist working in India, discovered that Marmite® was as effective as liver extract in the treatment of 'tropical macrocytic anaemia' in pregnancy. From this she deduced that both liver and Marmite® must contain a common factor, the administration of which corrected a dietary deficiency.

Back in England, Dr Wills contributed to a discussion on 'Diet in Pregnancy' which took place at the Royal Society of Medicine on 17 May 1935, a report of which was published in this journal (*J Obstet Gynaecol Br Emp* 1935;42:725–32). At that stage the 'exact nature of this intrinsic factor' remained unknown until it was isolated from spinach in 1941, hence being named 'folic acid' (from the Latin word *folium* for *leaf*). By the mid-1940s a team of biochemists, 'the folic acid boys', working at the Lederle Laboratory in Pearl River, New York, USA were able to synthesise folic acid in a pure crystalline form, allowing more detailed evaluation of its properties. In addition to ill effects on maternal health, animal studies in the 1950s suggested that deficiency of folic acid in pregnancy was also associated with fetal abnormalities. Later studies in humans (Smithells et al. *Arch Dis Child* 1976;51:44–50) showed a higher incidence of neural tube defects in women with low first trimester levels of red-cell folate. The authors then went on to demonstrate that recurrent abnormalities could be prevented by multivitamin supplementation. A subsequent placebo-controlled trial undertaken by the Medical Research Council (MRC) confirmed that folic acid was responsible for this beneficial effect (*Lancet* 1991;338:131–7).

On the strength of these findings the Department of Health (DoH), in 1992, recommended folic acid supplementation for all women planning a pregnancy and for the first 12 weeks of gestation. However, the evidence suggests that this advice has not been widely heeded. A recent systematic review published in this journal reported that preconception folic acid supplementation is only taken by around 25% of women and that there was a significant reduction in small-for-gestational age fetuses in this subgroup (Hodgetts et al. *BJOG* 2014;122:478–90). Evidence is also emerging about possible long-term benefits of taking folic acid. Krikke et al. (*BJOG* 2015;123:384–292) found that low levels of maternal folic acid in pregnancy are associated with raised body mass index (BMI) in their children at 5–6 years of age, adding support for the hypothesis that a mother's nutrition in early pregnancy may affect the future health of her offspring.

The well-established benefits of folic acid supplementation and the difficulty implementing the recommendations proposed by the DoH over 20 years ago should prompt us to reconsider the option of fortification of food with folic acid.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information. ■