

## Maternal first-trimester dietary intake and childhood blood pressure: the Generation R Study

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### Abstract

Suboptimal maternal dietary intake during pregnancy might lead to fetal cardiovascular adaptations and higher blood pressure in the offspring. The aim of the present study was to investigate the associations of maternal first-trimester dietary intake with blood pressure in children at the age of 6 years. We assessed first-trimester maternal daily dietary intake by a FFQ and measured folate, homocysteine and vitamin B<sub>12</sub> concentrations in the blood, in a population-based prospective cohort study among 2863 mothers and children. Childhood systolic and diastolic blood pressure was measured using a validated automatic sphygmomanometer. First-trimester maternal daily intake of energy, fat, protein and carbohydrate was not associated with childhood blood pressure. Furthermore, maternal intake of micronutrients was not associated with childhood blood pressure. Also, higher maternal vitamin B<sub>12</sub> concentrations were associated with a higher diastolic blood pressure (0.31 mmHg per standard deviation increase in vitamin B<sub>12</sub> (95% CI 0.06, 0.56)). After taking into account multiple testing, none of the associations was statistically significant. Maternal first-trimester folate and homocysteine concentrations were not associated with childhood blood pressure. The results from the present study suggest that maternal Fe intake and vitamin B<sub>12</sub> concentrations during the first trimester of pregnancy might affect childhood blood pressure, although the effect estimates were small and were not significant after correction for multiple testing. Further studies are needed to replicate these findings, to elucidate the underlying mechanisms and to assess whether these differences in blood pressure persist in later life.

**Key words:** Maternal diet: Childhood blood pressure: Iron: Calcium: Folate: Homocysteine: Vitamin B<sub>12</sub>

Suboptimal maternal and fetal nutrition might lead to fetal cardiovascular developmental adaptations and subsequent CVD in later life<sup>(1–3)</sup>. Support for this hypothesis is largely based on experimental animal studies and historical cohort studies in human subjects showing associations of maternal exposure to extreme famine during pregnancy with the development of hypertension in later life<sup>(4–6)</sup>. An imbalance in nutrient intake during pregnancy might also be an explanation for the development of hypertension<sup>(4)</sup>. Not much is known about the associations of less extreme variations in maternal dietary intake with CVD in the offspring. Several studies have focused on the associations of maternal micro- or macronutrient intake with childhood blood pressure but the results are inconclusive<sup>(7–17)</sup>. For example, two previous studies suggested that higher maternal fat intake during pregnancy was associated with diastolic blood pressure in the offspring, although the studies observed opposite effects<sup>(7,8)</sup>. Also, maternal intake

of micronutrients during pregnancy, such as Ca, Na and Fe, has been suggested to be associated with blood pressure in children, but results were not consistent<sup>(9–11,13–15)</sup>. Other micronutrients, including folate, homocysteine and vitamin B<sub>12</sub>, might affect vascular development<sup>(18)</sup>. High homocysteine and low folate levels are associated with endothelial dysfunction<sup>(19,20)</sup> and might subsequently lead to higher blood pressure in later life. Since vitamin B<sub>12</sub> lowers homocysteine levels, this might also influence blood pressure.

We assessed in a large population-based prospective cohort study among 2863 Dutch mothers and their children the associations of maternal daily total energy intake and intake of macronutrients and micronutrients in the first trimester of pregnancy with blood pressure in children at the age of 6 years. We also assessed whether first-trimester folate, homocysteine and vitamin B<sub>12</sub> concentrations in maternal blood were associated with childhood blood pressure.

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**Subjects and methods**

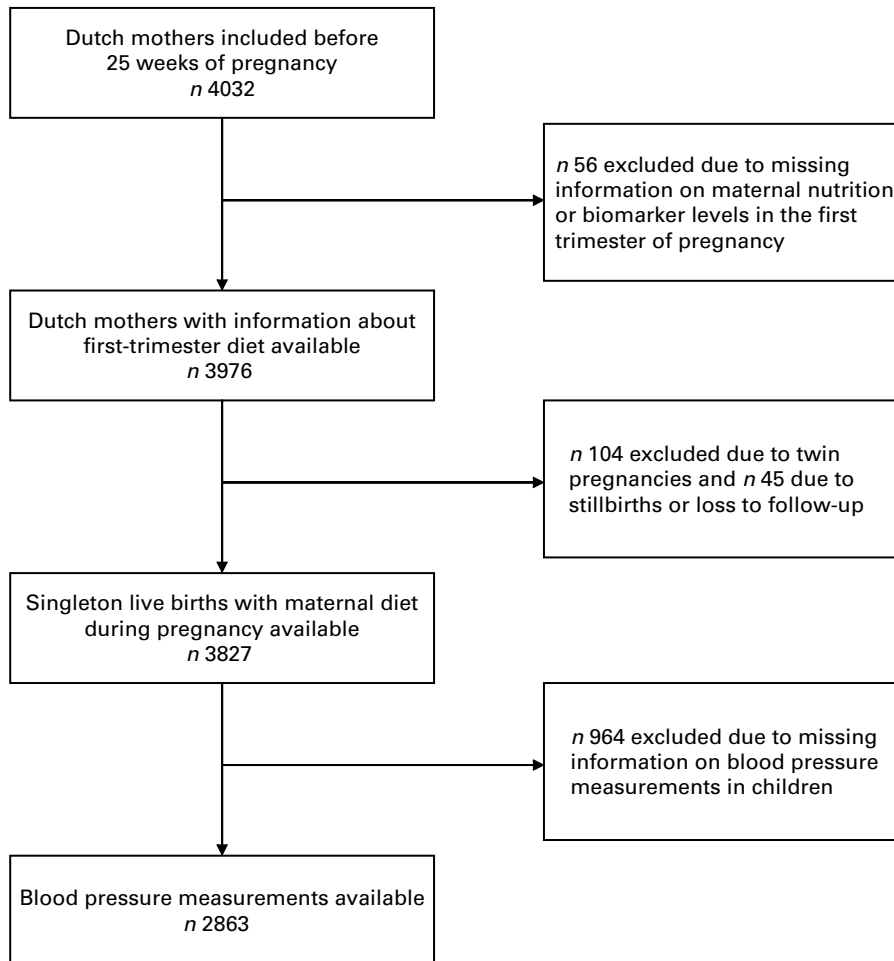
*Design*

The present study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, The Netherlands<sup>(21,22)</sup>. Enrolment in the study was aimed at early pregnancy, but was allowed until the birth of the child. Information about maternal diet and other lifestyle-related variables during pregnancy was collected at enrolment. At the age of 6 years, all participating children and their mothers were invited to a dedicated research centre, to participate in detailed hands-on measurements. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents of the participants. The present analysis was limited to Dutch mothers, since the FFQ was validated for the assessment of dietary intake in a Dutch population. We selected mothers who were enrolled in the study before a gestational age of 25 weeks, because we aimed to assess dietary intakes in the first trimester of pregnancy. In total, 4032 Dutch mothers were enrolled before a gestational age of 25 weeks with a median of 13.6 weeks of gestation (90% range 11.5–20.2

weeks; see Fig. 1). Data on maternal dietary intake or biomarker concentrations were available in 3960 (98%) of these mothers. We excluded multiple births (*n* 104) and stillbirths (*n* 29) from the analyses, leaving 3827 Dutch mothers. Of the 3827 singleton live-born children, 2949 (77%) children attended the follow-up visit at the age of 6 years. Blood pressure was successfully measured in 2863 (97%) of the children who visited the research centre.

*Maternal daily dietary intake*

We assessed maternal dietary intake at enrolment in the study in Dutch mothers using a modified version of the validated semi-quantitative FFQ of Klipstein-Grobusch *et al.*<sup>(23)</sup>. The FFQ considered food intake over the prior 3 months, thereby covering the dietary intake in the first trimester of pregnancy. The FFQ consisted of 293 items structured according to the meal pattern. Questions included consumption frequency, portion size, preparation method and additions. Portion sizes were estimated using Dutch household measures and photographs of foods showing different portion sizes<sup>(24)</sup>. We used the Dutch food composition table for calculating daily intake of nutritional values<sup>(25)</sup>.



**Fig. 1.** Flow chart of the participants included for analysis.

### Folic acid supplement intake

Information on folic acid supplement use (0.4–0.5 mg) and the initiation of supplementation was obtained by questionnaires at the enrolment of the study. We categorised folic acid supplement use into three groups: (1) periconceptual use; (2) start when pregnancy was known; (3) no use during pregnancy. Self-reported folic acid use was validated in a subgroup by serum folate levels in the first trimester, i.e. before 12 weeks of gestation. Within the group of mothers who reported using folic acid supplements ( $n$  204), the median of serum folate was 23.5 (range 4.3–45.3) nmol/l, whereas the median serum folate concentration of mothers who did not report folic acid supplement use ( $n$  68) was 11.1 (range 4.7–29.6) nmol/l. The difference in distribution function (Mann–Whitney test) was statistically significant ( $P < 0.001$ )<sup>(26)</sup>. Information about folic acid supplement use was available in 2505 subjects (87.5%).

### Folate, homocysteine and vitamin B<sub>12</sub> concentrations

In early pregnancy (median 12.9 weeks of gestation, 90% range 10.6–16.8), venous samples were drawn and stored at room temperature before being transported to the regional laboratory for processing and storage for future studies. Processing was aimed to be completed within a maximum of 3 h after venous puncture. The samples were centrifuged and thereafter stored at  $-80^{\circ}\text{C}$ <sup>(21)</sup>. To analyse folate, homocysteine and vitamin B<sub>12</sub> concentrations, EDTA plasma samples (folate and homocysteine) and serum samples (vitamin B<sub>12</sub>) were picked and transported to the Department of Clinical Chemistry at the Erasmus University Medical Center, Rotterdam in 2008. Folate, homocysteine and vitamin B<sub>12</sub> concentrations were analysed using an immunoelectrochemoluminescence assay on the Architect System (Abbott Diagnostics B.V.). The between-run CV for plasma folate were 8.9% at 5.6 nmol/l, 2.5% at 16.6 nmol/l and 1.5% at 33.6 nmol/l, with an analytic range of 1.8–45.3 nmol/l. The same CV for plasma homocysteine were 3.1% at 7.2  $\mu\text{mol/l}$ , 3.1% at 12.9  $\mu\text{mol/l}$  and 2.1% at 26.1  $\mu\text{mol/l}$ , with an analytic range of 1–50  $\mu\text{mol/l}$ . This CV for serum vitamin B<sub>12</sub> was 3.6% at 142 pmol/l, 7.5% at 308 pmol/l and 3.1% at 633 pmol/l, with an analytic range of 44–1476 pmol/l. Plasma concentrations of maternal folate, homocysteine and serum concentrations of vitamin B<sub>12</sub> in the first trimester of pregnancy were available in 2305 (80.5%) of the mothers of the children included in the present study. Missing data were mainly due to logistical reasons.

### Blood pressure measurements

Blood pressure measurements in children were conducted around the age of 6 years in a dedicated research centre in the Erasmus Medical Center, Rotterdam, The Netherlands. The child was lying quietly, while systolic blood pressure and diastolic blood pressure were measured at the right brachial artery in a supine position, four times with 1 min intervals. A cuff was selected with a cuff width approximately

40% of the arm circumference and long enough to cover 90% of the arm circumference. We used the validated automatic sphygmomanometer Datascope Accutorr Plus™ (Mindray DS USA, Inc.)<sup>(27)</sup>. Of all children visiting the research centre, 91.3% had four successful blood pressure measurements available.

### Covariates

Information on maternal age, pre-pregnancy BMI, parity, alcohol use and smoking habits during pregnancy, and educational level was obtained from questionnaires. Maternal education was defined as highest followed education according to the classification of Statistics Netherlands and categorised into primary, secondary and higher<sup>(28)</sup>. Child sex, gestational age at birth and birth weight were obtained from midwife and hospital registries. Breast-feeding (ever/never) was assessed using questionnaires. Current height and weight were measured without shoes and heavy clothing at the visit at 6 years, and BMI ( $\text{kg/m}^2$ ) was calculated.

### Statistical methods

Differences in child characteristics at the age of 6 years between boys and girls were assessed using the  $t$  test and  $\chi^2$  tests for independent samples. Maternal dietary intake variables were categorised into quintiles. This approach was used for all dietary exposures (total energy, carbohydrate, fat, protein intake and protein:carbohydrate ratio; Ca, Fe and Na intake; folate, homocysteine and vitamin B<sub>12</sub> concentrations). We used mixed models to assess the associations between predictors and blood pressure<sup>(29)</sup>. The mixed-model method fits each of the as many as four blood pressure measurements of every child as repeated outcome measures. An advantage of this modelling approach over using the average measure of blood pressure for each child as an outcome is that children with more measurements and less variability in their measurements are assigned more weight than those with fewer measurements, more variability or both<sup>(9,30)</sup>. We used similar mixed models to assess the association of folic acid supplement use with blood pressure at the age of 6 years. All analyses were adjusted for child's sex and age at blood pressure measurement (crude model). Potential covariates were selected based on previous literature<sup>(31,32)</sup>. We assessed crude associations, adjusted for age and sex, of possible covariates with childhood blood pressure. Only the covariates that were significantly associated with systolic or diastolic blood pressure in the present study population were included in our fully adjusted model. The fully adjusted model included maternal age, pre-pregnancy BMI, alcohol use and smoking during pregnancy, educational level, gestational age at birth, birth weight, current BMI and month in which blood pressure measurement was taken. Tests for trends were conducted by using maternal dietary intake variables as the continuous variable in the linear mixed models. We provided the effect per standard deviation increase in the dietary intake variable. In the macronutrient analyses, we used the energy partition method to adjust for energy intake of the other

macronutrients, since total energy intake and macronutrient intakes were strongly correlated<sup>(33)</sup>. The actual energy derived from the macronutrients was used in the analyses. In the micronutrient analyses, we used the residual nutrient method, and additionally adjusted for total energy intake<sup>(33)</sup>.

Missing values in covariates (ranging from 0 to 15%) were multiple-imputed to reduce potential bias associated with missing data<sup>(34)</sup>. We created five imputed datasets and each dataset was analysed separately to obtain the effect sizes and standard errors. The results of all the five imputed analyses were pooled and are presented in the present study. We investigated maternal macronutrient intake, micronutrient intake and maternal biomarker concentrations in pregnancy. Within these exposure groups, variables are correlated (Table S1, available online). To take into account multiple testing, we applied a Bonferroni correction and considered a *P* value lower than 0.017 (0.05/3 (three exposure groups)) as statistically significant. The mixed models were fitted

using the SAS version 9.2 (SAS Institute, Inc.). All other statistical analyses were performed using SPSS version 17.0 for Windows (SPSS, Inc.).

### Results

Table 1 presents the maternal and birth subject characteristics. The mean intake of total energy was 8982.8 (SD 2117.3) kJ/d, Ca 1222 (SD 420) mg/d, Fe 12.1 (SD 3.3) mg/d and Na 3314 (SD 931) mg/d. The mean birth weight was 3497 (SD 548) g. Child characteristics at the age of 6 years for boys and girls separately are shown in Table 2. Systolic blood pressure and diastolic blood pressure were significantly higher in girls compared with boys (*P*<0.05).

Maternal total daily energy intake and daily intake of carbohydrates, fat and proteins were not associated with childhood systolic and diastolic blood pressure (Table 3). The protein: carbohydrate intake ratio was not associated with childhood

**Table 1.** Maternal and birth characteristics: the Generation R Study Cohort, Rotterdam, The Netherlands\*  
(Mean values and standard deviations; medians, 95% ranges and percentages)

	Not imputed				Imputed			
	Mean	SD	Median	95% range	Mean	SD	Median	95% range
<b>Maternal characteristics</b>								
Age (years)			31.8	22.2–39.6				
Height (cm)	170.8	6.4						
Weight (kg)	68.1	12.3			68.1	12.2		
BMI (kg/m <sup>2</sup> )			22.3	18.2–34.3			22.4	18.2–34.5
Parity ≥ 1 (%)			38.4				38.4	
Alcohol use (%)								
No			31.8				31.9	
First trimester only			16.7				16.5	
Continued			51.5				51.6	
Smoking during pregnancy (%)								
Never			75.1				75.6	
First trimester only			9.6				9.6	
Continued			15.3				14.8	
Educational level (%)								
Primary			2.5				2.5	
Secondary			36.6				36.6	
Higher			60.9				60.9	
Energy intake (kJ/d)	8982.8	2117.3						
Carbohydrate intake (g/d)	262.2	74.1						
% of total energy intake			48.3	37.2–61.3				
Fat intake (g/d)	86.5	24.0						
% of total energy intake			36.4	25.3–46.4				
Protein intake (g/d)	79.7	18.9						
% of total energy intake			14.9	10.7–19.9				
Ca intake (mg/d)	1222.3	419.9						
Fe intake (mg/d)	12.1	3.3						
Na intake (mg/d)	3314.3	931.3						
Folic acid use (%)								
No			9.6					
Started when pregnancy was known			33.7					
Started periconceptional			56.7					
Folate levels (nmol/l)	20.2	8.7						
Homocysteine levels (μmol/l)	7.3	2.1						
Vitamin B <sub>12</sub> levels (pmol/l)	193.2	87.4						
<b>Birth characteristics</b>								
Birth weight (g)	3497	548						
Gestational age at birth (weeks)			40.3	35.9–42.3				
Sex								
Male (%)			49.8					
Breast-feeding (never) (%)			8.7				9.2	

\* Only covariates with missing data were multiple-imputed.

**Table 2.** Child characteristics: the Generation R Study Cohort, Rotterdam, The Netherlands†

(Mean values and standard deviations; medians, 95% ranges and percentages)

	Boys (n 1427)		Girls (n 1436)	
	Mean	SD	Mean	SD
Age (years)				
Median	6.0		6.0	
95% range	5.6–7.5		5.6–7.1	
Height (cm)	119.9	5.7	119.1	5.6
Weight (kg)	23.0	3.5	22.7	3.7
Underweight (%)	6.4		4.0	
Normal weight (%)	84.6		82.6	
Overweight (%)	9.0		13.3	
BMI (kg/m <sup>2</sup> )				
Median	15.7		15.6	
95% range	13.7–18.9		13.7–19.8	
Systolic blood pressure (mmHg)	102.2	7.3	103.3*	8.2
Diastolic blood pressure (mmHg)	60.2	6.4	61.6*	6.5

\* Mean values were significantly different from those of boys ( $P < 0.05$ ).

† Only covariates with missing data were multiple-imputed.

blood pressure (Table 3). After adjustment for confounders, maternal intake of Fe tended to be inversely associated with childhood systolic blood pressure ( $-0.30$  mmHg per standard deviation increase in Fe intake (95% CI  $-0.61, 0.01$ ),  $P$  for trend  $0.06$ ), but not with diastolic blood pressure (Table 4). Maternal intake of Ca and Na was not consequently associated with childhood systolic or diastolic blood pressure.

We did not find an association of folic acid supplement use during pregnancy with childhood systolic or diastolic blood pressure (Table 5). The associations of maternal first-trimester folate, homocysteine and vitamin B<sub>12</sub> concentrations with childhood blood pressure are presented in Table 6. We found no consistent associations of maternal first-trimester folate and homocysteine concentrations with childhood systolic blood pressure and diastolic blood pressure in 6-year-old children. Lower maternal first-trimester vitamin B<sub>12</sub> concentrations were associated with lower childhood diastolic blood pressure (difference  $0.31$  mmHg per standard deviation increase in vitamin B<sub>12</sub> concentration (95% CI  $0.06, 0.56$ ),  $P$  value for trend  $0.02$ ), but not with systolic blood pressure. However, the associations between Fe intake and vitamin B<sub>12</sub> concentrations with childhood blood pressure did not reach the significance threshold after adjustment for multiple testing.

## Discussion

The results from the present population-based prospective cohort study suggest that maternal daily macronutrient and micronutrient intake in the first trimester of pregnancy is not associated with childhood blood pressure at the age of 6 years. We observed that higher Fe intake and vitamin B<sub>12</sub> concentrations may be associated with a lower childhood systolic and higher diastolic blood pressure, respectively, but we should also consider the effect of multiple testing. After multiple testing correction, none of the results remained significant.

## Strengths and limitations

The main strength of the present study is the prospective design from early life onwards and the large sample size of this population-based cohort. To our knowledge, this is one of the largest prospective studies that examined the associations between the daily maternal intake of micronutrients and macronutrients in the first trimester of pregnancy with childhood blood pressure. We used a FFQ, previously validated in a Dutch population<sup>(23)</sup>, to assess dietary intake of the mothers. Although we have used a validated questionnaire, misclassification of dietary intake can still occur, which might have led to an underestimation of the effect estimates. We measured blood pressure in 6-year-old children using a validated automatic sphygmomanometer<sup>(27)</sup> and acquired multiple blood pressure measurements to minimise measurement error. Furthermore, information about a large number of potential confounders was available. However, some limitations need to be addressed. Of all mothers included before a gestational age of 25 weeks, information on maternal dietary intake was missing for only 1.4% of all mothers. This non-response would lead to biased effect estimates if the associations of maternal dietary intake and childhood blood pressure would be different between mothers included and not included in the analyses. This seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from a non-response at baseline<sup>(35)</sup>. Of all Dutch singleton live-born children with available data on maternal dietary intake during the first trimester of pregnancy, 74.3% participated in the follow-up measurements at the age of 6 years. Overall, mothers who did not visit the research centre were younger, less well educated, smoked more frequently and used less alcohol during pregnancy. This selective loss to follow-up might have led to biased effect estimates. We also have to acknowledge that the present study population included in the analyses is comprised of relatively healthy women with a higher proportion of folic acid supplement use compared with other populations. This might have resulted in smaller observed differences and might limit generalisability to other populations. Also, maternal dietary intake was only assessed in the first trimester of pregnancy, while dietary intake might change during pregnancy. Although it has been demonstrated that maternal nutritional intake did not change significantly during pregnancy<sup>(36)</sup>, second- or third-trimester maternal diet might be associated with cardiovascular development, and influence childhood blood pressure. Unfortunately, we were not able to assess these associations in the present study. Finally, although we have performed adjustment for various potential confounders, residual confounding might still be an issue due to the observational design of the study.

## Maternal macronutrient intake

Low birth weight is associated with higher blood pressure and CVD in later life<sup>(37,38)</sup>. It has been suggested that these associations might be explained by suboptimal maternal diet<sup>(1–3)</sup>. Historical cohort studies in The Netherlands and China

**Table 3.** Associations of maternal macronutrient intake during pregnancy with blood pressure in children at the age of 6 years in a Dutch population†  
(Regression coefficients and 95 % confidence intervals)

Macronutrients (n 2554)	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	Crude model		Adjusted model		Crude model		Adjusted model	
	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI
<b>Total energy intake (kJ)</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	0.07	-0.87, 1.01	0.09	-0.83, 1.00	0.53	-0.25, 1.31	0.55	-0.23, 1.32
Third quintile	-0.15	-1.08, 0.79	0.04	-0.87, 0.96	0.11	-0.67, 0.89	0.19	-0.58, 0.96
Fourth quintile	-0.44	-1.38, 0.50	-0.17	-1.09, 0.75	-0.20	-0.99, 0.58	-0.10	-0.88, 0.68
Fifth quintile	-0.14	-1.08, 0.80	-0.03	-0.95, 0.90	0.30	-0.48, 1.09	0.33	-0.45, 1.11
Trend‡	-0.04	-0.34, 0.26	0.02	-0.27, 0.31	0.06	-0.19, 0.31	0.07	-0.17, 0.32
<b>Carbohydrate intake (kJ)</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	-0.00	-0.94, 0.94	0.20	-0.75, 1.14	0.49	-0.29, 1.27	0.52	-0.28, 1.31
Third quintile	0.41	-0.53, 1.35	0.61	-0.38, 1.60	0.39	-0.39, 1.17	0.33	-0.50, 1.16
Fourth quintile	-0.14	-1.08, 0.80	0.06	-0.99, 1.11	-0.10	-0.88, 0.68	-0.20	-1.09, 0.68
Fifth quintile	0.18	-0.75, 1.12	0.37	-0.76, 1.50	0.65	-0.13, 1.44	0.47	-0.48, 1.42
Trend‡	0.07	-0.23, 0.36	0.11	-0.26, 0.49	0.15	-0.10, 0.40	0.10	-0.21, 0.41
<b>Fat intake (kJ)</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	0.09	-0.85, 1.02	0.19	-0.76, 1.14	0.47	-0.31, 1.25	0.36	-0.44, 1.16
Third quintile	-0.44	-1.38, 0.50	-0.24	-1.27, 0.79	0.19	-0.59, 0.98	0.06	-0.81, 0.93
Fourth quintile	-0.55	-1.50, 0.39	-0.27	-1.37, 0.82	-0.44	-1.23, 0.34	-0.68	-1.61, 0.25
Fifth quintile	-0.18	-1.12, 0.75	0.19	-1.05, 1.43	0.18	-0.61, 0.96	-0.10	-1.15, 0.94
Trend‡	-0.12	-0.42, 0.17	0.02	-0.41, 0.44	-0.07	-0.32, 0.18	-0.21	-0.57, 0.14
<b>Protein intake (kJ)</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	-0.75	-1.68, 0.19	-0.62	-1.58, 0.34	-0.19	-0.97, 0.59	-0.04	-0.85, 0.77
Third quintile	-0.87	-1.81, 0.07	-0.68	-1.71, 0.36	-0.58	-1.36, 0.20	-0.32	-1.19, 0.55
Fourth quintile	-0.60	-1.54, 0.34	-0.34	-1.48, 0.80	-0.30	-1.08, 0.49	0.06	-0.91, 1.02
Fifth quintile	-0.56	-1.50, 0.38	-0.45	-1.76, 0.84	-0.02	-0.81, 0.76	0.35	-0.75, 1.44
Trend‡	-0.15	-0.43, 0.17	-0.12	-0.57, 0.33	0.04	-0.21, 0.28	0.21	-0.17, 0.59
<b>Protein:carbohydrate ratio</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	-1.11*	-2.05, -0.17	-0.90	-1.82, 0.02	-0.63	-1.42, 0.14	-0.38	-1.16, 0.40
Third quintile	-0.88	-1.81, 0.05	-0.54	-1.47, 0.39	-0.80*	-1.58, -0.02	-0.47	-1.25, 0.31
Fourth quintile	-0.87	-1.81, 0.06	-0.50	-1.43, 0.43	-0.64	1.42, 0.14	-0.22	-1.01, 0.57
Fifth quintile	-0.99*	-1.93, -0.06	-0.65	-1.58, 0.29	-0.61	-1.39, 0.17	-0.21	-1.00, 0.58
Trend‡	-0.21	-0.51, 0.08	-0.11	-0.41, 0.19	-0.16	-0.41, 0.09	-0.05	-0.30, 0.20

Maternal diet and offspring blood pressure

\*P < 0.05.

† Values reflect the difference in blood pressure (mmHg) at the age of 6 years for each quintile of macronutrient intake (kJ derived from macronutrients). The crude model is adjusted for child sex and age at blood pressure measurement. The adjusted model is additionally adjusted for maternal age, pre-pregnancy BMI, alcohol use and smoking during pregnancy, educational level, gestational age at birth, birth weight, current BMI and month in which blood pressure measurement was taken and additionally adjusted for energy from the other macronutrients following the energy partition method<sup>(1)</sup>.

‡ Tests for trends were conducted using the maternal dietary intake variables as the continuous variable in the linear mixed models. Values reflect the difference in blood pressure (mmHg) per standard deviation increase in macronutrient intake.

**Table 4.** Associations of maternal micronutrient intake during pregnancy with blood pressure at the age of 6 years in a Dutch population†  
(Regression coefficients and 95 % confidence intervals)

Micronutrients (n 2554)	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	Crude model		Adjusted model		Crude model		Adjusted model	
	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI
<b>Ca</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	0.44	-0.50, 1.37	0.47	-0.45, 1.40	0.80*	0.02, 1.58	0.90*	0.13, 1.68
Third quintile	0.10	-0.83, 1.04	0.25	-0.68, 1.18	0.29	-0.49, 1.07	0.52	-0.26, 1.30
Fourth quintile	-0.25	-1.18, 0.69	-0.05	-0.98, 0.87	-0.34	-1.12, 0.44	-0.10	-0.88, 0.68
Fifth quintile	0.32	-0.62, 1.26	0.38	-0.54, 1.31	0.75	-0.04, 1.53	0.92*	0.14, 1.71
Trend‡	0.09	-0.21, 0.38	0.10	-0.20, 0.39	0.15	-0.10, 0.40	0.20	-0.04, 0.45
<b>Fe</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	-0.05	-0.99, 0.88	0.31	-0.63, 1.25	0.32	-0.47, 1.10	0.63	-0.16, 1.43
Third quintile	-0.57	-1.51, 0.37	-0.06	-1.02, 0.90	0.18	-0.60, 0.96	0.70	-0.11, 1.51
Fourth quintile	-0.94*	-1.87, -0.00	-0.34	-1.31, 0.62	-0.22	-1.00, 0.56	0.32	-0.49, 1.13
Fifth quintile	-1.38**	-2.32, -0.44	-0.76	-1.73, 0.21	-0.71	-1.50, 0.07	-0.14	-0.96, 0.68
Trend‡	-0.48**	-0.78, -0.18	-0.30	-0.61, 0.01	-0.23	-0.47, 0.02	-0.06	-0.32, 0.20
<b>Na</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	-0.71	-1.65, 0.23	-0.57	-1.50, 0.35	-0.41	-1.19, 0.38	-0.34	-1.12, 0.44
Third quintile	-0.98*	-1.92, -0.04	-0.90	-1.82, 0.02	-0.49	-1.27, 0.30	-0.44	-1.22, 0.33
Fourth quintile	-0.17	-1.10, 0.77	-0.24	-1.16, 0.68	-0.11	-0.89, 0.67	-0.12	-0.89, 0.66
Fifth quintile	-0.08	-1.02, 0.85	-0.08	-1.00, 0.85	-0.30	-1.08, 0.48	-0.27	-1.04, 0.52
Trend‡	0.07	-0.22, 0.37	0.06	-0.24, 0.35	-0.01	-0.25, 0.24	-0.00	-0.25, 0.24

\**P*<0.05, \*\**P*<0.01.

† Values reflect the difference in blood pressure (mmHg) at the age of 6 years for each quintile of micronutrient intake. The crude model is adjusted for child sex and age at blood pressure measurement. The adjusted model is additionally adjusted for maternal age, pre-pregnancy BMI, alcohol use and smoking during pregnancy, educational level, gestational age at birth, birth weight, current BMI and month in which blood pressure measurement was taken.

‡ Tests for trends were conducted using the maternal dietary intake variables as the continuous variable in the linear mixed models. Values reflect the difference in blood pressure (mmHg) per standard deviation increase in micronutrient intake.

**Table 5.** Associations of maternal folic acid supplement use during pregnancy with blood pressure at the age of 6 years in a Dutch population\* (Regression coefficients and 95 % confidence intervals)

	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	Crude model		Adjusted model		Crude model		Adjusted model	
	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI
Folic acid supplement use (n 2379)								
No (n 227)	0.64	-0.34, 1.61	0.09	-0.90, 1.07	0.46	-0.35, 1.27	-0.03	-0.85, 0.79
Started when pregnancy was known (n 797)	0.04	-0.59, 0.66	-0.04	-0.68, 0.60	-0.40	-0.92, 0.12	-0.52	-1.05, 0.04
Started periconceptional (n 1355)	Reference		Reference		Reference		Reference	

\* Values reflect the difference in blood pressure (mmHg) at the age of 6 years for each group of maternal folic acid supplement use. The crude model is adjusted for child sex and age at blood pressure measurement. The adjusted model is additionally adjusted for maternal age, pre-pregnancy BMI, alcohol use and smoking during pregnancy, educational level, gestational age at birth, birth weight, current BMI and month in which blood pressure measurement was taken.

showed that maternal exposure to famine is associated with a higher blood pressure in adult offspring<sup>(4,39)</sup>. The effect of the normal variation in maternal daily energy intake and macronutrient intake on childhood blood pressure has been examined in several studies, but showed inconsistent results<sup>(7,8,16)</sup>. Consistent with previous studies, we did not observe associations of maternal total energy intake during pregnancy and childhood blood pressure<sup>(8,40)</sup>. As suggested by Roseboom *et al.*<sup>(4)</sup>, it might be that an imbalance of maternal macronutrients, rather than total energy intake, is associated with childhood blood pressure. Using the energy partition method<sup>(35)</sup>, we did not observe associations of maternal daily carbohydrate, fat or protein intake with childhood blood pressure. Previously, two smaller studies from Finland and the Philippines showed conflicting results regarding the associations of maternal fat intake with childhood blood pressure<sup>(7,8)</sup>. The present results suggest that there is no association of maternal fat intake with childhood blood pressure. Also, a previous study showed that a higher maternal protein intake is associated with a lower blood pressure in boys, but these findings were not confirmed in other studies<sup>(8,40,41)</sup>. The inconsistent results might be explained by differences in study populations.

#### Maternal calcium, iron and sodium intake

Several studies, including follow-up studies of randomised clinical trials, have shown an association of Ca supplement use during pregnancy with a lower blood pressure in children<sup>(11,14)</sup>. However, maternal Ca intake from normal dietary intake was not associated with infant and childhood blood pressure<sup>(9,14)</sup>. In line with these studies, we did not find an association between maternal Ca intake in the first trimester of pregnancy and offspring blood pressure. It should be noted that our population had a high dietary Ca intake. This could have affected our power to investigate the effects of low Ca intake on childhood blood pressure. Another explanation might be that mothers who used Ca supplements also used other micronutrient supplements, and the combined effect of these supplements might lead to a lower blood pressure in the offspring<sup>(14)</sup>.

Animal studies have shown that maternal Fe restriction during pregnancy was associated with a higher blood pressure in the offspring<sup>(42,43)</sup>. In human subjects, Brion *et al.*<sup>(13)</sup> observed in a prospective cohort study among 7484 subjects that maternal Fe supplement use and Fe intake from food sources, assessed in the third trimester of pregnancy, were not associated with offspring blood pressure. Belfort *et al.*<sup>(10)</sup> showed in a prospective longitudinal cohort study among 1098 American children an association of maternal Fe supplement use with a higher systolic blood pressure in children. However, maternal Fe dietary intake from food sources, assessed in the first and second trimesters of pregnancy, was not associated with offspring blood pressure at both time points<sup>(10)</sup>. We observed an association of higher maternal Fe intake from food with a lower blood pressure, although the effect size was small and borderline significant. We assessed dietary intake in the first trimester of pregnancy. It might be that part of the differences in results between the present study and other studies can be explained by different timings of the exposure assessment. Animal studies have shown that offspring of Fe-restricted mothers have a higher blood pressure in later life, possibly mediated by cardiovascular adaptations in response to anaemia, which is in line with the present findings<sup>(42,44)</sup>. Previous studies in animals described the associations of maternal Na intake with childhood blood pressure and suggested that high Na intake was associated with a higher blood pressure<sup>(15,45)</sup>. In the present study, maternal Na intake was not associated with childhood blood pressure. However, Na intake is poorly assessed by the FFQ, which could have led to an underestimation of the effect. Further experimental studies in animals and observational studies in human subjects are needed to assess the associations of maternal micronutrient intake with offspring blood pressure in later life, and to investigate the possible underlying mechanisms.

#### Maternal folate, homocysteine and vitamin B<sub>12</sub> concentrations

Low folate and high homocysteine concentrations during pregnancy have been associated with lower birth weight and pregnancy complications, such as spontaneous abortion



**Table 6.** Associations of maternal first-trimester folate, homocysteine and vitamin B<sub>12</sub> levels with blood pressure at the age of 6 years in a Dutch population† (Regression coefficients and 95 % confidence intervals)

	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	Crude model		Adjusted model		Crude model		Adjusted model	
	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI	Regression coefficient	95 % CI
<b>Maternal folate (n 2266)</b>								
First quintile	0.68	-0.26, 1.63	0.27	-0.68, 1.23	0.46	-0.33, 1.25	0.14	-0.65, 0.94
Second quintile	0.50	-0.44, 1.44	0.41	-0.52, 1.35	0.46	-0.33, 1.24	0.41	-0.38, 1.18
Third quintile	-0.36	-1.29, 0.57	-0.35	-1.27, 0.58	-0.22	-1.00, 0.56	-0.19	-0.96, 0.59
Fourth quintile	0.46	-0.47, 1.39	0.36	-0.56, 1.29	0.60	-0.18, 1.38	0.56	-0.21, 1.34
Fifth quintile	Reference		Reference		Reference		Reference	
Trend‡	0.06	-0.24, 0.35	0.11	-0.18, 0.40	-0.03	-0.28, 0.21	0.03	-0.21, 0.27
<b>Maternal homocysteine (n 2244)</b>								
First quintile	Reference		Reference		Reference		Reference	
Second quintile	0.35	-0.55, 1.26	0.23	-0.66, 1.12	-0.07	-0.83, 0.68	-0.16	-0.90, 0.59
Third quintile	0.42	-0.50, 1.35	0.22	-0.69, 1.13	-0.21	-0.99, 0.56	-0.33	-1.09, 0.43
Fourth quintile	0.25	-0.68, 1.19	0.08	-0.83, 0.99	-0.19	-0.96, 0.58	-0.28	-1.04, 0.48
Fifth quintile	0.58	-0.35, 1.51	0.08	-0.84, 1.00	-0.23	-1.01, 0.54	-0.56	-1.33, 0.21
Trend‡	-0.00	-0.30, 0.29	-0.00	-0.29, 0.28	-0.19	-0.43, 0.05	-0.18	-0.42, 0.06
<b>Maternal vitamin B<sub>12</sub> (n 2158)</b>								
First quintile	-0.07	-1.03, 0.88	-0.50	-1.46, 0.45	-0.44	-1.24, 0.35	-0.75	-1.54, 0.05
Second quintile	-0.75	-1.70, 0.21	-0.96*	-1.91, -0.02	-0.70	-1.50, 0.09	-0.88*	-1.66, -0.09
Third quintile	-0.42	-1.37, 0.54	-0.48	-1.42, 0.47	-0.38	-1.18, 0.41	-0.42	-1.21, 0.36
Fourth quintile	-0.11	-1.06, 0.85	-0.24	-1.18, 0.71	-0.15	-0.95, 0.64	-0.24	-1.03, 0.55
Fifth quintile	Reference		Reference		Reference		Reference	
Trend‡	0.06	-0.24, 0.36	0.18	-0.11, 0.48	0.22	-0.03, 0.47	0.31*	0.06, 0.56

\*  $P < 0.05$ .

† Values reflect the difference in childhood blood pressure (mmHg) at the age of 6 years for each quintile of biomarker concentrations. The highest quintile in maternal folate and vitamin B<sub>12</sub> levels and the lowest quintile in homocysteine levels were used as reference groups. The crude model is adjusted for child sex and age at blood pressure measurement. The adjusted model is additionally adjusted for maternal age, pre-pregnancy BMI, alcohol use and smoking during pregnancy, educational level, gestational age at birth, birth weight, current BMI and month in which blood pressure measurement was taken.

‡ Tests for trends were conducted using the maternal dietary intake variables as the continuous variable in the linear mixed models. Values reflect the difference in blood pressure (mmHg) per standard deviation increase in micro-nutrient intake.

and pre-eclampsia<sup>(46–49)</sup>. Low folate and vitamin B<sub>12</sub> levels and high homocysteine levels might affect vascular development and subsequently lead to endothelial dysfunction and higher blood pressure<sup>(18)</sup>. A previous study described the associations of supplement intake, including Fe, folic acid and vitamin B<sub>12</sub>, during pregnancy with a lower systolic blood pressure in children at the age of 2 years<sup>(50)</sup>. However, since the supplement contained a combination of these nutrients, they could not infer which nutrient contributed to the effect.

We did not observe the associations of maternal folate and homocysteine levels during the first trimester of pregnancy with childhood blood pressure in their offspring. Since higher vitamin B<sub>12</sub> levels are associated with lower homocysteine levels, and lower homocysteine levels are associated with a lower blood pressure in adults, we expected that higher maternal vitamin B<sub>12</sub> concentrations in the blood would be associated with a lower childhood blood pressure. However, the present results show an opposite effect; higher maternal vitamin B<sub>12</sub> levels were associated with a higher childhood diastolic blood pressure, although the effect size was small and borderline significant. However, after correction for multiple testing, this association was not statistically significant. To the best of our knowledge, this association has not been described before and the underlying mechanisms are not known.

### Conclusions

The present study shows within a population-based cohort that the normal variation in the maternal intake of macronutrients and micronutrients during the first trimester of pregnancy is not associated with childhood blood pressure. We found some indications that high maternal Fe intake and low maternal vitamin B<sub>12</sub> levels seemed to be associated with a lower blood pressure in children at the age of 6 years. However, we investigated multiple exposures, the effect sizes are small and the associations were not significant after correction for multiple testing. Further studies are needed to replicate these findings, to elucidate the underlying mechanisms and to assess whether these differences persist in later life.

### Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114513000676>

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