

Supplementary Materials

Maternal and fetal folate, vitamin B₁₂ and homocysteine concentrations and childhood kidney outcomes.

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Supplementary Figure S1. Flowchart of the study participants

Supplementary Table S1. Subjects characteristics in the observed and imputed dataset (N = 4,226)

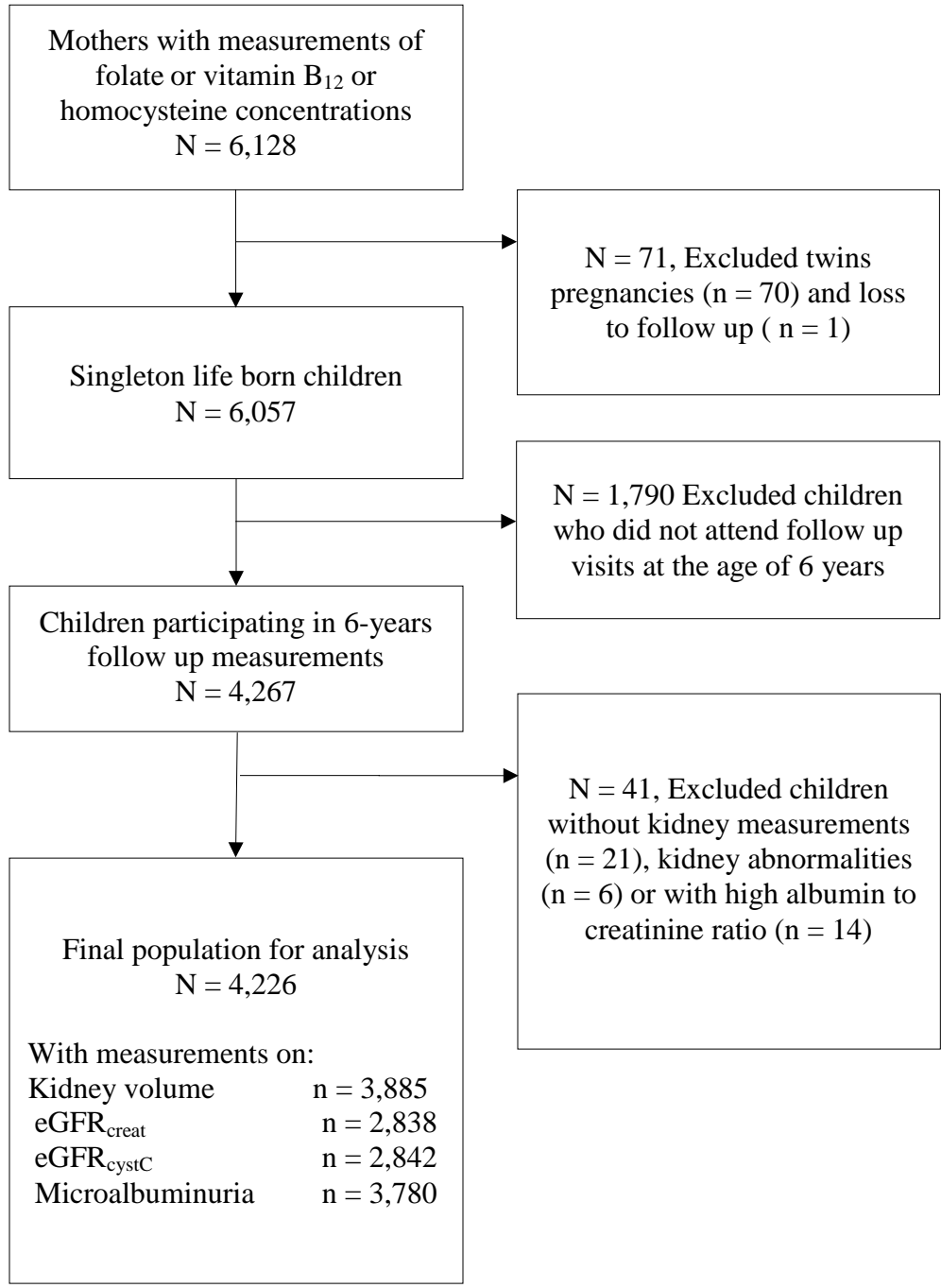
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Supplementary Figure S1. Flowchart of study participants



Supplementary Table S1. Subject characteristics (N = 4,226)

	Observed	Imputed
Maternal characteristics		
Maternal age (y)	30.4 (4.9)	30.4 (4.9)
Pre-pregnancy body mass index(kg/m ²)	22.6 (18.2, 34.6)	22.6 (18.1, 34.6)
Gestational age at intake (wk)	13.5 (2.0)	13.5 (2.0)
Education level (%)		
- No higher education	51.3	52.9
- Higher education	48.7	47.1
Ethnicity (%)		
- European	64.9	64.8
- Non-European	35.1	35.2
Smoking during pregnancy (%)		
- Never & until pregnancy was known	83.0	83.6
- Continued	17.0	16.4
Alcohol during pregnancy (%)		
- Never & until pregnancy was known	57.3	57.5
- Continued	42.7	42.5
Folic acid supplements use (%)		
- No	21.1	21.1
- Start 1st to 10 weeks	32.4	32.3
- Start periconceptional	46.5	46.6
Maternal calories intake (kcal)	2,045 (553)	2,044 (499)
Folate plasma concentrations (nmol/l)	16.8 (5.7, 37.6)	NI
Homocysteine plasma concentrations (μmol/l)	6.9 (4.6, 11.6)	NI
Vitamin B ₁₂ serum concentrations (pmol/l)	171 (74.0, 406.0)	NI
Infant characteristics		
Girls (%)	50.2	50.2
Gestational age at birth (wk)	40.1 (35.9, 42.3)	40.1 (35.9, 42.3)
Birth weight (g)	3,437 (551)	3,437 (551)
Breastfeeding in the first 4 months (%)		
- No	7.5	7.7
- Yes	92.5	92.3

Cord blood folate concentrations (nmol/l)	20.8 (10.4, 38.2)	NI
Cord blood vitamin B ₁₂ concentrations (pmol/l)	299 (120.8, 894.4)	NI
Cord blood homocysteine concentrations (μmol/l)	9.0 (5.2, 16.4)	NI
Child characteristics at 6y visit		
Age (y)	6.0 (5.6, 7.9)	6.0 (5.6, 7.9)
Height (cm)	119.3 (5.9)	119.3 (5.9)
Weight (kg)	22.4 (17.6, 33.7)	22.4 (17.6, 33.7)
Body mass index (kg/m ²)	15.8 (13.6, 21.3)	15.8 (13.6, 21.3)
Body surface area (m ²)	0.9 (0.1)	0.9 (0.1)
Combined kidney volume (cm ³)	120.0 (23.6)	NI
Creatinine (μmol/l)	37.4 (5.6)	NI
Cystatin C (mg/l)	784.4 (81.1)	NI
eGFR _{creat} (ml/min/1.73m ²)	119.1 (16.3)	NI
eGFR _{cystC} (ml/min/1.73m ²)	102.4 (14.6)	NI
Microalbuminuria (%)	7.5	NI

* Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution. Abbreviations: GFR_{creat} estimated glomerular filtration rate calculated based on creatinine blood levels; eGFR_{cystC} estimated glomerular filtration rate calculated based on cystatin C blood levels.

Supplementary Table S2. Biomarkers concentrations per supplement group of folic acid (N = 3,291)

Concentrations during 1st trimester			
	Folate (nmol/l)	Vitamin B₁₂ (pmol/l)	Homocysteine (μmol/l)
Folic Acid Supplement Use			
No (n = 696)	8.70 (4.90, 20.8)	158.00 (61.00, 397.05)	7.40 (4.78, 13.80)
Started when pregnancy was known (n = 1,065)	17.70 (7.02, 36.00)	177.00 (75.01, 397.00)	6.90 (4.60, 11.10)
Started periconceptual (n = 1,530)	22.70 (8.40, 39.63)	177.00 (82.00, 427.10)	6.70 (4.60, 10.57)
Cord blood concentrations			
	Folate (nmol/l)	Vitamin B₁₂ (pmol/l)	Homocysteine (μmol/l)
Folic Acid Supplement Use			
No (n = 425)	18.80 (9.95, 35.36)	289.00 (112.60, 840.60)	9.40 (5.80, 19.44)
Started when pregnancy was known (n = 684)	20.20 (10.38, 40.00)	297.00 (115.85, 914.10)	9.05 (5.30, 15.83)
Started periconceptual (n = 980)	22.70 (11.20, 39.23)	304.00 (134.00, 874.13)	8.80 (5.10, 16.20)

Values are medians (95% range).

Supplementary Table S3. Subject characteristics with and without kidney measurements (N = 6,043)

	<i>Kidney measurements available</i> <i>N = 4,226</i>	<i>Without kidney measurements</i> <i>N = 1,817</i>	<i>p-value</i>
Maternal characteristics			
Maternal age (y)	30.4 (4.9)	28.4 (5.2)	< 0.01
Pre-pregnancy body mass index(kg/m ²)	22.6 (18.1, 34.6)	22.6 (17.6, 35.2)	0.47
Gestational age at intake	13.5 (2.0)	13.4 (2.1)	< 0.01
Education level (%)			< 0.01
- No higher education	52.9	66.6	
- Higher education	47.1	33.4	
Ethnicity (%)			< 0.01
- European	64.8	52.2	
- Non-European	35.2	47.8	
Smoking during pregnancy (%)			< 0.01
- Never & until pregnancy was known	83.6	67.4	
- Continued	16.4	19.6	
Alcohol during pregnancy (%)			< 0.01
- Never & until pregnancy was known	57.5	69.5	
- Continued	42.5	30.5	
Folic acid supplements use (%)			< 0.01
- No	21.1	36.6	
- Start 1st to 10 weeks	32.3	30.4	
- Start periconceptional	46.6	33.0	
Maternal calories intake (kcal)	2,045 (553)	2,012 (485)	0.03
Folate plasma concentrations (nmol/l)	16.8 (5.8, 37.6)	13.2 (5.0, 37.1)	< 0.01
Vitamin B ₁₂ serum concentrations (pmol/l)	171 (74.0, 406.0)	165 (68.0, 419.0)	0.06
Homocysteine plasma concentrations (μmol/l)	6.9 (4.6, 11.6)	7.0 (4.6, 12.9)	0.03
Infant characteristics			
Girls (%)	50.2	47.5	
Gestational age at birth (wk)	40.1 (35.9, 42.3)	40.0 (34.2, 42.3)	< 0.01
Birth weight (g)	3,437 (551)	3,379 (592)	< 0.01
Breastfeeding (%)			0.01
- No	7.7	8.9	
- Yes	92.3	91.1	

Cord blood folate concentrations (nmol/l)	20.8 (10.4, 38.2)	20.3 (10.4, 38.7)	0.08
Cord blood vitamin B ₁₂ concentrations (pmol/l)	299 (120.8, 894.4)	301 (115.9, 931, 9)	0.76
Cord blood homocysteine concentrations (μmol/l)	9.0 (5.2, 16.4)	9.3 (5.1, 17.2)	< 0.01

Values are percentages for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

Supplementary Table S4. Associations of maternal folate, vitamin B₁₂ and homocysteine concentrations during pregnancy with kidney outcomes at the age of 6 years (N = 4,226)

First trimester maternal concentrations	Difference in outcome measure (95% Confidence Interval)			
	Kidney volume (cm ³)	eGFR _{creat} (ml/min/1.73m ²)	eGFR _{cystC} (ml/min/1.73m ²)	Microalbuminuria (odds ratio)
Folate (n = 4,149)	(n = 3,818)	(n = 2,788)	(n = 2,792)	(n = 4,011)
Basic Model	0.83 (0.10, 1.55)*	0.30 (-0.31, 0.91)	0.21 (-0.35, 0.76)	1.01 (0.90, 1.14)
Vitamin B₁₂ (n = 3,983)	(n = 3,666)	(n = 2,659)	(n = 2,663)	(n = 3,849)
Basic Model	0.21 (-0.52, 0.94)	0.28 (-0.34, 0.89)	1.11 (0.55, 1.67)**	1.07 (0.96, 1.20)
Homocysteine (n = 4,105)	(n = 3,779)	(n = 2,755)	(n = 2,751)	(n = 3,969)
Basic Model	-1.66 (-2.40, -0.93)**	-0.78 (-1.37, -0.18)*	-0.75 (-1.29, -0.20)**	1.07 (0.97, 1.19)

Values are linear and logistic regression coefficients (95% confidence interval). Basic model is adjusted for child's sex and age at 6 year visit. * p < 0.05, **p<0.01. Maternal folate, vitamin B₁₂ and homocysteine concentrations were analyzed per 1 standard deviation in folate, vitamin B₁₂ and homocysteine. Abbreviations: eGFR_{creat}, estimated glomerular filtration rate based on creatinine concentrations; eGFR_{cystC}, estimated glomerular filtration rate based on cystatin C concentrations.

Supplementary Table S5. Associations of cord blood folate, vitamin B₁₂ and homocysteine concentrations with kidney outcomes at the age of 6 years (N = 2,674)

Cord blood concentrations	Difference in outcome measure (95% Confidence Interval)			
	Kidney volume (cm ³)	eGFR _{creat} (ml/min/1.73m ²)	eGFR _{cystC} (ml/min/1.73m ²)	Microalbuminuria (odds ratio)
Folate (n = 2,599)	(n = 2,384)	(n = 1,750)	(n = 1,753)	(n = 2,517)
Basic Model	0.04 (-0.87, 0.94)	0.51 (-0.24, 1.25)	0.72 (0.03, 1.41)*	0.98 (0.84, 1.14)
Vitamin B₁₂ (n = 2,631)	(n = 2,413)	(n = 1,772)	(n = 1,776)	(n = 2,548)
Basic Model	-0.93 (-1.82, -0.04)*	-0.43 (-1.18, 0.33)	0.41 (-0.28, 1.10)	1.01 (0.88, 1.17)
Homocysteine (n = 2,522)	(n = 2,311)	(n = 1,702)	(n = 1,705)	(n = 2,443)
Basic Model	1.25 (0.33, 2.16)**	-1.06 (-1.85, -0.28)**	-1.22 (-1.95, -0.50)**	1.07 (0.93, 1.23)

Values are linear and logistic regression coefficients (95% confidence interval). Basic model is adjusted for child's sex and age at 6 year visit. * p < 0.05, **p<0.01. Cord blood folate, vitamin B₁₂ and homocysteine concentrations were analyzed per 1 standard deviation in folate, vitamin B₁₂ and homocysteine. Abbreviations: eGFR_{creat}, estimated glomerular filtration rate based on creatinine concentrations; eGFR_{cystC}, estimated glomerular filtration rate based on cystatin C concentrations.