First trimester maternal protein intake and childhood kidney outcomes. The Generation R Study

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Supplementary Table 1. Observed and imputed subjects characteristics (n = 3,650)

	Observed	Imputed
Maternal characteristics		
Maternal age (y)	31.1 (4.7)	31.1 (4.7)
Gestational age at intake (weeks)	13.8 (9.8, 23.5)	13.5 (9.8, 22.9)
Maternal body mass index at enrolment (kg/m2)	22.5 (18.3, 34.2)	22.6 (18.4, 34.4)
Missing, (%)	*15.3*	
Nulliparous (%)	59.5	59.4
Missing, (%)	*0.2*	
Educational level, (%)		
- No higher education	44.4	44.9
- Higher education	55.6	55.1
Missing,(%)	*2.7*	
Ethnicity (%)		
- Dutch	64.3	63.9
- Turkish	5.0	5.0
- Moroccan	3.5	3.5
- Surinamese or Dutch Antilles	7.9	7.8
- Other western	12.5	12.5
- Other non-western	6.7	6.7
Missing, (%)	*0.6*	
Household total income, (%)		
- < 1400 euro	14.8	13.4
- 1400-2200 euro	18.3	14.5
- > 2200 euro	66.9	60.7
Missing, %	*9.3*	
Smoking during pregnancy, (%)		
- Never smoked	76.2	76.0
- until pregnancy was known	9.7	9.6
- Continued	14.1	14.2
Missing, (%)	*9*	
Alcohol during pregnancy, (%)		
- Never alcohol in pregnancy	38.1	37.9
- Until pregnancy was known	14.5	14.5
- Continued	47.4	47.3
Missing, (%)	*9.8*	
Folic acid supplement use, (%)		
- No	17.4	22.4
- Start 1st to 10 weeks	31.9	31.1
- Start periconceptional	50.7	45.8
Missing, (%)	*20.8*	
Prepregnancy comorbidities (%)	1.6	NI
Missing, (%)	*28.7*	

Maternal diet		
Total energy intake (kcal)	2,073 (543)	NI
Protein (g/d)[1]		
- Total	76.6 (20.4)	NI
- Animal	46.9 (15.4)	NI
- Vegetable	29.9 (9.2)	NI
Protein (E%)	15.0 (2.5)	NI
Carbohydrates (E%)	48.5 (6.3)	NI
Fat (E%)	36.3 (5.5)	NI
Missing, %	-	
Infant characteristics		
Girls, (%)	50.1	NI
Dutch ethnicity (%)	66.2	65.8
Missing, (%)	0.5	
Gestational age at birth (wk)	40.1 (36.1, 42.4)	40.1 (36.1, 42.4)
Missing, (%)	-	
Birth weight, (g)	3,469 (538)	3,469 (538)
Missing, (%)	*0.02*	
Breastfeeding (%)		
- Exclusive ≥ 4 months	25.7	25.5
- Partial ≥ 4 months	65.5	64.7
- Never or ≤ 4 months	8.8	9.9
Missing, (%)	15.8	
Child protein intake at 1 y (g/d)	26.6 (29.6)	NI
Missing, (%)	*39.9*	
Child characteristics at 6 y visit		
Age (years)	6.1 (5.6 - 7.3)	NI
Height (cm)	119.1 (5.6)	119.1 (5.6)
Missing, %	*0.2*	
Weight (kg)	22.9 (3.8)	22.9 (3.8)
Missing,(%)	*0.2*	
Body mass index, (kg/m^2)	16.1 (1.7)	16.1 (1.7)
Missing, (%)	*0.2*	
Body surface area, (m^2)	0.90 (0.08)	0.90 (0.08)
Missing, (%)	*0.2*	
Screen time (hour/day)	1.3 (0.3, 4.7)	1.3 (0.3, 4.6)
Missing, (%)	*20.1*	
Kidney volume combined, (cm^3)	119 (23)	NI
Missing,(%)	*8.4*	
Creatinine, (μmol/l)	37.1 (5.4)	NI
Missing, %	*31.5*	
Cystatin C (μg/l)	784 (83)	NI
Missing, %	*31.5*	
eGFR, (ml/min/1.73m^2)	120 (16)	NI
Missing, (%)	*31.7*	
eGFR, (ml/min/1.73m^2)	103 (15)	NI
Missing, (%)	*31.5*	
Microalbuminuria, (%)	7.2	NI

Missing %	3.7

Values are means (SD), percentages (%), or medians (95% range) for variables with skewed distribution. Abbreviations: $eGFR_{creat}$, estimated glomerular filtration rate based on creatinine levels; $eGFR_{cyst C}$, estimated glomerular filtration rate based on cystatin C levels. [1] unadjusted for energy intake. NI - not imputed. In the observed dataset, percentages are valid percentage.

Supplementary Table 2. Associations of maternal protein intake during pregnancy with childhood creatinine and cystatin C [1]

	Serum creatinine (umol/l) n = 2,500	Serum creatinine (umol/l) adjusted for children protein intake at the age of 1 year n = 1,205	Serum cystatin C (µg/l) n = 2,500	Serum cystatin C (µg/l) adjusted for children protein intake at the age of 1 year n = 1,206
Total protein intake (g)[2]	**- 0.02 (- 0.04, - 0.01)**[3]	**-0.03 (- 0.05, - 0.01)**[3]	- 0.12 (- 0.41, 0.17)	- 0.03 (- 0.46, 0.39)
Animal protein intake (g)[2]	- 0.01 (- 0.03, 0.004)	**- 0.03 (- 0.06, - 0.01)**[3]	- 0.13 (- 0.42, 0.16)	- 0.11 (- 0.49, 0.27)
Vegetable protein intake (g)[2]	**- 0.07 (- 0.11, - 0.03)**[3]	**- 0.08 (- 0.13, - 0.03)**[3]	0.05 (- 0.60, 0.69)	0.14 (- 0.68, 0.45)

[1]Values are based on multivariable linear or logistic regression models and reflect differences or odds ratios and 95% confidence intervals in kidney volume and function measures for an increase in protein intake (g/d). The model is adjusted for child's sex, age at 6 year visit, maternal characteristics (age, body mass index before pregnancy, weight gain during pregnancy, gestational age at intake), maternal lifestyle (alcohol consumption, smoking during pregnancy, folic acid intake during pregnancy), socio-demographic factors (ethnicity, education), prepregnancy comorbidities, and child characteristics (breastfeeding, birthweight adjusted for gestational age, body surface area, and screen time at the age of 6y, child protein intake at 1 year). Models with animal protein intake were additionally adjusted for vegetable protein intake and vice versa. Protein intake is used as continuous variable in the regression models. [2]Protein intakes are energy-adjusted using the nutrient residual method. [3]$p < 0.05$. Abbreviations: $eGFR_{creat}$, estimated glomerular filtration rate based on creatinine levels; $eGFR_{cyst C}$, estimated glomerular filtration rate based on cystatin C levels.

Supplementary Table 3. Associations of maternal protein intake during pregnancy with childhood kidney outcomes, additionally adjusted for child protein intake at the age of 1 year (N = 2,193)[1]

	Kidney volume Difference (95% Confidence Interval) in cm^3 n = 1,603	eGFR$_{creat}$ Difference (95% Confidence Interval) in ml/min/1.73 m² n = 1,204	eGFR$_{cyst C}$ Difference (95% Confidence Interval) in ml/min/1.73 m² n = 1,206	Microalbuminuria Odds ratio (95% Confidence Interval) n = 1,713
Total protein intake (g)[2]	0.03 (-0.06, 0.11)	**0.11 (0.02, 0.19)**[3]	0.02 (-0.06, 0.09)	1.00 (0.98, 1.02)
Animal protein intake (g)[2]	0.04 (-0.03, 0.12)	**0.12 (0.05, 0.20)**[3]	0.02 (-0.05, 0.10)	1.00 (0.99, 1.02)
Vegetable protein intake (g)[2]	-0.01 (-0.17, 0.16)	**0.30 (0.13, 0.45)**[3]	0.001 (-0.17, 0.17)	1.00 (0.99, 1.03)

[1]Values are based on multivariable linear or logistic regression models and reflect differences or odds ratios and 95% confidence intervals in kidney volume and function measures for an increase in protein intake (g/d). The model is adjusted for child's sex, age at 6 year visit, maternal characteristics (age, body mass index before pregnancy, weight gain during pregnancy, gestational age at intake), maternal lifestyle (alcohol consumption, smoking during pregnancy, folic acid intake during pregnancy), socio-demographic factors (ethnicity, education), pre-pregnancy comorbidities, and child characteristics (breastfeeding, birthweight adjusted for gestational age, body surface area, and screen time at the age of 6y, child protein intake at 1 year). Models with animal protein intake were additionally adjusted for vegetable protein intake and vice versa. Protein intake is used as continuous variable in the regression models. [2]Protein intakes are energy-adjusted using the nutrient residual method. [3]$p < 0.05$. Abbreviations: eGFR$_{creat}$, estimated glomerular filtration rate based on creatinine levels; eGFR$_{cyst C}$, estimated glomerular filtration rate based on cystatin C levels.

Supplementary Table 4. Crude and multivariable adjusted associations of maternal protein intake during pregnancy with childhood kidney outcomes, in Dutch mothers only (N = 2,332)

	Kidney volume	Serum creatinine	Serum cystatin C	eGFR$_{creat}$	eGFR$_{cyst\ C}$	Microalbuminuria
	Difference (95% Confidence Interval) in cm^3 n = 2,137	Difference (95% Confidence Interval) in μmol/l n = 1,601	Difference (95% Confidence Interval) in μg/l n = 1,602	Difference (95% Confidence Interval) in ml/min/1.73m^2 n = 1,597	Difference (95% Confidence Interval) in ml/min/1.73m^2 n = 1,602	Odds ratio (95% Confidence Interval) n = 2,233
Basic model[1]						
Total protein intake (g)[3]	**0.08 (0.004, 0.17)**[4]	**-0.04 (-0.06, -0.01)**[4]	-0.36 (-0.72, 0.01)	**0.13 (0.06, 0.19)**[4]	**0.08 (0.01, 0.15)**[4]	1.00 (0.98, 1.02)
Animal protein intake (g)[3]	**0.08 (0.001, 0.17)**[4]	**-0.03 (-0.05, -0.007)**[4]	-0.37 (-0.74, 0.01)	**0.11 (0.04, 0.18)**[4]	**0.08 (0.01, 0.15)**[4]	1.00 (0.99, 1.02)
Vegetable protein intake (g)[3]	0.09 (-0.09, 0.26)	**-0.07 (-0.12, -0.02)**[4]	-0.21 (-0.98, 0.56)	**0.22 (0.07, 0.36)**[4]	0.06 (-0.09, 0.20)	0.99 (0.96, 1.02)
Multivariable adjusted model[2]						
Total protein intake (g)[3]	0.02 (-0.05, 0.10)	**-0.02 (-0.05, -0.004)**[4]	-0.26 (-0.64, 0.12)	**0.08 (0.01, 0.15)**[4]	0.07 (-0.001, 0.14)	1.03 (0.61, 1.73)
Animal protein intake (g)[3]	0.03 (-0.05, 0.10)	-0.02 (-0.05, 0.002)	-0.29 (-0.68, 0.09)	**0.07 (0.002, 0.15)**[4]	**0.07 (0.002, 0.14)**[4]	1.00 (0.98, 1.01)
Vegetable protein intake (g)[3]	-0.05 (-0.22, 0.11)	-0.04 (-0.09, 0.01)	0.09 (-0.74, 0.93)	0.10 (-0.05, 0.26)	0.02 (-0.14, 0.17)	0.97 (0.94, 1.01)

Values are based on multivariable linear or logistic regression models and reflect differences or odds ratios and 95% confidence intervals in kidney volume and function measures for maternal protein intake. Basic model[1] is adjusted for child's sex and age at 6 y visit. Multivariable adjusted model[2] is adjusted for child's sex, age at 6 y visit, maternal characteristics (age, body mass index before pregnancy, weight gain during pregnancy, gestational age at intake), maternal lifestyle (alcohol consumption, smoking during pregnancy, folic acid intake during pregnancy), socio-demographic factors (education), pre-pregnancy comorbidities, and child characteristics (breastfeeding, birth weight adjusted for gestational age, body surface area, and screen time at the age of 6y). Models with animal protein intake were additionally adjusted for vegetable protein intake and vice versa. [3] Protein intakes are energy-adjusted using the nutrient residual method. [4]$p < 0.05$.
Abbreviations: eGFR$_{creat}$, estimated glomerular filtration rate based on creatinine levels, eGFR$_{cyst\ C}$, estimated glomerular filtration rate based on cystatin C levels.