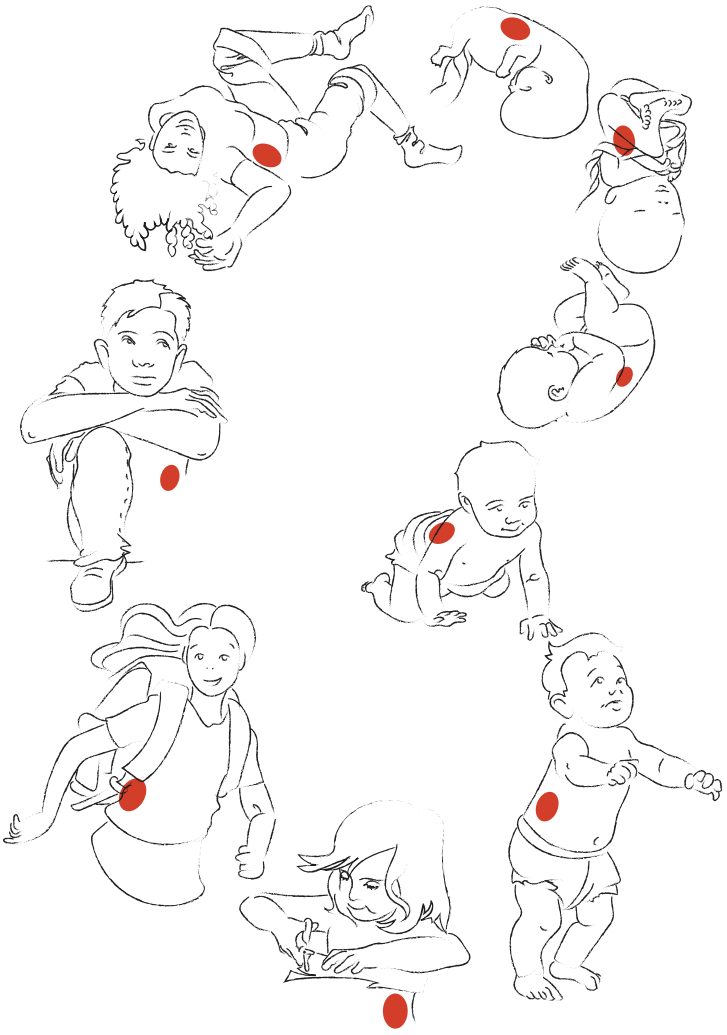
Fetal and Infant Origins of Childhood Kidney Function



Hanneke Bakker

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The Generation R Study

Hanneke Bakker

Acknowledgements

The work present in this thesis was conducted within the Generation R Study Group. The general design of the Generation R Study was supported by Erasmus Medical Center, Rotterdam; Erasmus University, Rotterdam; the Dutch Ministry of Health, Welfare and Sport; the Dutch Ministry of Youth and Families; the Netherlands Organization for Scientific Research (NWO); and the Netherlands Organization for Health Research and Development (ZonMw). The funders had no role in design or conduct of the studies; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscripts described in this thesis.

Publication of this thesis was kindly supported by the Department of Epidemiology, the Generation R Study Group and the Erasmus University Rotterdam.

ISBN: 978-94-92801-12-8

Cover design: Anne Mérat, www.annemerat.nl
Printing: Guus Gijben, proefschrift-aio.nl

Fetal and Infant Origins of Childhood Kidney Function
The Generation R Study

Foetale- en vroege postnatale origine van de nierfunctie op de kinderleeftijd
De Generation R Studie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

En volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Woensdag 29 november 2017 om 13.30 uur

door

Johanna Bakker

geboren te Kampen

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

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Chapter 1

Introduction and design

Chapter 1.1

General introduction

Introduction

Background

Chronic kidney disease (CKD) is a major public health problem with an increasing prevalence.(1) CKD affects around 10% of the adult population worldwide and is associated with increased risk of end-stage renal disease (ESRD) mortality, as well as cardiovascular mortality.(2) In the last decades, an accumulating body of evidence suggests that the risk of CKD originates in early life.(3,4) The developmental origins of health and disease (DOHaD) hypothesis suggests that adverse exposures in fetal life and infancy might lead to permanent developmental adaptations which might be beneficial on the short term, but lead to an increased risk of renal and cardiovascular disease in later life.(5,6)

In line with this DOHaD hypothesis, previous observational studies show that low birth weight is associated with higher risks of CKD and high blood pressure in adulthood.(7-9) It has been hypothesized that a reduced number of nephrons in low birth weight children underlie these associations.(3) Brenner et al. suggested that adverse circumstances in utero lead to lead to fetal growth restriction and smaller kidneys with a lower number of nephrons, which in turn may lead to glomerular hyperfiltration and finally glomerulosclerosis. This might predispose an individual for CKD in later life. It is not possible to count absolute nephron number *in vivo*. Since nephron number is correlated with kidney volume, kidney volume can be used as proxy for nephron number in population studies.(10) Previous studies report a positive relationship between kidney volume and kidney function in adults and in children.(11-13) The association of kidney volume with blood pressure has not been established yet.(12) The importance of early life kidney volume and function is emphasized by studies showing that variation in kidney function and blood pressure in childhood track into adulthood.(14,15)

Identifying critical periods and specific exposures relevant for kidney volume and function is important and may be helpful in development of preventive strategies to prevent CKD in the future. Subclinical differences in kidney volume and function in childhood might predispose individuals for an increased risk of CKD in later life. Therefore, the studies in this thesis aim to explore the associations between growth periods and patterns, and maternal and child factors, and kidney function in childhood (**Figure 1.1**).

Fetal and infant growth

Previous studies have shown associations of low birth weight with higher risks of CKD in later life.(7,9) Multiple post mortem studies showed a positive correlation between birth weight and nephron number.(16,17) Also, gestational age is positively associated with nephron number. Nephron number is lower in preterm born infants as compared to term born infants.(18)

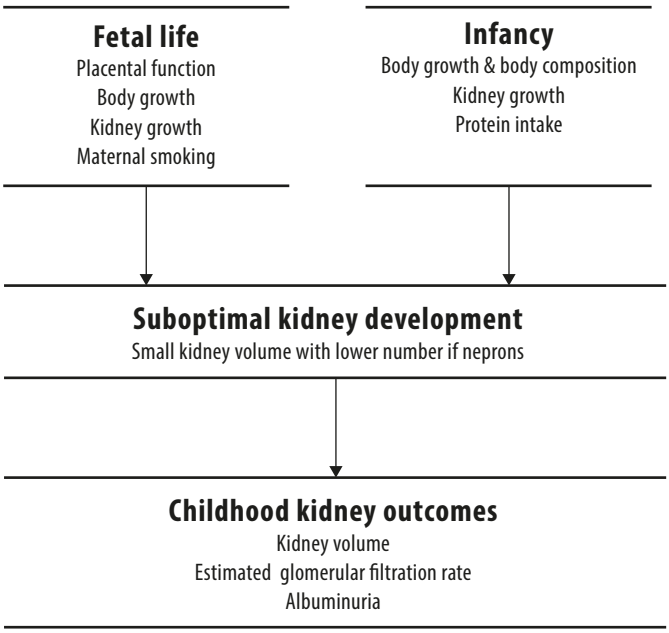
To date, it is not exactly known which growth periods are critical for kidney development. Human kidney development starts in the 9th week and ends around the 36th week of gestation.(19) In extremely premature born infants nephrogenesis was

observed until 40 days after birth.(18) Birth weight is used as a proxy for nephron number endowment.(20) However, birth weight is the outcome from various exposures and growth patterns in utero. Not only birth weight or gestational, but size for gestational age may be important for kidney development.(21) Size for gestational age is an endpoint from growth patterns in different trimesters in fetal life. Which periods are critical for kidney development is currently not exactly known. Moreover, fetal body growth and birth weight reflect overall growth. Specific kidney growth, in relation to body growth, might be important for kidney function in later life. Placental insufficiency is an important risk factor for impaired fetal growth(22) and is also associated with a reduced nephron number.(23) It has been shown that impaired fetal growth and placental insufficiency are associated with smaller fetal kidney volume.(24) Thus both fetal growth and placental function may affect kidney size.

Maternal and child factors

Multiple maternal and child factors may influence nephron endowment and preservation of glomerular number after birth. Most previous studies used birth weight as a proxy for both an adverse fetal environment and lower nephron number.(3) Maternal smoking is an important, modifiable risk factor for low birth weight.(25-27) Animal studies previously showed that kidney morphology can change after fetal smoking exposure.(28,29) It has been previously shown that maternal smoking is negatively associated with kidney development in third trimester.(30) It is not known whether this effect persists in postnatal life and what might be the effect of smoking on kidney function in childhood. Maternal and infant diet are factors that might influence kidney function in later life.(20) Animal studies show that increased maternal and infant protein intake leads to increased kidney growth.(31,32) Also, postnatal protein intake might be associated with kidney function and risk of hypertension in adulthood.(32,33) However, little is known about the association of early childhood diet and kidney volume and function in a healthy population. Estimation of glomerular filtration rate is an important tool in screening for and diagnosing kidney disease. Current estimations based on serum creatinine levels might have some limitations, such as the influence of body composition on creatinine levels.(34) Previous suggest that cystatin C based formula's might give more accurate estimations of glomerular filtration rate.(35,36)

Figure 1.1 Overview of the hypothesis on fetal and early childhood factors and childhood kidney function studied in this thesis



Overall aims

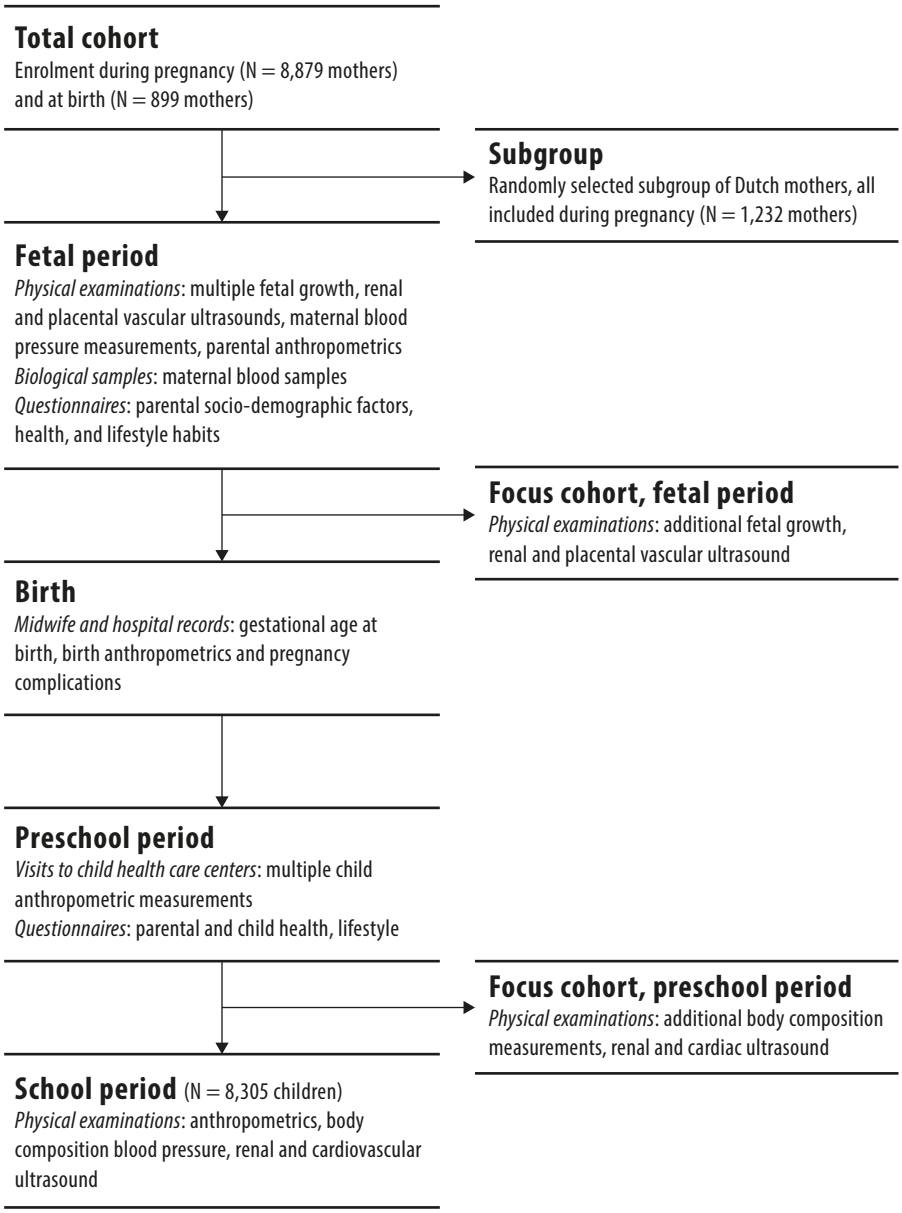
The overall aims of the studies presented in this thesis are:

- to examine which specific periods and patterns of body growth and kidney growth in fetal life and early infancy are associated with kidney function in childhood;
- to identify early life exposures related to kidney volume and function in childhood.

General design

The studies presented in this thesis were embedded in the Generation R Study, a population based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands.(37) The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrollment was aimed at early pregnancy, but was possible until the birth of the child. In total, 9,778 mother were enrolled in the study, of whom 8,880 (91%) were included during pregnancy (**Figure 1.2**). Assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18 -25 weeks of gestation) and late pregnancy (≥ 25 weeks of gestation), and included parental physical examinations, maternal blood and urine collection, fetal ultrasound examinations, and self-administered questionnaires. In the preschool periods, from birth to 4 years of age, data collection was performed with all children by questionnaires and visits to the routine child health care centers. All children were invited to a dedicated research center in the Erasmus MC – Sophia's Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements at the age of 6 years. Measurements during this visit included anthropometrics, body composition, cardiovascular development and body fluid specimen collection.

Figure 1.2 Design and data collection in the Generation R Study



Outline of thesis

In **Chapter 1.2**, the cross sectional associations of childhood characteristics and ethnicity with kidney volume and -function are evaluated.

Chapter 2 focusses on the associations of growth in early life with kidney volume and function in childhood. In **Chapter 2.1** studies on the associations of fetal first trimester growth with kidney outcomes in childhood are described. **Chapter 2.2** evaluates the associations of fetal blood flow and fetal kidney size with childhood kidney volume and function. Longitudinal, repeated fetal and infant growth measures and their association with kidney volume and function are presented in **Chapter 2.3. Chapter 2.4** describes specific growth periods and growth patterns of kidney volume and the association with kidney function in childhood.

In **Chapter 3**, studies focused on early life exposures and kidney volume and function in childhood are presented. In **Chapter 3.1**, studies on the associations of fetal smoke exposure with childhood kidney outcomes are described. **Chapter 3.2** describes the association of protein intake in infancy with kidney outcomes in childhood. The associations of childhood body composition with estimates of glomerular filtration rate based on creatinine and cystatin C concentration presented in **Chapter 3.3**.

Finally, **Chapter 4** provides a general discussion of the main findings presented in thesis. We will discuss possible underlying mechanisms, implications and give suggestions for future studies.

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Chapter 1.2

Kidney size and function in a multi-ethnic population-based cohort of school-age children

Adapted from Pediatr Nephrol. 2014;29(9):1589-98

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Abstract

Background Subclinical impaired kidney growth and function in childhood may lead to kidney diseases and high blood pressure in adulthood. We assessed the cross-sectional associations of childhood characteristics with kidney size and function in a multi-ethnic cohort.

Methods This study was embedded in a population-based cohort study of 6,397 children at the median age of 6.0 years. We measured kidney volume, creatinine and cystatin C blood levels, microalbuminuria and blood pressure. Glomerular filtration rate was estimated.

Results Childhood anthropometrics were positively associated with kidney volume, creatinine level and blood pressure (all p-values< 0.05). We observed sex and ethnic differences in all kidney size and function measures (all p-values< 0.05). Children with smaller kidneys had higher creatinine and cystatin C blood levels, leading to a lower estimated glomerular filtration rate (difference 5.68 ml/min per 1.73m^2 (95% confidence interval 5.14 to 6.12) per 1 SD increase in kidney volume). Larger kidney volume was associated with an increased risk of microalbuminuria.

Conclusions Childhood kidney volume and function are influenced by sex, anthropometrics, and ethnicity. Kidney volume is related with kidney function but not with blood pressure. This study results may help to identify individuals at risk for kidney disease in an early stage.

Introduction

An accumulating body of evidence suggests that chronic kidney disease and hypertension have at least part of their origins in early life.(1) Various studies showed associations of low birth weight with kidney disease and hypertension in later life.(2) These associations may be explained by adverse exposures in early life that lead to smaller kidneys with a reduced number of nephrons, and subsequently to glomerular hyperfiltration and sclerosis.(3,4) These adaptations may predispose the individual to higher risks of impaired kidney function and eventually end-stage kidney disease in adulthood.(3,4) The importance of early life for later kidney outcomes is also shown by studies reporting that variations in kidney function and blood pressure in childhood track into adulthood.(5,6) Thus, subclinical differences in kidney function may already be present in childhood, and predispose the individual to kidney disease. Identification of these subclinical differences and their determinants is needed to develop preventive strategies focused on the earliest phase of life. Not much is known about the normal variation of measures of kidney size and function in population-based samples of children. Studies in adolescents suggest that anthropometrics and specific ethnic backgrounds correlate with kidney size and function.(7) Whether and to what extend childhood anthropometrics and ethnicity influence kidney size and function is not known. Also, the relations between kidney size and function in childhood have not been studied in large populations.

Therefore, we assessed in a large population-based large-scale cohort study among 6,397 school-age children, the variations of kidney size and function outcomes. We also evaluated the associations of kidney size measures with kidney function and blood pressure.

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.(8) The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. All participants or their parents gave written consent to the study. In total 9,778 mothers were enrolled in the study, and 8,305 of their children participated in the follow-up measurements in children atthe age of 6 years (median 6.0 years, 95% range 5.6-7.9). In total, 6,690 (81%) of these children visited the research center for renal follow-up measurements. We excluded children with echocardiographic evidence of congenital heart disease and kidney abnormalities, which may influence blood pressure (N=28) or kidney function (N=12). Kidney volume or blood pressure measurements were successfully performed in 6,397 children (**Figure 1**). Blood and urine samples for kidney function measurements were available in 4,291 and 6,173 of those children, respectively.

Child characteristics

Anthropometrics At the age of 6 years, child height and weight were measured once without shoes and heavy clothing. Height was measured with a stadiometer. Body mass index (BMI) (kg/m^2) and body surface area (BSA) (m^2) were calculated.

Ethnicity As described previously, ethnic background of the child was defined by the country of birth of the parents.(9) All children were born in the city of Rotterdam, the second largest city in the Netherlands. Rotterdam, situated in the Western part of the Netherlands. The city has a strong multi-ethnic population. As a result of the colonial and immigrant worker history, the largest ethnic minority groups in the Netherlands are Cape Verdean, Dutch Antillean, Moroccan, Turkish, Surinamese-Creole and Surinamese-Hindustani.(10) Ethnic background of the child was defined by the country of birth of the parents, which is the commonly used demographic approach in the Netherlands.(9) We described the approach previously in detail.(8) Information about countries of birth was obtained by questionnaires from the parent(s) of the child. Children with both parents born in the Netherlands were classified as Dutch (N=3,537). The child was of non-Dutch ethnic origin if one of the parents was born abroad. If the parents were born in different countries, the country of birth of the mother determined the ethnic background.(9) We defined the following non-Dutch groups: Cape Verdean (N=194), Dutch Antillean (N=192), Moroccan (N=367), Surinamese (N=384) and Turkish (N=482). Since children with a Surinamese background are of mixed ethnic origin, Surinamese were further classified as: Surinamese-Creole (N=192) or Surinamese-Hindustani (N=192) based on the ethnic origin of the Surinamese parent of the child. (11) In the analyses on ethnic background, we excluded 1,044 children with a, non-Dutch European, African, Asian Western, Asian non-Western, American Western, American non-Western and Surinamese-other ethnicity because of the small country specific sample sizes.

Kidney outcome variables

Kidney dimensions: Left and right kidney biometrics were assessed with an ATL-Philips HDI 5000 instrument (Seattle, WA, USA) equipped with a 2.0–5.0 MHz curved array transducer or with a General Electric Logiq E9 (Milwaukee, WI, USA) equipped with a 2.0-7.0 MHz curved array transducer. During the examination the child was awake in a quiet room and calm in a standardized prone position. We identified the left and right kidney in the sagittal plane along its longitudinal axis. We performed measurements of maximal bipolar kidney length, width and depth. At the level of the hilum kidney width and depth were measured. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).(12) Combined kidney volume was calculated by summing right and left kidney volume. Quality checks were frequently carried out and feedback was provided to minimize inter-operator differences. Good reproducibility was pursued with intraobserver interclass correlation coefficients (ICC) ranged from 0.93 (left and right kidney width and right renal thickness) to 0.99 (left kidney length) and interobserver ICC ranged from 0.64 (right kidney thickness) to 0.90 (right kidney length).(13)

Kidney function: Blood samples were drawn by antecubital venipuncture. Missing samples were mainly due to no consent or crying of the child. Blood samples were collected in K2-EDTA tubes and temporally stored at the research centre in the fridge for a maximum of 4 hours. After completing the transport and collection, all blood samples underwent centrifugation for 10 minutes and stored at -80 °C. Serum creatinine was measured with the enzymatic method, on a Cobas c 502 analyser (Roche Diagnostic, Germany). We additionally measured serum cystatin C by a particle enhanced immunoturbidimetric assay on Cobas c 702 analyser (Roche Diagnostic, Germany). The intra-assay en inter-assay coefficients of variation and analytical ranges for serum creatinine and cystatin C are given in the supplementary materials (**Table S1**). Estimated glomerular filtration rate (eGFR) was calculated according to the revised Schwartz formula from 2009(14); eGFR = 36.5 * (height (cm)/creatinine (µmol/l). This formula has been validated in children.(14) Urine creatinine and albumin were determined on a Beckman-Coulter AU analyser. Urine creatinine levels were measured according to the Jaffe method. Albumin-creatinine ratio was calculated by dividing albumin concentration (mg/l) by creatinine concentration (mmol/l). Microalbuminuria was defined as an albumin-creatinine ratio between > 2.5 and 25 mg/mmol for boys and between > 3.5 and 25 mg/mmol for girls.(15)

Blood pressure: Systolic and diastolic blood pressure was measured at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus ™ (Paramus, NJ, USA).(16) The measurements were conducted while the child was quietly lying in supine position. A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference.

Statistical analyses

First, we performed multiple linear regression models to explore the cross-sectional associations of child sex, age, anthropometrics and ethnicity with combined kidney volume, kidney function measures and blood pressure. These analyses were adjusted for child sex, current age and body surface area. To enable comparison of effect estimates, we present the result as difference in outcome per standard deviation scores (Z-scores), for continuous variables and change per category for categorical variables. The associations of child characteristics with microalbuminuria were evaluated with logistic regression models. Second, we assessed the correlations of left and right kidney volume and total combined kidney volume with kidney function outcomes with Pearson correlation coefficients. We evaluated the associations of left and right kidney volume and total combined kidney volume with kidney function measures with multiple linear regression models for kidney function and blood pressure and logistic regression models for microalbuminuria. These regression models were adjusted for child sex, current age and body surface area. Appropriateness of fit for regression models was evaluated by checking residuals. All statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 for Windows (SPSS, Chicago, IL, USA).

Results

Subject characteristics

Child characteristics are shown in **Table 1**. Boys were taller and had higher weight and larger body surface area than girls. At the age of 6 years (95% range 5.6 – 7.9 years), mean (SD) combined left and right kidney volume was 120.3 (23.5) cm^3. Mean creatinine levels were 37.5 (5.6) µmol/l, mean cystatin C levels were 784 (82) µg/l and the mean glomerular filtration rate was 118.8 (16.5) ml/min per 1.73 m^2. Microalbuminuria was present in 7.5% of the participants, with a higher percentage in girls. Mean (SD) systolic and diastolic blood pressure were 102.7 (8.2) mmHg, and 60.7 (6.7) mmHg, respectively. The corresponding histograms of the kidney and blood pressure measurements are given in the supplementary materials (**Figure S1**). Children who did not provide blood samples (23%) had smaller combined kidney volumes and higher systolic and diastolic blood pressure than children who did provide blood samples (**Table S2**).

Child characteristics and kidney related outcomes

Table 2 shows that girls had smaller kidneys, a higher prevalence of microalbuminuria and higher blood pressure than boys (all p-values <0.05). In contrast, girls had lower cystatin C levels than boys (difference -11 (95% CI -16 to -6) µg/l). In the whole study population, each SD (0.5 years) older age led to higher creatinine levels (1.07 (95% Confidence Interval (CI) 0.89 to 1.25) µmol/l), a lower glomerular filtration rate (-1.75 (95% CI -2.21 to -1.28) ml/min per 1.73 m^2) and higher diastolic blood pressure (0.30 (95% CI 0.10 to 0.50) mmHg). Childhood height and weight were positively associated with several kidney size and function measures (all p-values <0.05). Each SD (1.9 kg/m^2) increase in body mass index was associated with a larger combined kidney volume (8.04 (95% CI 7.48 to 8.61) cm^3), higher blood creatinine levels (0.49 (95% CI 0.32 to 0.66) µmol/l), higher blood cystatin C levels (4 (95% CI 1 to 7) µg/l) and higher systolic and diastolic blood pressure (1.90 (95% CI 1.69 to 2.10), and 0.52 (95% CI 0.35 to 0.70) mmHg, respectively). As compared to Dutch children, Moroccan children had a larger combined kidney volume (difference 4.84 (95% CI 2.67 to 7.01) cm^3) whereas Surinamese-Hindustani children had a smaller combined kidney volume (-8.16 (95% CI -11.11 to -5.20) cm^3). As compared to Dutch children, Moroccan and Turkish children had a higher glomerular filtration rate, wheras Dutch Antillean and Surinamese-Creole children had a lower glomerular filtration rate (p-values <0.05). Also, systolic and diastolic blood pressure were higher in Cape Verdean (differences 1.77 (95% CI 0.60 to 2.95) mmHg, and (differences 1.67 (95% CI 0.67 to 2.66) mmHg, respectively) and Turkish children (differences 2.73 (95% CI 1.96 to 3.50) mmHg and 2.23 (95% CI 1.58 to 2.88) mmHg, respectively) than in Dutch children. These associations were all independent from child sex, current age and body surface area.

Table 1. Subject characteristics (N= 6,397)

	Total group N= 6,397	Boys N= 3,216	Girls N= 3,181	P value
Age and anthropometrics				
Age (years)	6.0 (5.6 – 7.9)	6.0 (5.6 – 8.0)	6.0 (5.6 – 7.8)	0.015
Height (cm)	119.5 (6.1)	119.9 (6.1)	119.0 (6.0)	<0.001
Weight (kg)	23.3 (4.3)	23.5 (4.1)	23.2 (4.5)	0.010
Body mass index (kg/m^2)	16.2 (1.9)	16.2 (1.8)	16.3 (2.0)	0.399
Body surface area (m^2)	0.88 (0.09)	0.88 (0.09)	0.87 (0.10)	<0.001
Ethnic background (%)				
Cape Verdean	3.8 (194)	3.8 (98)	3.8 (96)	0.981
Dutch	56.7 (3 537)	56.9 (1 784)	56.6 (1 753)	0.821
Dutch Antilles	3.7 (192)	3.3 (85)	4.2 (107)	0.074
Moroccan	7.1 (367)	7.2 (189)	7.0 (178)	0.721
Surinamese - Creoles	3.7 (192)	4.1 (106)	3.4 (86)	0.192
Surinamese - Hindustani	3.7 (192)	3.4 (88)	4.1 (104)	0.178
Turkish	9.3 (482)	9.9 (259)	8.8 (223)	0.148
Kidney size and function				
Kidney volume left (cm^3)	61.3 (13.2)	62.3 (13.6)	60.3 (12.8)	<0.001
Kidney volume right (cm^3)	59.0 (12.2)	60.0 (12.7)	58.0 (11.7)	<0.001
Kidney volume combined (cm^3)	120.3 (23.5)	122.3 (24.2)	118.3 (22.6)	<0.001
Creatinine (µmol/l)	37.5 (5.6)	37.6 (5.5)	37.3 (5.7)	0.073
Cystatin C (µg/l)	784 (82)	790 (81)	778 (83)	<0.001
eGFR (ml/min per 1.73m^2)	118.8 (16.4)	118.7 (16.2)	119.0 (16.6)	0.481
Urinary albumin (mg/l)	6.1 (18.8)	4.7 (12.9)	7.5 (23.3)	<0.001
Urinary creatinine (mmol/l)	5.4 (3.3)	5.8 (3.3)	5.1 (3.3)	<0.001
Microalbuminuria (%)	7.5 (462)	6.9 (215)	8.2 (247)	<0.001
Systolic blood pressure (mmHg)	102.7 (8.2)	102.2 (7.9)	103.3 (8.4)	<0.001
Diastolic blood pressure (mmHg)	60.7 (6.8)	60.0 (6.7)	61.4 (6.8)	<0.001

Values are means (standard deviation), median (95% range) or percentage (number). T-tests were used for continuous variables, chi-square tests for categorical variables.

eGFR, glomerular filtration rate

Table 2. Associations of child characteristics and ethnicity with kidney volume and function

		Difference (95% CI) in kidney size	Difference (95% CI) in kidney function				Difference (95% CI) in blood pressure	
		Combined kidney volume (cm^3)	Creatinine (µmol/l)	Cystatin C (µg/l)	eGFR (ml/min per 1.73m^2)	Micro-albuminuria (OR)†	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Girls compared to boys	(N= 6,397)	-2.60** (-3.60 to -1.60)	-0.16 (-0.47 to 0.16)	-11** (-16 to -6)	0.24 (-0.74 to 1.22)	1.21** (1.00 to 1.46)	1.28** (0.88 to 1.67)	1.54** (1.20 to 1.88)
Age (years) (1 SD = 0.5 yrs)	(N= 6,397)	-0.44 (-1.02 to 0.14)	1.07** (0.89 to 1.25)	1 (-1 to 4)	-1.75** (-2.21 to -1.28)	1.15* (1.03 to 1.28)	0.12 (-0.12 to 0.35)	0.30** (0.10 to 0.50)
Anthropometrics	**(N= 6,389)**							
Height (cm) (1 SD = 6.1 cm)	(N= 6,389)	11.30** (10.68 to 11.92)	1.02** (0.83 to 1.21)	3 (0 to 6)	2.65** (2.07 to 3.23)	1.03 (0.92 to 1.15)	1.65** (1.41 to 1.89)	0.48** (0.27 to 0.68)
Weight (kg) (1 SD = 4.3 kg)	(N= 6,389)	12.35** (11.77 to 12.93)	0.90** (0.72 to 1.09)	5** (2 to 7)	1.10** (0.54 to 1.67)	0.84** (0.75 to 0.95)	2.36** (2.14 to 2.59)	0.66** (0.46 to 0.86)
Body mass index (kg/m2) (1 SD = 1.9 kg/m2)	(N= 6,389)	8.04** (7.48 to 8.61)	0.49** (0.32 to 0.66)	4** (1 to 7)	-0.37 (-0.89 to 0.15)	0.80** (0.71 to 0.89)	1.90** (1.69 to 2.10)	0.52** (0.35 to 0.70)
Ethnicity	(N= 5,156)							
Dutch	(N= 3,537)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Cape Verdean	(N= 194)	-1.33 (-4.25 to 1.58)	0.31 (-0.61 to 1.23)	-23** (-38 to -9)	-1.27 (-4.15 to 1.60)	1,18 (0.70 to 2,01)	1.77** (0.60 to 2.95)	1.67** (0.67 to 2.66)
Dutch Antilles	(N= 192)	-0.77 (-3.75 to 2.21)	1.40** (0.49 to 2.32)	-15* (-29 to 0)	-5.27** (-8.13 to -2.40)	1,10 (0.64 to 1.91)	0.45 (-0.73 to 1.63)	1.21* (0.20 to 2.21)
Morrocan	(N= 367)	4.84** (2.67 to 7.01)	-2.66** (-3.34 to -1.98)	-16** (-26 to -5)	7.20** (5.07 to 9.32)	1.10 (0.74 to 1.65)	0.87 (-0.00 to 1.73)	-0.10 (-0.83 to 0.64)

Table 2. Continued

Surinamese - Creoles	(N= 192)	-2.06 (-5.04 to 0.92)	1.79** (0.87 to 2.72)	-18* (-33 to -3)	-5.56** (-8.45 to -2.66)	0.89 (0.49 to 1.62)	1.37* (1.87 to 2.55)	1.15* (1.15 to 2.16)
Surinamese - Hindustani	(N= 192)	-8.16** (-11.71 to -5.20)	0.46 (-0.45 to 1.36)	17* (3 to 31)	-1.53 (-4.36 to 1.30)	0.47 (0.22 to 1.02)	0.29 (-0.89 to 1.47)	1.29* (0.29 to 2.29)
Turkish	(N= 482)	1.67 (-0.29 to 3.62)	-1.50** (-2.12 to -0.89)	-2 (-12 to 8)	2.86** (0.92 to 4.80)	0.98 (0.67 to 1.43)	2.73** (1.96 to 3.50)	2.23** (1.58 to 2.88)

Values are regression coefficients (95% Confidence interval (CI) based on multiple regression models and reflect the difference for each outcome for child characteristics. Analyses focused on ethnic differences in kidney outcomes were performed among children from Dutch, Dutch-Antilles, Moroccan, Surinamese and Turkish children only, because the other groups were too small (see text).

† Odd's ratio's (95% Confidence interval) based on logistic regression models and reflect the difference for each outcome for child characteristics.

Models are adjusted for child sex and current age and body surface area.

eGFR, estimated glomerular filtration rate

* $p<0.05$, ** $p<0.01$

Kidney dimensions, kidney function and blood pressure

Correlation coefficients between kidney volume, kidney function and blood pressure are presented in the Supplementary material (**Table S3**) and show that combined kidney volume was inversely correlated with blood creatinine (r=-0.30, p-value < 0.001) and cystatin C (r=-0.27, p-value < 0.001) levels and positively correlated with glomerular filtration rate (r=0.31, p-value< 0.001) and urinary albumin–creatinine ratio (r=0.10, p-value < 0.001). Kidney volume and kidney function were not correlated with systolic and diastolic blood pressure. **Table 3** shows that each SD (23.5 m^3) increase in combined kidney volume was related with a lower blood creatinine level (-2.06 µmol/l (95% CI -2.26 to -1.85)), lower blood cystatin C level (-29 (95% CI -32 to -26) µg/l) and a higher glomerular filtration rate (5.68 (5.14 to 6.12) ml/min per 1.73m^2). A larger combined kidney volume was associated with a higher risk of microalbuminuria (OR 1.19 (95% CI 1.05 to 1.36 per SD). Kidney length, width and depth were all associated with the kidney function outcomes (**Table 3**). The effect estimates for the associations of left and right kidney width with creatinine, cystatin C, glomerular filtration rate and microalbuminuria were larger than the effect estimates for the associations of left and right kidney depth with kidney function measures with these outcomes. Right kidney depth was negatively associated with diastolic blood pressure, left kidney width was positively associated with systolic and diastolic and left kidney depth was negatively associated with diastolic blood pressure (all p-values <0.05, **Table 3**). Kidney volume was not consistently associated with systolic and diastolic blood pressure.

Table 3. Associations of kidney dimensions with kidney function and blood pressure

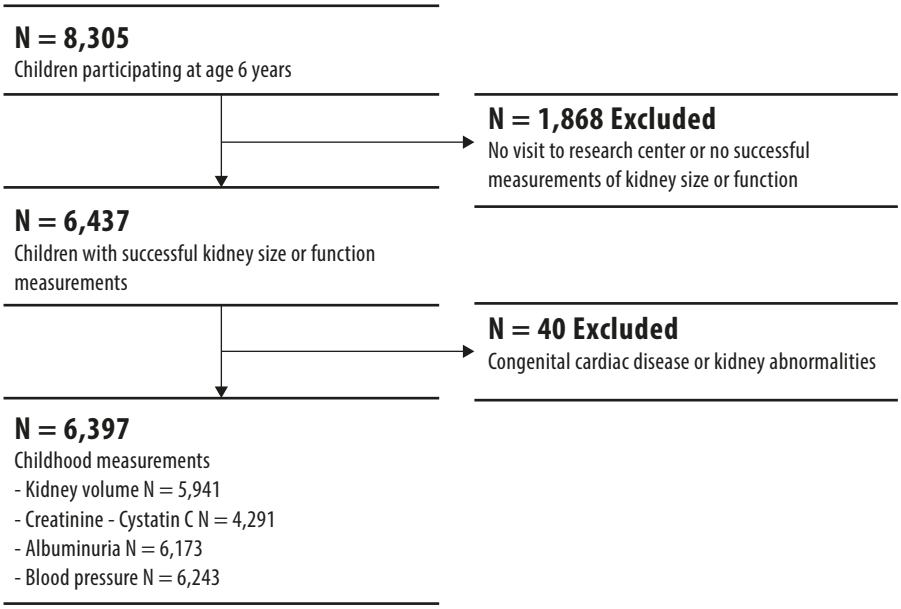
	Difference (95%) kidney function				Difference (95%) blood pressure	
Kidney characteristics	**Creatinine (µmol/l)**	**Cystatin C (µg/l)**	**eGFR (ml/min per 1.73m^2)**	**Microalbuminuria (OR) †**	**Systolic blood pressure (mmHg)**	**Diastolic blood pressure (mmHg)**
Right kidney						
Length (1SD = 5.7mm)	-1.49** (-1.67 to -1.30)	-19** (-22 to -17)	4.77** (4.26 to 5.27)	1.17* (1.04 to 1.31)	-0.13 (-0.37 to -0.11)	-0.03 (-0.22 to 0.182)
Width (1SD = 3.3mm)	-1.23** (-1.41 to -1.06)	-17** (-19 to -14)	3.84** (3.34 to 4.34)	1.07 (0.95 to 1.19)	0.09 (-0.14 to 0.32)	0.05 (-0.14 to 0.25)
Depth (1SD = 4.57mm)	-0.90** (-1.09 to -0.70)	-12** (-15 to -9)	3.12** (2.54 to 3.69)	1.08 (0.96 to 1.22)	-0.23 (-0.48 to 0.02)	-0.23* (-0.44 to -0.01)
Volume (1SD = 12.2cm^3)	-1.85** (-2.05 to -1.65)	-25** (-28 to -21)	5.33** (4.80 to 5.86)	1.16* (1.02 to 1.31)	-0.12 (-0.38 to 0.14)	-0.11 (-0.34 to 0.12)
Left kidney						
Length (1SD = 6.35mm)	-1.13** (-1.31 to -0.95)	-15** (-18 to -121)	3.86** (3.35 to 4.37)	1.17** (1.04 to 1.31)	0.07 (-0.17 to 0.30)	-0.01 (-0.21 to 0.19)
Width (1SD = 3.29mm)	-1.03** (-1.21 to -0.86)	-18** (-20 to -15)	3.35** (2.84 to 3.86)	1.13* (1.02 to 1.27)	0.31** (0.09 to 0.54)	0.23* (0.03 to 0.43)
Depth (1SD = 4.71mm)	-0.91** (-1.10 to -0.71)	-12** (-15 to -9)	3.19** (2.62 to 3.76)	1.02 (0.90 to 1.15)	-0.19 (-0.43 to 0.06)	-0.23* (-0.44 to -0.02)
Volume (1SD = 13.3cm^3)	-1.56** (-1.76 to -1.37)	-23** (-26 to -20)	4.74** (4.21 to 5.27)	1.16* (1.02 to 1.31)	0.10 (-0.16 to 0.35)	-0.02 (-0.24 to 0.20)
Combined kidney volume (1SD = 23.5cm^3)	-2.06** (-2.26 to -1.85)	-29** (-32 to -26)	5.68** (5.14 to 6.12)	1.19** (1.05 to 1.36)	-0.02 (-0.30 to 0.25)	-0.09 (-0.33 to 0.15)

Values are regression coefficients (95% CI) based on multiple regression models and reflect the difference for each outcome for different kidney dimensions.
† Odd's ratio's (95% CI) based on logistic regression models and reflect the difference for each outcome for child characteristics.
All models are adjusted for child sex and current age, body surface area, sonographer and ultrasound device.

eGFR, estimated glomerular filtration rate

* p<0.05, **p<0.01

Figure 1.


N = 8,305
Children participating at age 6 years
N = 1,868 Excluded
No visit to research center or no successful measurements of kidney size or function
N = 6,437
Children with successful kidney size or function measurements
N = 40 Excluded
Congenital cardiac disease or kidney abnormalities
N = 6,397
Childhood measurements
- Kidney volume N = 5,941
- Creatinine - Cystatin C N = 4,291
- Albuminuria N = 6,173
- Blood pressure N = 6,243

Discussion

We described a population-based large-scale cohort study, as resource for further studies focussed on early risk factors and later life consequences of kidney size and function variation in childhood. We observed that among school-age children, anthropometrics, sex and ethnicity are associated with kidney volume and function. Smaller kidney volume is associated with lower kidney function but not with blood pressure in childhood.

Methodological considerations

A major strength of this study is the large size of the population-based cohort. Our analyses were based on more than 6,000 children with kidney volume, kidney function and blood pressure measurements. Of the children who participated in the study at 6 years, more than 75% did participate in the kidney follow-up studies. Of all children who visited the research center at the age of 6 years, 96% provided kidney size, blood pressure and microalbuminuria measurements, and 67% provided blood samples for measurements of creatinine and cystatin C levels. Not all participants in the study gave consent for collecting blood samples. Children without blood samples had smaller kidney dimensions and higher blood pressure. These differences might have led to underestimation of the observed associations between kidney dimensions and kidney function. However, differences in characteristics between participants with and without blood samples were small, and not clinically relevant. Our observed mean values for creatinine, cystatin C, estimated glomerular filtration rate, microalbuminuria and blood pressure are within the range of previous population-based studies in the same age range.(17-19) We used well-established methods to measure kidney size and function.(13,20) We used kidney size as proxy for of kidney development. The number of nephrons cannot be measured *in vivo*. Post-mortem studies in humans showed that a lower nephron number is associated with lower weight at birth and hypertension in adults.(21,22) Hinchliffe et al. demonstrated in eleven spontaneously aborted foetuses a strong correlation between kidney volume and glomerular number up to 40 weeks of gestation.(23) Other post-mortem studies in humans showed consistent positive associations between kidney size and glomerular number.(24,25) Therefore, kidney volume seems to be a good surrogate for nephron number. Still, glomerular enlargement due to glomerulosclerosis and hyperfiltration may attenuate the observed differences in kidney volume, this might have underestimated the associations.(26) Since exact measurement of glomerular filtration rate in a population-based sample is not feasible, we used plasma creatinine levels to estimate glomerular filtration rate. Limitations of this approach is the active creatinine secretion by the proximal tubule and the relation of blood creatinine levels with muscle mass.(27) Blood cystatin C levels might be a better protein to estimate glomerular filtration rate, because the production rate is constant and it is freely filtered.(28) It has been suggested that cystatin C is less dependent from children's body weight, height and sex than creatinine.(29) Studies focused on the question whether creatinine or cystatin C is most accurate for estimating glomerular filtration in children

are inconclusive.(17,30) In adults with and without chronic kidney disease cystatin C seems more accurate.(31) We used the revised Schwartz 2009 formula to estimate the glomerular filtration rate 6 year old children. This formula is validated in children with chronic kidney disease, but tends to underestimate the estimated glomerular filtration rate in adolescents. Whether, this underestimation is also present in healthy children at the of 6 years is not known yet.(32) We used the urine albumin-creatinine ratio to evaluate albuminuria in a random urine sample.(33) We collected urine samples random during the day. The variability would probably be lower if we collected first morning void samples. This might have caused a random and error and loss of power.(34) We used a shygmamometer for blood pressure measurement, which might be different from manual blood pressure measurement. However, we used a valid instrument and we do not think that blood pressure measurement influences our main results.(35)

Childhood characteristics and kidney outcomes

Girls had smaller kidneys than boys at the age of 6 years. This finding is consistent with previous findings in our study in fetal life and early childhood,(12) but results seem not consistent.(36) In adults, kidney volume seems to be larger in men than in women.(37) Creatinine level and glomerular filtration rate did not differ between sexes. We observed higher cystatin C levels in boys than in girls. A similar difference was found in a study among 719 adolescents aged 12 to 19 years.(29) However, a study among 135 healthy children aged from 1 to 15 years found no differences in cystatin C levels between boys and girls.(38) This might be due to the broader age range and the limited sample size. The sex differences may arise from the school-age period. Studies in adults showed that males have higher cystatin C levels than females.(39) The underlying mechanisms for these sex differences may include differences in body composition, other than body surface area. Also, prevalence of microalbuminuria was higher in girls compared to boys. This difference was explained by lower urine albumin levels and higher urine creatinine levels in boys compared to girls. Previous studies in childhood and adolescents found similar associations.(40,41) These differences may be explained by higher urine creatinine levels in boys caused by a larger muscle mass. Several studies in adults showed higher urine creatinine levels in men than in women and lower urine creatinine levels in adults with lower muscle mass.(29,42) Girls had higher systolic and diastolic blood pressure than boys. Similar differences have been reported in others studies, but these seem inconclusive.(43,44)

In adults, a high body mass index is associated with kidney disease and hypertension.(7,45) Various previous studies reported a positive association of body mass index with blood pressure(46,47) in childhood, which is in line with the results of our study. We found a positive association of body mass index with creatinine and cystatin C levels but not with glomerular filtration rate. A recent study among 107 children aged 11 years found an increased prevalence of hypertension in obese children, only a small difference in albuminuria, but no changes in creatinine, cystatin C and glomerular filtration rate.(48) We observed an inverse association of body mass index and the risk of microalbuminuria,

which was mainly driven by an increase in urine creatinine excretion. We are not aware of other studies that observed similar associations and could not fully explain this association. Further studies focused on body composition rather than on body mass index may further elucidate the mechanisms underlying this observed association.

Kidney function and chronic kidney disease prevalence in adults varies across ethnic groups.(49) Studies in the Netherlands demonstrated that hypertension and kidney dysfunction are more prevalent among African, Surinamese and Dutch Antilles adults compared to Dutch adults.(50) The prevalence of hypertension is lower in Turkish and Moroccan than in Dutch adults.(51) Ethnic background is associated with blood pressure distribution.(52) Not much is known about the differences in variation in kidney size and function and blood pressure between these specific ethnic groups in childhood. A recent study among 6,643 children aged 11 to 16 years in the United Kingdom found small differences in blood pressure between different ethnic groups.(53) The highest blood pressure was observed in black African and Caribbean individuals. Another study in the United States among 534 children aged 6 to 10 years found higher blood creatinine levels in blacks than in whites and Hispanics and higher urinary creatinine levels in blacks and Hispanics than in whites.(41) Also, a study in the United States among 719 subjects showed that cystatin C levels are higher in white than in black and Mexican adolescents.(29) The ethnic populations in our study are most frequent in the Netherlands. We observed ethnic differences in kidney volume, kidney function and blood pressure in school-age children. We are not aware of other studies in these groups. We observed small and subclinical ethnic differences in kidney volume, kidney function and blood pressure in children aged 6 years. The observed differences reflect a 4% to 7% change in combined kidney volume and a 1% to 6% change in estimated glomerular filtration rate. Although these differences were without clinical relevance at young age, our results suggest that children from Cape Verdean, Dutch Antilles and Surinamese groups living in the Netherlands have higher risks of development of renal and cardiovascular disease in later life. Further studies are needed to evaluate whether these ethnic differences predispose individuals for impaired kidney function in later life. Kidney volume and function differed across ethnic groups. It has been suggested that these ethnic differences might be explained by differences in body composition.(29) However, in the present study, we analyzed the associations of ethnic background with kidney function with adjustment for body surface area. The observed ethnic differences in kidney outcomes may be explained by both environmental and genetic factors related to kidney growth and function.(54,55) Further studies are needed to examine whether the observed ethnic differences lead to ethnic differences in kidney disease in adulthood. The ethnic populations in our study are most frequent in the Netherlands, and strongly related to the specific colonial and workers migration patterns. The observed ethnic distribution reflect the specific populations in the largest cities in the Netherlands, but are different from large cities in other countries. We used a demographic definition of ethnicity. This might not fully reflect the ethnic differences. Further studies are needed to evaluate kidney function differences in other ethnic populations.

Kidney size and function

To our knowledge, this is the first study with this large sample size to find an association between kidney volume and kidney function in childhood. We observed inverse associations of kidney volume with creatinine and cystatin C levels and positive associations with glomerular filtration rate. Another study among 257 healthy children older than 6 months, indicated that renal mass is inversely associated with creatinine levels in serum.(56) A study in 116 healthy children aged 1 to 15 years found a strong posistive correlation between ultrasonographic kidney size and glomerular filtration rate.(57) A previous study among 672 Australian Aborigines with a high prevalence of kidney disease, suggested that smaller kidneys predisposed to higher blood pressure and albuminuria.(1) A recent study among 1,748 Italian children, older than 6 months with and without urethral or kidney diseases, suggested that variation in serum creatinine levels were partially explained by renal mass.(56) In the same study, no correlation between kidney size and blood pressure was found.(56) These findings are in line with the hyperfiltration hypothesis which state that smaller kidneys lead to hyperfiltration, sclerosis and impaired renal function. We did not observe associations of childhood kidney size with blood pressure. It might be that kidney dimensions do not influence blood pressure in childhood yet. Further research is needed to study the associations between kidney volume in childhood and kidney function in adulthood. In contrast with our hypothesis, in our study kidney volume is associated with an increased risk of microalbuminuria. We cannot explain this finding. We observed inverse associations of kidney volume with creatinine and cystatin C levels and positive associations with estimated glomerular filtration rate. Differences in kidney function at the age of 6 years were small but might predispose individuals to increased risk of kidney diseases in later life.

Conclusion and perspectives

The present study in a multi-ethnic cohort showed that childhood anthropometrics, sex and ethnicity were associated with kidney size and function measures. Also, kidney volume, was negatively associated with kidney function but not with blood pressure at the age of 6 years. A larger kidney volume was associated with an increased risk of microalbuminuria. This study provides a unique resource for future studies on the early risk factors and later life consequences of kidney size and function variation in childhood.

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Supplementary table 1. Creatinine and cystatin C assay coefficient of variation and analytical ranges

	Intra-assay coefficient of variation	Inter-assay coefficient of variation	Analytical range
Creatinine (µmol/l)	0.92% at 84.2 µmol/l 0.51% at 368.4 µmol/l	1.37% at 84.2 µmol/l 0.71% at 368.4 µmol/l	5-2 700 µmol/l
Cystatin C (mg/l)	1.65% at 1.09 mg/l 1.25% at 4.33 mg/l	1.65% at 1.09 mg/l 1.13% at 4.33 mg/l	0.4-8.0 mg/l

Supplementary table 2. Subject characteristics (N=6,397)

	Group with blood samples N=4,300	Group without blood samples N=2,097	P value
Age and anthropometrics			
Age (years)	6.0 (5.7 – 8.0)	6.0 (5.6 – 7.6)	0.001
Height (cm)	119.7 (6.1)	119.0 (6.0)	<0.001
Weight (kg)	23.4 (4.2)	23.1 (4.3)	0.023
Body mass index (kg/m2)	16.2 (1.8)	16.2 (2.0)	0.671
Body surface area (m2)	0.88 (0.09)	0.87 (0.09)	<0.001
Ethnic background (%)			
Cape Verdean	3.7 (128)	3.9 (66)	0.685
Dutch	57.4 (2 403)	55.3 (1 134)	0.122
Dutch Antilles	3.8 (132)	3.6 (60)	0.667
Moroccan	7.1 (248)	7.1 (119)	0.914
Surinamese - Creoles	3.7 (128)	3.8 (64)	0.844
Surinamese - Hindustani	3.8 (132)	3.6 (60)	0.667
Turkish	8.6 (300)	10.8 (182)	0.013
Kidney growth and function			
Kidney volume left (cm^3)	61.6 (13.4)	60.8 (13.0)	0.038
Kidney volume right (cm^3)	59.2 (12.4)	58.6 (11.9)	0.043
Kidney volume combined (cm^3)	120.8 (23.7)	119.4 (23.1)	0.032
Creatinine (µmol/l)	37.5 (5.6)	NA	NA
Cystatin C (µg/l)	784 (82)	NA	NA
eGFR (ml/min per 1.73m^2)	118.8 (16.4)	NA	NA
Micro albuminuria (%)	7.6 (316)	7.4 (146)	0.803
Systolic blood pressure (mmHg)	102.4 (8.0)	103.4 (8.6)	<0.001
Diastolic blood pressure (mmHg)	60.5 (6.7)	61.1 (7.1)	0.001

Values are means (standard deviation), median (95% range) or percentage (number) T-tests were used for continuous variables, chi-square tests for categorical variables eGFR, estimated glomerular filtration rate. NA, not applicable

Supplementary table 3. Correlations between kidney volume, kidney function and blood pressure

	Combined kidney volume (cm^3)	Creatinine (µmol/l)	Cystatin C (µg/l)	eGFR (ml/min per 1.73m^2)	Albumin – creatinine ratio (mg/mmol)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Combined kidney volume (cm^3)	1	-	-	-	-	-	-
Creatinine (µmol/l)	-0.30**	1	-	-	-	-	-
Cystatin C (µg/l)	-0.27**	0.33**	1	-	-	-	-
eGFR (ml/min per 1.73m^2)	0.31**	-0.96**	-0.35**	1	-	-	-
Albumin – creatinine ratio (mg/mmol)	0.10**	-0.10**	-0.07**	0.11**	1	-	-
Systolic blood pressure (mmHg)	-0.01	0.01	-0.01	-0.01	-0.00	1	-
Diastolic blood pressure (mmHg)	-0.01	-0.02	-0.03	0.02	0.03	0.62**	1

Values are correlation coefficients. Models are adjusted for child sex, current age and body surface area.

eGFR, glomerular filtration rate

* p<0.05, **p<0.01

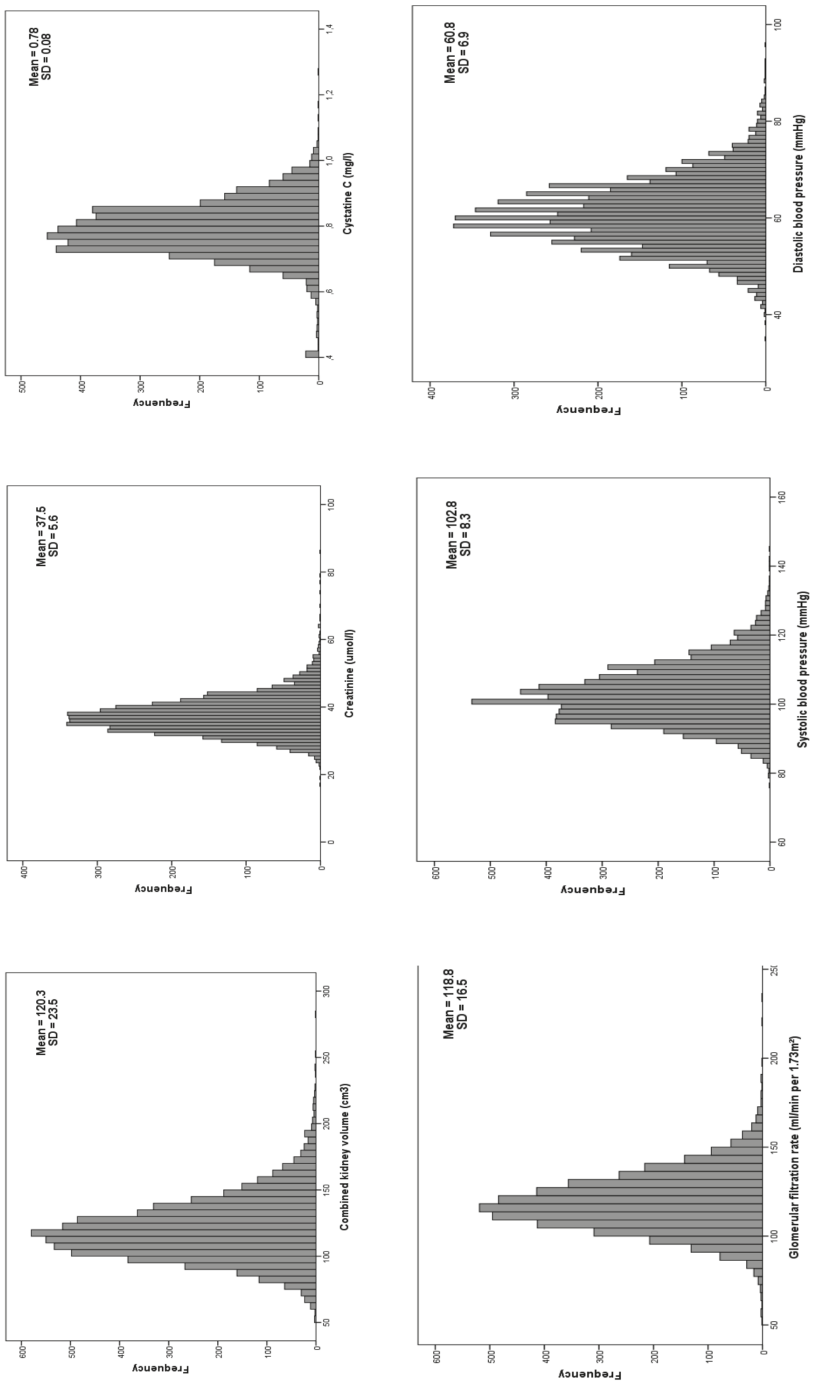

Figure S1. Histograms of kidney measurements

Chapter 2

Fetal and infant growth

Chapter 2.1

Childhood kidney outcomes in relation to fetal blood flow and kidney size

Adapted from Pediatr Nephrol. 2014;29(9):1589-98

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Abstract

Background Fetal restricted abdominal blood flow may lead to smaller kidneys with a reduced number of nephrons, and subsequently to impaired kidney function in later life.

Methods In a population-based prospective cohort study among 923 pregnant women and their children, we measured third trimester fetal growth characteristics and kidney dimensions. We assessed fetal blood flow distribution by umbilical and cerebral artery resistance indices. At the median age of 5.9 years (95% range 5.7–6.6), we measured kidney dimensions, creatinine and cystatin C blood levels, microalbuminuria and blood pressure, and estimated the glomerular filtration rate.

Results A preferential fetal blood flow to the upper body parts at expense of the abdominal organs, reflected by higher ratio of fetal umbilical/cerebral artery pulsatility indexes, was associated with a smaller kidney volume and lower risk of microalbuminuria. Larger fetal kidney volume was associated with smaller childhood kidney volume, lower creatinine and cystatin C levels and higher estimated glomerular filtration rate, but not with microalbuminuria and blood pressure. As compared to children with a normal fetal blood flow and fetal combined kidney volume, children with a high U/C ratio and small fetal combined kidney volume had the highest creatinine levels (difference 2.16µmol/l, 95% confidence interval 0.52, 3.81), and lowest estimated glomerular filtration rate (difference -6.36ml/min per 1.73m^2, 95% confidence interval -11.78, -0.94).

Conclusions and Relevance Fetal blood flow redistribution at expense of the abdominal organs, and smaller fetal kidney size are associated with lower kidney function in school-age children, independent of later kidney growth.

Introduction

Suboptimal development of the fetal kidney, in response to an adverse intrauterine environment, leads to common kidney diseases in later life.(1) Late pregnancy is a critical period for kidney development and diseases. Nephrogenesis continues until 36 weeks of gestation, after which the induction of nephron numbers ceases.(2) A permanent reduction of kidney size and number of nephrons leads to a smaller glomerular filtration surface area, which might predispose the individual to decreased kidney function in childhood and development of kidney disease and hypertension in adulthood.(3,4) This hypothesis is mainly supported by studies showing consistent associations of low birth weight with higher risks of kidney disease and hypertension in later life. Although the observed effects from these studies were small, they are important from an etiological perspective.(5,6) The potential role of smaller kidneys with a reduced number of nephrons in the observed associations of birth weight with kidney disease in later life is further supported by animal and human post mortem studies. A post-mortem study in 20 humans showed that individuals with hypertension had fewer glomeruli than normotensive controls.(7) Also, birth weight is positively correlated with kidney size and glomerular number.(8,9)

Animal studies demonstrated a reduction in glomeruli number due to vascular placental insufficiency.(10) Placental insufficiency is an important risk factor for fetal growth restriction and low birth weight.(11) Also, we recently demonstrated that increased third trimester placental insufficiency is associated with a higher blood pressure in childhood.(12) Fetal growth restriction in response to placental insufficiency is characterized by a preferential blood flow to the brain at expense of the trunk.(13) This fetal blood flow redistribution is caused by a lower cerebral arterial resistance,(11) and can be measured as a higher umbilical artery pulsatility index (PI) and lower cerebral artery PI, which leads to a higher ratio of these measures (higher U/C ratio).(14) Whether and to what extend impaired abdominal or more specifically, kidney blood flow and kidney growth restriction during fetal life lead to risk factors for kidney disease in later life is unknown.

We evaluated in a population-based prospective cohort study among 923 pregnant women and their children, the associations of blood flow redistribution, at expense of the abdominal organs, and impaired kidney growth during fetal life with kidney function in school-age children. We also explored whether any association was explained by childhood kidney size.

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands. All children were

born between April 2002 and January 2006. Enrollment was aimed at early pregnancy at the routine fetal ultrasound examination in pregnancy but was allowed until birth of the child. In total, 9,778 mothers and their children were included in the study. More detailed assessments of fetal and postnatal growth and development were conducted in a random subgroup of 1,232 Dutch mothers and children (response 80%).(15) Twin pregnancies (n = 15) and pregnancies leading to perinatal death (n = 2) were excluded from the analysis, leading to 1,215 singleton live births. Third trimester blood flow distribution and fetal kidney measurements were successfully performed in 1,201 singleton live born children, of whom 925 children (77%) visited the research center for follow up measurements at the median age of 5.9 (95% range 5.7 – 6.6) years. Childhood kidney measurements were successfully performed in 923 children (Flow chart is given in **Figure 1**). Written informed consent was obtained from all parents. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

Third trimester fetal measurements

Third trimester fetal ultrasound examinations were performed at a median gestational age of 30.3 (95% range 28.5 – 32.7) weeks.

Fetal growth: Gestational age was established by first trimester ultrasound measurements.(16) Fetal head circumference, abdominal circumference, and femur length were measured and estimated fetal weight was calculated using the formula by Hadlock et al.(17)

Fetal blood flow distribution: We measured fetal blood flow distribution as inverse of the corresponding resistance indices in the umbilical and cerebral artery by pulsed-wave Doppler, as described previously.(11) The pusatility index (PI) in a fetal artery reflects the difference between the peak systolic and minimum diastolic velocities divided by the mean velocity during the cardiac cycle and is inversely related to the flow in this artery. Thus, a higher PI reflects a lower flow in this artery. Colour imaging was used to optimize placement of the pulsed wave Doppler gate in every measurement. For each measurement three consecutive uniform waveforms, during fetal apnoea and without fetal movement, were recorded and the mean of these measurements was used for further analysis. Umbilical artery PI was determined in a free-floating loop of the umbilical cord. A raised umbilical artery PI indicates increased vascular resistance and lower blood flow in the lower body parts.(18) Middle cerebral artery Doppler measurements were performed with colour Doppler visualization of the circle of Willis in the fetal brain, and flow-velocity waveforms were obtained in the proximal part of the cerebral arteries. Reductions in the middle cerebral artery PI are a valid indicator of fetal blood redistribution in favour of the brains.(19) Fetal blood redistribution in favour to the brain at expense of the trunk, including the abdominal organs, is indicated by a higher ratio between the umbilical artery PI and the cerebral artery PI (higher U/C ratio).(14) Intra- and inter-observer analyses showed good reproducibility for all Doppler measurements, as described previously (all intra-class correlation coefficients > 0.80).(13) The mean (SD) PI observed in our study were in line with a previous longitudinal study focused on serial measurements.(20)

Fetal kidney dimensions: In a sagittal plane, the maximum longitudinal kidney lengths were measured placing the callipers on the outer edges of the caudal and cranial side(21). Antero-posterior and transverse kidney diameters were measured perpendicular to each other, outer tot outer, in an axial plane. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used.(22) Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).(23) Combined kidney volume was calculated by summing right and left kidney volume.

Childhood kidney outcomes

Childhood kidney dimensions: Left and right kidney biometrics were at the median age of 5.9 (95% range 5.7 – 6.6) years. We identified the left and right kidney in the sagittal plane along its longitudinal axis. We performed measurements of maximal bipolar kidney length, width and depth. Kidney width and depth were measured at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. We calculated left and right kidney volume by using the same ellipsoid equation as for fetal kidney volume.(23) We previously reported good intra-observer and inter-observer correlation coefficients.(24)

Childhood kidney function: Blood creatinine levels were measured with an enzymatic method on a Cobas c 502 analyzer (Roche Diagnostics, Germany), and cystatin C levels by a particle enhanced immunoturbidimetric assay on a Cobas c 702 analyzer (Roche Diagnostics, Germany). Quality control samples demonstrated intra- and inter-assay coefficients of variation ranging from 0.51% to 1.37% and from 1.13% to 1.65%, respectively. Estimated glomerular filtration rate (eGFR) was calculated according to the revised Schwartz 2009 formula(25); eGFR = 36.5 * (height (cm)/creatinine (µmol/l)(25) Urine creatinine (mmol/l) and urine albumin (mg/l) levels were determined on Beckman Coulter AU analyser, creatinine levels were measured according to the Jaffe method. We calculated the albumin-creatinine ratio. For boys microalbuminuria was defined as an albumin-creatinine ratio between 2.5 and 25 mg/mmol, for girls we used a ratio between 3.5 and 25 mg/mmol.(26)

Childhood blood pressure: Systolic and diastolic blood pressure were measured at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus ™ (Paramus, NJ, USA).(27) A cuff was selected which was long enough to cover 90% of the arm length and the cuff-width to cover approximately 40% of the arm circumference.

Covariates

Information on maternal age, pre-pregnancy body mass index, parity, educational level, smoking during pregnancy, folic acid use during pregnancy, gestational hypertensive complications was obtained by questionnaires and registries.(28) Maternal height was measured without shoes and pre-pregnancy body mass index (BMI) was calculated (kg/m^2). Date of birth, infant sex and birth weight were obtained from midwife and

hospital registries. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and body surface area was calculated.

Statistical analysis

First, we performed correlation analyses using scatterplots and Pearson correlation coefficients to explore the relation between gestational age with U/C ratio and fetal kidney volume. To explore whether fetal kidney size was disproportionally affected by fetal blood flow redistribution, we performed linear regression models with gestational age as independent variable and fetal kidney volume/estimated fetal weight as dependent variable, and stratified these analyses for U/C ratio. Second, the associations of fetal blood flow distribution and kidney growth with kidney function outcomes were analysed in a stepwise approach. We performed correlation analyses using scatterplots and Pearson correlation coefficients. Next we performed multiple linear regression models. The models were first adjusted for gestational age and estimated fetal weight at third trimester measurement, and child sex, current age and body surface area (Basic model). Analyses focused on estimated glomerular filtration rate were not further adjusted for body surface area since this is included in the Schwartz 2009 formula. These models were additionally adjusted for potential confounders including maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, pregnancy complications (pre-eclampsia and pregnancy induced hypertension), gestational age at birth and gestational age adjusted birth weight (Confounder model). This confounder model was considered as the main model. We additionally adjusted the Confounder model for childhood combined kidney volume to explore whether any association was explained by childhood combined kidney volume (Kidney volume model). Third, we tested potential combined effects and interactions between fetal blood flow and fetal kidney volume on childhood outcomes and performed stratified multiple linear regression analyses where the interaction terms were significant. We also tested the interaction between estimated fetal weight and umbilical/cerebral ratio with fetal kidney volume. We performed a sensitivity analysis using gestational age adjusted birth weight as an additional index of final nephron number instead of fetal kidney volume .To reduce the possibility of potential bias associated with missing data (less than 17%), missing values in maternal, fetal and child covariates were imputed using the multiple imputations procedure with five imputations and these datasets were analyzed together. Further information about the methods of multiple imputation are given in the **Supplemental file.** All statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal, fetal and child characteristics are shown in **Table 1.** Observed data before multiple imputations are presented in the Supplementary material (**Supplementary Table S1**). Non-response analysis showed that as compared to mothers of children not included in the analysis, mothers of children included in the study were older, higher educated, smoked less frequently during pregnancy, more frequently nulliparous, and more frequently users of folic acid supplements. Furthermore, children included in the study had a higher birth weight and gestational age at birth and were more often girls (**Supplementary Table S2**). Subject characteristics in different strata of U/C ratio and different strata of fetal kidney volume are given in **Supplementary Tables S3** and **S4**. Gestational age was slightly shorter in the high umbilical/cerebral ratio group as compared to the normal and low cerebral/umbilical ratio group, but differences were small (< 0.2 weeks). In **Supplemental Figure S4 and S5** the scatterplots and the correlations of gestational age and estimated fetal weight with fetal kidney volume/ estimated fetal weight are shown. There was no significant interaction of gestational age or estimated fetal weight with umbilical/cerebral ratio (p-value>0.05), suggesting that the associations of gestational age and estimated fetal weight with fetal kidney volume/ estimated fetal weight are not modified by the U/C ratio.

Fetal blood flow redistribution and childhood kidney outcomes

Scatterplots for the observed correlations between blood flow redistribution, fetal kidney dimensions and childhood kidney outcomes are shown in **Supplementary Figures S1, S2 and S3. Table 2** shows that in the main confounder model a higher U/C ratio, which reflects a preferential blood flow to the upper body parts at expense of the trunk and abdominal organs, was associated with a smaller childhood combined kidney volume (-2.58 cm^3 per 1 SDS increase in ratio, 95% Confidence Interval (CI) -4.01, -1.14) and a lower risk for microalbuminuria (Odds ratio 0.71 per 1 SDS increase in U/C ratio, 95% CI 0.51, 0.97). Fetal blood flow distribution was not associated with childhood creatinine and cystatin C levels, estimated glomerular filtration rate and blood pressure. Results from the basic model were similar as those from the confounder model. The effect estimates for associations of fetal blood flow distribution with microalbuminuria were attenuated towards zero by additional adjustment for childhood kidney size. The effect of stepwise adjustment of covariates in the associations of third trimester U/C ratio and third trimester fetal kidney dimensions with childhood kidney outcomes are given in **Supplementary Tables S5 and S6**. R squares for the effect estimates for the associations of third trimester U/C ratio and third trimester fetal kidney dimensions with childhood kidney outcomes were all between 0.20 and 0.25 (**Supplementary Tables S7 and S8**). We also evaluated the associations of estimated fetal weight and fetal kidney volume in different strata of U/C ratio and found no differences in effect estimates between high, normal and low U/C ratio (p-value > 0.05) (**Supplementary Table S9)**.

Table 1. Subject characteristics (N=923)

Maternal characteristics	
Age, (y)	32.2 (23.4 – 39.4)
Height, (cm)	171 (6.3)
Pre-pregnancy weight, (kg)	69.0 (13.0)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)
Parity ≥1, (%)	37.7 (348)
Educational level, (%)	
Primary/secondary	34.1(315)
Secondary or higher	65.9 (608)
Smoking during pregnancy, (%)	
Yes	21.5 (198)
No	78.5 (745)
Folic acid supplement use, (%)	
Yes	90.9 (839)
No	9.1 (84)
Pregnancy induced hypertension, (%)	
Yes	5.6 (52)
No	94.4 (871)
Preeclampsia, %	
Yes	3.1 (29)
No	96.9 (894)
Fetal characteristics	
General	
Gestational age at measurement, (wk)	30.3 (28.5 – 32.7)
Estimated fetal weight, (g)	1634 (263)
Blood flow distribution	
Umbilical artery PI	0.97 (0.16)
Middle cerebral artery PI	1.97 (0.33)
U/C ratio middle cerebral artery	0.50 (0.11)
Fetal kidney biometrics	
Right kidney volume, (cm^3)	10.64 (3.07)
Left kidney volume, (cm^3)	9.94 (2.76)
Combined kidney volume, (cm^3)	20.56 (5.48)
Birth and infant characteristics	
Gestational age at birth, (wk)	40.3 (36.4 – 42.4)
Birth weight, (g)	3534 (509)
Sex boys, (%)	50.3 (464)
Childhood characteristics	
Age at follow up, (y)	5.9 (5.7 – 6.6)
Height, (cm)	119 (5.2)

Table 1. Continued

Weight, (kg)	22.6 (3.2)
Body mass index, (kg/m^2)	15.9 (1.4)
Body surface area, (m^2)	0.86 (0.07)
Kidney volume left, (cm^3)	61.4 (12.6)
Kidney volume right, (cm^3)	59.5 (11.7)
Kidney volume combined, (cm^3)	120.9 (22.1)
Creatinine, (µmol/l)	36.8 (4.9)
Cystatin C, (µg/l)	790 (74)
Estimated glomerular filtration rate ml/min per 1.73m^2	120.2 (15.8)
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)
Microalbuminuria[a], (%)	7.1 (62)
Systolic blood pressure, (mmHg)	102.2 (7.7)
Diastolic blood pressure, (mmHg)	60.1 (6.3)

Values are means (SD), medians (95% range), or % (numbers).
[a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

Fetal kidney volume and childhood kidney outcomes

Table 3 shows that in the main confounder model a larger fetal combined kidney volume was associated with larger childhood combined kidney volume (3.96 cm^3 per 1 SDS increase in volume, 95% CI 2.43, 5.49), lower creatinine levels (-1.22 µmol/l per 1 SDS increase in volume, 95% CI -1.63, -0.80), lower cystatin C levels (-18 µg/l per 1 SDS increase in volume, 95% CI -25, -12) and higher eGFR (4.45 ml/min per 1.73m^2 per 1 SDS increase in volume, 95% CI 3.08, 5.83), but not with microalbuminuria and blood pressure at the age of 6 years. These effect estimates were largely similar for the basic models. Also, additional adjustment for childhood kidney volume did not materially change the effect estimates.

Fetal blood flow distribution, fetal kidney volume and childhood function

Figure 2 shows the combined effects of fetal blood flow and kidney volume on childhood kidney outcomes. The interaction terms between fetal blood flow and fetal kidney volume were significant for the analyses focused on creatinine levels and eGFR. **Figures 2a and 2b** show that as compared to children with a normal fetal blood flow and fetal combined kidney volume, children with a low U/C ratio and large fetal combined kidney volume had the lowest creatinine levels and highest eGFR. The linear association of fetal combined kidney volume with creatinine levels and eGFR was significant in children with a normal or low U/C ratio (p-values < 0.05), but not in children with higher U/C ratio (**Table S11**). When we used gestational age adjusted birth weight as an additional index of final nephron number instead of fetal kidney volume, we observed similar results (**Supplementary Figure S6)**. Lower gestational age adjusted birth weight was

Table 2. Associations of third trimester fetal blood flow parameters with kidney outcomes at the age of 6 years (N= 879)

	Kidney size	Kidney function				Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
Basic model							
Umbilical artery PI	-1.08	0.23	2	-1.13	0.99	0.22	-0.08
(SD = 0.16)	(-2.48, 0.33)	(-0.18, 0.63)	(-4, 9)	(-2.45, 0.19)	(0.75, 1.30)	(-0.31, 0.76)	(-0.53, 0.36)
M. cerebral artery PI	2.09	0.01	-2	-0.16	1.39	-0.14	-0.16
(SD = 0.33)	(0.74, 3.44)[b]	(-0.39, 0.40)	(-8, 4)	(-1.35, 1.04)	(1.12, 1.71)[a]	(-0.65, 0.39)	(-0.55, 0.23)
U/C ratio	-2.46	0.14	4	-0.67	0.75	0.27	0.04
(SD = 0.11)	(-3.89, -1.04)[b]	(-0.27, 0.55)	(-3, 10)	(-2.00, 0.68)	(0.55, 1.01)	(-0.26, 0.81)	(-0.41, 0.49)
Confounder model							
Umbilical artery PI	-1.01	0.33	3	-1.43	0.97	0.19	-0.12
(SD = 0.16)	(-2.42, 0.41)	(-0.08, 0.75)	(-4, 9)	(-2.78, -0.08)[a]	(0.73, 1.29)	(-0.35, 0.73)	(-0.57, 0.33)
M. cerebral artery PI	2.18	-0.03	-2	0.01	1.40	-0.16	-0.20
(SD = 0.33)	(0.83, 3.54)[b]	(-0.42, 0.37)	(-8, 4)	(-1.30, 1.32)	(1.07, 1.83)[a]	(-0.68, 0.37)	(-0.63, 0.23)
U/C ratio	-2.58	0.24	4	-1.03	0.71	0.30	0.05
(SD = 0.11)	(-4.01, -1.14)[b]	(-0.18, 0.66)	(-3, 11)	(-2.39, 0.34)	(0.52, 0.97)[a]	(-0.25, 0.85)	(-0.41, 0.50)
Childhood kidney volume model							
Umbilical artery PI	NA	0.14	2	-0.74	0.95	0.13	-0.21
(SD = 0.16)		(-0.27, 0.55)	(-4, 9)	(-2.08, 0.59)	(0.71, 1.28)	(-0.42, 0.68)	(-0.68, 0.25)
M. cerebral artery PI	NA	0.01	-2	-0.31	1.36	-0.07	-0.16
(SD = 0.33)		(-0.40, 0.39)	(-8, 5)	(-1.59, 0.97)	(1.03,1.79)[a]	(-0.61, 0.48)	(-0.61, 0.30)
U/C ratio	NA	0.10	3	-0.29	0.72	0.21	-0.02
(SD = 0.11)		(-0.32, 0.52)	(-3, 10)	(-1.66, 1.08)	(0.52, 1.00)	(-0.37, 0.78)	(-0.50, 0.45)

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight. Childhood kidney volume model is additionally adjusted for combined childhood kidney volume. Results for the analyses additionally adjusted for fetal kidney size are given in the supplementary material (**Table S13**).

eGFR, estimated glomerular filtration rate. BP, blood pressure

[a]$p<0.05$, [b]$p<0.01$

associated with smaller childhood kidneys with a lower eGFR. The interaction between U/C ratio and gestational age adjusted birth weight for the associated with childhood kidney outcomes were not significant. When we used GFR/cm^3 kidney volume instead of GFR, no differences in results were observed in the final models.

Figure 1. Associations of fetal blood flow, fetal combined kidney volume, and childhood kidney volume with childhood eGFR (N= 613)

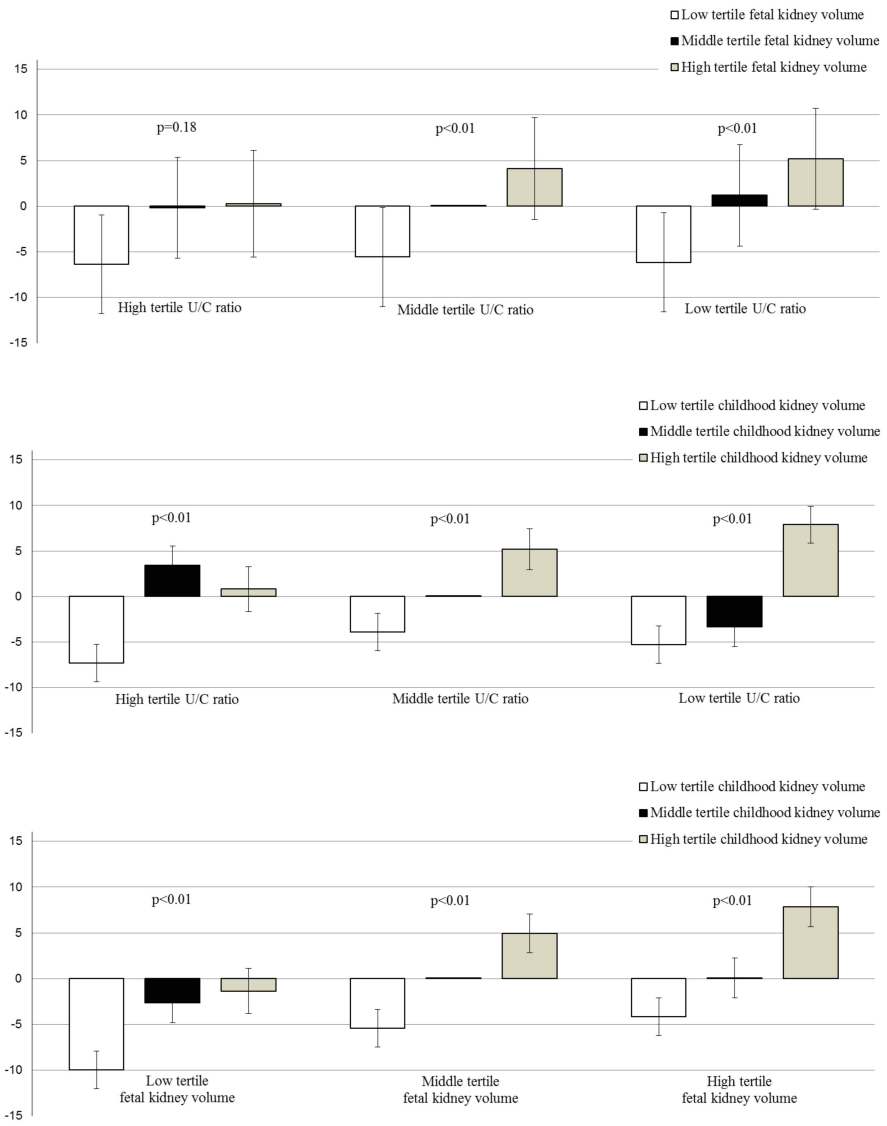

Table 3. Associations of third trimester fetal kidney dimensions with kidney outcomes at the age of 6 years (N=870)

	Kidney size	Kidney function				Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
Combined fetal kidney volume (SD = 5.48)							
Basic model	3.89 (3.61, 4.16)[a]	-1.18 (-1.60, -0.77)[a]	-18 (-25, -12)[a]	4.40 (3.06, 5.75)[a]	1.05 (0.90, 1.22)	-0.32 (-0.89, 0.26)	-0.24 (-0.49, 0.01)
Confounder model	3.96 (2.43, 5.49)[a]	-1.22 (-1.63, -0.80)[a]	-18 (-25, -12)[a]	4.45 (3.08, 5.83)[a]	1.06 (0.78, 1.44)	-0.29 (-0.89, 0.31)	-0.18 (-0.67, 0.32)
Childhood kidney volume model	NA	-0.87 (-1.29, -0.45)[a]	-16 (-22, -9)[a]	3.21 (1.85, 4.56)[a]	0.97 (0.70, 1.34)	-0.26 (-0.89, 0.38)	-0.08 (-0.60, 0.44)

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight. Childhood kidney volume model is additionally adjusted for combined childhood kidney volume.

eGFR, estimated glomerular filtration rate. BP, blood pressure

[a]p <0.01

Bars represent regression coefficients (95% CIs) based on multiple regression models and reflect the associations of fetal blood flow and kidney volume (A), fetal blood flow and childhood kidney volume (B), and fetal and childhood kidney volume (C) (tertiles) with childhood eGFR. Models are adjusted for maternal age, parity, education level, prepregnancy body mass index, smoking status during pregnancy, folic acid use during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, gestational age, and gestational age–adjusted birth weight, and current age. $P<0.01$ for the interaction between fetal blood flow and fetal combined kidney volume for the association with childhood eGFR. The *P* value for the interaction between fetal blood flow and childhood combined kidney volume and for interaction between fetal combined kidney volume and childhood combined kidney volume is not significant.

Discussion

We observed that that third trimester fetal blood flow redistribution, at expense of the trunk and abdominal organs, and restricted fetal kidney growth are associated with smaller kidney volume and lower kidney function in school-age children. Fetal blood flow and fetal kidney size was not associated with childhood blood pressure.

An adverse fetal environment may lead to smaller kidneys with a reduced number of nephrons.(29) These kidney adaptations may lead to a reduced glomerular filtration surface area, hyperfiltration and eventually glomerular sclerosis and chronic kidney disease.(2,3) Thus far, this hypothesis is mainly supported by studies showing associations of low birth weight with higher risks of kidney disease and hypertension in adulthood.(5,6) Also, post-mortem studies showed that nephron number is lower in children with a low birth weight, or small kidney size, and in adults with primary hypertension.(7-9) Studies in rats showed that fetal growth restriction, induced by bilateral uterine artery ligation, leads to an increased risk of kidney disease and higher blood pressure in adulthood.(30,31) In a study among 7,457 adults aged 20 to 30 years in Norway, intrauterine growth restriction was associated with low-normal kidney function. In this study the effect sizes were small and participation in follow-up measurements was less than 50%.(32) Another study among 82 young adults born before 32 weeks of gestation in the Netherlands, observed no consistent associations of preterm birth in combination with intra-uterine growth restriction with kidney function.(33)

Animal studies showed a reduction in glomerular and nephron number due to vascular placental insufficiency.(10,34) We observed in a previous study within the same cohort that fetal blood flow redistribution at expense of the abdominal organs, leads to smaller fetal kidneys.(21) In this study, we did not observe differences in the association of gestational age and fetal weight and fetal kidney volume between high, normal and low U/C ratio groups. In the present follow-up study, we observed that fetal blood flow redistribution is also associated with smaller kidneys and impaired kidney function in

children. Surprisingly, we observed that fetal blood redistribution at expense of the abdominal organs was associated with a lower risk of microalbuminuria, independent of potential confounders and childhood kidney size. We could not explain this finding. Blood flow through the umbilical arteries, reflects the arterial resistance and blood flow to the intra-abdominal arteries, including the descending aorta and renal artery.(19) More detailed studies focused on the directly measured renal artery blood flow might give more information about the fetal and childhood consequences of impaired fetal kidney blood flow.

A recent cross-sectional study, among 257 healthy children in Italy older than 6 months, indicated that childhood kidney size was inversely correlated with creatinine levels.(35) In line with that study, we observed an inverse association of fetal kidney size with creatinine and cystatin C levels in childhood, and a positive association with eGFR. Previously, we observed in the same cohort as the present study that small fetal kidney size tends to track in early childhood.(36) However, in the current study, the associations of fetal kidney volume with childhood kidney function outcomes were independent of childhood kidney size. These findings suggest that fetal kidney size may have permanent effects, independent of later kidney growth, on kidney function in later life. Within the group with the highest U/C ratio, reflecting blood flow redistribution at expense of the abdominal organs, there was no clear association between fetal kidney volume and childhood kidney function. This suggests that there might be another pathway, besides via fetal kidney volume, by which fetal blood redistribution affects kidney function in childhood. Fetal kidney size was not associated with childhood microalbuminuria or blood pressure. It might be that differences in these more clinical markers of kidney dysfunction appear at older ages.

The results from this study are important from an etiological perspective. They suggest that suboptimal abdominal blood flow and kidney growth in fetal life have persistent consequences. However, the observed effect estimates were small and reflect subclinical changes in kidney function in school-age children. None of the children had a known clinical kidney disease. Longitudinal studies reported tracking of risk factors for kidney and cardiovascular disease during childhood.(37,38) Also, the consequences of impaired kidney growth might not yet be fully detectable in early childhood, but might become more evident in later life. It has been suggested that fetal adverse adaptations can be compensated for many years until for example hypertension occurs.(5)

The biological mechanisms underlying the associations of low birth weight with kidney diseases in adulthood may also include other mechanisms than smaller kidneys with a lower number of nephrons with glomerular hyperfiltration.(4) Animal studies showed alterations in the renin angiotensin system in experimentally induced intrauterine growth restricted rats at adult age. These differences were not present at younger age.(39) Several markers of the renin angiotensin system were increased in intrauterine growth restricted subjects with hypertension.(39) An accumulating body of evidence suggests that an adverse intra-uterine environment might cause epigenetic alterations which in turn influence kidney growth and function.(4) Finally, a mismatch between

fetal and postnatal growth may also lead to insufficient kidney function for an individual metabolic load. Future studies are needed to identify possible underlying mechanisms.

The main strength of this study is the population-based prospective design from fetal life onwards. Follow-up measurements at the age of 6 years were obtained in 75% of the children. If the associations of fetal characteristics with childhood kidney outcomes would differ between those with and without follow-up measurements, the results would be biased. This seems unlikely, but cannot be excluded. Children without kidney measurements were on average smaller at birth, which might have led to an underestimation of our associations. We evaluated fetal blood flow and fetal kidney volume at one time point during late pregnancy. Although the intra- and interobserver variability are adequate and mean values are in line with previous studies, misclassification due to measurement error cannot be excluded. However, this would most likely have to random error, which reduces power of the study and may have led to an underestimation of the evaluated associations. Fetal kidney volume was evaluated around 30 weeks of gestational age. Since nephrogenesis continues until 36 weeks gestational age, our measurements did not reflect final nephron number.(2) Evaluation of fetal kidney size until 36 weeks of gestational age, might have been more representative for final nephron number. When we used gestational age adjusted birth weight as a surrogate for final nephron number instead of third trimester kidney volume, we observed similar results. It is not known whether birth weight is a better proxy for fetal nephron number than fetal kidney size.(40) We used kidney size as a measure of kidney development, since nephron number cannot be studied *in vivo*. Kidney size is correlated with the number of glomeruli and can be used in epidemiological studies as measure of kidney development.(40) However, glomerular enlargement due to hyperfiltration may attenuate the differences in childhood kidney volume and may lead to an underestimation of the associations of interest.(41) We estimated the glomerular filtration using blood creatinine levels. Blood cystatin C levels might be more accurate in estimating glomerular filtration. As compared to creatinine, cystatin C is freely filtered, produced more constantly and less dependent from children's body weight, height and sex.(42) In the current study, we observed similar results for creatinine and cystatin C levels. We used the urine albumin-creatinine ratio to evaluate albuminuria in a random urine sample.(43) Since the within subject variation in urinary albumin excretion is large, the variability would probably be lower if we collected first morning void samples instead of random during the day.(44) This was not possible in the current study. In the present study we evaluated multiple associations, this might have led to chance findings due to multiple testing. However, since the kidney related outcomes were correlated we did not adjust for multiple testing. Finally, although we had information about a large number of confounders, the influence of residual confounding should be considered, as in any observational study.

The findings from the present study are important from a developmental perspective. They strongly suggest that suboptimal third trimester fetal kidney development influences childhood kidney function. The observed small differences in kidney function are without direct clinical consequences in childhood, but may lead to impaired kidney function in later life.

Conclusion and perspectives

In conclusion, fetal blood redistribution at expense of the intra-abdominal organs and impaired fetal kidney growth have persistent consequences for kidney function in childhood. The observed associations suggest that fetal kidney developmental adaptations have affect kidney function throughout the life-course, and predispose individuals for kidney diseases in later life. Further studies are needed to identify the underlying biological mechanisms and the long-term consequences of the observed associations.

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Imputation procedure

To reduce the possibility of potential bias associated with missing data (less than 17%), missing values were imputed using the multiple imputations procedure.(1) For the multiple imputations, we used Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model, we included all covariates plus maternal pre-pregnancy weight, birth weight, height and weight of the child aged 6. Furthermore, we added the determinants and outcomes studied in the imputation model as prediction variables only; they were not imputed themselves. Five imputed datasets were created and analyzed together.

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Table S1. Subject characteristics in tertiles of U/C ratio (N=879)

	High tertile U/C ratio (N=297)	Middle tertile U/C ratio (N=285)	Low tertile U/C ratio (N=297)	P-value for trend
Maternal characteristics				
Age, (y)	31.8 (24.1, 39.5)	32.3 (23.6, 40.6)	32.2 (21.9, 39.0)	P=0.43
Height, (cm)	170.9 (6.2)	171.4 (6.3)	170.8 (6.1)	P=0.53
Pre-pregnancy weight, (kg)	68.0 (12.7)	69.5 (13.6)	69.6 (13.3)	P=0.39
Pre-pregnancy body mass index, (kg/m^2)	23.4 (4.1)	23.6 (4.2)	23.9 (4.4)	P=0.35
Parity ≥1, (%)	32.0 (95)	40.7 (116)	41.8 (124)	P<0.05
Educational level, (%)				
Primary/secondary	28.6 (85)	36.1 (103)	35.7 (106)	P=0.12
Secondary or higher	71.4 (212)	63.9 (182)	64.6 (191)	
Smoking during pregnancy, (%)				
Yes	19.9 (59)	21.8 (62)	22.2 (66)	P=0.74
No	80.1 (238)	78.2 (223)	77.8 (231)	
Folic acid supplement use, (%)				
Yes	89.9 (267)	93.3 (266)	87.9 (261)	P=0.10
No	10.1 (30)	6.7 (19)	12.1 (36)	
Pregnancy induced hypertension, (%)				
Yes	6.1 (18)	5.6 (16)	4.7 (14)	P=0.74
No	93.9 (279)	94.4 (269)	96.3 (283)	
Preeclampsia, (%)				
Yes	3.4 (10)	3.2 (9)	2.2 (6)	P=0.69
No	96.6 (287)	96.8 (276)	97.8 (291)	
Fetal characteristics				
Gestational age at measurement, (wk)	30.2 (28.5, 32.5)	30.4 (28.6, 32.5)	30.4 (28.4, 33.1)	P<0.05
Estimated fetal weight, (g)	1580 (237)	1647 (259)	1675 (279)	P<0.01
Umbilical artery PI	1.08 (0.16)	0.97 (0.12)	0.86 (0.12)	P<0.01
Middle cerebral artery PI	1.73 (0.28)	1.98 (0.26)	2.21 (0.28)	P<0.01
U/C ratio middle cerebral artery	0.63 (0.08)	0.49 (0.03)	0.39 (0.04)	P<0.01
Right kidney volume, (cm^3)	10.3 (3.1)	10.7 (2.9)	11.0 (3.2)	P<0.05
Left kidney volume, (cm^3)	9.5 (2.7)	10.1 (2.7)	10.4 (2.8)	P<0.01
Combined kidney volume, (cm^3)	19.8 (5.5)	20.8 (5.2)	21.3 (5.7)	P<0.01
Birth and infant characteristics				
Gestational age at birth, (wk)	40.4 (35.6, 42.6)	40.3 (37.0, 42.6)	40.3 (36.9, 42.1)	P=0.36
Birth weight, (g)	3450 (506)	3568 (510)	3601 (474)	P<0.01
Sex boys, (cm^3)	47.1 (140)	52.3 (149)	52.5 (156)	P=0.34
Childhood characteristics				
Height, (cm)	118.3 (5.2)	119.4 (5.1)	119.4 (5.1)	P<0.05
Weight, (kg)	22.1 (3.1)	22.9 (3.6)	22.8 (3.0)	P<0.01
Age at follow up, (y)	5.9 (5.6, 6.8)	5.9 (5.7, 6.5)	5.9 (5.7, 6.8)	P=0.69
Body mass index, (kg/m^2)	15.8 (1.37)	16.0 (1.62)	15.9 (1.28)	P=0.12

Table S1. Continued

Body surface area, (m^2)	0.85 (0.07)	0.87 (0.08)	0.87 (0.07)	P<0.01
Kidney volume left, (cm^3)	59.2 (11.5)	61.7 (12.7)	63.4 (13.2)	P<0.01
Kidney volume right, (cm^3)	56.8 (11.4)	60.5 (12.0)	61.3 (11.5)	P<0.01
Kidney volume combined, (cm^3)	115.9 (20.8)	122.1 (22.5)	124.8 (22.6)	P<0.01
Creatinine, (µmol/l)	36.8 (5.2)	36.8 (4.6)	37.0 (5.0)	P=0.91
Cystatin C, (µg/l)	793 (77)	794 (77)	784 (70)	P=0.32
Estimated glomerular filtration rate ml/min per $1.73m^2$	119.4 (15.5)	120.4 (15.2)	120.4 (16.8)	P=0.75
Estimated glomerular filtration rate/cm^3 kidney volume	1.06 (0.19)	1.01 (0.18)	0.97 (0.17)	P<0.01
Microalbuminuria[a], (%)	4.0 (12)	9.1 (26)	7.1 (21)	P=0.05
Systolic blood pressure, (mmHg)	101.9 (8.5)	102.7 (7.4)	101.8 (7.1)	P=0.28
Diastolic blood pressure, (mmHg)	59.8 (6.9)	60.3 (5.7)	60.0 (6.3)	P=0.64

Means were compared using ANOVA for continuous variables and chi-square test for categorical variables.

[a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

Table S2. Subject characteristics in tertiles of fetal kidney volume (N=870)

	Low tertile kidney volume (N=302)	Middle tertile kidney volume (N=292)	High tertile kidney volume (N=276)	P-value for trend
Maternal characteristics				
Age, (y)	31.8 (22.5, 39.6)	32.5 (23.8, 39.4)	31.9 (23.4, 39.5)	P=0.17
Height, (cm)	170.5 (6.1)	170.9 (6.2)	171.9 (6.3)	P<0.05
Pre-pregnancy weight, (kg)	67.7 (12.0)	64.2 (12.3)	70.8 (14.0)	P<0.05
Pre-pregnancy body mass index, (kg/m^2)	23.3 (3.9)	23.5 (4.1)	24.0 (4.6)	P=0.17
Parity ≥1, (%)	35.4 (107)	43.5 (127)	32.2 (89)	P=0.30
Educational level, (%)				
Primary/secondary	35.1 (106)	31.2 (91)	34.1 (94)	P=0.63
Secondary or higher	64.9 (196)	68.8 (201)	65.9 (182)	
Smoking during pregnancy, (%)				
Yes	23.2 (70)	21.5 (63)	21.0 (58)	P=0.74
No	76.8 (232)	78.5 (229)	79.0 (218)	
Folic acid supplement use, (%)				
Yes	90.1 (272)	90.8 (265)	90.6 (251)	P=0.59
No	9.9 (30)	9.2 (27)	9.4 (25)	
Pregnancy induced hypertension, (%)				
Yes	7.0 (21)	4.5 (13)	5.4 (15)	P=0.84
No	93.0 (281)	96.5 (279)	94.6 (261)	
Preeclampsia, %				
Yes	3.6 (11)	2.1 (6)	2.5 (7)	P=0.76
No	96.4 (291)	97.9 (286)	97.5 (269)	
Fetal characteristics				
Gestational age at measurement, (wk)	30.1 (28.1, 32.0)	30.4 (28.6, 32.5)	30.7 (28.9, 33.1)	P<0.01
Estimated fetal weight, (g)	1511 (230)	1635 (232)	1768 (264)	P<0.01
Umbilical artery PI	1.00 (0.17)	0.97 (0.16)	0.94 (0.16)	P<0.01
Middle cerebral artery PI	1.96 (0.36)	1.99 (0.33)	1.95 (0.31)	P=0.20
U/C ratio middle cerebral artery	0.52 (0.12)	0.49 (0.10)	0.49 (0.11)	P<0.01
Right kidney volume, (cm$^{3)}$	7.9 (1.3)	10.4 (1.1)	13.9 (2.7)	P<0.01
Left kidney volume, (cm$^{3)}$	7.4 (1.2)	9.7 (1.1)	12.9 (2.3)	P<0.01
Combined kidney volume, (cm$^{3)}$	15.3 (2.0)	20.1 (1.3)	26.8 (4.2)	P<0.01
Birth and infant characteristics				
Gestational age at birth, (wk)	40.1 (37.0, 42.3)	40.4 (35.7, 42.6)	40.4 (36.8, 42.3)	P=0.52
Birth weight, (g)	3384 (489)	3561 (478)	3682 (502)	P<0.01
Sex boys, (%)	54.3 (164)	52.1 (152)	55.1 (152)	P=0.37
Childhood characteristics				
Age at follow up, (y)	5.9 (5.7, 6.7)	5.9 (5.7, 6.6)	6.0 (5.7, 6.7)	P=0.19
Height, (cm)	118.3 (5.4)	118.7 (5.1)	120.2 (4.9)	P<0.01
Weight, (kg)	22.2 (3.4)	22.3 (2.9)	23.3 (3.4)	P<0.01
Body mass index, (kg/m^2)	15.8 (1.5)	15.8 (1.2)	16.1 (1.5)	P<0.05

Table S2. Continued

Body surface area, (m^2)	0.85 (0.08)	0.86 (0.07)	0.88 (0.07)	P<0.01
Kidney volume left, (cm^3)	58.9 (11.9)	61.4 (12.6)	63.9 (12.6)	P<0.01
Kidney volume right, (cm^3)	56.5 (11.1)	59.3 (11.2)	62.8 (12.1)	P<0.01
Kidney volume combined, (cm^3)	115.4 (21.0)	120.7 (21.6)	126.7 (22.4)	P<0.01
Creatinine, (µmol/l)	37.8 (4.7)	36.4 (4.8)	36.1 (5.1)	P<0.01
Cystatin C, (µg/l)	812 (74)	780 (75)	776 (71)	P<0.01
Estimated glomerular filtration rate ml/min per $1.73m^2$	116.0 (14.0)	121.3 (15.2)	124.0 (17.1)	P<0.01
Estimated glomerular filtration rate/cm^3 kidney volume	1.04 (0.01)	1.02 (0.01)	1.00 (0.01)	P=0.16
Microalbuminuria[a], (%)	6.6 (20)	5.1 (15)	8.3 (23)	P=0.62
Systolic blood pressure, (mmHg)	102.2 (8.2)	101.9 (7.7)	102.4 (7.2)	P=0.88
Diastolic blood pressure, (mmHg)	60.3 (6.2)	60.2 (6.5)	59.8 (6.0)	P=0.70

Means were compared using ANOVA for continuous variables and chi-square test for categorical variables.

[a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

Table S3. Associations of third trimester umbilical/cerebral artery resistance ratio and covariates with kidney outcomes at the age of 6 years (N=879)

	Kidney size	Kidney function				Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
U/C ratio	-3.88	-0.04	4	-0.58	0.75	0.15	0.06
(SD = 0.11)	(-5.44, -2.32)[b]	(-0.46, 0.38)	(-2, 10)	(-1.91, 0.75)	(0.56, 1.01)	(-0.39, 0.68)	(-0.38, 0.50)
Maternal age	-3.88	-0.04	4	-0.59	0.75	0.14	0.06
	(-5.44, -2.32)[b]	(-0.45, 0.38)	(-2, 10)	(-1.92, 0.75)	(0.56, 1.01)	(-0.39, 0.68)	(-0.39, 0.50)
Ppre-pregnancy body	-3.87	-0.03	4	-0.59	0.75	0.19	0.07
mass index	(-5.44, 2.31)[b]	(-0.45, 0.38)	(-2, 10)	(-1.93, 0.74)	(0.56, 1.00)	(-0.35, 0.72)	(-0.37, 0.52)
Parity	-3.96	-0.03	4	-0.63	0.74	0.12	0.05
	(-5.46, -2.46)[b]	(-0.25, 0.18)	(1, 7)	(-1.92, 0.66)	(0.55, 1.00)	(-0.16, 0.39)	(-0.18, 0.27)
Maternal educational level	-4.01	-0.03	4	-0.61	0.74	0.18	0.09
	(-5.55, -2.47)[b]	(-0.44, 0.39)	(-2, 11)	(-1.94, 0.72)	(0.56, 0.99)[a]	(-0.36, 0.71)	(-0.34, 0.53)
Maternal smoking	-3.90	-0.04	4	-0.58	0.74	0.16	0.07
	(-5.46, 2.34)[b]	(-0.25, 0.17)	(-2, 10)	(-1.91, 0.75)	(0.55, 1.00)	(-0.38, 0.70)	(-0.38, 0.51)
Maternal folic acid use	-3.81	0.03	4	-0.61	0.75	0.14	0.06
	(-5.37, -2.25)[b]	(-0.45, 0.38)	(-2, 10)	(-1.94, 0.73)	(0.55, 1.01)	(-0.40, 0.68)	(-0.38, 0.50)
Gestational hypertension	-3.89	-0.05	4	-0.57	0.75	0.15	0.06
	(-5.44, -2.33)[b]	(-0.46, 0.37)	(-2, 11)	(-1.88, 0.75)	(0.56, 1.01)	(-0.39, 0.68)	(-0.38, 0.50)
Preeclampsia	-3.86	-0.04	4	-0.83	0.75	0.14	0.06
	(-5.43, 2.30)[b]	(-0.45, 0.38)	(-3, 10)	(-1.93, 0.75)	(0.56, 1.01)	(-0.40, 0.68)	(-0.39, 0.50)

Table S3. Continued

Gestational age at measurement	-3.86 (-5.43, -2.30)[b]	0.00 (-0.42, 0.42)	4 (-3, 10)	-0.71 (-2.05, 0.63)	0.74 (0.55, 1.00)	0.17 (-0.37, 0.71)	0.05 (-0.39, 0.50)
Third trimester fetal weight	-3.52 (-5.08, -1.97)[b]	0.01 (-0.40, 0.43)	3 (-3, 10)	-0.63 (-1.97, 0.72)	0.75 (0.56, 1.01)	0.19 (-0.09, 0.47)	0.03 (-0.39, 0.46)
Gestational age at birth	-3.89 (-5.45, -2.33)[b]	-0.03 (-0.45, 0.39)	4 (-2, 11)	-0.62 (-1.95, 0.72)	0.75 (0.55, 1.00)	0.15 (-0.39, 0.69)	0.06 (-0.39, 0.50)
SDS birth weight	-3.34 (-4.90, -1.77)[b]	0.05 (-0.36, 0.47)	4 (-3, 10)	-0.68 (-2.03, 0.67)	0.74 (0.55, 0.99)	0.13 (-0.42, 0.68)	0.01 (-0.44, 0.46)
Child sex	-3.80 (-5.35, -2.25)[b]	-0.04 (-0.46, 0.38)	4 (-2, 10)	-0.58 (-1.91, 0.75)	0.75 (0.56, 1.01)	0.12 (-0.41, 0.66)	0.04 (-0.40, 0.48)
Age at 6	-3.71 (-5.25, -2.17)[b]	0.00 (-0.41, 0.41)	4 (-2, 11)	-0.62 (-1.95, 0.71)	0.75 (0.55, 1,00)	0.17 (-0.37, 0.70)	0.07 (-0.38, 0.51)
hild body surface area	-2.58 (-3.99, -1.16)[b]	0.12 (-0.29, 0.53)	4 (-2, 11)	-0.41 (-1.75, 0.94)	0.74 (0.56, 0.98)[a]	0.30 (-0.23, 0.83)	0.10 (-0.35, 0.54)

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics.

eGFR, estimated glomerular filtration rate. BP, blood pressure

[a]p<0.05, [b]p<0.01

Table S4. Associations of third trimester fetal kidney size and covariates with kidney outcomes at the age of 6 years (N=870)

	Kidney size	Kidney function				Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
Fetal kidney volume (SD = 5.48)	5.22 (3.71, 6.73)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.24, 4.68)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.44)	-0.25 (-0.67, 0.18)
Maternal age	5.21 (3.70, 6.72)[b]	-0.70 (-1.08, -0.31)[b]	-16 (-22, -10)[b]	3.44 (2.21, 4.66)[b]	1.07 (0.82, 1.39)	-0.08 (-0.61, 0.45)	-0.23 (-0.66, 0.20)
Pre-pregnancy body mass index	5.22 (3.71, 6.73)[b]	-0.72 (-1.10, -0.33)[b]	-16 (-22, -10)[b]	3.48 (2.26, 4.71)[b]	1.08 (0.83, 1.40)	-0.14 (-0.67, 0.39)	-0.27 (-0.70, 0.16)
Parity	5.23 (4.46, 6.00)[b]	-0.71 (-0.90, -0.51)[b]	-16 (-19, -13)[b]	3.46 (2.84, 4.08)[b]	1.08 (0.94, 1.23)	-0.09 (-0.36, 0.18)	-0.25 (-0.47, 0.03)
Mal educational level	5.20 (3.73, 6.70)[b]	-0.70 (-1.33, -0.14)[b]	-16 (-19, -13)[b]	3.42 (2.21, 4.63)[b]	1.07 (0.83, 1.39)	-0.08 (-0.61, 0.45)	-0.24 (-0.66, 0.19)
Maternal smoking	5.22 (3.73, 6.71)[b]	-0.71 (-1.09, -0.33)[b]	-16 (-22, -10)[b]	3.45 (2.23, 4.68)[b]	1.08 (0.83, 1.40)	-0.08 (-0.62, 0.45)	-0.24 (-0.67, 0.19)
Maternal folic acid use	5.24 (3.74, 6.74)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.44 (2.22, 4.67)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.45)	-0.24 (-0.67, -0.19)
Gestational hypertension	5.22 (3.57, 5.87)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.24, 4.68)[b]	1.08 (0.84, 1.39)	-0.09 (-0.62, 0.44)	-0.25 (-0.46, -0.03)
Preeclampsia	5.21 (3.70, 6.71)[b]	-0.74 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.29, 4.63)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.44)	-0.25 (-0.46, -0.03)
Gestational age at measurement	5.85 (4.26, 7.44)[b]	-0.91 (-1.32, -0.50)[b]	-17 (-23, -11)[b]	4.22 (2.93, 5.51)[b]	1.08 (0.82, 1.43)	-0.18 (-0.74, 0.39)	-0.27 (-0.73, 0.18)

Table S4. Continued

Third trimester fetal weight	4.96 (3.29, 6.63)[b]	-1.02 (-1.44, -0.59)[b]	-17 (-23, -11)[b]	4.21 (2.85, 5.56)[b]	1.04 (0.90, 1.21)	-0.25 (-0.84, 0.35)	-0.26 (-0.72, 0.19)
Gestational age at birth	5.22 (3.71, 6.73)[b]	-0.70 (-1.09, -0.31)[b]	-16 (-22, -10)[b]	3.43 (2.20, 4.65)[b]	1.08 (0.83, 1.40)	-0.08 (-0.62, 0.45)	-0.25 (-0.68, 0.18)
SDS birth weight	4.41 (2.86, 5.96)[b]	-0.87 (-1.27, -0.48)[b]	-16 (-22, -10)[b]	3.71 (2.45, 4.96)[b]	1.09 (0.83, 1.43)	-0.08 (-0.63, 0.48)	-0.24 (-0.63, 0.27)
Child sex	-3.22 (-6.21, -0.24)[a]	-0.71 (-1.10, -0.33)[b]	-16 (-22, -11)[b]	3.47 (2.24, 4.69)[b]	1.07 (0.82, 1.39)	-0.02 (-0.55, 0.51)	-0.19 (-0.62, 0.24)
Age at 6	4.97 (3.47, 6.48)[b]	-0.82 (-1.19, -0.44)[b]	-16 (-22, -11)[b]	3.61 (2.39, 4.84)[b]	1.09 (0.84, 1.41)	-0.14 (-0.67, 0.40)	-0.26 (-0.69, 0.17)
Child body surface area	3.52 (2.14, 4.89)[b]	-0.89 (-1.27, -0.51)[b]	-17 (-23, -11)[b]	3.33 (2.10, 4.57)[b]	1.09 (0.84, 1.42)	-0.34 (-0.87, 0.20)	-0.30 (-0.73, 0.14)

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics.

eGFR, estimated glomerular filtration rate; BP, blood pressure

[a]$p<0.05$, [b]$p<0.01$

Table S5. Associations of third trimester umbilical/cerebral artery resistance ratio with childhood kidney volume at the age of 6 years (N=834)

	Combined kidney volume difference (95%CI) (cm^3)	R square
U/C ratio (SD = 0.11)		
Basic model	-2.46 (-3.89, -1.04)[a]	0.221
Confounder model	-2.58 (-4.01, -1.14)[a]	0.223

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the difference for kidney volume for fetal blood flow characteristics. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight.

[a]p<0.01

Table S6. Associations of third trimester fetal kidney volume with childhood kidney volume at the age of 6 years (N=799)

	Combined kidney volume difference (95%CI) (cm^3)	R square
Combined fetal kidney volume (SD = 5.48)		
Basic model	3.89 (3.61, 4.16)[a]	0.246
Confounder model	3.96 (2.43, 5.49)[a]	0.247

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the difference for kidney volume for fetal kidney volume. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight.

[a]p<0.01

Table S7. Subject characteristics of participants with and without follow-up data (N=1,201)

	Childhood data available (N=923)	Childhood data unavailable (N=278)
Maternal characteristics		
Age, (y)	32.2 (23.4 – 39.4)	31.1 (19.2 – 38.2)[a]
Height, (cm)	171 (6.3)	170 (6.4)
Pre-pregnancy weight, (kg)	69.3 (13.0)	67.1 (11.3)
Pre-pregnancy body mass index, (kg/m$^{2)}$)	23.6 (4.2)	23.1 (3.7)
Parity ≥1, (%)	37.7 (348)	44.2 (123)[a]
Missing	0.2 (2)	0.7 (2)
Educational level, (%)		
Primary/secondary	33.7 (311)	45.3 (126)[b]
Secondary or higher	65.3 (603)	51.1 (142)
Missing	1 (9)	3.6 (10)
Smoking during pregnancy, (%)		
Yes	19.4 (179)	35.3 (89)[b]
No	70.7 (653)	58.2 (171)
Missing	9.9 (91)	6.5 (18)
Folic acid, (%)		
Yes	76.6 (707)	69.4 (193)[b]
No	6.5 (60)	11.9 (33)
Missing	16.9 (156)	18.7 (52)
Pregnancy induced hypertension, (%)		
Yes	4.9 (45)	4.0 (11)
No	89.7 (828)	89.5 (249)
Missing	5.4 (50)	6.5 (18)
Preeclampsia, (%)		
Yes	2.5 (23)	1.8 (5)
No	89.7 (828)	89.5 (249)
Missing	7.8 (72)	8.7 (24)
Gestational age at measurement, (wk)	30.3 (28.5 – 32.7)	30.4 (28.1 – 32.5)
Estimated fetal weight, (g)	1634 (263)	1616 (285)
Blood flow distribution		
Umbilical artery PI	0.97 (0.16)	0.99 (0.18)
Middle cerebral artery PI	1.97 (0.33)	1.99 (0.33)
U/C ratio, middle cerebral artery	0.50 (0.11)	0.51 (0.12)

Table S7. Continued

Fetal kidney biometrics		
Right kidney volume, (cm^3)	10.64 (3.07)	10.84 (3.13)
Left kidney volume, (cm^3)	9.94 (2.76)	10.01 (2.86)
Combined kidney volume, (cm^3)	20.56 (5.48)	20.85 (5.61)
Birth and infant characteristics		
Gestational age at birth, (wk)	40.3 (36.4 – 42.4)	40.0 (34.7 – 42.4)[b]
Birth weight, (g)	3534 (509)	3446 (628)[a]
Sex boys, (%)	50.3 (464)	57.6 (160)[a]
Missing	0	
Childhood characteristics		
Age at follow up, (y)	5.9 (5.7 – 6.6)	N.A.
Height, (cm)	119 (5.2)	N.A.
Weight, (kg)	22.6 (3.2)	N.A.
Body mass index, (kg/m^2)	15.9 (1.4)	N.A.
Body surface area, (m^2)	0.86 (0.07)	N.A.
Kidney volume left, (cm^3)	61.4 (12.6)	N.A.
Kidney volume right, (cm^3)	59.5 (11.7)	N.A.
Kidney volume combined, (cm^3)	120.9 (22.1)	N.A.
Creatinine, (µmol/l)	36.8 (4.9)	N.A.
Cystatin C, (µg/l)	790 (74)	N.A.
Estimated glomerular filtration rate ml/min per $1.73m^2$	120.2 (15.8)	N.A.
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)	
Micro albuminuria[c], (%)	7.1 (62)	N.A.
Systolic blood pressure, (mmHg)	102.2 (7.7)	N.A.
Diastolic blood pressure, (mmHg)	60.1 (6.3)	N.A.

Values are means (SD), medians (95% range), or % (numbers). Participants were compared using independent samples t-test for continuous variables and chi-square test for categorical variables.

[c]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

N.A. not applicable

[a]$p<0.05$, [b]$p<0.01$

Table S8. Subject characteristics of observed and imputed data (N=923)

	Observed data	Imputed data
Maternal characteristics		
Age, (y)	32.2 (23.4 – 39.4)	32.2 (23.4 – 39.4)
Height, (cm)	171 (6.3)	171 (6.3)
Pre-pregnancy weight, (kg)	69.3 (13.0)	69.0 (13.0)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)	23.6 (4.2)
Parity ≥1, (%)	37.7 (348)	37.7 (348)
Missing	0.2 (2)	
Educational level, (%)		
Primary/secondary	33.7 (311)	34.1(315)
Secondary or higher	65.3 (603)	65.9 (608)
Missing	1 (9)	
Smoking during pregnancy, (%)		
Yes	19.4 (179)	21.5 (198)
No	70.7 (653)	78.5 (745)
Missing	9.9 (91)	
Folic acid, (%)		
Yes	76.6 (707)	90.9 (839)
No	6.5 (60)	9.1 (84)
Missing	16.9 (156)	
Pregnancy induced hypertension, (%)		
Yes	4.9 (45)	5.6 (52)
No	89.7 (828)	94.4 (871)
Missing	5.4 (50)	
Preeclampsia, (%)		
Yes	2.5 (23)	3.1 (29)
No	89.7 (828)	96.9 (894)
Missing	7.8 (72)	
Fetal characteristics		
General		
Gestational age at measurement, (wk)	30.3 (28.5 – 32.7)	30.3 (28.5 – 32.7)
Estimated fetal weight, (g)	1634 (263)	1634 (263)
Blood flow distribution		
Umbilical artery PI	0.97 (0.16)	Not imputed
Middle cerebral artery PI	1.97 (0.33)	Not imputed
U/C ratio, middle cerebral artery	0.50 (0.11)	Not imputed

Table S8. Continued

Fetal kidney biometrics		
Right kidney volume, (cm^3)	10.64 (3.07)	Not imputed
Left kidney volume, (cm^3)	9.94 (2.76)	Not imputed
Combined kidney volume, (cm^3)	20.56 (5.48)	Not imputed
Birth and infant characteristics		
Gestational age at birth, (wk)	40.3 (36.4 – 42.4)	40.3 (36.4 – 42.4)
Birth weight, (g)	3534 (509)	3534 (509)
Sex boys, (%)	50.3 (464)	50.3 (464)
Missing	0	
Childhood characteristics		
Age at follow up, (y)	5.9 (5.7 – 6.6)	5.9 (5.7 – 6.6)
Height, (cm)	119 (5.2)	119 (5.2)
Weight, (kg)	22.6 (3.2)	22.6 (3.2)
Body mass index, (kg/m^2)	15.9 (1.4)	15.9 (1.4)
Body surface area, (m^2)	0.86 (0.07)	0.86 (0.07)
Kidney volume left, (cm^3)	61.4 (12.6)	Not imputed
Kidney volume right, (cm^3)	59.5 (11.7)	Not imputed
Kidney volume combined, (cm^3)	120.9 (22.1)	Not imputed
Creatinine, (µmol/l)	36.8 (4.9)	Not imputed
Cystatin C,(µg/l)	790 (74)	Not imputed
Estimated glomerular filtration rate ml/min per $1.73m^2$	120.2 (15.8)	Not imputed
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)	Not imputed
Micro albuminuria[a], (%)	7.1 (62)	Not imputed
Systolic blood pressure, (mmHg)	102.2 (7.7)	Not imputed
Diastolic blood pressure, (mmHg)	60.1 (6.3)	Not imputed

Values are means (SD), medians (95% range), or % (numbers)

[a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)
Missing values for continuous maternal characteristics are: age (N=0), height (N=0), pre-pregnancy weight (N=138), pre-pregnancy body mass index (N=138), for fetal characteristics: gestational age at measurement (N=0), estimated fetal weight (N=6), for infant characteristics: gestational age at birth (N=0), birth weight (N=0), for childhood characteristics: age (N=0), height (N=1), weight (N=1), body mass index (N=1), body surface area (N=1).

Figure S1. Scatterplots: gestational age, estimated fetal weight and fetal kidney outcomes (N=879)

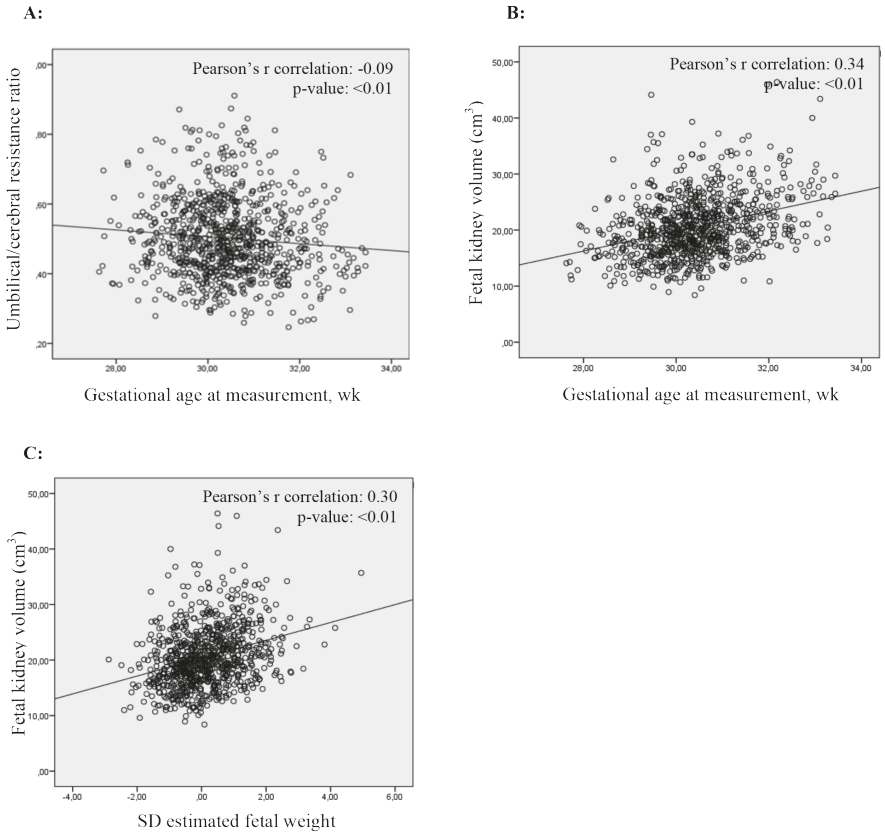


Figure S2. Scatterplots: gestational age, estimated fetal weight and fetal kidney volume/ estimated fetal weight (N=870)

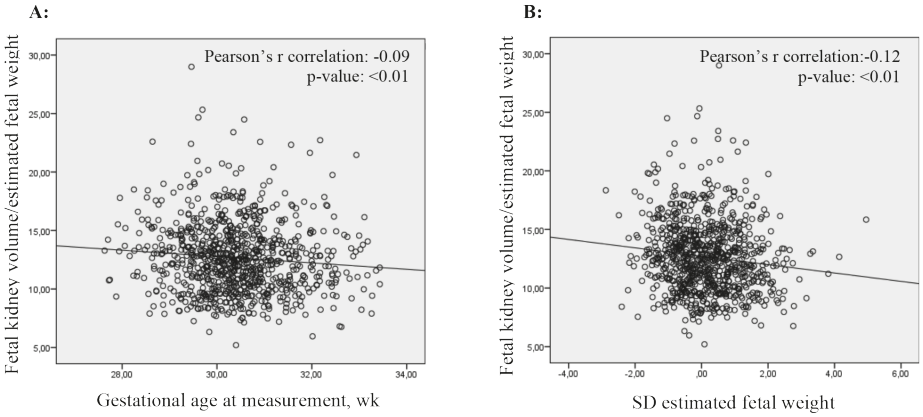


Figure S3. Scatterplots: third trimester umbilical/cerebral resistance ratio, fetal kidney volume and childhood kidney volume (N=923)

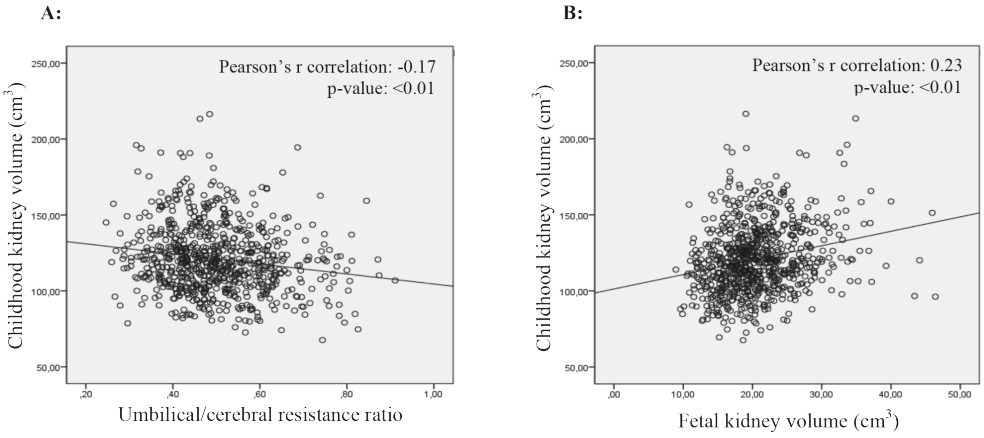


Figure S4. Scatterplots: third trimester umbilical/cerebral resistance ratio and childhood kidney outcomes (N=879)

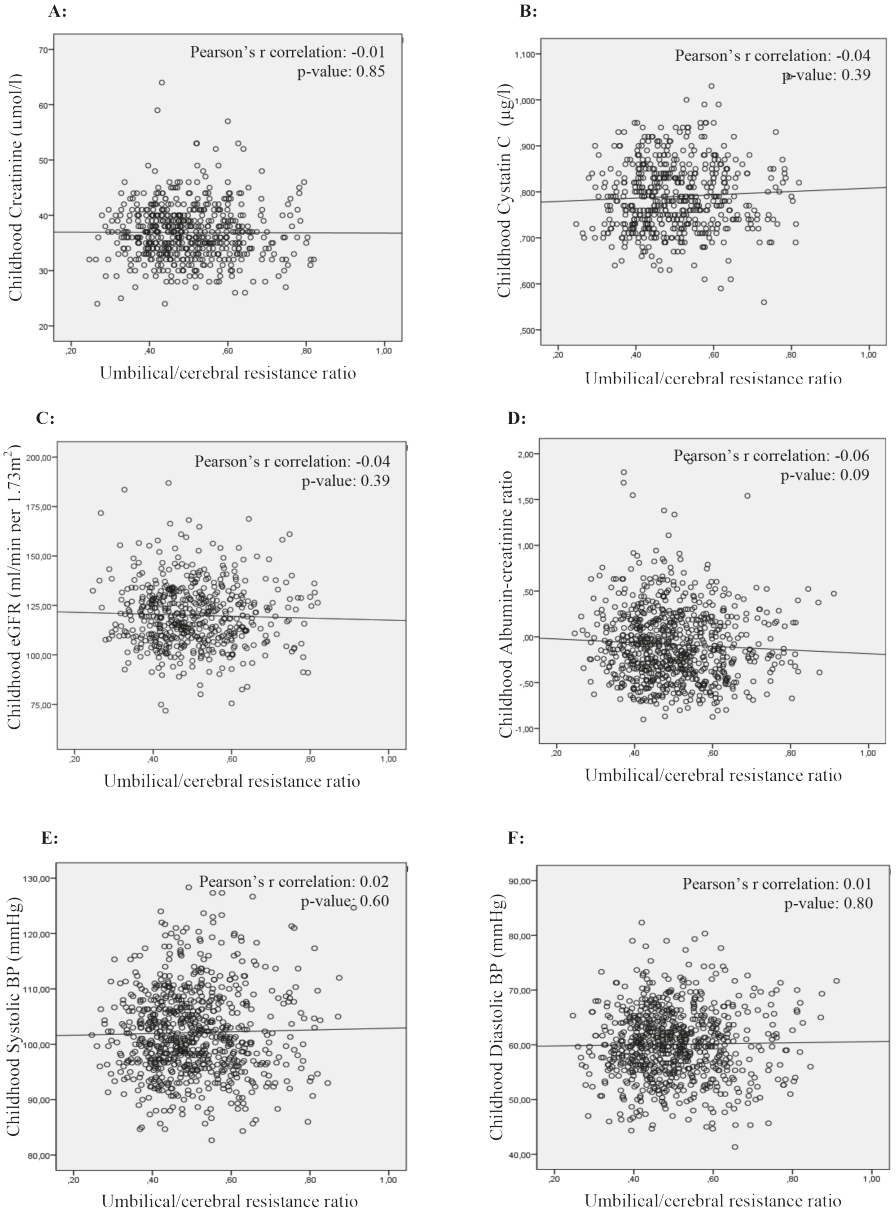


Figure S5. Scatterplots: fetal kidney volume and kidney outcomes (N=870)

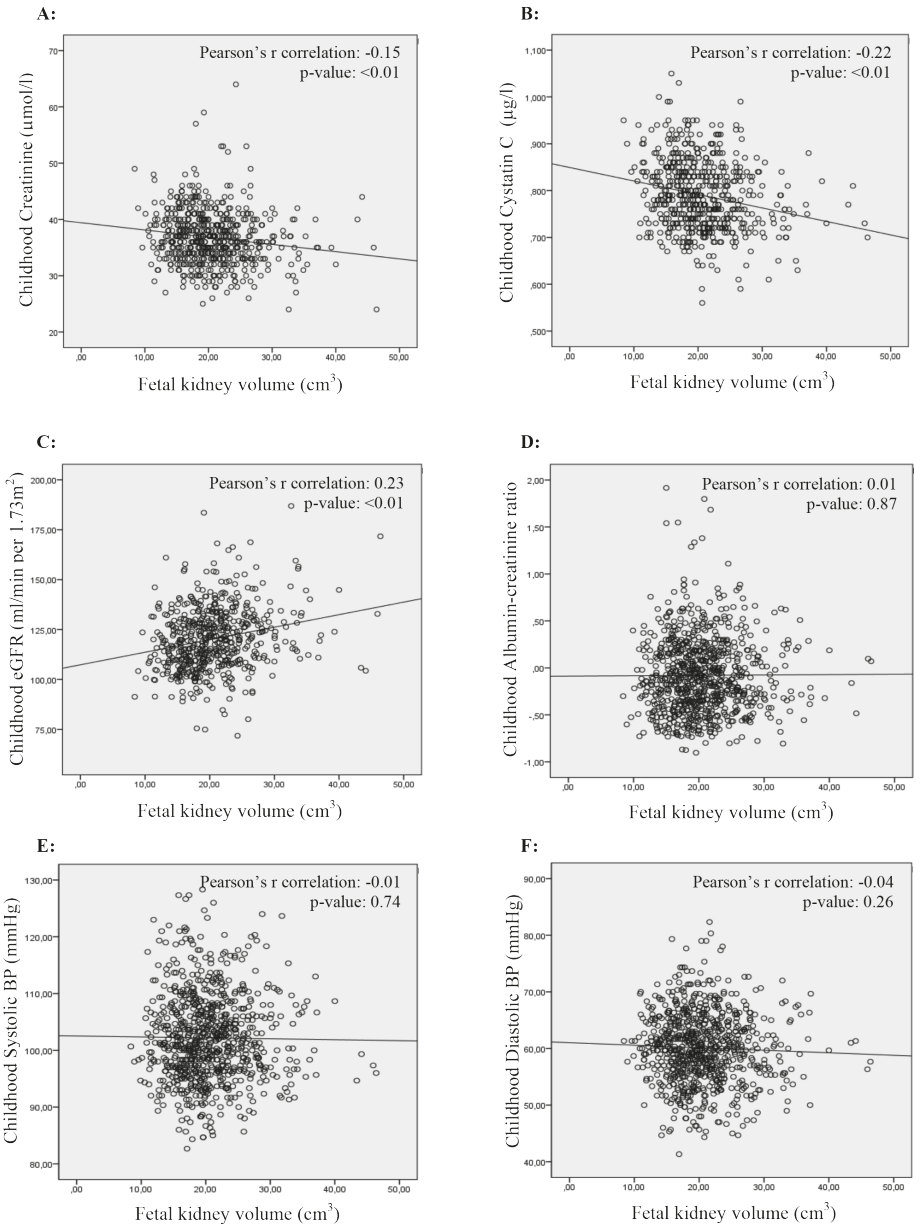
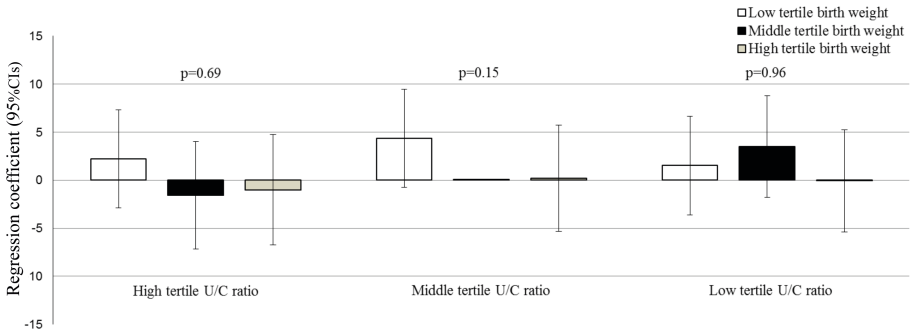
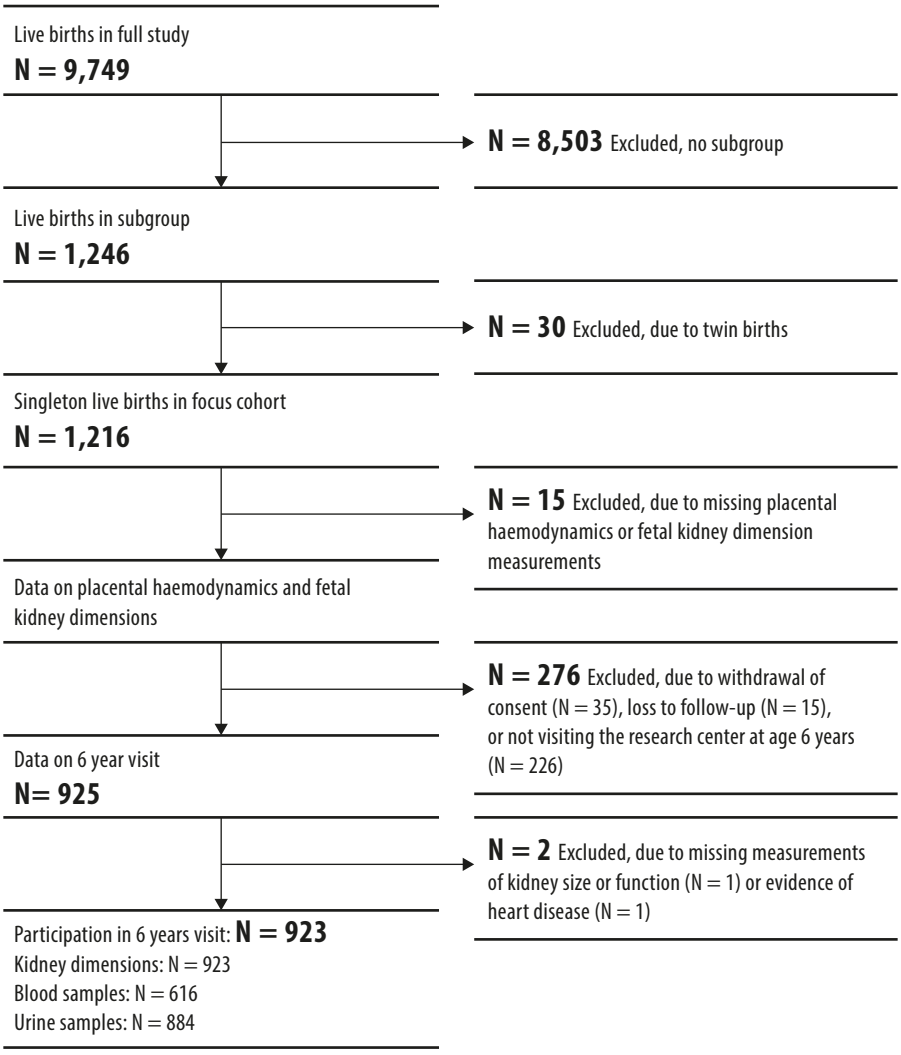


Figure S6. Associations of gestational age adjusted birth weight with childhood estimated glomerular filtration rate (N=613)



Bars represent regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the associations of gestational age adjusted birth weight with kidney outcomes in tertiles of U/C ratio. Models are adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, folic acid use during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, gestational age, current age and body surface are. P-value not significant for interaction of fetal blood flow and birth weight with all childhood kidney outcomes.

Figure S7. Flow chart of participants included in the analysis



Chapter 2.2

Fetal first trimester growth is not associated with kidney outcomes in childhood

Adapted from Pediatr Nephrol. 2017;32(4):651-658

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Abstract

Background Impaired fetal growth is associated with increased risks of kidney diseases in later life. Because of the high development rates, the first trimester might be a specific critical period for kidney outcomes. We examined the associations of fetal first trimester growth with kidney outcomes in childhood.

Methods This study was embedded in a prospective population-based cohort study among 1,176 pregnant women and their children. We measured fetal first trimester crown to rump length as growth measure among mothers with a regular menstrual cycle and a known first day of the last menstrual period. At the age of 6 years (median 5.7-6.8), we measured combined kidney volume, microalbuminuria and estimated glomerular filtration rate based on serum creatinine and cystatin C concentrations.

Results We did not observe consistent associations of fetal first trimester crown to rump length with childhood combined kidney volume, estimated glomerular filtration rate and microalbuminuria. As compared to children with a fetal first trimester crown to rump length in the highest quintile, those in the lowest quintile had a larger childhood combined kidney volume (difference 5.32 cm^3, 95% confidence interval 1.06 to 9.57), but no differences in kidney function.

Conclusion Our results do not support the hypothesis that fetal first trimester growth restriction affects kidney size and function in childhood. Further studies are needed to focus on critical periods in early life for kidney function and disease in later life.

Introduction

Chronic kidney disease may originate in the earliest phase of life.(1) It has been hypothesized that adverse environmental exposures in utero may lead to kidney developmental adaptations, including lower nephron numbers leading to glomerular hyperfiltration.(2-4) These adaptations may subsequently lead to glomerulosclerosis, impaired kidney function and increased risks of chronic kidney disease in adulthood.(5,6) This hypothesis is supported by observational studies showing associations of preterm birth or small-size for gestational age at birth with smaller kidneys and increased risk of kidney disease later in life.(7-9) Previously, we have shown that decreased second and third trimester fetal growth and lower infant growth rates are associated with smaller kidneys in childhood. Also, decreased second and third trimester fetal weight growth was associated with lower kidney function in childhood.(8) Not much is known about the influence of first trimester fetal development on later life kidney outcomes. Nephrogenesis starts around the 8th week of gestation and ceases around 36 weeks of gestation.(10) Because of the relatively high developmental rates, first trimester might also be a critical period for kidney development. Fetal first trimester crown to rump measurement is often used for determination of gestational age in obstetric care practices which suggests there is no variation in early fetal growth.(11) However, we have previously shown that in women with a regular cycle and a known first day of their menstrual period, fetal first trimester crown to rump length can be used to assess differences in embryonic growth rate.(11,12)

We assessed, in a population-based prospective cohort study among 1,176 mothers and their children, the associations of fetal first trimester crown to rump length with kidney outcomes in childhood. Kidney outcomes included combined kidney volume, estimated glomerular filtration rate (eGFR) based on creatinine and cystatin C blood levels, and microalbuminuria. Since kidney function tracks from childhood into adulthood, subclinical variations at young age might already be associated with renal impairment in later life.(7,8)

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.(13) Written informed consent was obtained from all parents. The study has been approved by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam. As previously described, inclusion in the study was aimed at early pregnancy but enrollment was allowed until birth.(14) Of the total cohort of 9,901 mothers, 1,630 mothers had an available fetal first trimester crown to rump measurement within the range of 10 weeks 0 days to 13 weeks 6 days and had a reliable gestational age based on the last menstrual

period and a regular menstrual cycle, and were therefore eligible for this study.(12) We included only mothers who gave birth to singleton live born child (N=1,619). In total, 1,176 mothers and children were included in the detailed follow-up measurements at age of 6 years. Blood and urine samples for kidney function measurements were available in 793 (67%) and 1,141 (98%) children, respectively. Missing blood samples were mainly because of lack of consent. A flow chart is given in **Figure 1**. Differences in subject characteristics between children with and without blood samples are shown in **Supplemental Table 1**. There were no differences in fetal first trimester crown to rump length between children with and without blood sample measurements.

Fetal first trimester crown to rump length

As previously described, fetal first trimester crown to rump length measurements were carried out in the gestational age range of 10 week 0 days to 13 weeks 6 days in a true mid-sagittal plane with genital tubercle and the fetal spine longitudinally in view.(12) Intraclass correlation coefficients were 0.995 and higher.(15) Information about the first day of the last menstrual cycle was obtained from the referring letter from the midwife or hospital and was confirmed at enrollment.(12) Mothers gave additional information on the regularity and duration of the menstrual cycle at the ultrasound visit. Gestational age adjusted standard deviation scores for first trimester crow to rump length were constructed, as described before.(12)

Childhood kidney outcomes

We measured left and right kidney biometrics at the median age of 6 years (90% range 5.7-6.8). Measurements were conducted as described previously.(8,16) The child was awake and calm in a standardized prone position during the ultrasound measurements. Maximal bipolar kidney length, width and depth were measured. We measured kidney width and depth at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. We calculated the kidney volume as the equation of an ellipsoid: volume (cm^3) = 0.523 x length (cm) x width (cm) x depth (cm).(17) Combined kidney volume was calculated by summing right and left kidney volume. We previously reported intra-observer and inter-observer correlation coefficients.(18)

Blood samples were drawn by antecubital venipuncture. Serum creatinine levels were measured by an enzymatic method on a Cobas c 502 analyzer (Roche Diagnostics, Germany), and serum cystatin C levels were measured using a particle-enhanced immunoturbidimetric assay on Cobas c 702 analyzer. Intra- and interassay coefficients were used a described previously.(19) eGFR was calculated according to the revised Schwartz 2009 formula(20): $eGFR_{creat}$ = 36.5 * (height (cm)/creatinine (µmol/l).(20) Additionally, eGFR based on cystatin C levels according to the Zappitelli's formula(21): $eGFR_{Cyst}$ = 75.94 / [$CysC^{1.17}$].(16) Urine creatinine (mmol/l) and urine albumin (mg/l) levels were determined on Beckman Coulter AU analyser, creatinine levels were measured according to the Jaffe method. The albumin-creatinine ratio was calculated. We defined

microalbuminuria as an albumin-creatinine ratio between 2.5 and 25 mg/mmol for boys, and for girls we used a ratio between 3.5 and 25 mg/mmol.(22)

Covariates

We obtained information on maternal age, pre-pregnancy weight, parity, ethnicity, educational level, smoking during pregnancy, alcohol consumption during pregnancy, folic acid supplementation during pregnancy, and breastfeeding by questionnaires and registries. Maternal height was measured without shoes and pre-pregnancy body mass index (BMI) was calculated. Date of birth, infant sex and birth weight were obtained from midwife and hospital registries. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and body surface area (BSA) was calculated using the DuBois formula (BSA = weight (kg) $^{0.425}$ x height (cm) $^{0.725}$ x 0.007184).(23)

Statistical analyses

First, we analysed the associations of fetal first trimester crown to rump length with childhood kidney outcomes (kidney volume, eGFR based on creatinine and cystatin C levels and microalbuminuria) by using multiple linear and logistic regression models. We used first trimester crown to rump length standard deviation scores as continuous variables and as quintiles to explore non-linear associations. We performed sensitivity analyses using tertiles of fetal first trimester growth. To explore the associations of lower and higher growth as compared to average growth, we compared the lowest and the highest tertile with the middle tertile. The fully adjusted model was adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking during pregnancy, alcohol consumption during pregnancy, folic acid supplement use, breastfeeding and current body surface area. To take into account body composition, analyses focussed on total kidney volume were indexed for body surface area. Analyses focused on eGFR were not adjusted for childhood body surface area (BSA) since height is included in the Schwartz 2009 formula. Potential confounders were based on their associations with kidney outcomes or a change in effect estimate of more than 10%. To reduce the possibility of potential bias due to missing data, we imputed missing data of the fetal, child and maternal covariates with five imputations and analysed these datasets together.(24) Additional information on the imputation procedure is given in the **Supplementary material.** All statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal, fetal and child characteristics are shown in **Table 1**. Mean fetal first trimester crown to rump length was 61.1 (SD 11.4) mm at a median gestational age of 12.4 (90%

range 11.0-13.7) weeks. At the median age of 6.0 (90% range 5.7-6.8) years, mean combined kidney volume was 119.7 (SD 22.0) cm^3, creatinine based eGFR was 119.4 (SD 15.4) ml/min per 1.73m^2 and cystatin C based eGFR was 102.8 (SD15.9) ml/min/1.73m2. Microalbuminuria was present in 7.0 % of all children. Correlations of total kidney volume and BSA-related kidney volume with estimated glomerular filtration rate based on creatinine and cystatin levels are given in **Supplemental Tables 4 and 5.** In total N=117 children were born with a small size for gestational age at birth (<10%) and N 54 (4.6%) children were born preterm (<37 weeks).

Fetal first trimester crown to rump length and kidney outcomes in childhood

Table 2 presents the analyses focused on the associations between fetal first trimester crown to rump length and kidney volume and function. Fetal first trimester crown to rump length SDS was not associated with kidney volume and eGFR. Also, there was no association of fetal first trimester crown to rump length SDS with the risk of microalbuminuria (all p-values > 0.05).

To investigate non-linearity, we created quintiles of fetal first trimester growth. **Table 2** shows that as compared to the highest quintile of fetal first trimester crown to rump length, the lowest quintile was associated with a larger childhood combined kidney volume (difference 5.32cm^3, 95% confidence interval 1.06 to 9.57), but not with eGFR and microalbuminuria. Sensitivity analyses using tertiles of fetal first trimester crown to rump length showed no associations with childhood kidney outcomes. Results of this sensitivity analyses are presented in **Supplementary table 3.** Observed data before multiple imputations are presented in **Supplementary Table 2.** The association of fetal first trimester crown to rump length with childhood kidney volume was not observed in non-imputed data. Also, analyses of non-imputed data showed no associations with other kidney outcomes in childhood. The lack of significant associations in non-imputed datasets may be due to smaller numbers. Analyses in imputed data were based on N=1,176, whereas analyses in the non-imputed data were based on N=934.

Table 1. Maternal and child characteristics (N=1,176)

	Values
Maternal characteristics	
Age, median (90% range), (yr)	31.8 (22.8-38.1)
Height, mean (SD,) (cm)	168.8 (7.1)
Pre-pregnancy weight, mean (SD), (kg)	67.0 (11.8)
Pre-pregnancy body mass index, (kg/m^2)	23.5 (3.9)
Parity, nulliparous, No. (%)	715 (60.8)

Table 1. Continued

Ethnicity, No. (%)	
European	853 (72.5)
Non -European	323 (27.5)
Educational level, No. (%)	
No higher education	525 (44.6)
Higher education	651 (55.4)
Smoking, No. (%)	
Non-smoking	881 (74.9)
Continued smoking	295 (25.1)
Folic acid supplement use, No. (%)	
No use	156 (13.3)
First 10 weeks use	376 (32.0)
Preconception use	644 (54.8)
Fetal characteristics	
Gestational age at fetal crown to rump length, median (90% range), weeks	12.4 (11.0-13.7)
First trimester fetal crown to rump length, mean (SD), (mm)	61.1 (11.4)
Birth and infant characteristics	
Males, No. (%)	570 (48.5)
Gestational age, median, (90% range) weeks	40.1 (37.1-42.0)
Birth weight, (g)	3,459.2 (549.9)
Breastfeeding, (%)	
No	92 (7.8)
Yes	1,084 (92.2)
Child characteristics	
Age, (median 990% range), (yr)	6.0 (5.7-6.8)
Height, mean (SD), (cm)	119.0 (5.5)
Weight, mean (SD), (kg)	22.9 (3.7)
Body mass index, mean (SD), (kg/m^2)	16.1 (1.7)
Kidney volume combined, (cm^3)	119.7 (22.0)
eGFR, (Schwartz, creatinine based) (ml/min per $1.73m^2$)	119.4 (15.4)
eGFR, (Zappitelli, cystatin C based), (ml/min per $1.73m^2$)	102.8 (15.9)
Microalbuminuria, No. (%)	82 (7)

Values are means (standard deviation), median (90% range) or number of subjects (valid %).

eGFR, estimated glomerular filtration rate

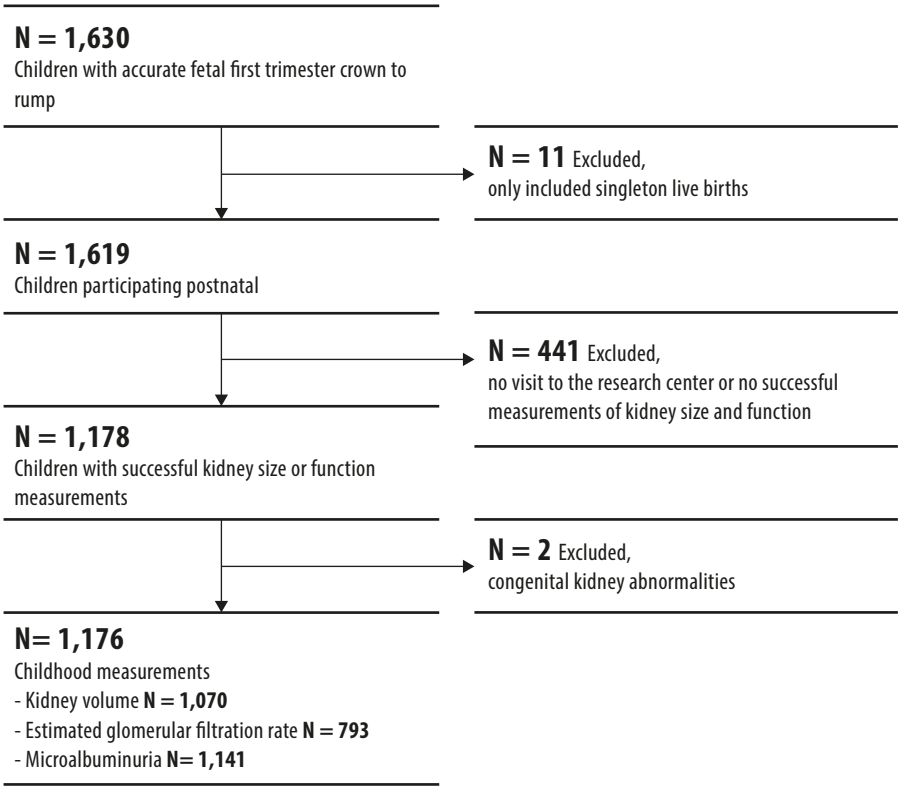
Table 2. Fetal First Trimester Growth Quintiles and Childhood Kidney Volume and Function (N=1,176)[1]

CRL quintiles in SDS	BSA-adjusted Combined kidney volume (cm^3)	GFRcreat (ml/min per 1.73m^2)	GFR cys C (ml/min per 1.73m^2)	Micro albuminuria (mg/mmol) (OR)
1 N=238	5.32 (1.06, 9.57)*	0.24 (-3.33, 3.80)	-0.67 (-4.47, 3.14)	0.60 (0.25, 1.44)
2 N=234	-0.88 (-5.14, 3.38)	-0.34 (-3.93, 3.26)	-2.69 (-6.53, 1.15)	0.92 (0.42, 2.02)
3 N=234	0.42 (-0.85, 4.68)	-0.26 (-3.83, 3.31)	0.33 (-3.48, 4.14)	1.13 (0.52, 2.44)
4 N=237	0.54 (-3.75, 4.83)	1.04 (-2.54, 4.64)	-1.11 (-4.96, 2.73)	1.41 (0.66, 2.96)
5 N=233	reference	reference	reference	reference
p-value for trend	0.10	0.58	0.83	0.51

[1]Values are regression coefficients (95% confidence interval) that reflect the difference in childhood kidney outcomes between first-trimester crown to rump length fifths, highest fifth is reference group. Model is adjusted for duration of last menstrual cycle, and child sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, breastfeeding and current childhood body surface area.

* P value < 0.05

Figure 1. Flow chart: inclusion of participants in analyses



Discussion

In this population-based prospective cohort study, we evaluated the associations of fetal first trimester crown to rump length with kidney growth and function in childhood. We did not observe consistent associations of fetal first trimester growth with kidney outcomes in childhood.

Some methodological issues need to be addressed. We used a subgroup of a large population based prospective cohort study to examine the kidney consequences of fetal first trimester growth restriction. Only mothers with a first trimester crown to rump measurement and a reliable first day of their last period were eligible, which was only a small subgroup of the full study. We used the first day of the last menstrual period in women with a regular menstrual cycle to date gestational age. Since we could not measure timing of ovulation and implantation, misclassification of gestational age could have occurred.(25) Of the eligible subgroup, 67% of all children had blood sample collections. Our results would be biased if results would differ between children with and without follow up measurements at the age of 6 years. This seems unlikely, but we cannot exclude it. Children without blood samples had lower mean birth weight as compared to children with blood samples. Our results would be biased if the associations of first trimester growth with childhood kidney function differ between children with and without blood samples. Since smaller numbers of blood samples were available in children with lower birth weight, our observed effect estimates may be underestimated. Glomerular number cannot be evaluated *in vivo* but kidney size and glomerular number correlate in pathological studies in childhood and adulthood.(26-28) We estimated glomerular filtration rate based on the Schwartz formula (20) which is based on serum creatinine levels and based on the Zappitelli formula (21) which is based on serum cystatin C levels, both are validated in pediatric populations. Estimated glomerular filtration rate was higher when calculated based on creatinine concentrations than on cystatin C concentrations. This difference is in line with previous studies.(29) However, no difference in results were observed when we used eGFR based on creatinine concentrations compared to cystatin C concentrations. In the present study we did not find differences in outcomes between those formulas. It has been suggested that serum cystatin C levels might be superior in evaluating kidney function to serum creatinine levels.(30) However, to date it is not clear which formula provides the best estimation of the eGFR.(30) We used a random urine sample to determine the albumin to creatinine ratio to evaluate microalbuminuria. Since intra-individual variation in urinary albumin secretion might be large, the variability would probably have been lower if we collected first morning void samples.(31) Finally, although we adjusted for several potential confounders, residual confounding might still be a problem because of the observational design of the study.

Growth and development rates are higher in fetal life than in childhood. Human organogenesis has the highest development rates in the first trimester. The first trimester might be a critical period for developing risk factors for diseases in later life. Similar as in previous studies focused on fetal first trimester growth in relation to birth outcomes and

cardiovascular outcomes, we used quintiles for fetal first trimester growth.(12,14) No major differences in results were observed when we used tertiles of fetal first trimester growth instead of quintiles. Longitudinal studies, including studies from the same cohort as the present study, showed associations of impaired first trimester growth with increased risks of premature birth, low birth weight and being small for gestational age at birth.(12,32,33) Also, results from this cohort study have previously shown that fetal first trimester growth restriction was associated with cardiovascular risk factors in childhood.(14) The analyses in this study were performed in a healthy population with the majority born at term and normal weight for gestational age. To the best of our knowledge, no other human studies evaluated the associations of first trimester growth with kidney function in later life. Nephrogenesis starts around week 5 of gestation, at approximately week 9 formation of the first nephrons begins.(34) Nephrogenesis continues during gestation and stops around week 34-36.(10) Against this background, we aimed to identify the role of first trimester fetal development for kidney development.

Using data from the same cohort as the present study, we have previously reported that lower fetal growth from second trimester onwards was associated with lower combined kidney volume and eGFR in childhood.(8) Also, a previous study among children born preterm showed that size for gestational age was correlated with kidney volume at the ages of 0, 3 and 18 months which implies that impaired fetal growth has consequences for kidney growth in infancy.(35) Some studies on fetal growth impairment showed slight catch up kidney growth postnatally in small for gestational age infants but results are inconclusive.(35,36) The current study extends these previous findings since it is focused on first trimester of pregnancy, a period of which little is known in relation to kidney outcomes. We did not observe consistent associations of fetal first trimester growth with kidney outcomes in childhood.

Smaller kidneys with fewer nephrons will lead to compensatory glomerular hyperfiltration, which might be beneficial in the short term but can lead to glomerulosclerosis and impaired kidney function in later life.(1) Hyperfiltration might increase renal mass while glomerular number is relatively low.(37) However, it is not possible to distinguish hypertrophy or normal growth by ultrasound. Also, it is not known when hypertrophy exactly occurs and it is difficult to determine whether glomerular enlargement is caused by glomerular hyperfiltration.(38,39) In the present study, we observed an inverse association of fetal first trimester crown to rump length and kidney volume in childhood, we cannot fully explain this finding. This finding is in line with a previous study from the same cohort which showed that the lowest tertile of gestational age-adjusted abdominal circumference in third trimester was associated with a larger relative fetal kidney volume.(40) Renal hypertrophy might be an explanation for this inverse association. More studies are needed to replicate our findings and to identify the underlying mechanisms.

We performed multiple statistical tests. However, since the kidney outcomes are highly correlated, we did not adjust analyses for multiple testing. Therefore, the observed association of the lowest of first trimester crown to rump length with kidney volume

should be interpreted carefully and may be a chance finding. Decreased fetal growth might lead to impaired kidney function by other mechanisms than smaller kidneys. For example, multiple studies suggested that epigenetic changes in response to adverse fetal exposures lead to developmental adaptations.(6,41,42)

In conclusion, we did not observe associations of fetal first trimester growth restriction with kidney size and function in childhood. These findings do not support the hypothesis that first trimester is a critical period for kidney function in later life, further studies on this hypothesis are needed.

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Imputation procedure

To reduce the possibility of potential bias associated with missing data and to maintain statistical power, missing values were imputed using the multiple imputations procedure. (1) For the multiple imputations, we used Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model for the analyses focused on the associations of early growth outcomes with kidney outcomes in childhood, we included all covariates except childhood body surface area plus maternal weight gain during pregnancy and height and weight of the child aged 6. Furthermore, we added the determinants and outcomes studied in the imputation model as prediction variables only. Determinants and outcomes were not imputed themselves. Five imputed datasets were created and analyzed together. For the conditional analyses only, we additionally imputed fetal and childhood growth characteristics using a similar imputation model.

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Table S1. Maternal and child characteristics in subjects with and without blood samples (N=1,176)

	Subjects with blood samples N=794	Subjects without blood samples N=382
Maternal characteristics		
Age, median (90% range), (yr)	31.8 (23.1-38.4)	31.6 (22.0-37.6)
Height, mean (SD,) (cm)	168.8 (7.1)	168.3 (6.8)
Pre-pregnancy weight, mean (SD), (kg)	66.9 (11.8)	67.2 (12.0)
Pre-pregnancy body mass index, (kg/m^2)	23.4 (3.8)	23.7 (4.1)
Parity, nulliparous, No. (%)	467 (58.9)	247 (64.7)
Ethnicity, No. (%)		
European	584 (73.6)	268 (70.2)
Non -European	209 (26.4)	111 (29.3)
Educational level, No. (%)		
No higher education	344 (43.4)	182 (47.6)
Higher education	449 (56.6)	200 (52.4)
Smoking, No. (%)		

Table S1. Continued

Non-smoking	590 (74.4)	292 (76.4)
Continued smoking	206 (26.6)	90 (23.6)
Folic acid supplement use, No. (%)		
No use	103 (13.0)	42 (13.5)
First 10 weeks use	239 (30.1)	99 (31.8)
Preconception use	451 (56.9)	170 (54.70
Fetal characteristics		
Gestational age at fetal crown to rump length, median (90% range), weeks	12.4 (10.6-13.9)	12.4 (10.9-13.6)
First trimester fetal crown to rump length, mean (SD), (mm)	61.3 (11.4)	60.7 (11.7)
Birth and infant characteristics		
Males, No. (%)	392 (49.4)	178 (46.6)
Gestational age, median, (90% range) weeks	40.1 (37.1-42.0)	40.2 (37.0-42.1)
Birth weight, (g)	3,490.6 (536.8)	3,394.8 (572.0)**
Ever breastfeeding, (%)		
No	61 (7.7)	27 (7.1)
Yes	732 (92.3)	355 (92.9)
Child characteristics		
Age, (median 90% range), (yr)	6.0 (5.7-7.0)	6.0 (5.7-6.5)**
Height, mean (SD), (cm)	119.2 (5.5)	1.19 (5.6)
Weight, mean (SD), (kg)	22.9 (3.6)	22.7 (3.8)
Body mass index, mean (SD), (kg/m^2)	16.0 (1.3)	16.1 (1.8)
Kidney volume combined, (cm^3)	120.1 (22.3)	N.A.
eGFR, (Schwartz, creatinine based) (ml/min per $1.73m^2$)	119.4 (15.4)	N.A.
eGFR, (Zapitelli, cystatin C based), (ml/min per $1.73m^2$)	102.8 (15.9)	N.A.
Microalbuminuria, No. (%)	52 (6.7)	30 (8.2)

Values are means (standard deviation), median (90% range) or number of subjects (valid %). T-tests were used for continuous variables, chi-square tests for categorical variables

eGFR, estimated glomerular filtration rate, N.A. not applicable

* p-value < 0.05, ** p-value < 0.01

Table S2. Fetal First Trimester Growth Quintiles and Childhood Kidney Volume and Function (N=934)[1] non-imputed analyses

CRL quintiles in SDS	Combined kidney volume (cm3)	GFRcreat (ml/min per 1.73m^2)	GFR cys C (ml/min per 1.73m^2)	Micro albuminuria (mg/mmol) (OR)
1	3.28 (-0.91, 7.47)	-0.32 (-4.63, 4.00)	0.08 (-4.89, 5.04)	0.53 (0.18, 1.54)
2	-3.52 (-7.70, 0.66)	-2.06 (-6.36, 2.25)	-3.43 (-8.39, 1.52)	0.94 (0.38, 2.34)
3	0.51 (-3.73, 4.76)	-0.80 (-5.08, 3.49)	0.85 (-4.07, 5.77)	0.99 (0.39, 2.52)
4	1.80 (-2.47, 6.06)	0.12 (-4.33, 4.57)	-0.84 (-5.97, 4.28)	1.12 (0.44, 2.85)
5	reference	reference	reference	reference
p-value for trend	0.69	0.38	0.79	0.45

[1]Values are regression coefficients (95% confidence interval) that reflect the difference in childhood kidney outcomes between first-trimester crown to rump length fifths, highest fifth is reference group. Model is adjusted for duration of last menstrual cycle, and child sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, breastfeeding and current childhood body surface area.

Table S3. Fetal First Trimester Growth Tertiles and Childhood Kidney Volume and Function (N=1,176)[1] non-imputed analyses

CRL quintiles in SDS	Combined kidney volume (cm^3)	GFRcreat (ml/min per 1.73m^2)	GFR cys C (ml/min per 1.73m^2)	Micro albuminuria (mg/mmol) (OR)
1	2.28 (-0.55, 5.10)	-0.45 (-3.20, 2.30)	-1.58 (-4.52, 1.36)	0.80 (0.43, 1.48)
2	Reference	Reference	Reference	reference
3	0.02 (-2.85, 2.89)	0.24 (-2.51, 2.99)	0.03 (-2.91, 2.98)	1.15 (0.64, 2.07)
p-value for trend	0.11	0.58	0.83	0.51

[1]Values are regression coefficients (95% confidence interval) that reflect the difference in childhood kidney outcomes between first-trimester crown to rump length fifths, highest fifth is reference group. Model is adjusted for duration of last menstrual cycle, and child sex and age at outcome measurements, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking during pregnancy, alcohol consumption during pregnancy and folic acid supplement use, breastfeeding and current childhood body surface area.

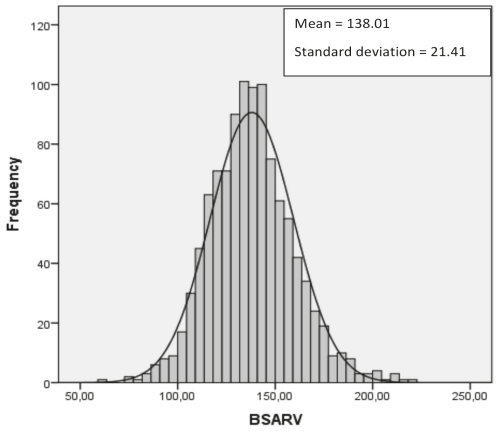
Table S4. Correlations between kidney volume and kidney function

	Combined kidney volume (cm^3)	eGFRcreat (ml/min per 1.73m^2)	eGFRcys C (ml/min per 1.73m^2)
Combined kidney volume (cm^3)	1	-	-
eGFRcreat (ml/min per 1.73m^2)	0.23**	1	-
eGFRcys C (ml/min per 1.73m^2)	0.10**	0.29**	1

Table S5. Correlations between body surface area-related kidney volume and kidney function

	BSA-related kidney volume (cm^3)	eGFRcreat (ml/min per 1.73m^2)	eGFRcys C (ml/min per 1.73m^2)
BSA-related kidney volume (cm^3)	1	-	-
eGFRcreat (ml/min per 1.73m^2)	0.28**	1	-
eGFRcys C (ml/min per 1.73m^2)	0.13**	0.29**	1

Figure S1. Normal distribution of of BSA-related renal volume



BSARV in cm^3/m^2

Chapter 2.3

Fetal and infant growth patterns and kidney function at school-age

Adapted from J Am Soc Nephrol. 2014;25(11):2607-15

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Abstract

Background Low birth weight is associated with end-stage kidney disease. To identify specific growth patterns in early life, we examined the associations of longitudinally measured fetal and infant growth with kidney function in school-age children.

Methods This study was embedded in a population-based prospective cohort study among 6,482 children followed from fetal life onwards. Fetal and childhood growth was measured during second and third trimester of pregnancy, at birth, and at 6, 12, 24, 36 and 48 months postnatally. At the age of 6 years, we measured kidney volume by ultrasound. The glomerular filtration rate was estimated using blood creatinine levels.

Results Higher gestational age adjusted birth weight was associated with higher combined kidney volume and higher estimated glomerular filtration rate (per 1 Standard deviation score (SDS) increase in birth weight 1.27 cm^3 (95 % Confidence Interval (CI) 0.61 to 1.93), and 0.78 ml/min per 1.73m^2 (95 % CI 0.16 to 1.39), respectively). Fetal weight, birth weight and weight at 6 months were positively associated with childhood kidney volume, whereas higher second trimester fetal weight was positively associated with higher glomerular filtration rate (all p-values < 0.05). Fetal and childhood length were not consistently associated with kidney function.

Conclusion Lower fetal and early infant weight growth is associated with smaller kidney volume in childhood, whereas only lower fetal weight growth is associated with lower kidney function in childhood, independent of childhood growth. Whether these associations lead to an increased risk of kidney disease needs to be further studied.

Introduction

Low birth weight is associated with higher risks of end-stage renal disease and hypertension in later life.(1-3) Clearly, low birth weight is not the causal factor per se leading to kidney diseases in later life. Birth weight is the result of various exposures and growth patterns in fetal life and the starting point of childhood growth. It has been hypothesized that especially third trimester fetal growth restriction lead to persistently smaller kidneys with a reduced number of nephrons, which may predispose the individual to kidney disease in adulthood.(4-6) This hypothesis is supported by both animal and human studies showing that kidney volume and nephron number are reduced in fetal growth restricted subjects and hypertensive subjects.(7-9) Although nephrogenesis is known to continue until 36 weeks of gestation, and to cease thereafter, not much is known about the specific critical periods and early growth patterns related to kidney function in later life.(10) Also, whether and to what extend the associations of low birth weight with chronic kidney disease are explained by preterm birth is not known.(1) Longitudinal studies suggested that the associations of low birth weight with hypertension were stronger in subjects with rapid weight gain in childhood, but results are inconclusive.(11,12) A similar growth pattern has not been identified as risk factor for kidney diseases yet. Prospective studies linking fetal and early childhood growth patterns to kidney outcomes in later life might help to identify critical periods in later life.

Prospective studies linking fetal and early childhood growth patterns to kidney outcomes in later life might help to identify early critical periods for developing impaired kidney function in later life.

Therefore, we examined, in a population-based prospective cohort study among 6,482 children followed from early fetal life onwards, the associations of birth weight, gestational age and birth weight for gestational age, and longitudinally measured fetal and early childhood growth patterns with kidney size and function at school-age. We used subclinical variation of kidney function in childhood as outcome, since they relate to kidney disease in later life.(13)

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.(14) All children were born between April 2002 and January 2006. Written informed consent was obtained from all parents. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. In total 8,305 children participated in the follow-up measurements at the age of 6 years, of whom 6,494 (78%) visited the research center with successful measurements of kidney size. We excluded children with kidney abnormalities (N=12). Blood samples for kidney function measurements were successfully obtained in 4,336 (67%) children (**Figure 1**). Missing blood samples were mainly due to non-consent.

Fetal and childhood growth measurements

Gestational age was established by first trimester ultrasound measurements.(15) Second and trimester fetal growth examinations were performed at median (90% range) gestational ages of 20.6 (18.9 – 22.9) weeks, and 30.4 (29.0 – 33.1) weeks, respectively. Fetal head circumference, abdominal circumference, and femur length were measured and estimated fetal weight was calculated using the formula by Hadlock et al (log10 EFW=1.5662-0.0108 (HC) +0.0468 (AC) +0.171 (FL) +0.00034 (HC) ^{2}-0.003685 (AC x FL)). (16) At birth, gestational age, length and weight were obtained from community midwife and hospital registries. Small and large size for gestational age was defined as the lower and the upper 5% of birth weight standard deviation score (SDS).

We measured childhood length and weight using standardized methods at the median (90% range) ages of 6.2 (5.4 to 7.5) months, 11.1 (10.2 to 12.3) months, 24.8 (23.5 to 27.6) months, 36.7 (35.6 to 39.7) months, 45.8 (44.8 to 48.0) months and 72.6 (68 to 95.5) months. All growth characteristics were converted into SDS using fetal,(15) birth weight(17) and childhood(18) reference growth charts (Growth Analyzer 3.5, Dutch Growth Research Foundation, Rotterdam, the Netherlands).

Childhood kidney outcomes

Left and right kidney biometrics were measured at the median age of 6.0 (90% range 5.7 – 7.5) years. We identified the left and right kidney in the sagittal plane along its longitudinal axis. We performed measurements of maximal bipolar kidney length, width and depth. Kidney width and depth were measured at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).(19) Combined kidney volume was calculated by summing right and left kidney volume. We previously reported good intra-observer and inter-observer correlation coefficients.(20)

Blood creatinine levels were measured with an enzymatic method on a Cobas c 502 analyzer (Roche Diagnostics, Germany). Quality control samples demonstrated intra- and inter-assay coefficients of variation ranging from 0.51% to 1.37% . Estimated glomerular filtration rate (eGFR) was calculated according to the revised Schwartz 2009 formula[21]; eGFR = 36.5 * (height (cm)/creatinine (µmol/l).(21) Urine creatinine (mmol/l) and urine albumin (mg/l) levels were determined on Beckman Coulter AU analyser, creatinine levels were measured according to the Jaffe method. We calculated the albumin-creatinine ratio. For boys microalbuminuria was defined as an albumin-creatinine ratio between 2.5 and 25 mg/mmol, for girls we used a ratio between 3.5 and 25 mg/mmol.(22)

Covariates

Information on maternal age, pre-pregnancy weight, parity, ethnicity, educational level, smoking during pregnancy, folic acid supplementation during pregnancy, and breastfeeding was obtained by questionnaires.(14) Maternal height was measured

without shoes and pre-pregnancy body mass index (BMI) was calculated (kg/m^2). Infant sex was obtained from midwife and hospital registries. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and body surface area was calculated.

Statistical analyses

First, we explored differences in characteristics between boys and girls by t-tests for continuous variables and chi-square tests for categorical variables. Second, we performed multiple linear or logistic regression models to explore the associations of birth outcomes (gestational age at birth; birth weight; gestational age adjusted birth weight) with childhood combined kidney volume, estimated glomerular filtration rate and microalbuminuria. These models were adjusted for sex and age only, and additionally for potential confounders. Potential confounders were based on their associations with kidney outcomes or a change in effect estimate of more than 10%. The associations with kidney function outcomes were additionally adjusted for kidney volume to explore whether any association was explained by kidney growth. We performed a sensitivity analysis using the lower and upper 10% as definition for small and large size for gestational age at children. Third, we assessed the associations of fetal (second and third trimester, birth) and childhood (6, 12, 24, 36, 48 and 72 months) weight and length measures with kidney outcomes at the age of 6 years using multiple linear regression models. Since fetal and childhood growth measurements at different ages are strongly correlated, we additionally performed conditional regression analyses to explore the independent associations of fetal and early childhood growth with kidney outcomes, taking account for their correlation.(23) For these analyses, we constructed length and weight variables for each timepoint, which are statistically independent from each other, by using standardized residuals obtained from regression of growth measures at a specific time point on prior growth measures.(23) As conditional growth measures are statistically independent of each other, this approach allows inclusion of growth measures simultaneously in one linear regression model. Thus, the associations of fetal and childhood growth measures at specific ages with kidney outcomes can be assessed adjusted for, and compared, with fetal and childhood growth measures at other ages.(24,25) Results from these datasets were pooled and presented in the conditional growth results. All statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal and child characteristics are shown in **Table 1.** At the age of 6.0 years (90 % range 5.7 to 7.4 years), mean (SD) total kidney volume was 120.3 (23.5) cm^3 and estimated glomerular filtration rate was 118.8 (16.4) ml/min per 1.73m^2, respectively.

Microalbuminuria was present in 7.6 % of all children. In **Table 2** all fetal, birth and childhood growth characteristics are presented. Observed data before multiple imputation are presented in **Supplementary Table S1**. Differences in subject characteristics between children with and without blood samples are shown in **Supplementary Table S2.**

Birth outcomes and childhood kidney outcomes

Figure 2 shows that 1 SD longer duration of gestational age at birth was associated with a larger combined kidney volume in childhood (p-value for trend < 0.05). As compared to term born children (38.0-39,9 weeks), children born very preterm (< 34 weeks) had a smaller kidney volume (difference: -10.48 cm^3, 95 % CI -17.74 to -3.22). Post term birth (> 42 weeks) was associated with higher estimated glomerular filtration rate in childhood (difference: 3.80 ml/min per 1.73m^2, 95 % 1.39 to 6.21). Gestational age at birth was not associated with the risk of childhood microalbuminuria. Birth weight not adjusted for gestational age was positively associated with combined kidney volume and with estimated glomerular filtration rate (p values for trend < 0.01) in childhood. Gestational age adjusted birth weight was positively associated with childhood combined kidney volume and estimated glomerular filtration rate (p values for trend < 0.05). As compared to children appropriate for gestational age, children born small for gestational age had smaller kidney volume (-3.74 cm^3 (95 % CI -6.89 to -0.89)). We performed a sensitivity analysis using the lower and upper 10% as definition for small and large size for gestational age at children. These analyses showed the same results as compared to 5% cut off (**Figure S1**). Birth weight was not associated with risk of microalbuminuria. Results from models adjusted for sex and age only were similar with some stronger effect estimates (**Supplementary Table S3)**. Results from analyses on the data before imputation are shown in **Supplementary Table S4.** After additional adjustment for childhood kidney volume, the associations of birth outcomes with kidney function attenuated towards non-significant (results presented in **Supplementary Table S5)**.

Fetal and early childhood growth and kidney outcomes

We explored whether the associations of fetal and early childhood growth characteristics with childhood kidney function outcomes were independent from growth measures at other ages using conditional growth analyses. **Figure 3** shows that higher second and third trimester fetal weight, birth weight and weight at the age of 6 months were all, independently associated with a larger combined kidney volume in childhood (all p values < 0.05). Also, higher second trimester and third trimester fetal weight tended to be independently from weight at other ages, associated with higher estimated glomerular filtration rate (p value < 0.05 and 0.05 respectively). When we additionally adjusted the models focused on estimated glomerular filtration rate for childhood kidney volume, we observed that these associations attenuated towards non-significant (Results shown in **Supplementary Figure S3**). Conditional analyses for fetal and childhood length growth did not show consistent associations with childhood kidney volume and function outcomes (Results shown in **Supplementary Figure S2)**.

Results from the normal multivariate regression models, which do not take into account growth measures at other ages, showed that larger length at different ages is associated with higher estimated glomerular filtration rate and an increased risk of microalbuminuria (all p values < 0.05). Higher weight at different ages is associated with larger kidney volume and higher estimated glomerular filtration rate (all p values < 0.05), but with the risk of microalbuminuria (basic and adjusted models are shown in **Supplementary Tables S6 and S7).**

Table 1.

	Boys N=3,257	Girls N=3,235	P value
Maternal characteristics			
Age, (yr)	31.1 (21.2 to 38.8)	31.0 (21.2 to 38.5)	0.284
Height, (cm)	167.6 (7.2)	167.5 (7.6)	0.129
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)	23.8 (4.3)	0.075
Parity ≥1, (%)	44.6 (1452)	43.1 (1390)	0.304
Ethnicity, (%)			0.104
European	61.2 (3964)	62.1 (2001	
Non -European	38.8 (2518)	37.9 (1224)	
Educational level, (%)			0.188
No higher education	53.9 (1756)	55.4 (1788)	
Higher education	46.1 (1501)	44.6 (1437)	
Smoking, (%)			0.222
Non-smoking	73.5 (2066)	75.9 (2133)	
Continued smoking	26.5 (745)	24.1 (678)	
Folic acid supplement use, (%)			0.086
No	25.7 (570)	24.5 (552)	
Preconceptional	42.0 (929)	44.5 (1001)	
Postconceptional	32.3 (715)	30.9 (696)	
Infant characteristics			
Gestational age, (week)	40.1 (37.0 to 42.1)	40.1 (36.9 to 42.0)	0.069
Birth weight, (g)	3488 (569)	3362 (534)	<0.001
Breastfeeding, (%)			0.805
No	7.5 (188)	7.8 (252)	
Yes	92.5 (2325)	92.2 (2973)	
Child characteristics			
Age, (years)	6.0 (5.7 to 7.5)	6.02 (5.7 to 7.2)	0.013
Height, (cm)	119.9 (6.1)	119.0 (6.0)	<0.001

Table 1. Continued

Weight, kg	23.4 (4.1)	23.2 (4.5)	0.011
Body mass index, (kg/m^2)	16.2 (1.8)	16.3 (2.0)	0.381
Kidney volume combined, (cm^3)	122.3 (24.2)	118.3 (22.6)	<0.001
eGFR, ml/min per 1.73m^2	118.7 (16.2)	118.9 (16.7)	0.611
Microalbuminuria (%)	6.8 (217)	8.3 (256)	0.204

Values are means (standard deviation), median (90% range) or percentage (number). T-tests were used for continuous variables, chi-square tests for categorical variables.

eGFR, estimated glomerular filtration rate

Table 2. Fetal and early childhood growth characteristics (N=6,482)

Growth characteristics	Boys N=3,257	Girls N=3,235	P value
Fetal growth			
Second trimester			
Gestational age (weeks)	20.6 (18.9 to 22.9)	20.5 (18.9 to 22.7)	< 0.001
Femur length (mm)	33.5 (3.6)	33.5 (3.6)	0.824
Estimated fetal weight (g)	387 (98)	378 (91)	0.000
Third trimester			
Gestational age (weeks)	30.4 (29.0 to 32.4)	30.3 (28.8 to 32.3)	0.001
Femur length (mm)	57.4 (3.1)	57.6 (3.1)	0.002
Estimated fetal weight (g)	1633 (260)	1618 (268)	0.033
Birth			
Gestational age (weeks)	40.1 (37.0 to 42.1)	40.1 (36.9 to 42.0)	0.075
Length (cm)	50.6 (2.4)	49.9 (2.3)	<0.001
Weight (g)	3488 (569)	3362 (534)	<0.001
Early childhood growth			
6 months			
Age (months)	6.2 (5.4 to 7.5)	6.2 (5.5 to 7.5)	0.650
Length (cm)	68.5 (2.5)	66.7 (2.4)	<0.001
Weight (g)	8176 (903)	7590 (832)	<0.001
12 months			
Age (months)	11.1 (10.2 to 12.3)	11.1 (10.2 to 12.3)	0.621
Length (cm)	75.1 (2.5)	73.5 (2.5)	<0.001
Weight (g)	9970 (1061)	9326 (991)	<0.001
24 months			
Age (months)	24.8 (23.5 to 27.6)	24.8 (23.6 to 27.4)	0.347
Length (cm)	88.9 (3.3)	87.7 (3.4)	<0.001

Table 2. Continued

Weight (kg)	13.2 (1.5)	12.7 (1.5)	<0.001
36 months			
Age (months)	36.7 (35.6 to 39.8)	36.7 (35.5 to 39.6)	0.174
Length (cm)	97.9 (3.8)	96.8 (3.8)	<0.001
Weight (kg)	15.4 (1.8)	15.0 (1.9)	<0.001
48 months			
Age (months)	45.8 (44.8 to 48.0)	45.8 (44.7 to 47.9)	0.334
Length (cm)	103.7 (4.1)	102.8 (4.2)	<0.001
Weight (kg)	17.1 (2.2)	16.7 (2.3)	<0.001
72 months			
Age (months)	72.6 (44.7 to 48.0)	72.6 (69.0 to 87.3)	0.013
Length (cm)	119.9 (6.1)	119.0 (6.0)	<0.001
Weight (kg)	23.4 (4.1)	23.1 (4.5)	0.011

Values are means (standard deviation), median (90% range) or percentage (number). T-tests were used for comparison between boys and girls.

Figure 1. Flow chart: inclusion of participants in analyses

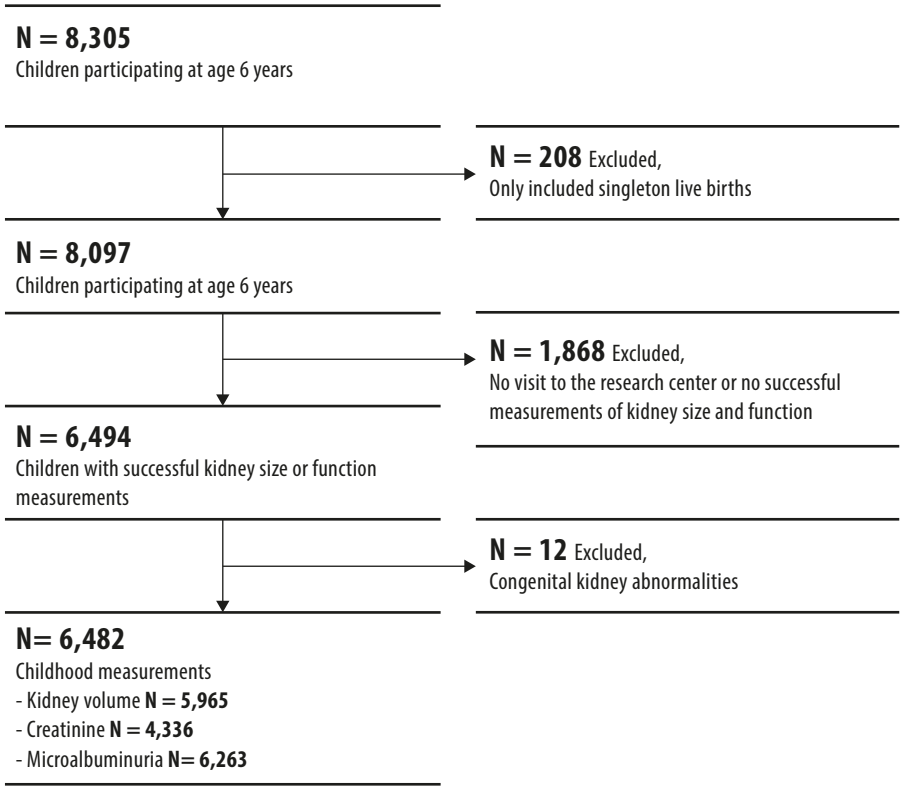
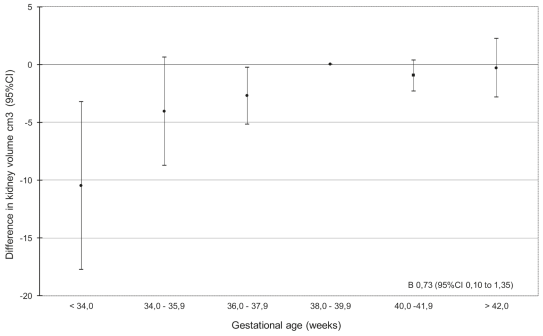
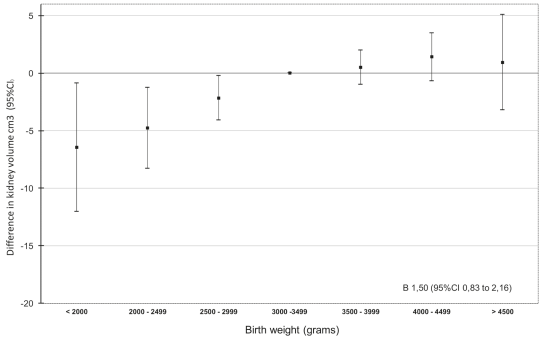


Figure 2. Birth outcomes are associated with kidney outcomes (N=6,482)

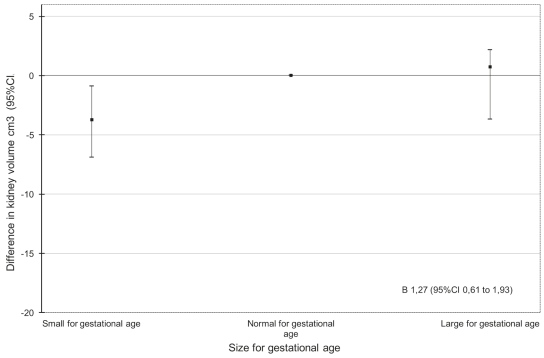
A



B



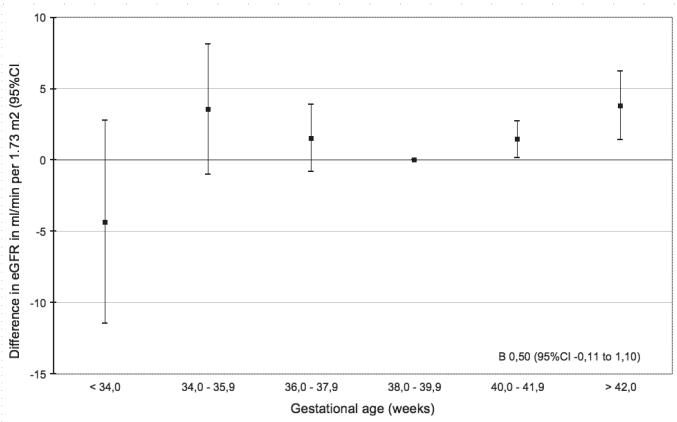
C



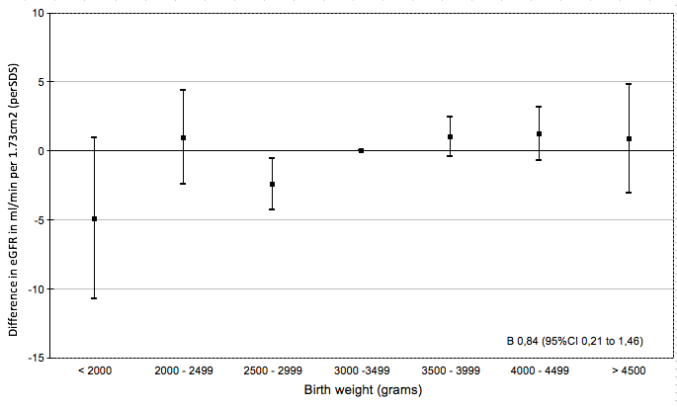
Bars represent regression coefficients (95% CI) based on multiple regression models and reflect the difference for each outcome for the birth weight or gestational age group, as compared to the reference group. Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age and body surface area. eGFR, estimated glomerular filtration rate. β for trend (95% confidence interval) Results from models adjusted for sex and age only are given in **Table S3**. Results for models additionally adjusted for kidney volume are given in **Table S5**.

Figure 2. Continued

D



E



F

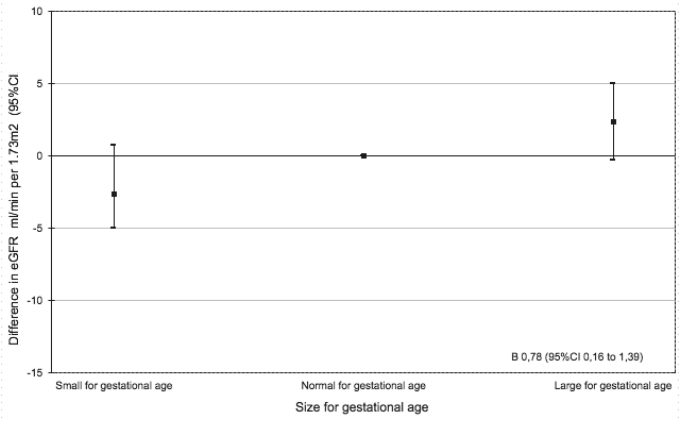
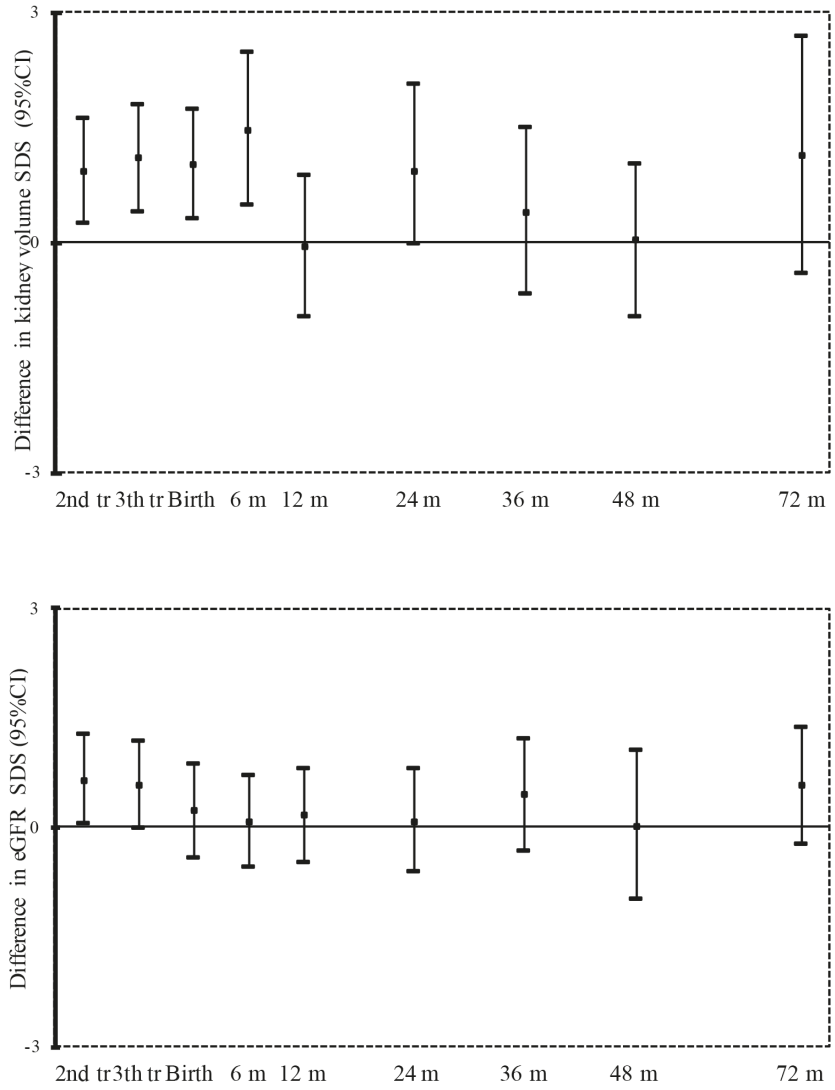


Figure 3. Fetal and childhood weight are associated with kidney outcomes at the age of 6 years (N=6,482)



Effect estimates ((95% Confidence Interval) represent regression coefficients based on multiple regression models and reflect the difference per 1 SD increase in standardized residual score for (estimated) weight measures at different time points (see details for conditional regression models in Supplementary material). Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age and body surface area. eGFR, estimated glomerular filtration rate. Results for models additionally adjusted for kidney volume are given **Figure S3.**

Discussion

In this population-based prospective cohort study, we aimed to identify critical periods during fetal life and childhood for development of impaired kidney function. We observed that preterm birth was associated with smaller kidney volume, and smaller size for gestational at birth was associated with a smaller kidney volume and lower estimated glomerular filtration rate. Higher fetal weight, birth weight and weight at 6 months were independently positively associated with childhood kidney volume, whereas only higher fetal weight was independently positively associated with higher estimated glomerular filtration rate. Fetal life and early infancy may be critical periods for kidney function in later life.

Strengths and limitations

A major strength of our study is its prospective design from fetal life onwards within a large population-based cohort. Our analyses were based on more than 6,000 children with kidney volume and function measurements available. The study population comprised a multi-ethnic group, comprising almost 40% of non-Western children. The largest non-Western groups were Moroccan, Surinamese, and Turkish children, which are the largest ethnic minority groups in the Netherlands.(14) Whether these results are generalizable to other populations should be further studied. Repeated fetal and childhood growth measures were available, which enabled us to identify critical growth periods that might influence kidney volume and function. We did take account for the repeated growth measures by performing conditional growth analyses. Of all children, more than 80% did participate in the kidney follow up studies. Since not all participants in the study gave consent for collecting blood samples, 67% of all children provided useful blood samples for measurements of creatinine levels. There were no differences in birth outcomes, fetal and early childhood growth measures between children with and without blood samples. However, children without blood samples had smaller kidney dimensions at the age of six years. These differences might have led to an underestimation of the observed associations. Furthermore, statistical power might have been reduced due to the missing data. We used kidney size as a measure of kidney development, since nephron number cannot be studied *in vivo*. Kidney size is correlated with the number of glomeruli and can be used in epidemiological studies as measure of kidney development.(4) However, glomerular enlargement due to hyperfiltration may attenuate the differences in

childhood kidney volume and may lead to an underestimation of the associations of interest.(26) In the present study, estimated glomerular filtration rate was based on one random creatinine value. This is a limitation to the study. However, measurement error due to only one creatinine value is likely to be random and might have underestimated the observed differences. Mean values for estimated glomerular filtration rate and overall prevalence of microalbuminuria are in line with results of previous population based-studies in children of the same age range.(27,28) We used the urine albumin-creatinine ratio to evaluate albuminuria in a random urine sample.(29) Since the within subject variation in urinary albumin excretion is large, the variability would probably be lower if we collected first morning void samples instead of random during the day.(30) Finally, although we had information about a large number of confounders, residual confounding might still be an issue due to the observational design of the study.

Fetal and early childhood growth and childhood kidney

To the best of our knowledge, the current study is the largest population-based prospective cohort study from fetal life onwards focused on the associations of early growth with kidney function at school-age. Several studies showed associations of low birth weight with renal disease and hypertension in later life.(1-3) Results from a systematic review based on 31 studies among 49,387 subjects showed that low birth weight is associated with a 1,73 higher risk of kidney disease.(1) Studies focused on the associations of birth weight with predictors of renal disease at younger ages are scarce. A Norwegian study among 7,457 subjects showed that young adults born with a small size for gestational age had an increased risk of low-normal creatinine clearance compared with children with appropriate birth weight for gestational age.(31) In contrast, a Dutch study among 82 severely growth restricted children, did not show associations of birth weight with renal function in young adulthood.(8) A study among 86 children aged 9 to 12-year-old found no differences in kidney volume or function between preterm born term children, children born small for gestational age and children born appropriate for gestational age.(32) A study among 73 9.5 year-old children reported a positive association of birth weight with estimated glomerular filtration rate.(33) An observational cohort study among 426 children with congenital kidney disease showed that low birth weight and being small for gestational age are risk factors for poor growth outcomes in children with mild and moderate chronic kidney disease.(34) We observed that that younger gestational age and lower gestational age adjusted birth weight are associated with both a lower kidney volume and a lower estimated glomerular filtration rate in school-age children. These findings are in line with previous studies showing that low birth weight for gestational age are associated with kidney function in childhood.(13) We are not aware of other large population-based studies focused on the associations of birth weight, taking into account gestational age, with kidney function at young age.

Since birth weight is the result of various exposures and growth patterns in fetal life and the starting point of childhood growth, longitudinal fetal and early childhood growth patterns might be stronger associated with increased risk of renal disease in

later life.(13) Longitudinal studies linking fetal and early childhood growth patterns to kidney outcomes in later life might help to identify critical periods in later life. A study among 50 children aged 7.6 years, showed that children with low birth weight and slow growth rates had slightly lower glomerular filtration rate as compared to children with appropriate growth.(35) It has been postulated that rapid weight gain and obesity in childhood are associated with an increased risk of hypertension and type 2 diabetes in adulthood, which are risk factors for kidney disease.(11,12) In a retrospective cohort study among 80 children with proteinuric kidney disease, obese children who were born preterm had an increased risk of progression of kidney disease as compared to obese who were born at term, suggesting an additive risk of obesity and prematurity in the risks for progression of kidney disease.(36) Experimental studies have shown that adequate feeding of low birth weight rats could restore nephron numbers to normal and overfeeding of these rats led to low nephron numbers, hypertension and renal injury. Overfeeding of normal birth weight rats had also adverse effects.(37-39) In line with these findings we observed that lower fetal weight gain and lower early infancy weight gain lead to an impaired kidney growth, whereas only lower fetal weight gain leads to impaired kidney function. The results from our present study suggest that both fetal life and early infancy may be critical periods for the development of kidney diseases in later life. To our knowledge, this is the first population-based study, which shows that fetal and early growth are associated with kidney function in childhood. The present study was focused on the associations of fetal and childhood growth in relation to kidney outcomes. Previous studies, including those from the same cohort as the present study, suggested that children with fetal growth restriction, followed by infant growth acceleration have higher blood pressure.(40,41) Both fetal growth in later pregnancy and growth in early infancy seem to be critical periods for childhood blood pressure.

The underlying mechanisms for the associations between early growth and kidney function are not known. An adverse fetal growth may lead to a persistently reduced congenital nephron number and smaller kidney volume, with glomerular hyperfiltration and subsequent glomerulosclerosis. These adaptations may predispose individuals to impaired renal function and hypertension.(4,5) Specifically third trimester growth is important in kidney development, since approximately 60 % of the total nephron number develops during the third trimester of gestation.(42) In line with this hypothesis, we observed that fetal growth was positively associated with kidney growth and estimated glomerular filtration rate. We also observed that after additional adjustment for kidney volume, most associations of birth outcomes focused on kidney function outcomes attenuated. Other mechanisms may also be involved. Experimental studies showed alterations in the renin angiotensin system in experimentally induced intrauterine growth restricted individuals at adult age, these differences were not present at younger age.(43) Several markers of the renin angiotensin system were increased in intrauterine growth restricted subjects with hypertension.(43) Future studies are needed to identify possible underlying mechanisms. The observed effect estimates in the present study are small and without clinical significance at young age. However, they are important

from a etiological developmental perspective. The presented results suggest that birth characteristics, fetal and early childhood growth influence kidney function throughout the life course. Whether and to what extend the observed variations in kidney function relate with kidney disease in later life is not known yet. Tracking of blood pressure from childhood to adulthood has been described previously,(44) but is less clear for kidney function. Studies showing that lower estimated glomerular filtration rate relates with renal disease development many decades later, suggest that subclinical variation in kidney function precede the developmental of renal disease.(13)

Conclusion and perspectives

In conclusion, lower fetal and lower early infant weight gain lead to smaller kidneys, whereas and only lower fetal weight gain led to a lower estimated glomerular filtration rate. Although the observed effect estimates are small, and without direct individual clinical consequence, they suggest that suboptimal early growth affects kidney function in later life. Future studies are needed to evaluate the long-term consequences of the observed associations.

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Imputation procedure

To reduce the possibility of potential bias associated with missing data and to maintain statistical power, missing values were imputed using the multiple imputations procedure.(1) For the multiple imputations, we used Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model for the analyses focused on the associations of early growth outcomes with kidney outcomes in childhood, we included all covariates except childhood body surface area plus maternal weight gain during pregnancy and height and weight of the child aged 6. Furthermore, we added the determinants and outcomes studied in the imputation model as prediction variables only. Determinants and outcomes were not imputed themselves. Five imputed datasets were created and analyzed together. For the conditional analyses only, we additionally imputed fetal and childhood growth characteristics using a similar imputation model.

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Table S1. Maternal and child characteristics for non-imputed and imputed data

	Non-imputed	Imputed
Maternal characteristics		
Age, (yr)	31.1 (21.2 to 38.8)	-
Height, (cm)	167.5 (7.4)	167.6 (7.4)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)	23.6 (4.3)
Parity ≥1, (%)	43.6 (2730)	44.0 (2852)
Ethnicity, (%)		
European	61.5 (3882)	61.2 (3964)
Non -European	38.5 (2428)	38.8 (2518)
Educational level, (%)		
No higher education	53.3 (3142)	54.5 (3534)
Higher education	46.7 (2750)	45.5 (2948)
Smoking, (%)		
No	74.7 (4199)	74.9 (4855)
Yes	25.3 (1423)	25.1 (1627)
Folic acid supplement use, (%)		
No	25.7 (1122)	23.4 (1601)
Preconceptional	43.2 (1930)	38.8 (2515)
Postconceptional	31.6 (1411)	37.8 (2189)
Infant characteristics		
Gestational age, (weeks)	40.1 (37.0 to 42.1)	-
Birth weight, (g)	3425 (558)	-
Breastfeeding, (%)		
No	7.6 (382)	7.9 (511)
Yes	92.4 (4662)	92.1 (5971)
Child characteristics		
Age, (years)	6.0 (5.7 to 7.5)	-
Height,(cm)	119.5 (6.1)	-
Weight, (kg)	23.3 (4.3)	-
Body mass index, (kg/m^2)	16.2 (1.9)	-
Kidney volume combined, (cm^3)	120.3 (23.5)	-
eGFR, ml/min per (1.73m^2)	118.8 (16.4)	-
Microalbuminuria (%)	6.8 (217)	-

Values are means (standard deviation), median (90% range) or percentage (number).

eGFR, estimated glomerular filtration rate

Table S2. Subject characteristics of subjects with and without childhood blood samples

	With blood sample (N=4,336)	Without blood sample (N=2,146)	P value
Maternal characteristics			
Age, (yr)	31.2 (21.4 to 38.7)	30.8 (20.9 to 38.4)	<0.001
Height, (cm)	167.7 (7.5)	167.1 (7.3)	0.002
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)	23.6 (4.4)	0.969
Parity ≥1, (%)	44.9 (1875)	41.0 (855)	0.003
Ethnicity, (%)			0.409
European	60.2 (2610)	60.8 (1272)	
Non -European	38.1 (1608)	39.2 (820)	
Educational level, (%)			0.007
No higher education	52.1 (2053)	55.8 (1089)	
Higher education	47.9 (1888)	44.2 (862)	
Smoking, (%)			0.763
No	75.0 (2820)	74.0 (1379)	
Yes	24.9 (336)	8.7 (162)	
Folic acid supplement use, (%)			0.432
No	24.8 (739)	25.8 (383)	
Preconceptional	31.2 (930)	32.4 (481)	
Postconceptional	44.0 (1309)	41.8 (621)	
Infant characteristics			
Gestational age, (weeks)	40.1 (36.9 to 42.0)	40.1 (36.9 to 42.0)	0.685
Birth weight, (g)	3439 (548)	3397 (566)	0.004
Breastfeeding, (%)			0.705
No	7.5 (255)	7.8 (127)	
Yes	92.5 (3156)	92.2 (1506)	
Child characteristics			
Age, (years)	6.0 (5.7 to 7.5)	6.0 (5.7 to 7.1)	<0.001
Height, (cm)	119.7 (6.0)	118.9 (6.0)	<0.001
Weight, (kg)	23.4 (4.3)	23.1 (4.3)	0.009
Body mass index, (kg/m^2)	16.2 (1.9)	16.2 (2.0)	0.838
Kidney volume combined, (cm^3)	120.8 (23.6)	119.3 (23.1)	0.021
eGFR, ml/min per 1.73m^2	118.8 (16.4)	-	-
Microalbuminuria (%)	7.7 (324)	7.3 (149)	0.585

Values are means (standard deviation), median (90% range) or percentage (number)
T-tests were used for continuous variables, chi-square tests for categorical variables.
eGFR, estimated glomerular filtration rate

Table S3. Associations of birth outcomes with kidney volume and function at the age of 6 years: basic model

Birth characteristics	Difference (95% CI) in combined kidney volume (cm^3)	Difference (95% CI) in eGFR (ml/min per 1.73m^2)	Risk (Odds Ratio (95%CI)) of micro albuminuria
Gestational age N=6,482			
< 34 weeks	-6.36**	-2.12	0.81
N=98	(-11.15 to -1.56)	(-6.24 to 1.99)	(0.35 to 1.88)
34.0 – 35.9 weeks	-9.08**	2.85	0.64
N=102	(-13.68 to -4.48)	(-1.06 to 6.76)	(0.26 to 1.60)
36.0 – 37.9 weeks	-3.58**	0.92	0.93
N=514	(-5.83 to -1.32)	(-1.01 to 2.85)	(0.64 to 1.36)
38.0 - 39.9 weeks	Reference	Reference	Reference
N=2,199			
40.0-41.9 weeks	0.11	1.62**	1.01
N=3,131	(-1.17 to 1.39)	(0.54 to 2.71)	(0.82 to 1.24)
≥ 42.0 weeks	0.64	3.82**	1.02
N=438	(-1.76 to 3.04)	(1.77 to 5.88)	(0.69 to 1.52)
Trend (SDS)	1.60	0.86	1.02
	(1.02 to 2.17)	(0.36 to 1.36)	(0.93 to 1.13)
P value	< 0.001	0.001	0.656
Birth weight N=6,482			
<2,000 grams	-10.85**	-7.21**	0.65
N=79	(-16.07 to -5.63)	(-11.95 to -2.48)	(0.24 to 1.80)
2,000-2,499 grams	-8.74**	0.39	0.79
N=213	(-11.97 to -5.50)	(-2.49 to 3.27)	(0.44 to 1.42)
2,500-2,999 grams	-4.57**	-1.98*	1.17
N=958	(-6.31 to -2.83)	(-3.51 to -0.45)	(0.89 to 1.54)
3,000-3,499 grams	Reference	Reference	Reference
N=2,241			
3,500-3,999 grams	4.14**	1.28*	0.85
N=2,072	(2.77 to 5.55)	(0.09 to 2.47)	(0.67 to 1.07)
4,000-4,499 grams	7.46**	1.81*	1.02
N=756	(5.55 to 9.37)	(0.19 to 3.43)	(0.75 to 1.40)
≥ 4,500 grams	11.69**	2.14	1.26
N=163	(8.01 to 15.37)	(-1.07 to 5.34)	(0.71 to 2.23)
Trend (SDS)	4.64	1.20	0.98
	(4.07 to 5.20)	(0.71 to 1.70)	(-0.89 to 1.08)
P value	< 0.001	<0.001	0.658
Birth weight for gestational age N=6,482			
Small for gestational age	-9.05**	-2.34	0.79
N=321	(-11.65 to -6.56)	(-4.69 to 0.02)	(0.49 to 1.26)
Normal for gestational age	Reference	Reference	Reference
N=5,779			

Table S3. Continued

Large for gestational age N=321	9.05 (6.42 to 11.68))	2.26* (0.05 to 4.47)	0.95 (0.61 to 1.49)
Trend (SDS)	4.52 (3.96 to 5.08)	0.96 (0.47 to 1.45)	0.93 (0.85 to 1.03)
P value	< 0.001	< 0.001	0.934

Values are regression coefficients (95% Confidence Interval) based on multiple regression models and reflect the difference for each outcome for the birth weight or gestational age group, as compared to the reference group. Values for analyses on microalbuminuria are Odds ratios (95% Confidence Interval) and reflect the Odds ratio for each outcome for the birth weight or gestational age group, as compared to the reference group. Models are adjusted for sex and current age.

eGFR, estimated glomerular filtration rate

*P<0.05, **P<0.01

Table S4. Associations of birth outcomes with kidney outcomes at the age of 6 years with non-imputed data

Birth characteristics	**Combined kidney volume (cm^3)**	**eGFR ($ml/min\ per\ 1.73m^2$)**	**Micro albuminuria (Odds Ratio)**
Gestational age N=6,482			
< 34 weeks N=98	-21.84** (-34.52 to -9.16)	-4.82 (-16.70 to 7.05)	1.68 (0.20 to 13.91)
34.0 – 35.9 weeks N=102	-5.36 (-11.28 to 0.56)	3.44 (-2.29 to 9.18)	0.90 (0.27 to 3.00)
36.0 – 37.9 weeks N=514	-2.52 (-5.50 to -0.46)	1.22 (-1.66 to 4.10)	1.06 (0.59 to 1.90)
38.0 - 39.9 weeks N=2,199	Reference	Reference	Reference
40.0-41.9 weeks N=3,131	-1.03 (-2.62 to 0.56)	1.12 (-0.41 to 2.66)	1.16 (0.85 to 1.59)
≥ 42.0 weeks N=438	-0.64 (-3.51 to 2.24)	2.65 (-0.15 to 5.45)	1.39 (0.83 to 2.34)
Trend (SDS)	0.83 (0.03 to 1.63)	0.36 (-0.41 to 1.13)	1.03 (0.88 to 1.20)
P values	0.041	0.360	0.730
Birth weight N=6,482			

Table S4. Continued

<2,000 grams N=79	-13.20** (-21.76 to -4.64)	-8.09 (-17.18 to 0.99)	0.49 (0.07 to 3.78)
2,000-2,499 grams N=213	-3.92 (-8.53 to 0.70)	2.49 (-2.07 to 7.06)	0.98 (0.41 to 2.36)
2,500-2,999 grams N=958	-1.01 (-3.34 to 1.33)	-2.14 (-4.41 to 0.14)	0.97 (0.63 to 1.49)
3,000-3,499 grams N=2,241	Reference	Reference	Reference
3,500-3,999 grams N=2,072	0.55 (-1.18 to 2.28)	1.14 (-0.52 to 2.80)	0.80 (0.58 to 1.12)
4,000-4,499 grams N=756	1.84 (-0.56 to 4.23)	1.14 (-1.10 to 3.37)	0.69 (0.42 to 1.12)
≥ 4,500 grams N=163	3.31 (-1.46 to 8.12)	0.68 (-3.99 to 5.35)	1.09 (0.45 to 2.64)
Trend (SDS) P value	1.58 (0.77 to 2.38) <0.001	0.63 (-0.12 to 1.39) 0.099	0.94 (0.81 to 1.10) 0.441
Birth weight for gestational age N= 6,482			
Small for gestational age N=321	-4.60* (-8.12 to -1.08)	-1.88 (-5.39 to 1.63)	0.66 (0.30 to 1.44)
Normal for gestational age N=5,779	Reference	Reference	Reference
Large for gestational age N=321	0.72 (-2.61 to 4.04)	2.31 (-0.69 to 5.31)	1.02 (0.53 to 1.93)
Trend (SDS) P value	1.26 (0.48 to 2.05) 0.002	0.61 (-0.11 to 1.34) 0.097	0.91 (0.79 to 1.06) 0.234

Values are regression coefficients (95% Confidence Interval) based on multiple regression models and reflect the difference for each outcome for the birth weight or gestational age group, as compared to the reference group. Values for analyses on microalbuminuria are Odds ratios (95% Confidence Interval) and reflect the Odds ratio for each outcome for the birth weight or gestational age group, as compared to the reference group. Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age and body surface area.

eGFR, estimated glomerular filtration rate

*P<0.05, **P<0.01

Table S5. Associations of birth outcomes with kidney outcomes at the age of 6 years adjusted for kidney volume

Birth characteristics	Difference (95% CI) in eGFR (ml/min per 1.73m^2)	Risk (Odds Ratio (95%CI)) of micro albuminuria
Gestational age N=6,482		
< 34 weeks	-1.22	0.45
N=98	(-8.63 to 6.19)	(0.06 to 3.36)
34.0 – 35.9 weeks	3.72	0.81
N=102	(-0.79 to 8.22)	(0.25 to 1.98)
36.0 – 37.9 weeks	2.10	1.03
N=514	(-0.24 to 4.45)	(0.81 to 1.32)
38.0 - 39.9 weeks	Reference	Reference
N=2,199		
40.0-41.9 weeks	1.92**	0.92
N=3,131	(0.64 to 3.20)	(0.70 to 1.21)
≥ 42.0 weeks	4.08**	1.07
N=438	(1.70 to 6.46)	(0.65 to 1.77)
Trend (SDS)	0.39	0.99
	(-0.22 to 1.00)	(0.87 to 1.12)
P value	0.205	0.827
Birth weight N=6,482		
<2,000 grams	-2.50	0.76
N=79	(-8.42 to 3.42)	(0.23 to 2.50)
2,000-2,499 grams	2.13	0.87
N=213	(-1.22 to 5.47)	(0.43 to 1.78)
2,500-2,999 grams	-1.61	1.06
N=958	(-3.72 to 0.25)	(0.73 to 1.52)
3,000-3,499 grams	Reference	Reference
N=2,241		
3,500-3,999 grams	0.36	0.75
N=2,072	(-1.06 to 1.78)	(0.55 to 1.03)
4,000-4,499 grams	-0.28	0.71
N=756	(-2.23 to 1.67)	(0.45 to 1.11)
≥ 4,500 grams	-1.05	1.17
N=163	(-5.04 to 2.95)	(0.52 to 2.65)
Trend (SDS)	0.09	0.93
	(-0.55 to 0.72)	(0.82 to 1.07)
P value	0.789	0.311
Birth weight for gestational age N=6,482		
Small for gestational age	-0.81	0.60
N=321	(-3.64 to 2.02)	(0.30 to 1.19)
Normal for gestational age	Reference	Reference
N=5,779		

Table S5. Continued

Large for gestational age	0.66	1.18
N=321	(-2.02 to 3.35)	(0.66 to 2.14)
Trend (SDS)	-0.09	0.91
	(-0.72 to 0.53)	(0.79 to 1.04)
P values	0.771	0.151

Values are regression coefficients (95% Confidence Interval) based on multiple regression models and reflect the difference for each outcome for the birth weight or gestational age group, as compared to the reference group. Values for analyses on microalbuminuria are Odds ratios (95% Confidence Interval) and reflect the Odds ratio for each outcome for the birth weight or gestational age group, as compared to the reference group. Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age, body surface area and childhood kidney volume.

eGFR, estimated glomerular filtration rate

*P<0.05, **P<0.01

Table S6. Associations of fetal and length and weight with kidney volume and function at the age of 6 years: basic model

Growth characteristics	Difference (95% CI) in combined kidney volume (cm^3)	Difference (95% CI) in eGFR (ml/min per 1.73m^2)	Risk (Odds Ratio (95%CI)) of micro albuminuria
Length			
Second trimester (SDS)	0.84**	-0.15	0.97
N=5,553	(0.23 to 1.46)	(-0.67 to 0.37)	((0.88 to 1.08)
Third trimester (SDS)	2.35**	0.37	0.98
N=5,711	(1.73 to 2.96)	(-0.16 to 0.89)	(0.89 to 1.08)
Birth (SDS)	2.53**	0.49	0.97
N=3,963	(1.91 to 3.15)	(-0.06 to 1.04)	(0.87 to 1.08)
6 months (SDS)	6.48**	2.09**	1.05
N=4,242	(5.77)	(1.45 to 2.73)	(0.93 to 1.19)
12 months (SDS)	6.98**	2.01**	1.10
N=4,369	(6.28 to 7.68)	(1.37 to 2.66)	(0.97 to 1.24)
24 months (SDS)	7.37**	2.08**	1.08
N=4,066	(6.70 to 8.05)	(1.46 to 2.69)	(0.96 to 1.21)
36 months (SDS)	8.47**	2.07**	1.05
N=3,890	(7.80 to 9.13)	(1.45 to 2.69)	(0.93 to 1.18)
48 months (SDS)	8.25**	1.85**	1.08
N=3,446	(7.53 to 8.96)	(1.19 to 2.51)	(0.96 to 1.23)
72 months (SDS)	9.40**	2.26**	1.02
N=6,473	(8.88 to 9.91)	(1.77 to 2.74)	(0.93 to 1.12)
Weight			
Second trimester (SDS)	2.03**	0.41	0.91
N=5,523	(1.40 to 2.66)	(-0.12 to 0.93)	(0.82 to 1.01)
Third trimester (SDS)	3.58**	0.97**	0.97
N=5,692	(2.99 to 4.18)	(0.47 to 1.48)	(0.88 to 1.07)
Birth (SDS)	4.52**	0.96**	0.93
N=6,421	(3.96 to 5.08)	(0.47 to 1.45)	(0.85 to 1.03)
6 months (SDS)	6.53**	0.50	0.98
N=4,734	(5.86 to 7.20)	(-0.12 to 1.11)	(0.87 to 1.11)
12 months (SDS)	7.17**	0.71*	1.05
N=4,376	(6.49 to 7.84)	(0.07 to 1.35)	(0.93 to 1.18)
24 months (SDS)	8.63**	1.25**	0.97
N=4,124	(7.97 to 9.29)	(0.64 to 1.87)	(0.86 to 1.09)
36 months (SDS)	9.06**	1.18**	0.95
N=3,934	(8.41 to 9.72)	(0.55 to 1.80)	(0.85 to 1.07
48 months (SDS)	9.22**	0.91**	0.93
N=3,459	(8.53 to 9.90)	(0.25 to 1.57)	(0.82 to 1.05)
72 months (SDS)	10.47**	1.02**	0.88
N=,3473	(9.98 to 10.95)	(0.55 to 1.50)	(0.81 to 0.97)

Values are regression coefficients (95% CI) based on multiple regression models and reflect the difference for each kidney volume and function outcome per change in SDS of fetal and early childhood length or weight. Values for analyses on microalbuminuria are Odds ratios (95% CI) and reflect the difference for each kidney volume and function outcome per change in SDS of fetal and early childhood length or weight. Models are adjusted for sex and current age.
eGFR, estimated glomerular filtration rate
*P<0.05, **P<0.01

Table S7. Associations of fetal and length and weight with kidney volume and function at the age of 6 years: confounder model

Growth characteristics	Difference (95% CI) in combined kidney volume (cm^3)	Difference (95% CI) in eGFR (ml/min per 1.73m^2)	Risk (Odds Ratio (95%CI)) of micro albuminuria
Length			
Second trimester (SDS)	-0.42	-0.30	0.98
N=5,553	(-1.14 to 0.04)	(-0.86 to 0.27)	(0.88 to 1.10)
Third trimester (SDS)	-0.39	0.11	0.94
N=5,711	(-0.98 to 0.21)	(-0.45 to 0.67)	(0.84 to 1.06)
Birth (SDS)	-0.11	0.39	0.98
N=3,963	(-0.74 to 0.53)	(-0.23 to 0.99)	(0.87 to 1.10)
6 months (SDS)	0.76	1.85**	1.08
	(-0.15 to 1.68)	(1.09 to 2.61)	(0.90 to 1.28)
N=4,242			
12 months (SDS)	0.23	1.73**	1.25*
N=4,369	(-0.72 to 1.17)	(0.99 to 2.48)	(1.05 to 1.50)
24 months (SDS)	-0.42	1.83**	1.30**
N=4,066	(-1.43 to 0.58)	(1.13 to 2.53)	(1.09 to 1.56)
36 months (SDS)	0.71	1.88**	1.37**
N=3,890	(-0.47 to 1.89)	(1.16 to 2.59)	(1.10 to 1.72)
48 months (SDS)	-0.91	1.79**	1.27
N=3,446	(-2.20 to 0.38)	(1.08 to 2.52)	(1.00 to 1.62)
72 months (SDS)	-1.00	1.94**	1.45**
N=6,473	(-2.14 to 0.14)	(1.39 to 2.49)	(1.16 to 1.81)
Weight			
Second trimester (SDS)	0.63*	0.37	0.91
N=5,523	(0.02 to 1.23)	(-0.21 to 0.94)	(0.81 to 1.02)
Third trimester (SDS)	1.13**	0.71*	0.92
N=5,692	(0.55 to 1.72)	(0.16 to 1.26)	(0.82 to 1.03)
Birth (SDS)	1.33**	0.84**	0.94
N=6,421	(0.72 to 1.94)	(0.27 to 1.42)	(0.83 to 1.05)
6 months (SDS)	1.51**	0.25	1.02
N=4,734	(0.69 to 2.32)	(-0.48 to 0.97)	(0.88 to 1.19)

Table S7. Continued

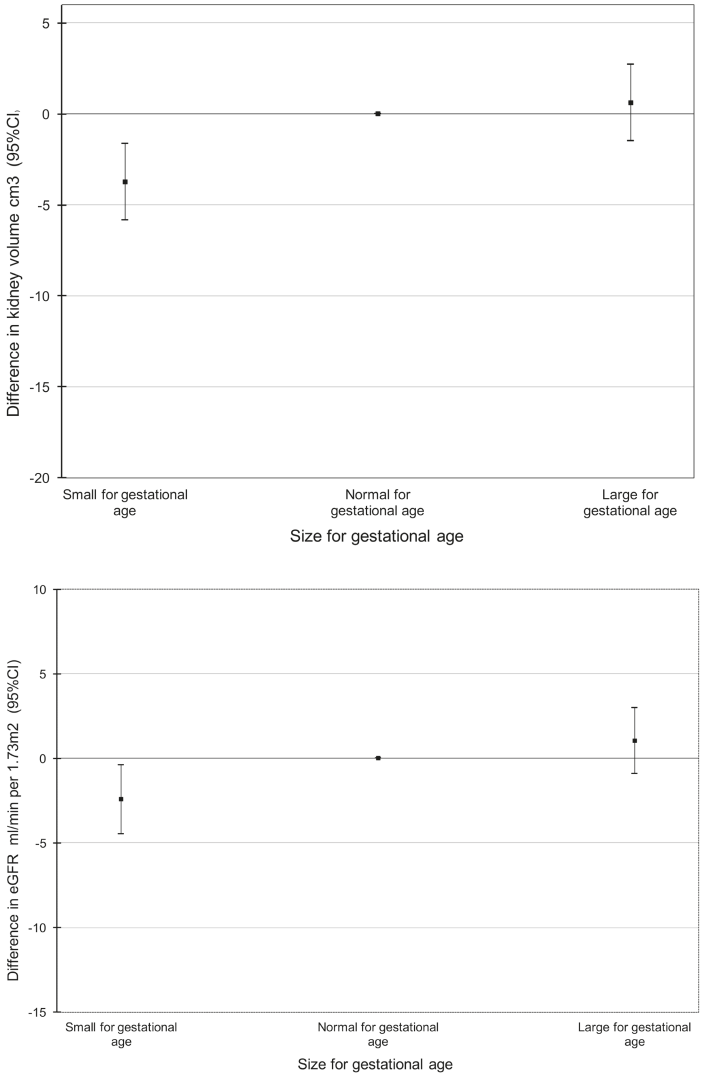
12 months (SDS) N=4,376	1.19* (0.28 to 2.10)	0.56 (-0.17 to 1.29)	1..09 (0.92 to 1.30)
24 months (SDS) N=4,124	1.28* (0.19 to 2.36)	1.06** (0.37 to 1.76)	1.01 (0.83 to 1.24)
36 months (SDS) N=3,934	1.81** (0.54 to 3.09)	1.02** (0.31 to 1.73)	0.98 (0.78 to 1.24)
48 months (SDS) N=3,459	0.95 (-0.53 to 2.44)	0.92* (0.17 to 1.67)	0.84 (0.65 to 1.09)
72 months (SDS) N=3,473	2.13* (0.01 to 4.24)	0.83** (0.29 to 1.38)	0.72 (0.48 to 1.07)

Values are regression coefficients (95% Confidence Interval) based on multiple regression models and reflect the difference for each kidney outcome per change in SDS of fetal and early childhood length or weight. Values for analyses on microalbuminuria are Odds ratios (95% Confidence Interval) and reflect the difference for each kidney volume and function outcome per change in SDS of fetal and early childhood length or weight. Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, smoking, total daily calorie intake and alcohol consumption during pregnancy, and gestational age, child sex, breastfeeding and current age and body surface area.

eGFR, estimated glomerular filtration rate

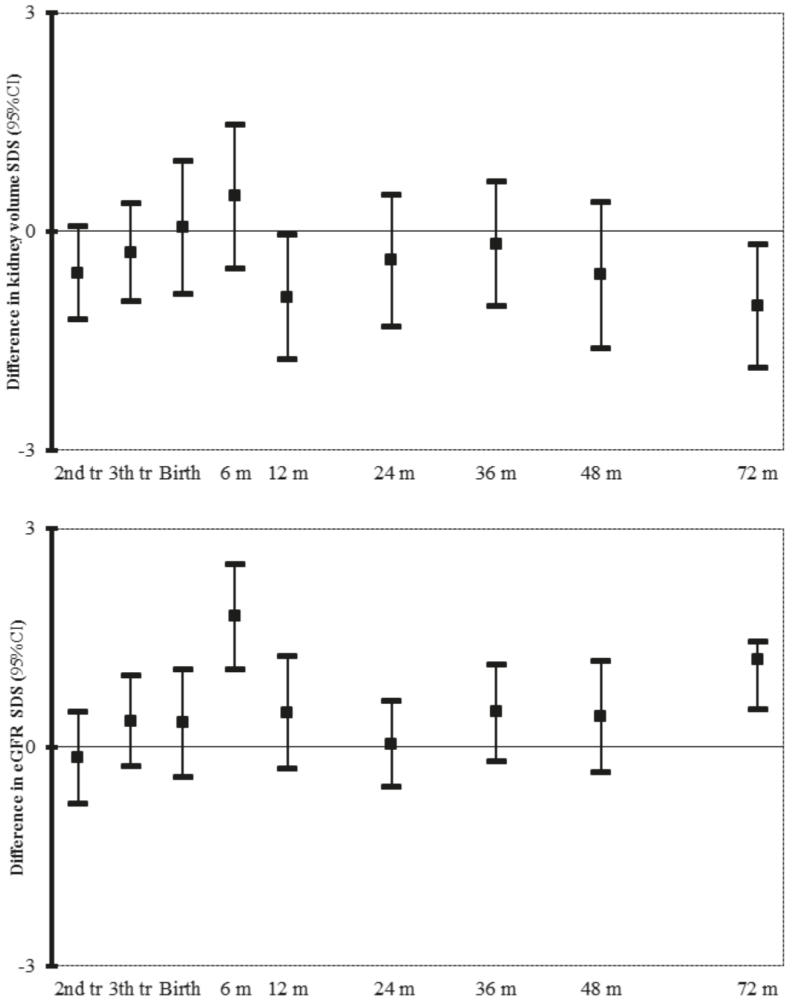
*P<0.05, **P<0.01

Figure S1. Association of small and large size size for gestational age with kidney volume and function at the age of 6 years



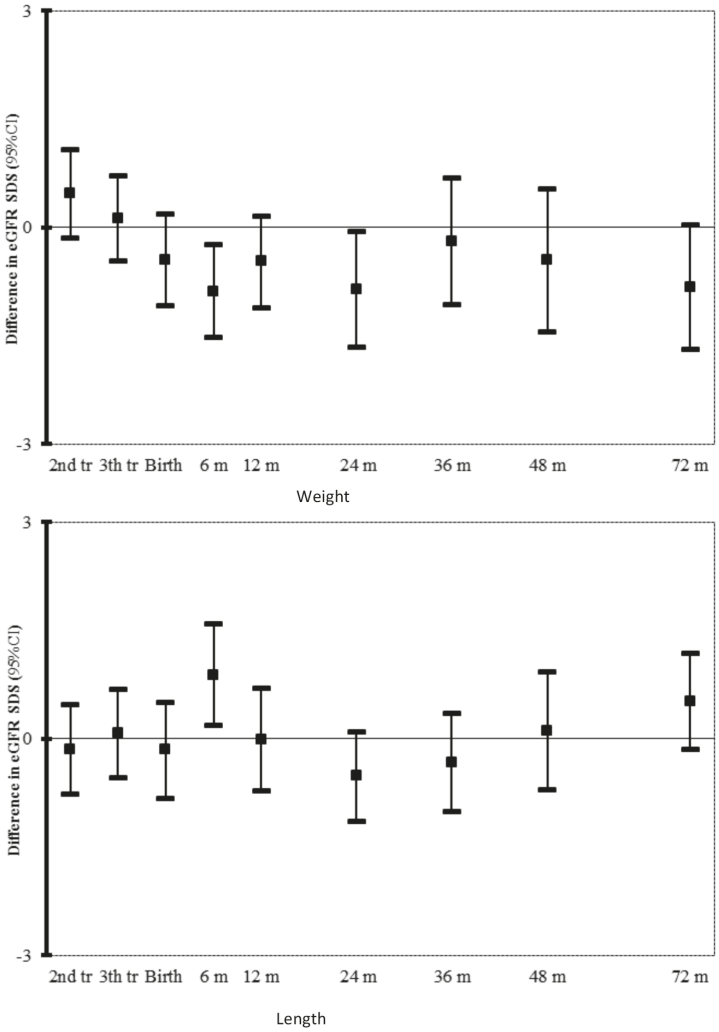
Bars represent regression coefficients (95% Confidence Interval) based on multiple regression models and reflect the difference for each kidney outcome between small (<10%), normal (10-90%) and large (>90%) size for gestational age born children. Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age and body surface area. eGFR, estimated glomerular filtration rate. β for trend (95% Confidence Interval).

Figure S2. Associations of fetal and childhood length with kidney outcomes at the age of 6 years



Effect estimates ((95% Confidence Interval) represent regression coefficients based on multiple regression models and reflect the difference per 1 SD increase in standardized residual score for (estimated) length measures at different time points (see details for conditional regression models in Supplementary Material). Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding and current age. Models focused on kidney volume were additionally adjusted for body surface area. eGFR, estimated glomerular filtration rate

Figure S3. Associations of fetal and childhood weight and length with estimated glomerular filtration rate at the age of 6 years adjusted for kidney volume



Effect estimates ((95% Confidence Interval) represent regression coefficients based on multiple regression models and reflect the difference per 1 SD increase in standardized residual score for (estimated) weight and length measures at different time points (see details for conditional regression models in Supplementary Material). Models are adjusted for maternal age, body mass index, parity, ethnicity, educational level, folic acid supplementation and smoking during pregnancy, and child sex, breastfeeding, current age and kidney volume at the age of 6 years. eGFR, estimated glomerular filtration rate

Chapter 2.4

Early longitudinal kidney growth patterns and glomerular filtration rate at school-age

Submitted

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Abstract

Background Suboptimal kidney development in early life may be associated with an increased risk of kidney disease in later life. We aimed to identify specific kidney growth patterns during fetal life and infancy associated with impaired kidney function in childhood.

Methods In a population-based prospective cohort study among 614 mothers and their children, combined kidney volume was measured by ultrasound at the ages of 30 weeks of gestation, and 6, 24 and 72 months. At the age of 6 years, estimated glomerular filtration rate (eGFR) was calculated from blood creatinine and cystatin C concentrations.

Results Children with small combined kidney volume during fetal life which persisted at 6 years had lower $eGFR_{creat}$, compared to children with persistent large combined kidney volume (differences $eGFR_{creat}$ -0.64 SD (95% CI -0.95 to -0.33) and $eGFR_{cyst}$ -0.39 SD (95% CI -0.71 to -0.07), respectively). Longitudinal analyses showed that children in the lowest tertile of $eGFR_{creat}$ and $eGFR_{cyst}$ had smaller combined kidney volume from fetal life onwards, compared to children in the highest tertile (all p values <0.01). Conditional regression analyses showed that early childhood kidney growth, independent from previous kidney growth, was positively associated with $eGFR_{creat}$ and $eGFR_{cyst}$ (all P values <0.05).

Conclusion Smaller combined kidney growth during both fetal life and early childhood is associated with lower eGFR at school-age.

Introduction

The prevalence of chronic kidney disease (CKD) is increasing and has a growing impact on morbidity and mortality.(1) An accumulating body of evidence suggests that the susceptibility for CKD is established in early life.(2) Multiple studies have shown that low birth weight is associated with increased risks of CKD in later life.(3-5) It has been hypothesized that adverse exposures in utero lead to impaired fetal growth and smaller kidneys with a persistently reduced number of nephrons.(6-8) A persistently reduced nephron number in smaller kidneys may subsequently lead to glomerular hyperfiltration and hypertrophy, which in turn leads to glomerulosclerosis and CKD in later life.(8) This hypothesis is supported by our recent studies showing that reduced fetal and early postnatal growth are associated with smaller kidney volume and lower kidney function in childhood.(9,10) Also, impaired third trimester fetal blood flow to the abdominal organs was associated with lower eGFR in childhood.(11) To the best of our knowledge, no previous studies have examined the associations of directly longitudinally measured kidney volume with kidney function in later life. Nephron numbers cannot be assessed *in vivo*. Kidney volume can be used as proxy for kidney development and nephron number.(6) Previous studies suggested that small kidney volume is associated with hypertension and renal disease.(9)

We evaluated, in a population based prospective cohort study involving 614 pregnant mothers and their children, the associations of longitudinally measured fetal and early childhood kidney volumes with eGFR at school age. We used different approaches to identify longitudinal patterns and critical periods for fetal and early childhood kidney growth related to impaired kidney function at school-age.

Methods

Design and study population

This study was nested in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.(10) All children were born between April 2002 and January 2006. Written informed consent was obtained from all parents. The study has been approved by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam. More detailed growth and development evaluations were conducted in a subgroup of 1,232 Dutch mothers and their children, of which 1,218 had at least 1 kidney measurement in fetal life or early childhood. Only singleton life births were included in the current study. In total, combined kidney volume measures were available from 922 children the age of 6 years, of whom 614 (67%) provided blood samples for creatinine and cystatin C measurements (**Figure 1)**. Missing blood samples were mainly due to non-consent. No kidney abnormalities were present in these 614 children.

Fetal and childhood combined kidney volume

Left and right kidney biometrics were measured at the median (90% range) age of 30.4 (28.8-32.3) weeks of gestational age, and 6.3 (5.5-7.8), 25.0 (23.8-27.5) and 71.5 (69.0-76.6) months. As previously described, we identified the left and right kidney in the sagittal plane along its longitudinal axis.(11) We performed measurements of maximal bipolar kidney length, width and depth. Kidney width and depth were measured at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).(12) Combined kidney volume was calculated by summing right and left kidney volume. We previously reported good intra-observer and inter-observer correlation coefficients.(13)

Childhood estimated glomerular filtration rate

Since creatinine concentrations might be influenced by muscle mass, ethnicity, age, sex and dietary factors, we calculated the eGFR based on creatinine and cystatin C blood concentrations.(14) Blood creatinine concentrations were measured with an enzymatic method and cystatin C concentrations with a particle enhanced immunoturbimetric assay on a Cobas c 502 analyzer (Roche Diagnostics, Germany). Quality control samples demonstrated intra-assay and inter-assay coefficients of variation of 0.51% for creatinine and 1.65% for cystatin C, and 1.37% for creatinine and 1.13% for cystatin C, respectively.(15) eGFR was calculated according to the revised Schwartz 2009 formula(16); $eGFR_{creat}$ = 36.5 * (height (cm)/creatinine (µmol/l).(16) We used the formula as proposed by Zappitelli in 2006: $eGFR_{cyst}$ = 75.94 / [CysC(mg/L)$^{1.17}$].(17)

Covariates

Information on parity, educational level, smoking during pregnancy and folic acid supplementation during pregnancy was obtained by questionnaires.(10) Infant sex, gestational age at birth and birth weight was obtained from midwife and hospital registries. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and body mass index (BMI) was calculated.

Statistical analyses

We performed a non-response analyses to compare characteristic between included and not-included mother-child pairs. We used different approaches to identify longitudinal patterns and critical periods for fetal and early childhood kidney growth related to impaired kidney function at school-age. First, we assessed the correlations of combined kidney volume in fetal life and early childhood with eGFR in childhood and performed multiple linear regression models to explore the associations of combined kidney volume (at the ages of 30 weeks of gestation, and 6, 24 and 72 months) with childhood combined kidney volume and eGFR. Second, we created tertiles of fetal

and early childhood combined kidney volume to compare the associations in different combined strata. We used linear regression models to assess whether the associations of fetal combined kidney volume with eGFR were modified by childhood combined kidney volume at 6 years. Additionally, we also used linear regression models to assess whether the associations of fetal combined kidney volume with eGFR were modified by infants combined kidney volume at the of 24 months. Third, we compared fetal and early childhood longitudinal combined kidney volume growth patterns between children in different tertiles of eGFR. For these analyses we used mixed-effects models. These regression models take into account the correlation between repeated measurements within the same participant and allowing for incomplete data.(18) Finally, to identify independent critical periods for combined kidney volume in relation to eGFR at school-age, we performed conditional regression taking account for the correlations between kidney volume measurements at different ages.(11,19) For these analyses, we constructed kidney volume variables for each time point, which are statistically independent from each other, by using standardized residuals obtained from regression of kidney volume measures at a specific time point on prior kidney volume measures.(19) As conditional kidney volume measures are statistically independent of each other, this approach allows inclusion of kidney volume measures simultaneously in one linear regression model. Thus, the associations of fetal and early childhood kidney volume measures at specific ages with kidney outcomes can be assessed adjusted for, and compared, with fetal and childhood kidney volume measures at other ages.(20,21) Results from these datasets were pooled and presented in the conditional kidney volume growth results.

The regression models were first adjusted for child sex and age and for potential confounders. Potential confounders were based on their associations with kidney outcomes or a change in effect estimate of more than 10% and included folic acid use, maternal smoking, parity, birth weight, gestational age at birth and body mass index at 6 years. To reduce the possibility of potential bias due to missing data, we imputed missing data of the fetal, child and maternal covariates with five imputations and analysed these datasets together.(22) Additional information on the imputation procedure is given in the **Supplementary material.** The repeated measurement analyses, including the Prox Mixed module for unbalanced repeated measurements, were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). All other statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal and child characteristics are shown in **Table 1.** At the age of 71.5 months (90 % range 69.0 to 76.6), and mean (SD) $eGFR_{creat}$ was 119.98 (16.4) ml/min per 1.73m^2 and $eGFR_{cyst}$ was 102.0 (14.6) ml/min per 1.73m^2, respectively. Non–response analyses

showed no differences in combined kidney volume measures between children included and children not included in the study. Mothers of children included in the current study were higher educated than mothers of children who were not included. Also, included children were born at older gestational age and had a higher birth weight as compared to children not included in the study. (**Supplementary Table S1).** Observed data before multiple imputation are presented in **Supplementary Table S2**.

Correlations between combined kidney volumes and $eGFR_{creat}$ and $eGFR_{cyst}$ are given in **Supplementary Table S3** and showed that all kidney measures were correlated. Also Supplementary **Table S4** shows that combined kidney volume at all fetal and early childhood ages was positively associated with $eGFR_{creat}$ and $eGFR_{cyst}$ (all P values < 0.01), independent of combined kidney volume at the age of 6 years (all P values < 0.01).

Early fetal and infant kidney growth and childhood eGFR

Figure 2 shows the eGFR of children in strata of combined kidney volume in fetal life and early childhood. Early childhood combined kidney volume was positively associated with $eGFR_{creat}$ in each tertile of fetal combined kidney volume, whereas no consistent associations of early childhood combined kidney volume with $eGFR_{cyst}$ were observed in different tertiles of fetal combined kidney volume. Children in the lowest tertile of both fetal and childhood combined kidney volume had the lowest $eGFR_{creat}$ and $eGFR_{cyst}$ at the age of 6 years (difference compared with children with normal fetal and early childhood combined kidney: -0.64 SD (95% Confidence Interval (CI) -0.95 to -0.33) and -0.39 SD (95% CI -0.71 to -0.07), respectively). Additional analyses in strata of fetal kidney volume and kidney at 24 months showed similar results (**Supplementary figure 1**).

Figure 3 gives the results from the longitudinal analyses and present the kidney growth patterns from fetal life into childhood for children with different tertiles of eGFR. Overall, analyses on $eGFR_{creat}$ and $eGFR_{cyst}$ showed similar results. As compared to children within the highest tertile of eGFR, those in the middle and lowest tertile tended to have smaller combined kidney volume in early childhood (all p values < 0.01).

Figure 4 gives the results from the conditional regression analyses, which were performed to identify the at which age combined kidney volume is associated with eGFR, independent of combined kidney volume at other ages. We observed that independent from combined kidney volume at other ages, combined kidney volumes at 6 months, 24 months and 72 months were positively associated $eGFR_{creat}$ and $eGFR_{cryst}$ (all P values < 0.05), except for combined kidney volume at 72 months and $eGFR_{cyst}$. The associations were similar across ages for $eGFR_{creat}$, but stronger for combined kidney volume at younger ages for $eGFR_{cyst}$.

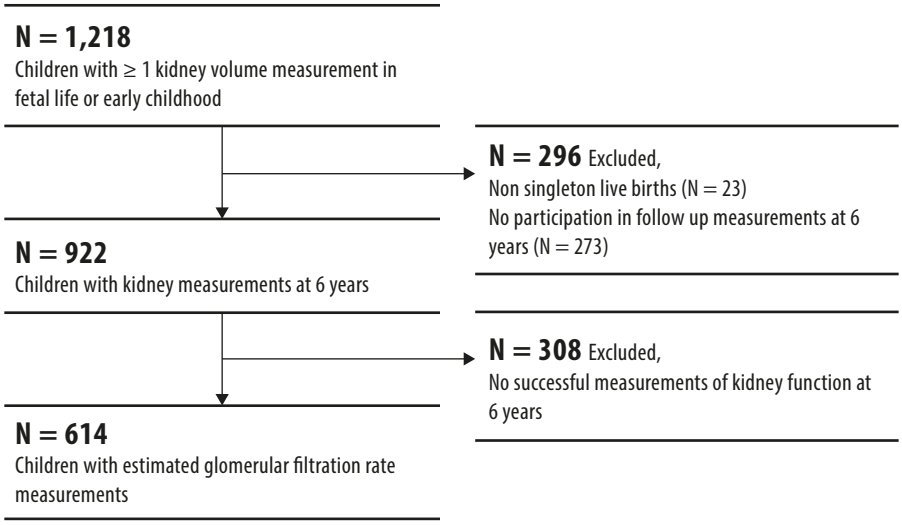
Table 1. Maternal and child characteristics (N=614)[1]

	Values
Maternal characteristics	
Age, (yr)	32.3 (25.1-37.9)
Height, (cm)	171.1 (6.2)
Pre-pregnancy body mass index, (kg/m^2)	23.5 (4.2)
Parity, nulliparous, (%)	383 (62.5)
Educational level, (%)	
No higher education	210 (34.7)
Higher education	396 (65.3)
Smoking, (%)	
No	427 (77.5)
Yes	124 (22.5)
Folic acid supplement use, (%)	
No use	49 (8.0)
First 10 weeks use	184 (30.0)
Preconception use	381 (62.1)
Fetal and infant characteristics	
Males, (%)	312 (50.8)
Gestational age, (weeks)	40.3 (37.3-42.1)
Birth weight, (g)	3549 (506)
Combined kidney volume	
30 weeks, (cm^3)	20.6 (5.5)
6 months, (cm^3)	46.1 (9.5)
24 months, (cm^3)	66.5 (13.2)
Child characteristics	
Age, (months)	71.5 (69.0-76.6)
Height, (cm)	119.2 (5.0)
Weight, (kg)	22.7 (3.2)
Body mass index, (kg/m^2)	15.9 (1.4)
Combined kidney volume at 72 months ,(cm^3)	120.8 (22.1)
eGFR, (Schwartz, creatinine based) ml/min per 1.73m^2	119.9 (16.1)
eGFR, (Zapitelli, cystatin C based) ml/min per 1.73m^2	102.0 (14.6)

[1]Values are means (standard deviation), median (90% range) or number of subjects (valid %).

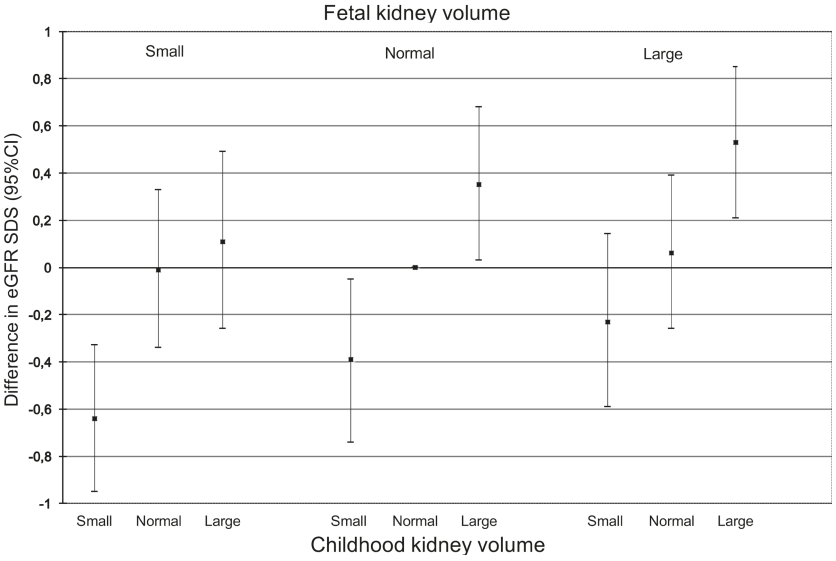
eGFR, estimated glomerular filtration rate

Figure 1. Flow chart: inclusion of participants in analyses



Values are regression coefficients (95% confidence interval) based on multiple regression models and reflect the difference for standard deviation score of eGFR compared to with children with normal fetal and infant kidney volume. Tertiles of fetal kidney volume at 30 weeks gestational age and early childhood kidney volume at the age of 6 years were created. Models are adjusted for child sex and age at outcome measurements, folic acid use, maternal smoking, parity, birth weight, gestational age at birth and BMI at 6 years.

Figure 2. Associations of fetal and early childhood combined kidney volume with eGFR at school-age (N=614)



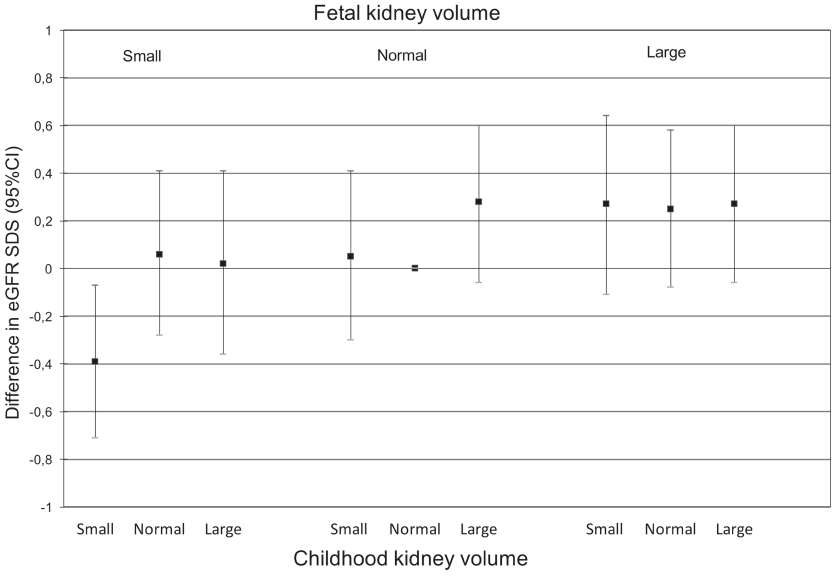
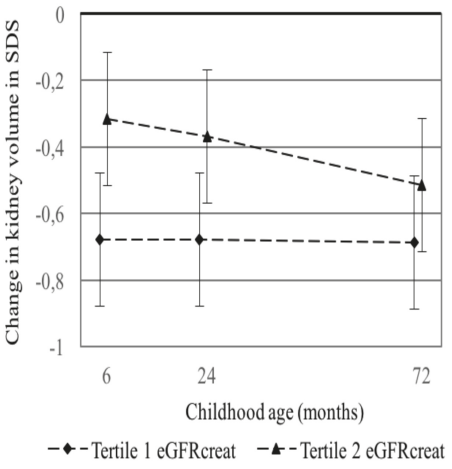
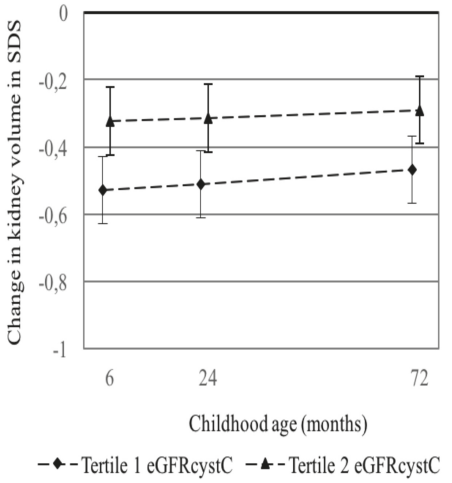


Figure 3. Combined kidney volume growth patterns during fetal life and infancy from children in eGFRcreat tertiles (N=614)

A. eGFRcreat

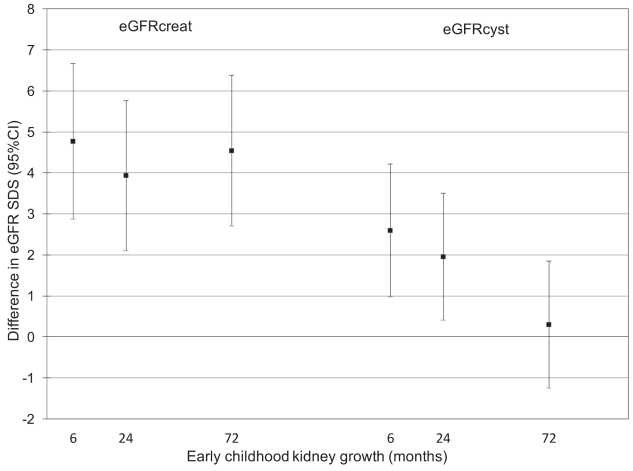


B. eGFRcyst



Values are estimates based on repeated linear regression models and reflext the standard deviation score for each kidney growth measure in children who were in first and second tertile of eGFRcreat and eGFRcyst compared to children in the third tertile of eGFRcreat and eGFRcyst at the age of 6 years.

Figure 4. Associations of independent early childhood kidney growth measures and eGFRcreat and eGFRcyst in childhood (N=614)



Values are regression coefficients (95% confidence interval) based on multiple regression models and reflect the difference for each kidney volume and function outcome per increase of 1 SDS of standardized residual score for kidney volume in a specific time period.

Model is adjusted for child sex and age at outcome measurements, folic acid use, maternal smoking, parity, birth weight, gestational age at birth and BMI at 6 years.

Discussion

In this low-risk population-based prospective cohort study, we aimed to identify longitudinal growth patterns and critical periods for kidney growth related to kidney function at school age. Smaller fetal kidney volume which persisted in childhood was associated with impaired eGFR at school age. Also, children with the lowest and the middle tertile of eGFR in childhood had smaller combined kidney volume growth from fetal life onwards. Furthermore, early life kidney growth, independent from previous kidney growth, is positively associated with eGFR in childhood.

Strengths and limitations

A major strength of our study is its population-based prospective design from fetal life onward and the large number of repeated kidney volume measurements available. By performing repeated measurements analyses and conditional growth analyses, we were able to identify kidney growth patterns and critical periods that might influence kidney function in childhood. Not all participants at baseline participated in the follow-up studies at the age of 6 years. Mothers of children included in the current study were higher educated than mothers of children who were not included. Also, included children were born at older gestational age and had a higher birth weight as compared to children not included in the study. These differences might have led to bias if the associations between early kidney growth and childhood kidney function differ between the children with and without follow-up measurements. We consider this unlikely but cannot explore this in further detail. Since nephron number cannot be studied *in vivo*, we used combined kidney volume as a proxy for kidney development. Kidney size is correlated with glomerular number in childhood and adulthood, and can be used in epidemiological studies as measure of kidney development.(6) GFR was estimated based on the Schwartz formula,(16) which is based on serum creatinine concentrations and on the Zappitelli formula(17) which is based on serum cystatin C concentrations. Both have been validated in pediatric populations. In line with previous studies, GFR estimations were higher when calculated based on creatinine concentrations than on cystatin C concentrations.(24) Overall, results for analyses on $eGFR_{creat}$ were comparable with results from analyses on $eGFR_{cyst}$. However, effect estimates were higher for eGFRcreat in the stratified and conditional analyses. We have recently shown that creatinine based eGFR is stronger influenced by body composition than cystatin C based eGFR(Miliku et al Submitted). Since body growth is strongly correlated with kidney growth, this might have influenced our results. The stronger associations with creatinine based eGFR might be explained by relatively lower creatinine levels in high body growth. Confounder models were adjusted for childhood BMI, this might limit the effect of body composition on the observed associations. Currently, it is not clear which formula estimates GFR the most accurate. Previous studies suggested that serum cystatin C concentrations might be superior in evaluating kidney function to serum creatinine concentrations.(25) Although we had information about a large number of confounders, residual confounding might still be an issue due to the observational design of the study.

Fetal and early childhood kidney growth and childhood eGFR

To the best of our knowledge, this study is the first population-based prospective cohort study focused on the associations of fetal and early childhood kidney growth and kidney function in childhood. Kidney development starts in early fetal life and ends arounds the 36th week of gestation.(26) To date, it is not known whether early childhood kidney growth is associated with the risk of lower kidney function in childhood. The longitudinally measured kidney volumes in fetal life and early childhood in the current study provided the oppurtunity to identify specific periods and patterns of kidney growth which might be associated with childhood eGFR.

Low birth weight is associated with an increased risk of chronic kidney disease in adulthood. Only a few studies evaluated nephron numbers and birth weight, since nephron number can only be counted post mortem. These studies showed a lower nephron number in infants with lower birth weight.(3,27-30) Results of studies focused on birth weight and kidney function in childhood are inconclusive.(31-34) We have previously shown that suboptimal fetal and infant body growth is associated with smaller kidney volume and lower eGFR in childhood.(11). A previous study in small groups of 9 to 12-year-old children did not find a difference in kidney volume or kidney function between groups of children born preterm, born term but small for gestational age or born term appropriate for gestational age.(32) A recent study in 100 low birth weight (LBW) and 66 normal birth weight (NBW) children showed that combined kidney volume was lower in LBW children as compared to NBW children during infancy. However despite the lower kidney volumes there were no differences in eGFR at the end of infancy.(35) Impaired fetal growth and low birth weight might be associated with smaller kidney volume and lower kidney function. The present study showed that longitudinally impaired kidney growth patters in fetal life and in early childhood is associated with lower eGFR in childhood.

Nephron endowment takes place in fetal life, new nephrons are formed until 36 weeks of gestation, thereafter nephrogenesis ceases.(28) Multiple factors are suggested to be important for nephron mass endowment, for example genetic and epigenetic factors, maternal nutritional status during pregnancy and maternal medication use.(6,9,36) Also, multiple factor are identified which might be associated with maintaining or decreasing nephron number in postnatal life, for example breastfeeding, body growth and protein intake.(6,9) Normal nephron number in human varies widely, it might be around 1.000.000, but there is a 10-fold range in this spectrum.(37) However, people at the lower end of this nephron number might be at a higher risk for kidney disease in later life.(37) Chronic kidney disease occurs when there are too few nephrons to maintain kidney function. The hyperfiltration theory hypothesizes that a reduction in nephron number leads to compensatory hyperfiltration which in turn might lead to glomerulosclerosis and decreased kidney function.(8) Previous studies in rats showed that when the total nephron number is decreased, the volume and the glomerular filtration rate of a single nephron increases. This might be beneficial on the short term, but on the long term total glomerular filtration rate decreases and progressive renal injury develops.(38,39) In

contrast with this hypothesis, and with animal studies and studies focused on early life low nephron number, the loss of one kidney does not lead to impaired kidney function per se. Studies in living kidney donors show that this reduction in nephron number does not have to lead to hypertension and/or impaired kidney function.(40,41) The current study shows for the first time that in a low risk population, fetal and early childhood are critical periods for kidney volume and are associated with lower eGFR in childhood. These findings might support the hypothesis that a smaller nephron number, reflected by smaller kidney volume, may lead to lower eGFR. Since nephrogenisis ceases before birth in term born children, only a decrease in nephron number can occur postnatal. The smaller kidney volumes in early childhood associated with lower eGFR in childhood in the present study might reflect a decrease in nephron number.

In conclusion, smaller kidney growth in fetal life and during infancy is associated with lower eGFR in childhood. Together with previous studies, this study supports the hypothesis that kidney development in the early phases of life has persistent consequences for kidney function in later life. Future studies are needed to identify factors that can optimize early kidney development to improve kidney function throughout the life course.

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Imputation procedure

To reduce the possibility of potential bias associated with missing data and to maintain statistical power, missing values were imputed using the multiple imputations procedure.(1) For the multiple imputations, we used Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model for the analyses focused on the associations of early growth outcomes with kidney outcomes in childhood, we included all covariates. Furthermore, we added the determinants and outcomes studied in the imputation model as prediction variables only. Determinants and outcomes were not imputed themselves. Five imputed datasets were created and analyzed together.

1. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ (Clinical research ed. 2009;338:b2393.

Supplementary Table 1. Maternal and child characteristics included and not included[1]

	Included N=614	Not included N=604
Maternal characteristics		
Age, (yr)	32.3 (25.1-37.9)	31.6 (22.4-37.9)
Height, (cm)	171.1 (6.2)	170.7 (6.3)
Pre-pregnancy body mass index, (kg/m^2)	23.5 (4.2)	23.4 (4.0)
Parity, nulliparous, (%)	383 (62.5)	350 (58.2)
Educational level, (%)		
No higher education	210 (34.7)	240 (40.6)*
Higher education	396 (65.3)	351 (59.4)
Smoking, (%)		
No	427 (77.5)	405 (72.8)
Yes	124 (22.5)	151 (27.2)
Folic acid supplement use, (%)		
No use	49 (8.0)	57 (11.4)
First 10 weeks use	184 (30.0)	140 (28.1)
Preconception use	381 (62.1)	301 (60.4)
Fetal and infant characteristics		
Males, (%)	312 (50.8)	323 (53.5)
Gestational age, (weeks)	40.3 (37.3-42.1)	40.1 (36.0-42.0)**
Birth weight, (g)	3549 (506)	3535.7 (507.8)**
Combined kidney volume		
30 weeks, (cm^3)	20.6 (5.5)	20.7 (5.3)
6 months, (cm^3)	46.1 (9.5)	46.6 (9.6)
24 months, (cm^3)	66.5 (13.2)	65.0 (12.4)
Child characteristics		
Age, (months)	71.5 (69.0-76.6)	5.9 (5.7-6.4)
Height, (cm)	119.2 (5.0)	118.5 (5.4)
Weight, (kg)	22.7 (3.2)	22.2 (3.2)*
Body mass index, (kg/m^2)	15.9 (1.4)	15.7 (1.3)
Combined kidney volume at 72 months ,(cm^3)	120.8 (22.1)	120.3 (22.2)
eGFR, (Schwartz, creatinine based) ml/min per 1.73m^2	119.9 (16.1)	N.A.
eGFR, (Zapitelli, cystatin C based) ml/min per 1.73m^2	102.0 (14.6)	N.A.

[1]Values are means (standard deviation), median (90% range) or number of subjects (valid %).

eGFR, estimated glomerular filtration rate, N.A. not applicable

*P<0.05 **P<0.01

Supplementary Table 2. Maternal and child characteristics imputed vs non-imputed (N=614)[1]

	Values Non imputed	Values imputed
Maternal characteristics		
Age, (yr)	32.2 (24.9-38.0)	32.2 (24.9-38.0)
Height, (cm)	171.0 (6.2)	171.0 (6.2)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.2)	23.6 (4.3)
Parity, nulliparous, (%)	575 (62.5)	576 (62.5)
Educational level, (%)		
No higher education	311 (34.1)	314 (34.1)
Higher education	602 (65.9)	608 (65.9)
Smoking, (%)		
No	654 (78.7)	726(78.7)
Yes	177 (21.3)	196(21.3)
Folic acid supplement use, (%)		
No use	61 (7.9)	79 (8.6)
First 10 weeks use	218 (28.4)	268 (29.1)
Preconception use	489 (63.7)	575 (62.4)
Fetal and infant characteristics		
Males, (%)	464 (50.3)	464 (50.3)
Gestational age, (weeks)	40.3 (37.5-42.1)	40.3 (37.5-42.1)
Birth weight, (g)	3535.7 (507.8)	3535.7 (507.8)
Combined kidney volume		
30 weeks, (cm^3)	20.6 (5.5)	20.6 (5.5)
6 months, (cm^3)	46.1 (9.5)	46.1 (9.5)
24 months, (cm^3)	68.3 (27.2)	68.3 (27.2)
Child characteristics		
Age, (months)	5.9 (5.7-6.4)	5.9 (5.7-6.4)
Height,(cm)	119.0 (5.2)	119.0 (5.2)
Weight, (kg)	22.6 (3.2)	22.6 (3.2)
Body mass index, (kg/m^2)	15.9 (1.4)	15.9 (1.4)
Combined kidney volume at 72 months ,(cm^3)	120.8 (22.1)	120.8 (22.1)
eGFR, (Schwartz, creatinine based) ml/min per 1.73m^2	119.9 (16.1)	119.9 (16.1)
eGFR, (Zapitelli, cystatin C based) ml/min per 1.73m^2	102.0 (14.6)	102.0 (14.6)

[1]Values are means (standard deviation), median (90% range) or number of subjects (valid %).

eGFR, estimated glomerular filtration rate

Supplementary Table 3. Correlations between Kidney Growth and Childhood Kidney Volume and Function (N=614)[1]

	Kidney volume (cm^3) 30 weeks gestation	Kidney volume (cm^3) 6months	Kidney volume (cm^3) 24months	Kidney volume (cm^3) 60months	eGFR creat (ml/min per 1.73m^2)	eGFR cys C (ml/min per 1.73m^2)
Kidney volume (cm^3) 30 weeks gestation	1.00	0.34**	0.30**	0.17**	0.22**	0.18**
Kidney volume (cm^3) 6months		1.00	0.32**	0.29**	0.26**	0.18**
Kidney volume (cm^3) 24months			1.00	0.30**	0.39**	0.25**
Kidney volume (cm^3) 60months				1.00	0.32**	0.13**
eGFR creat (ml/min per 1.73m^2)					1.00	0.31**
eGFR cys C (ml/min per 1.73m^2)						1.00

[1]Values are Pearson correlation coefficients and reflect correlation between kidney volume at specific ages and kidney function at the age of 6 years.
Model is adjusted for child sex and age at outcome measurements.

*P<0.05, **P<0.01

Supplementary Table 4. Combined kidney volume in fetal life and early childhood and childhood eGFR (N=614)[1]

	Change in GFR (ml/min per 1.73m^2)			
	Confounder model		Kidney volume model	
Combined kidney volume (age)	**GFRcreat**	**GFRcyst**	**GFRcreat**	**GFRcyst**
30 weeks gestation	3.59 (2.30 - 4.88)**	2.74(1.54 - 3.95)**	2.33(1.06 – 3.61)**	2.74(1.53 - 3.94)**
6 months	4.53(2.97 – 6.10)**	3.21(1.71 - 4.71)**	2.99(1.34 – 4.65)**	2.73(1.17 - 4.29)**
24 months	6.14(4.58 – 7.69)**	2.90(1.68 – 4.12)**	4.14(2.42 – 5.87)**	2.69(1.26 – 4.12)**
72 months	5.87(4.59 – 7.15)**	1.83(0.64 – 3.02)**	N.A.	N.A.

[1]Values are regression coefficients (95% confidence interval) based on multiple regression models and reflect the difference for each kidney volume and function outcome per change in SDS of combined kidney volume. Confounder model is adjusted for child sex and age at outcome measurements, folic acid use, maternal smoking, parity, birth weight, gestational age at birth and BMI at 6 years.
Kidney volume model is additionally adjusted for combined kidney volume at the age of 6 years

N.A. not applicable

*P<0.05, **P<0.01.

Supplementary Table 5. Conditional analyses on Kidney growth patterns and childhood kidney function in childhood[1] Crude model

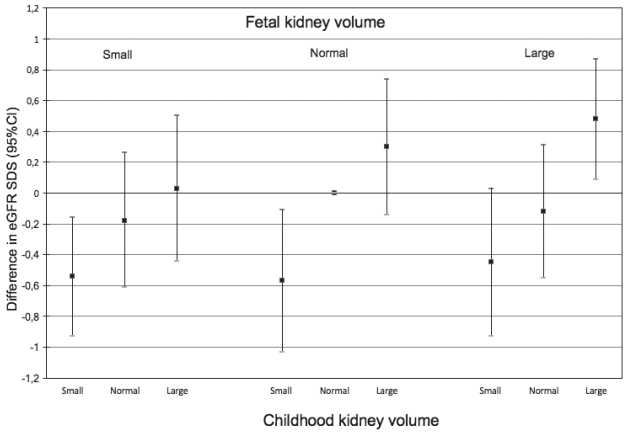
Kidney volume (age)	**Combined kidney volume (cm^3)**	**GFR creat (ml/min per 1.73m^2)**	**GFR cys C (ml/min per 1.73m^2)**
30 weeks gestation to 6 months (N=560)	6.66(6.04 – 7.29)**	4.32(2.43 – 6.22)**	2.39(0.81 – 3.98)**
6 months to 24 months (N=365)	6.10(5.50 – 6.71)**	3.76(1.93 – 5.58)**	2.00(0.47 – 3.52)*
24 to 60 months (N=329)	N.A.	3.98(2.15 – 5.81)**	0.07(-1.46 – 1.60)

[1]Values are regression coefficients (95% confidence interval) based on multiple regression models and reflect the difference for each kidney volume and function outcome per change in SDS of kidney volume in a specific time period.
Model is adjusted for child sex and age at outcome measurements.

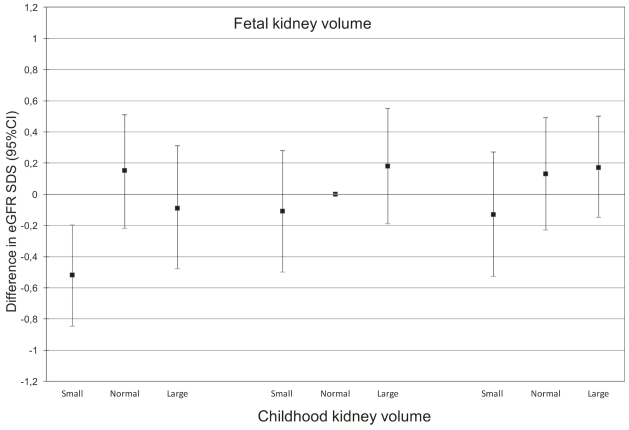
*P<0.05, **P<0.01

Supplemental Figure 1. Associations of fetal and infant combined kidney volume with eGFR at school-age (N=614)

A. eGFRcreat



B. eGFRcyst



Values are regression coefficients (95% confidence interval) based on multiple regression models and reflect the difference for standard deviation score of eGFR compared to with children with normal fetal and infant kidney volume. Tertiles of fetal kidney volume at 30 weeks gestational age and early childhood kidney volume at the age of 24 months were created. Models are adjusted for child sex and age at outcome measurements, folic acid use, maternal smoking, parity, birth weight, gestational age at birth and BMI at 6 years.

Chapter 3

Fetal, infant and childhood life style related factors

Chapter 3.1

Fetal smoke exposure and kidney outcomes in school-age children

Adapted from Am J Kidney Dis. 2015;66(3):412-20

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Abstract

Background Fetal smoke exposure might lead to developmental adaptations that permanently affect the developing kidney. We assessed the associations of maternal and paternal smoking during pregnancy with childhood kidney size and function.

Methods Prospective cohort study from fetal life onwards. This study was conducted in a group of 5,622 children in Rotterdam, the Netherlands. Maternal and paternal smoking were assessed during pregnancy by questionnaires. At a median age of 6.0 years (95% range 5.6 – 7.9), we measured childhood kidney volumes, estimated glomerular filtration rate (eGFR) and microalbuminuria.

Results Compared to children from mothers who did not smoke during pregnancy, those from mothers who continued smoking during pregnancy had a smaller combined kidney volume at the age of 6 years. The strongest effect estimate was observed for mothers who smoked 5 or more cigarettes per day during pregnancy (difference for combined kidney volume -2.80 cm^3 (95% CI: -5.15, -0.45)). Similarly, continued maternal smoking during pregnancy was also associated with a lower eGFR in childhood (difference -2.09 ml/min per 1.73m^2 (95% CI: -3.55, -0.63)). First trimester only smoking was associated with a higher risk of microalbuminuria (Odds Ratio 1.45, (95% CI: 1.05, 2.01)). Among mothers who did not smoke during pregnancy, paternal smoking was associated with smaller childhood combined kidney volume (difference -1.78 cm^3 (95% (CI): -3.48, -0.07)), but not with childhood kidney function measures. Smoking behavior was measured with questionnaires. Follow-up measurements were only available in 70% of the children.

Conclusions Continued maternal smoking during pregnancy is associated with smaller combined kidney volume and lower eGFR in school-age children. Stronger effect estimates for maternal smoking than paternal smoking suggest intra-uterine adaptive responses might be involved as underlying mechanisms.

Introduction

Fetal kidney development can be adversely affected by adverse exposures. Since nephrogenesis continues until 36 weeks of gestation and largely ceases thereafter, adverse exposures during this critical period may lead to impaired kidney development.(1,2) Impaired fetal kidney growth with a reduced number of nephrons might lead to glomerular hyperfiltration and sclerosis, subsequently predisposing the individual to impaired kidney function and chronic kidney disease in adulthood.(3,4) This hypothesis is supported by various studies showing associations of low birth weight, as a result of an adverse fetal environment, with chronic kidney disease in later life.(5) Thus far, not much is known about the specific adverse fetal exposures leading to impaired kidney function in later life. Maternal smoking during pregnancy is an important modifiable adverse fetal exposures, and strongly related with increased risks of low birth weight and preterm birth.(6-8) Maternal smoking during pregnancy may also have direct adverse effects on fetal kidney and vascular development.(9) Previously, we have shown that maternal smoking during pregnancy is associated with third trimester fetal kidney development.(10) Other studies showed that fetal kidney growth, measured by ultrasound or MRI, was affected by prenatal cigarette exposure.(9,11) Also, several animal studies showed structural changes in kidney morphology after prenatal cigarette exposure.(12,13) Whether these changes in early life persist and affect kidney function in later life is not known. Although any observational association between maternal smoking and kidney development may be the result of direct intra-uterine effects of fetal smoke exposure on kidney development, they may also reflect family-, socioeconomic-based, or life style related characteristics. Comparing the strength of effects of maternal and paternal smoking could help in disentangling the underlying mechanisms. Stronger effects for maternal compared to paternal smoking during pregnancy would suggest direct intra-uterine effects, whereas similar effects for paternal and maternal smoking are more likely to imply a role for shared family life style related characteristics or genetic factors.(14,15)

We assessed in a large population-based prospective cohort study among 5,622 children, the associations of maternal and paternal smoking during pregnancy with kidney size and function, measured in blood and urine samples, in school-age children. We used subclinical changes in kidney function as outcomes since they precede clinical disease at later life.(3)

Methods

Study design and population for analysis

The study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.(16) Enrolment in the study was aimed at early pregnancy, but was allowed until the birth of the child. 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 participants. In total, 8,879 mothers were enrolled in the study during pregnancy, of whom 8,244 (84.3%) provided information about their smoking habits. For the present study, only singleton life births were included (N=8,024), of whom 5,658 (70.5%) children attended the follow-up visit between March 2008 and January 2012. Children with evidence of congenital kidney abnormalities on ultrasound examination were excluded from the study (N=10). Kidney ultrasound, blood samples or urine samples were successfully obtained in 5,622 (99.3%) children (**Figure 1**).

Maternal and paternal smoking during pregnancy

As we have described before,(17) we asked each mother at enrolment, whether she smoked during pregnancy (no smoking; smoking until pregnancy was acknowledged (first trimester only smoking); continued smoking during pregnancy). Mothers who were enrolled before a gestational age of 18 weeks and between 18 and 25 weeks of gestation, also received a second and third trimester questionnaire, respectively. Mothers who reported in the first questionnaire that they smoked during the first trimester only (N=921), but still reported to smoke in the second or third trimester questionnaire (N=312) were reclassified into the 'continued smoking during pregnancy' category. The same strategy was used for women who reported no smoking in the first questionnaire, but reported smoking in the second or third questionnaire (N=80). Paternal smoking was assessed in the first questionnaire by asking the mother whether the father smoked during pregnancy (yes, no, or do not know). Among mothers and fathers who smoked, the number of cigarettes smoked daily was categorized as: <5 cigarettes/day ≥5 cigarettes/day. Similar information completed by the father was available in 3,558 (64%) participants. Agreement between these assessments by the mother and the father was good (sensitivity: 91%; specificity: 95%).(17) Tobacco smoke exposure at the child's home was assessed by questionnaire around the age of 6 (no, seldom or never; yes, but less than once a week; yes, more than once a week).

Kidney outcomes in children

Childhood kidney dimensions: Left and right kidney biometrics were at the median age of 6.0 (95% range 5.6 – 7.9) years. As we described earlier,(18,19) we identified the left and right kidney in the sagittal plane along its longitudinal axis. We performed measurements of maximal bipolar kidney length, width and depth. Kidney width and depth were measured at the level of the hilum. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used. Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).(20) Combined kidney volume was calculated by summing right and left kidney volume. We previously reported good intra-observer and inter-observer correlation coefficients.(21)

Childhood kidney function: Blood creatinine levels were measured with an enzymatic method on a Cobas c 502 analyzer (Roche Diagnostics, Germany), as previously

described.(18,19) Quality control samples demonstrated intra- and inter-assay co-efficients of variation ranging from 0.51% to 1.37%. Estimated glomerular filtration rate (eGFR) was calculated according to the revised Schwartz 2009 formula(22); eGFR = 36.5 * (height (cm)/creatinine (µmol/l).(22) Urine creatinine (mmol/l) and urine albumin (mg/l) levels were determined on Beckman Coulter AU analyser, creatinine levels were measured according to the Jaffe method. We calculated the albumin-creatinine ratio. For boys microalbuminuria was defined as an albumin-creatinine ratio between 2.5 and 25 mg/mmol, for girls we used a ratio between 3.5 and 25 mg/mmol.(23)

Covariates

Information on maternal age, parity, educational level, pre-pregnancy body mass index (BMI) and maternal and paternal ethnicity was obtained from questionnaires.(24) Maternal and paternal blood pressure at intake was measured with the validated Omron 907 automated digital oscillometric sphygmanometer (OMRON Healthcare B.V. Hoofddop, the Netherlands).(25) Child sex, gestational age at birth and birth weight were obtained from midwife and hospital registries. Breastfeeding (yes/no) was assessed using questionnaires. Current height and weight were measured without shoes and heavy clothing at the visit at 6 years, and body surface area (m^2) (BSA) was calculated. We assessed the associations of the covariates with the outcome measurements in a univariate model including child age and sex. All covariates, except ethnicity, were associated ($P<0.10$) with one or more outcome measurements (Supplementary Material **Table S1**).

Statistical analysis

We assessed differences in baseline characteristics between the categories of maternal smoking during pregnancy using independent samples t-tests and Chi Square tests. We used multiple linear regression models to assess the associations of maternal smoking with combined kidney volume and eGFR in childhood. For analyses on the risk of microalbuminuria, we used logistic regression models. We investigated the associations of the quantity of maternal cigarettes smoked with kidney outcomes using similar models. Tests for trend were performed using multiple linear regression models in which the categories of the number of cigarettes smoked were included as a continuous variable, using the non-smoking mothers as reference group. The models were first adjusted for sex and age of the child (basic model). These models were additionally adjusted for potential confounders including maternal age, ethnicity, parity, educational level, pre-pregnancy body mass index, blood pressure at intake, gestational age at birth, birth weight and childhood BSA (confounder model). Analyses focused on eGFR were not further adjusted for BSA since this is included in the Schwartz 2009 formula. In addition we adjusted the confounder model for smoke exposure at home around the age 6 (childhood smoke exposure model). We additionally adjusted the childhood smoke exposure model for childhood kidney volume (childhood kidney volume model). With the childhood models we try to explore whether any association was explained

by childhood smoke exposure or childhood kidney volume. The confounder model was considered as the main model. We used similar models to assess the associations of paternal smoking during pregnancy on childhood kidney outcomes. These analyses were performed among mothers who did not smoke during pregnancy only. Since maternal and paternal smoking were strongly correlated, investigating paternal smoking among all mothers would overestimate the effect of paternal smoking if direct intra-uterine mechanisms are present. In these analyses we adjusted for paternal ethnicity and blood pressure at intake. Furthermore, we tested potential combined effects and interactions between maternal smoking, size at birth, gestational age at birth for the associations with childhood kidney volume and eGFR by performing stratified regression analyses. The interactions between smoking of the mother and birth outcomes with childhood kidney outcomes were not significant and no further stratified analyses were performed. Missing values in covariates (ranging from 0 to 39%, see Supplementary Material **Table S2**), were multiple-imputed, to reduce potential bias associated with missing data.(26) We created five imputed datasets and each dataset was analyzed separately to obtain the effect sizes and standard errors. The results of all 5 imputed analyses were pooled and are presented in this paper. Measures of association are presented with their 95% confidence intervals (CI). All P-values are 2-sided and considered a p-value of <0.05 as statistically significant. All statistical analyses were performed using the Statistical Package of Social Sciences version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Subject characteristics are presented in **Table 1**. The mean estimated glomerular filtration rate (eGFR) was 118.7 ml/min per 1.73m^2 (SD 16.0) and 7.4 percent of our sample had microalbuminuria. The observed, non-imputed, baseline characteristics are given in the Supplementary Material (**Table S2**). Mothers who continued smoking during pregnancy were more often younger, lower educated, from a non-European ethnicity, and more often had partners who also smoked. Also, their children had a lower birth weight, were less often breastfed and had a higher body mass index (BMI) at the age of 6 than children of mothers who did not smoke. Non-response analysis (**Table S3**) showed that compared with mothers of children not included in the analysis, mothers of children included in the analysis were older, were more frequently higher educated and of the Dutch or European ethnicity.

Maternal smoking during pregnancy and childhood kidney outcomes

Table 2 shows that compared to children from mothers who did not smoke during pregnancy, those from mothers who continued smoking during pregnancy had a smaller combined kidney volume at the age of 6 years. The strongest effect estimate was observed for mothers who smoked 5 or more cigarettes per day during pregnancy (difference for combined kidney volume -2.80 cm^3 (95% Confidence Interval (CI): -5.15,

-0.45)). Similarly, continued maternal smoking during pregnancy was also associated with a lower eGFR in childhood (difference -2.09 ml/min per 1.73m^2 (95% CI: -3.55, -0.63)). The association of maternal smoking during pregnancy with eGFR was stronger for a higher number of cigarettes (p-value for trend <0.05). First trimester only smoking was associated with a higher risk of microalbuminuria (Odds Ratio (OR) 1.45, (95% CI: 1.05, 2.01)). Additional adjustment for smoke exposure during childhood did not materially changed the effect estimates. The effects of maternal smoking on childhood eGFR were only slightly explained by childhood kidney volume. Models that were only adjusted for sex and age of the child (basic models) show slightly stronger effect estimates (**Table 2**).

Paternal smoking during pregnancy and childhood kidney outcomes

Table 3 shows that among children of mothers who did not smoke during pregnancy, paternal smoking tended to be associated with a smaller childhood combined kidney volume. Similarly to maternal smoking during pregnancy, the strongest association was observed for fathers who smoked 5 or more cigarettes per day (difference -1.77 cm^3 (95% CI: -3.47, -0.07)). Paternal smoking was not associated with childhood eGFR and microalbuminuria. Additional adjustment for smoke exposure during childhood and childhood kidney volume did not materially change the associations between paternal smoking during pregnancy and measures of childhood kidney volume and function. Models that were only adjusted for sex and age of the child show similar results (**Table 3**). The effect estimates for maternal smoking on childhood kidney volume were not significantly stronger compared with paternal smoking.

Figure 1. Flowchart

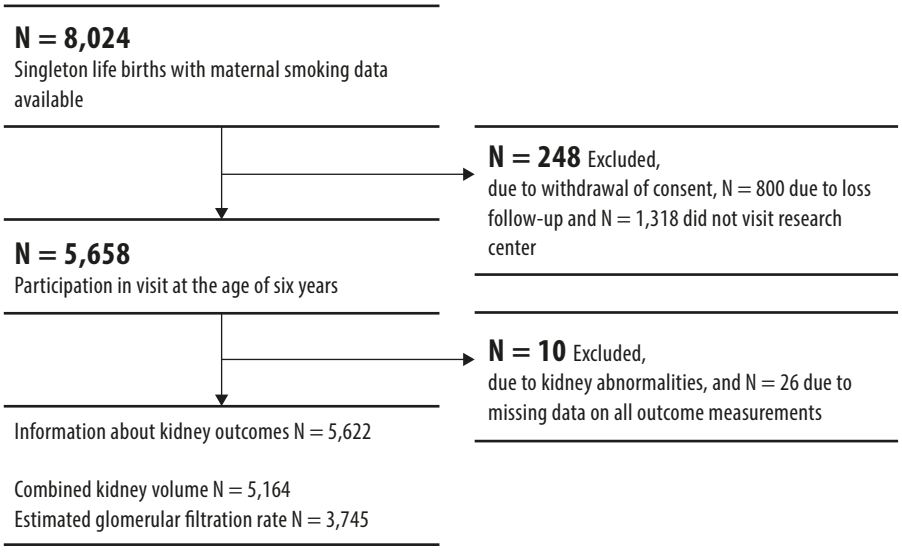

Table 1. Subject characteristics (N=5,622)

	Maternal smoking during pregnancy		
	Non smoking N=4,199 (74.7%)	**Stopped when pregnancy was known N=498 (8.9%)**	**Continued smoking N=925 (16.4%)**
Maternal characteristics			
Age, (y)	31.4 (20.4, 39.9)	30.7 (19.4, 39.5)†	29.6 (18.7, 39.2)†
Gestational age at enrollment (wks)	13.9 (9.8, 24.4)	13.4 (9.8, 22.3)†	14.1 (9.5, 23.8)
Height, (cm)	167.7 (7.4)	168.8 (7.0)†	166.9 (7.1)†
Pre-pregnancy weight, (kg)	66.5 (12.4)	66.1 (11.5)	67.1 (9.6)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (3.9)	23.2 (4.0)*	23.8 (4.8)
Parity ≥1, (%)	44.2 (1855)	29.3 (146)†	44.2 (409)
Systolic blood pressure (mmHg)	115.8 (12.3)	116.0 (12.1)	115.9 (12.1)
Diastolic blood pressure (mmHg)	68.6 (9.4)	67.7 (9.5)	67.1 (9.7)†
Education, (%)			
Primary/secondary	48.8 (2051)	54.0 (269)*	77.6 (718)†
Secondary or higher	51.2 (2148)	46.0 (228)*	22.4 (207)†
Ethnicity, (%)			
Dutch or European	63.7 (2673)	66.9 (333)	55.7 (515)†
Non-European	36.3 (1526)	33.1 (165)	44.3 (410)†
Paternal characteristics			
Age, (y)	33.6 (22.8, 46.7)	32.4 (22.1, 46.8)†	31.9 (19.8, 44.6)†
Height, (cm)	182.0 (7.6)	182.6 (8.1)	180.5 (7.9)†
Weight, (kg)	83.8 (11.2)	83.6 (13.0)	82.8 (11.8)
Body mass index, (kg/m^2)	25.3 (3.1)	25.1 (3.2)	25.4 (3.6)
Ethnicity, (%)			
Dutch or European	64.6 (2714)	68.5 (341)*	56.2 (520)†
Non-European	35.4 (1485)	31.5 (157)*	43.8 (405)†
Systolic blood pressure (mmHg)	130.0 (13.5)	130.1 (13.6)	129.7 (13.4)
Diastolic blood pressure (mmHg)	73.6 (10.6)	72.9 (10.3)	72.7 (10.7)*
Smoking, (%)			
Yes	35.5 (1350)	65.3 (301)†	77.1 (608)†
No	64.5 (2454)	34.7 (160)†	23.0 (180)†
Birth characteristics			
Sex boys, (%)	49.2 (2066)	47.4 (236)	55.0 (509)†
Gestational age, (wk)	40.1 (36.0, 42.3)	40.1 (35.9, 42.2)	40.0 (34.7, 42.3)†
Preterm, (%) (n)	4.5 (191)	4.2 (21)	6.1 (56)
Birth weight, (g)	3457 (545)	3453 (551)	3282 (556)†
Small for gestational age, (%) (n)	5.1 (216)	5.8 (29)	11.6 (107)†
Breastfed yes, (%)	93.4 (3922)	92.6 (461)	84.4 (781)†

Table 1. Continued

Child characteristics			
Age, (y)	6.0 (5.6, 7.8)	6.0 (5.6, 8.1)	6.1 (5.6, 8.1)†
Height, (cm)	119.4 (5.9)	119.8 (6.2)	119.5 (6.4)
Weight, (kg)	23.1 (4.1)	23.3 (4.2)	24.0 (5.1)†
Body mass index, (kg/m^2)	16.1 (1.8)	16.1 (1.9)	16.7 (2.2)†
Body surface area, (m^2)	0.87 (0.09)	0.88 (0.09)	0.89 (0.11)†
Smoke at home, (%)			
No, seldom or never	91.0 (3821)	80.5 (401)	46.8 (433)
Yes, but less than once a week	3.0 (127)	6.2 (31)	9.7 (90)
Yes, more than once a week	6.0 (251)	13.3 (66)	43.5 (402)
Combined kidney volume, (cm^3)	120.1 (23.2)	119.8 (21.4)	119.9 (25.0)
Creatinine, (µmol/l)	37.3 (5.3)	37.5 (5.5)	38.5 (5.7)†
eGFR, ml/min 1.73 m^2	119.3 (16.0)	119.3 (15.3)	116.0 (16.3)†
Microalbuminuria‡, (%)	7.2 (291)	9.9 (48)*	7.1 (63)

Values are means (SD) or medians (95% range) or % (numbers). Differences between categories of maternal smoking were compared using T-test and chi-square, non-smoking category was used as reference.

‡Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls).

eGFR, estimated glomerular filtration rate

*P<0.05, †P<0.01

Table 2. Maternal smoking during pregnancy and kidney size and function in 6 year old children (N=5,622)

	Combined kidney volume difference (95% CI) (cm^3)	eGFR difference (95% CI) (ml/min per 1.73m^2)	Microalbuminuria Odds Ratio (95% CI)
Maternal smoking during pregnancy			
Basic model†			
Non smoking during pregnancy (N=4,199)	Reference	Reference	Reference
Stopped when pregnancy was known (N=498)	-0.27 (-2.46, 1.91)	0.10 (-1.71, 1.91)	1.41 (1.02, 1.95)*
Continued smoking during pregnancy (N=925)	-1.91 (-3.59, -0.22)*	-2.94 (-4.35, -1.54)†	0.99 (0.74, 1.31)
< 5 cigarettes per day (N=355)	-0.73 (-3.28, 1.82)	-2.29 (-4.44, -0.13)*	1.08 (0.71, 1.65)

Table 2. Continued

≥5 cigarettes per day (N=334)	-4.28 (-6.91, -1.65)†	-3.36 (-5.51, -1.20)†	1.01 (0.71, 1.67)
P-value for trend	P<0.01	P<0.01	P=0.64
Confounder model[2]			
Non smoking during pregnancy (N=4,199)	Reference	Reference	Reference
Stopped when pregnancy was known (N=498)	-1.08 (-2.99, 0.83)	-0.06 (-1.88, 1.76)	1.45 (1.05, 2.01)*
Continued smoking during pregnancy (N=925)	-0.89 (-2.41, 0.63)	-2.09 (-3.55, -0.63)†	1.08 (0.80, 1.44)
< 5 cigarettes per day (N=355)	0.22 (-2.02, 2.45)	-1.68 (-3.85, 0.49)	1.16 (0.76, 1.77)
≥5 cigarettes per day (N=334)	-2.80 (-5.15, -0.45)*	-2.22 (-4.42, -0.02)*	1.21 (0.78, 1.88)
P-value for trend	P<0.05	P<0.05	P=0.33
Childhood smoke exposure model[3]			
Non smoking during pregnancy (N=4,199)	Reference	Reference	Reference
Stopped when pregnancy was known (N=498)	-1.02 (-2.94, 0.90)	-0.11 (-1.94, 1.72)	1.44 (1.04, 2.00)*
Continued smoking during pregnancy (N=925)	-0.70 (-2.37, 0.96)	-2.04 (-3.71, -0.38)*	1.03 (0.74, 1.43)
< 5 cigarettes per day (N=355)	0.30 (-2.01, 2.60)	-1.79 (-4.05, 0.48)	1.14 (0.74, 1.77)
≥5 cigarettes per day (N=334)	-2.72 (-5.22, -0.21)*	-1.92 (-4.33, 0.50)	1.17 (0.73, 1.88)
P-value for trend	P=0.11	P<0.05	P=0.53
Childhood kidney volume model[4]			
Non smoking during pregnancy (N=4,197)	NA	Reference	Reference
Stopped when pregnancy was known (N=498)	NA	0.65 (-1.17, 2.47)	1.43 (1.01, 2.02)*
Continued smoking during pregnancy (N=925)	NA	-1.90 (-3.57, 0.22)*	1.04 (0.74, 1.47)
< 5 cigarettes per day (N=355)	NA	-1.28 (-3.54, 0.97)	1.20 (0.76, 1.90)
≥5 cigarettes per day (N=334)	NA	-1.52 (-3.95, 0.91)	1.36 (0.84, 2.20)
P-value for trend	NA	P=0.11	P=0.26

Values are based on multiple linear and logistic regression models and reflect regression coefficients (95% Confidence interval (CI)) and Odds Ratio's (95% CI) for microalbuminuria. [1]Model is adjusted for child sex and current age. [2]Model is additionally adjusted for maternal age, parity, educational level, ethnicity, pre-pregnancy body mass index, blood pressure at intake, child's gestational age at birth, birth weight, breastfeeding status and childhood body surface area. [3]Model is additionally adjusted for child smoke exposure. [4]Model is additionally adjusted for combined childhood kidney volume. Children of non-smoking mothers were used as reference group.

eGFR, estimated glomerular filtration rate

*P<0.05, †P<0.01

Table 3. Paternal Smoking during pregnancy and kidney size and function in 6 year old children (N=3,804)

	Combined kidney volume difference (95% CI, cm^3)	eGFR difference (95% CI, ml/min per 1.73m^2)	Microalbuminuria Odds Ratio (95%CI)
Paternal smoking			
Basic model[1]			
No (N=2,454)	Reference	Reference	Reference
Yes (N=1,350)	-0.15 (-1.72, 1.42)	-0.45 (-1.74, 0.85)	0.92 (0.71, 1.20)
< 5 cigarettes per day (N=590)	0.89 (-1.22, 2.99)	-1.16 (-2.91, 0.59)	0.90 (0.62, 1.29)
≥5 cigarettes per day (N=731)	-1.40 (-3.33, 0.54)	0.20 (-1.43, 1.83)	0.96 (0.70, 1.33)
P-value for trend	P=0.28	P=0.92	P=0.72
Confounder model[2]			
No (N=2,454)	Reference	Reference	Reference
Yes (N=1,350)	-0.77 (-2.16, 0.61)	-0.36 (-1.66, 0.94)	0.95 (0.73, 1.24)
< 5 cigarettes per day (N=590)	0.06 (-1.80, 1.92)	-1.24 (-2.99, 0.52)	0.89 (0.62, 1.29)
≥5 cigarettes per day (N=731)	-1.78 (-3.48, -0.07)*	0.31 (-1.33, 1.95)	1.01 (0.73, 1.40)
P-value for trend	P=0.06	P=0.96	P=0.92
Childhood smoke exposure model[3]			
No (N=2,454)	Reference	Reference	Reference
Yes (N=1,350)	-0.77 (-2.24, 0.70)	-0.30 (-1.67, 1.08)	0.94 (0.70, 1.25)
< 5 cigarettes per day (N=590)	0.05 (-1.83, 1.92)	-1.22 (-3.00, 0.55)	0.89 (0.61, 1.29)
≥5 cigarettes per day (N=731)	-1.80 (-3.62, 0.02)	0.46 (-1.30, 2.21)	1.00 (0.70, 1.43)
P-value for trend	P=0.08	P=0.79	P=0.85
Childhood kidney volume model[4]			
No (N=2,454)	NA	Reference	Reference
Yes (N=1,350)	NA	-0.26 (-1.63, 1.11)	1.08 (0.80, 1.46)
< 5 cigarettes per day (N=590)	NA	-1.44 (-3.21, 0.33)	1.03 (0.71, 1.51)
≥5 cigarettes per day (N=731)	NA	0.67 (-1.04, 2.46)	1.14 (0.79, 1.66)
P-value for trend	NA	P=0.64	P=0.49

Values are based on multiple linear and logistic regression models and reflect regression coefficients (95% Confidence interval (CI)) and Odds Ratio's (95% CI) for microalbuminuria. The associations of paternal smoking were assessed among children of mothers who did not smoke during pregnancy. [1]Model is adjusted for child sex and current age. [2]Model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, paternal ethnicity and blood pressure at intake, child's gestational age at birth, birth weight, breastfeeding status and childhood body surface area. [3]Model is additionally adjusted for child smoke exposure. [4]Model is additionally adjusted for combined childhood kidney volume. Children of non-smoking fathers were used as reference group.

eGFR, estimated glomerular filtration rate

*P<0.05

Discussion

The results of this study suggest that continued maternal smoking during pregnancy is associated with a smaller childhood kidney size and a lower eGFR. A higher risk of microalbuminuria was found in children of mothers who smoked only in first trimester. Among mothers who did not smoke during pregnancy, paternal smoking was associated with smaller childhood combined kidney volume, but not with childhood kidney function measures.

Adverse fetal exposures, such as exposure to cigarette smoking, may influence fetal kidney development. Since nephrogenesis continues until 36 weeks of gestation and largely ceases thereafter, adverse exposures during this critical period may lead to impaired kidney development.(1,2) It has been hypothesized that impaired fetal kidney growth with a reduced number of nephrons leads to glomerular hyperfiltration and sclerosis, subsequently predisposing the individual to impaired kidney function and chronic kidney disease in adulthood.(3,4) Several studies, both in animals and in humans, have investigated the association of cigarette exposure during pregnancy with short-term kidney development outcomes in early life. We have previously shown in 1,072 pregnant mothers and their unborn children that maternal smoking during pregnancy was associated with an altered kidney development in third trimester of pregnancy in a time- and dose-dependent manner.(10) Two human studies using ultrasound or MRI during fetal life showed diminished kidney growth in fetuses of mothers who smoked during pregnancy compared to non-smoking mothers.(9,11)

To the best of our knowledge the present study is the first that examines the associations of parental smoking during pregnancy with childhood kidney outcomes. We observed associations of maternal smoking with smaller childhood kidney volume and a lower eGFR. These associations were not explained by childhood smoke exposure. Our results suggest that the changes observed in fetal life, persist in childhood and may have effect on kidney function. Animal studies showed changes in kidney morphology after prenatal exposure to maternal cigarette smoking.(12,13) Our results are in line with an earlier study from the same cohort as the present study, which showed that maternal smoking during pregnancy is associated with an increase in diastolic blood pressure and fractional shortening in 6 year old children, with stronger effects for maternal smoking than paternal smoking.(17) Another study showed an effect of maternal smoking during pregnancy on childhood systolic blood pressure, dependent of birth weight.(27) Surprisingly, we observed that first trimester smoking only was associated with a higher risk of microalbuminuria, independent of potential confounders, childhood smoke exposure and kidney size. Continued smoking was not associated with a higher risk of microalbuminuria. We could not explain why only first-trimester maternal smoking would be related with childhood microalbuminuria.

In the current study, we tried to disentangle the causality for the association of maternal smoking during pregnancy with childhood kidney outcomes. Next to adjusting for multiple potential confounders, we assessed the association of both maternal and paternal smoking during pregnancy with kidney outcomes to further explore the role of confounding.

Adjustment for potential confounders did not fully explain the associations. The adverse effects of maternal smoking on the fetus are likely to be much larger than the effects of paternal smoking. Stronger effects on kidney outcomes for maternal compared to paternal smoking during pregnancy would suggest direct intra-uterine effects, whereas similar effects for paternal and maternal smoking are more likely to imply a role for shared family-based and life style related factors.(14,15) Earlier we showed continued maternal smoking during pregnancy leads to a 100-200 grams lower birth weight, whereas only paternal smoking leads to a 29-44 grams lower birth weight.(6) Although the differences in effect estimates of maternal and paternal smoking were not significant, we observed stronger effect sizes for the associations of maternal smoking than for paternal smoking with childhood kidney volume. Maternal smoking was related to kidney function, but paternal smoking was not. These results suggest a role of direct intra-uterine mechanisms following tobacco exposure. However, we should be careful with concluding intrauterine causal mechanisms since the effect estimates were small, and of borderline significance.

The biological mechanisms underlying the association of fetal smoke exposure with childhood kidney development are unknown. It might be explained by various smoking substances, such as teratogen and toxins, which involve nicotine, carbon monoxide and cadmium. Nicotine induces vasoconstriction, which leads to reduced placental blood flow and lower oxygen levels during fetal life,(28) which may result in a decreased hemodynamic stimulus for fetal vascular development.(29,30) Carbon monoxide and cadmium exposure lead to lower placental and fetal perfusion.(17,18) Subsequently, these vascular changes might lead to kidney developmental adaptations. The possible mechanism underlying the associations between fetal smoke exposure and kidney function in later life need to be studied in further research.

The results of this study are important from an etiological perspective. Fetal exposure to maternal smoking have persistent subclinical consequences on kidney outcomes. The associations of smoking with childhood eGFR were independent of childhood kidney size. This might imply that fetal smoke exposure has a permanent subclinical effect, independent of later kidney growth, on kidney function in later life. Longitudinal studies report the risks factors for kidney diseases track from childhood to adulthood.(31,32) We did not find associations of maternal smoking with a higher risk of childhood microalbuminuria. The effects of impaired kidney growth on microalbuminuria may not be detectable during childhood, but may become more evident later in life. For example, it also has been suggested that fetal adverse exposures can be compensated for many years before high levels of blood pressure are present.(33)

The main strength of our study is the prospective data collection from early fetal life onwards and the size of the population-based cohort. Our analyses were based on more than 5,600 children with kidney outcome measurements. The detailed information on maternal and paternal smoking during pregnancy enabled us to assess both trimester specific and dose-response relationships. We used ultrasound measurements to calculate kidney volume and measured kidney function using blood and urine samples. Also, we had information about a large number of potential confounders. We also need to address some limitations.

Information about smoking during pregnancy was missing for 16% of all mothers. This non-response would lead to biased effect estimates if associations of maternal smoking during pregnancy with kidney outcomes would be different between those mothers included and not included in the analyses. This seems unlikely, but cannot be excluded. Biased estimates in large cohort studies mainly arise from loss to follow-up rather than from a non-response at baseline.(34) Of all children with available data on maternal smoking during pregnancy, 70% participated in the follow-up measurements at the age of six years. Overall, mothers who did not visit the research center for follow-up measurements were younger, did more frequently smoke during pregnancy, were less educated and did less often breastfed their child than the total sample. Their children had also a lower birth weight. This selective loss to follow-up might have led to an underestimation of the effect estimates observed. Furthermore, the selection towards a healthier group of mothers might affect the generalizability of the results towards more high-risk populations. Smoking behavior was measured with questionnaires. This might have resulted in an underreporting of smoking behavior in some individuals. Because nephron number cannot be studied *in vivo*, kidney size was used as a measure of kidney development. Kidney size is correlated with the number of glomeruli and can, in epidemiology studies, be used as a measure of kidney development.(35) The glomerular filtration rate was calculated using blood creatinine levels. More accurate might be the use of Cystatin C blood levels, Cystatin C is produced more constantly and less dependent on children's body weight, height, and sex.(36) However, when using Cystatin C instead of eGFR, we observed quite similar results. The albumin/creatinine ratio was used to assess albuminuria in a random urine sample.(37) For urinary albumin excretion the within subject variation is large, variability will be lower if we collected first morning void samples instead of random samples during the day;(38) unfortunately this was not possible in the current study. Finally, although we have performed adjustment for various potential confounders, residual confounding should be considered due to the observational design of the study.

Conclusion and perspectives

In conclusion, we observed that maternal smoking during pregnancy is associated with smaller childhood kidney volume and lower eGFR. Maternal smoking during pregnancy is modifiable and could present an opportunity for prevention of chronic kidney disease in later life. Further research is needed to assess whether the associations between fetal smoke exposure and kidney function persist during later life and to establish whether reduction of maternal smoking during pregnancy might lead to a better kidney development and improved kidney function during the life course.

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Table S1. Univariate associations of covariates with kidney size and function in six year old children

	Combined kidney volume difference (95% CI) (cm^3)	eGFR difference (95% CI) (ml/min per 1.73m^2)	Microalbuminuria Odds Ratio (95% CI)
Covariates, maternal characteristics			
Maternal age, (y)	-0.00 (-0.12, 0.12)	0.09 (-0.01, 0.19)#	1.02 (1.00 , 1.04)
Pre-pregnancy body mass index, (kg/m^2)	0.43 (0.28, 0.59)†	0.07 (-0.07, 0.21)	0.99 (0.97 , 1.02)
Parity (multiparous vs primiparous)	-1.21 (-2.45, 0.04)#	-1.37 (-2.41, -0.34)†	0.97 (0.79 , 1.19)
Systolic blood pressure (mmHg)	0.12 (0.07, 0.18)†	-0.01 (-0.05, 0.04)	1.01 (1.00, 1.02)
Diastolic blood pressure (mmHg)	0.04 (-0.03, 0.11)	0.02 (-0.04, 0.08)	1.01 (1.00, 1.02)#
Educational level (higher versus primary/secondary)	2.36 (1.10, 3.62)†	1.75 (0.69 , 2.82)†	1.22 (0.99 , 1.50)#
Ethnicity (European vs Non-European)	-0.91 (-2.20, 0.38)	0.18 (-0.90, 1.26)	0.95 (0.77, 1.17)
Covariates, paternal characteristics			
Ethnicity (European vs Non-European)	0.40 (-1.09, 1.89)	-0.04 (-1.25, 1.17)	1.15 (0.92, 1.44)
Systolic blood pressure (mmHg)	-0.01 (-0.08, 0.05)	-0.04 (-0.10, 0.02)	1.00 (0.99, 1.01)
Diastolic blood pressure (mmHg)	-0.10 (-0.18, -0.01)*	-0.06 (-0.12, 0.01)	1.00 (0.99, 1.01)
Covariates, birth characteristics			
Gestational age, (wk)	0.76 (0.41, 1.12)†	0.33 (0.03, 0.62)*	1.01 (0.98, 1.04)
Birth weight, (kg)	8.16 (7.06, 9.27)†	1.85 (0.90, 2.79)†	0.95 (0.79, 1.14)
Breastfed (yes vs no)	3.98 (1.14, 6.82)†	2.90 (0.77, 5.04)†	1.41 (0.76, 2.62)
Covariates, child characteristics			
Body mass index, (kg/m^2)	4.21 (3.88, 4.53)†	-0.20 (-0.48, 0.08)	0.90 (0.85, 0.96)†
Body surface area, (m^2)	139.17 (132.33, 146.02)†	18.64 (12.20, 25.08)†	0.24 (0.06, 0.88)*

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds Ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference in kidney size and function at the age of six years for different categories of maternal smoking during pregnancy. All regression analyses were adjusted for child sex and current age.

#<p<0.10, *P<0.05, †P<0.01

Table S2. Subject characteristics of observed and imputed data (N=5,622)

	Maternal smoking during pregnancy					
	Non smoking N=4,199 (74.7%)		Stopped when pregnancy was known N=498 (8.9%)		Continued smoking N=925 (16.4%)	
	Observed	Imputed	Observed	Imputed	Observed	Imputed
Maternal characteristics						
Age, (y)	31.4 (20.4, 39.9)	31.4 (20.4, 39.9)	30.7 (19.4, 39.5)	30.7 (19.4, 39.5)	29.6 (18.7, 39.2)	29.6 (18.7, 39.2)
Missing, (%)	0 (0)		0 (0)		0 (0)	
Gestational age at enrollment (wks)	13.9 (10.2, 24.4)	13.9 (9.8, 24.4)	13.4 (9.8, 22.3)	13.4 (9.8, 22.3)	14.1 (9.5, 23.7)	14.1 (9.5, 23.8)
Missing, (%)	8.0 (338)		6.4 (32)		3.7 (34)	
Height, (cm)	167.7 (7.4)	167.7 (7.4)	168.8 (7.0)	168.8 (7.0)	166.9 (7.1)	166.9 (7.1)
Missing, (%)	8.3 (347)		6.6 (33)		3.8 (35)	
Pre-pregnancy weight, (kg)	66.5 (12.4)	66.5 (12.4)	66.1 (11.5)	66.1 (11.5)	66.5 (14.2)	67.1 (9.6)
Missing, (%)	17.6 (737)		15.5 (77)		18.6 (172)	
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.1)	23.6 (3.9)	23.2 (4.0)	23.2 (4.0)	23.8 (4.8)	23.8 (4.8)
Missing, (%)	17.7 (745)		15.5 (78)		18.6 (172)	
Parity ≥1, (%)	42.7 (1795)	44.2 (1855)	28.5 (142)	29.3 (146)	43.4 (401)	44.2 (409)
Missing	2.5 (103)		2.6 (13)		1.2 (11)	
Systolic blood pressure (mmHg)	115.7 (12.3)	115.8 (12.3)	115.9 (11.9)	116.2 (12.1)	115.8 (12.0)	115.9 (12.1)
Missing, (%)	9.0 (377)		7.2 (36)		5.0 (46)	
Diastolic blood pressure (mmHg)	68.5 (9.5)	68.6 (9.4)	67.6 (9.4)	67.7 (9.5)	67.0 (9.6)	67.1 (9.7)
Missing, (%)	9.0 (377)		7.2 (36)		5.0 (46)	
Education, (%)						
Primary/secondary	47.3 (1988)	48.8 (2051)	53.4 (266)	54.0 (269)*	71.6 (662)	77.6 (718)

Table S2. Continued

Secondary or higher	50.5 (2119)	51.2 (2148)	45.8 (228)	46.0 (228)*	21.4 (198)	22.4 (207)
Missing	2.2 (92)		0.8 (4)		7.0 (65)	
Ethnicity, (%)						
Dutch or European	63.6 (2672)	63.7 (2673)	66.9 (333)	66.9 (333)	55.2 (511)	55.7 (515)
Non-European	36.2 (1522)	36.3 (1526)	32.9 (164)	33.1 (165)	43.2 (400)	44.3 (410)
Missing	0.2 (5)		0.2 (1)		1.5 (14)	
Paternal characteristics						
Age,(y)	33.6 (22.9, 46.8)	33.6 (22.8, 46.7)	32.5 (22.2, 46.9)	32.4 (22.1, 46.8)	40.0 (19.8, 44.5)	31.9 (19.8, 44.6)
Missing, (%)	12.3 (518)		9.8 (49)		20.3 (188)	
Height, (cm	182.3 (7.8)	182.0 (7.6)	183.0 (8.0)	182.6 (8.1)	180.4 (8.1)	180.5 (7.9)
Missing, (%)	27.6 (1159)		23.6 (118)		33.3 (308)	
Weight, (kg)	84.0 (12.7)	83.8 (11.2)	83.7 (13.0)	83.6 (13.0)	82.9 (13.9)	82.8 (11.8)
Missing, (%)	27.6 (1161)		23.6 (118)		33.4 (309)	
Body mass index, (kg/m^2)	25.3 (3.4)	25.3 (3.1)	25.0 (3.4)	25.1 (3.2)	25.4 (3.6)	25.4 (3.6)
Missing, (%)	27.6 (1161)		23.6 (118)		33.4 (309)	
Ethnicity, (%)						
Dutch or European	50.9 (2139)	64.6 (2714)	54.6 (272)	68.5 (341)	41.3 (382)	56.2 (520)
Non-European	20.1 (846)	35.4 (1485)	20.3 (101)	31.5 (157)	22.7 (210)	43.8 (405)
Missing	29.0 (1214)		25.1 (125)		36.0 (333)	
Systolic blood pressure (mmHg)	130.3 (13.5)	130.0 (13.5)	130.1 (14.1)	130.1 (13.6)	130.2 (13.6)	129.7 (13.4)
Missing, (%)	31.7 (1333)		27.9 (139)		37.5 (347)	
Diastolic blood pressure (mmHg)	73.7 (10.5)	73.6 (10.6)	72.8 (10.3)	72.9 (10.3)	72.8 (11.1)	72.7 (10.7)
Missing, (%)	31.7 (1333)		27.9 (139)		37.5 (348)	
Smoking, (%)						

Table S2. Continued

Yes	32.2 (1350)	*Not imputed*	60.4 (301)	*Not imputed*	65.7 (608)	*Not imputed*
No	58.4 (2454)	*Not imputed*	32.1 (160)	*Not imputed*	19.5 (180)	*Not imputed*
Missing	9.4 (395)	*Not imputed*	7.4 (37)	*Not imputed*	14.8 (137)	*Not imputed*
Birth characteristics						
Sex boys, (%)	49.2 (2066)	49.2 (2066)	47.4 (236)	47.4 (236)	55.0 (509)	55.0 (509)
Missing	0 (0)		0 (0)		0 (0)	
Gestational age, (wk)	40.1 (36.0, 42.3)	40.1 (36.0, 42.3)	40.1 (35.9, 42.2)	40.1 (35.9, 42.2)	40.0 (34.7, 42.3)	40.0 (34.7, 42.3)
Missing, (%)	0 (2)		0 (0)		0.1 (1)	
Preterm, (%)	4.5 (191)	4.5 (191)	4.2 (21)	4.2 (21)	6.1 (56)	6.1 (56)
Birth weight, (g)	3458 (544)	3457 (545)	3453 (551)	3453 (551)	3281 (556)	3282 (556)
Missing, (%)	0.1 (6)		0 (0)		0.2 (2)	
Small for gestational age, (%)	5.1 (216)	5.1 (216)	5.8 (29)	5.8 (29)	11.6 (107)	11.6 (107)
Breastfed yes, (%)	76.3 (3202)	93.4 (3922)	76.3 (380)	92.6 (461)	59.4 (549)	84.4 (781)
Missing	18.6 (779)		17.7 (88)		30.6 (283)	
Child characteristics						
Age, (y)	6.0 (5.6, 7.8)	6.0 (5.6, 7.8)	6.0 (5.6, 8.1)	6.0 (5.6, 8.1)	6.1 (5.6, 8.1)	6.1 (5.6, 8.1)
Missing, (%)	0 (0)		0 (0)		0 (0)	
Height, (cm)	119.4 (5.9)	119.4 (5.9)	119.8 (6.2)	119.8 (6.2)	119.5 (6.4)	119.5 (6.4)
Missing, (%)	0.2 (8)		0.4 (2)		0 (0)	
Weight, (kg)	23.1 (4.1)	23.1 (4.1)	23.3 (4.2)	23.3 (4.2)	24.0 (5.1)	24.0 (5.1)
Missing, (%)	0.2 (8)		0.4 (2)		0 (0)	
Body mass index, (kg/m^2)	16.1 (1.8)	16.1 (1.8)	16.1 (1.9)	16.1 (1.9)	16.7 (2.2)	16.7 (2.2)
Body surface area, (m^2)	0.87 (0.09)	0.87 (0.09)	0.88 (0.09)	0.88 (0.09)	0.89 (0.11)	0.89 (0.11)
Smoke at home, (%)						
No, seldom or never	70.3 (2953)	91.0 (3821)	61.2 (305)	80.5 (401)	30.6 (283)	46.8 (433)
Yes, but less than once a week	1.9 (78)	3.0 (127)	3.9 (19)	6.2 (31)	5.6 (52)	9.7 (90)

Table S2. Continued

Yes, more than once a week	3.8 (162)	6.0 (251)	7.0 (35)	13.3 (66)	24.6 (227)	43.5 (402)
Missing	24.0 (1006)		27.9 (139)		39.2 (363)	
Combined kidney volume, (cm^3)	120.1 (23.2)	*Not imputed*	119.8 (21.4)	*Not imputed*	119.9 (25.0)	*Not imputed*
Creatinine, (µmol/l)	37.3 (5.3)	*Not imputed*	37.5 (5.5)	*Not imputed*	38.5 (5.7)	*Not imputed*
eGFR, ml/min 1.73 (m^2)	119.3 (16.0)	*Not imputed*	119.3 (15.3)	*Not imputed*	116.0 (16.3)	*Not imputed*
Microalbuminuria*,(%)	7.2 (291)	*Not imputed*	9.9 (48)	*Not imputed*	7.1 (63)	*Not imputed*

Values are means (SD), medians (95% range), or % (numbers)
*Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

eGFR, estimated glomerular filtration rate

Table S3. Subject characteristics of participants with and without follow-up data (N=8,024)

	Maternal smoking during pregnancy					
	Non smoking		Stopped when pregnancy was known		Continued smoking	
	Childhood data available N=4,199	Childhood data unavailable N=1,697	Childhood data available N=498	Childhood data unavailable N=183	Childhood data available N=925	Childhood data unavailable N=522
Maternal characteristics						
Age, (y)	31.4 (20.4, 39.9)	29.4 (18.7, 38.9)†	30.7 (19.4, 39.5)	28.3 (16.9, 32.2)†	29.6 (18.7, 39.2)	26.8 (18.1, 39.2)†
Gestational age at enrollment (wks)	13.9 (10.2, 24.4)	14.4 (10.2, 24.4)†	13.4 (9.8, 22.3)	13.6 (9.2, 23.1)	14.1 (9.5, 23.7)	14.2 (9.1, 29.9)
Height, (cm)	167.7 (7.4)	166.4 (7.4)†	168.8 (7.0)	166.9 (7.1)†	166.9 (7.1)	166.9 (7.0)
Pre-pregnancy weight, (kg)	66.5 (12.4)	65.8 (13.4)	66.1 (11.5)	66.1 (14.7)	66.5 (14.2)	65.1 (12.9)
Pre-pregnancy body mass index, (kg/m^2)	23.6 (4.1)	23.8 (4.6)	23.2 (4.0)	23.8 (5.3)	23.8 (4.8)	23.3 (4.5)
Parity ≥1, (%)	42.7 (1795)	46.6 (790)*	28.5 (142)	37.7 (69)*	43.4 (401)	46.0 (240)
Systolic blood pressure (mmHg)	115.7 (12.3)	114.8 (12.7)*	115.9 (11.9)	115.6 (11.9)	115.8 (12.0)	115.3 (11.6)
Diastolic blood pressure (mmHg)	68.5 (9.5)	67.8 (9.7)*	67.6 (9.4)	67.9 (8.7)	67.0 (9.6)	66.5 (10.1)
Education, (%)						
Primary/secondary	47.3 (1988)	58.0 (985)†	53.4 (266)	67.8 (124)†	71.6 (662)	82.0 (428)†
Secondary or higher	50.5 (2119)	38.3 (650)†	45.8 (228)	30.1 (55)†	21.4 (198)	10.9 (57)†
Ethnicity, (%)						
Dutch or European	63.6 (2672)	46.6 (790)†	66.9 (333)	49.7 (91)†	55.2 (511)	55.0 (287)
Non-European	36.2 (1522)	52.5 (891)†	32.9 (164)	49.7 (91)†	43.2 (400)	40.6 (212)
Paternal characteristics						
Age, (y)	33.6 (22.9, 46.8)	32.1 (20.7, 45.5)†	32.5 (22.2, 46.9)	31.1 (18.3, 41.7)†	40.0 (19.8, 44.5)	29.7 (20.2, 45.0)†
Height, (cm)	182.3 (7.8)	180.9 (7.7)†	183.0 (8.0)	181.0 (6.8)*	180.4 (8.1)	180.3 (7.3)
Weight, (kg)	84.0 (12.7)	83.2 (13.1)	83.7 (13.0)	84.2 (13.8)	82.9 (13.9)	81.5 (13.9)
Body mass index, (kg/m^2)	25.3 (3.4)	25.4 (3.6)	25.0 (3.4)	25.7 (4.0)	25.4 (3.6)	25.0 (3.9)
Ethnicity, (%)						

Table S3. Continued

Dutch or European	50.9 (2139)	37.6 (638)†	54.6 (272)	40.4 (74)	41.3 (382)	34.3 (179)
Non-European	20.1 (846)	24.0 (408)†	20.3 (101)	19.2 (35)	22.7 (210)	21.1 (110)
Systolic blood pressure (mmHg)	130.3 (13.5)	129.5 (13.8)	130.1 (14.1)	131.1 (14.3)	130.2 (13.6)	130.6 (12.8)
Diastolic blood pressure (mmHg)	73.7 (10.5)	72.9 (11.2)*	72.8 (10.3)	73.3 (11.5)	72.8 (11.1)	73.2 (11.0)
Smoking, (%)						
Yes	32.2 (1350)	35.0 (594)	60.4 (301)	61.7 (113)	65.7 (608)	67.2 (351)
No	58.4 (2454)	58.7 (996)	32.1 (160)	34.5 (63)	19.5 (180)	21.5 (112)
Birth characteristics						
Sex boys, (%)	49.2 (2066)	51.2 (869)	47.4 (236)	48.1 (88)	55.0 (509)	54.4 (284)
Gestational age, (wk)	40.1 (36.0, 42.3)	40.1 (35.0, 42.4)*	40.1 (35.9, 42.2)	39.9 (34.8, 42.1)	40.0 (34.7, 42.3)	39.9 (34.6, 42.4)
Preterm, (%)	4.5 (191)	5.6 (95)	4.2 (21)	6.6 (12)	6.1 (56)	8.4 (44)
Birth weight, (g)	3458 (544)	3441 (582)	3453 (551)	3366 (578)	3281 (556)	3199 (543)†
Small for gestational age, (%)	5.1 (216)	5.4 (90)	5.8 (29)	6.0 (11)	11.6 (107)	12.1 (63)
Breastfed yes, (%)	76.3 (3202)	53.1 (901)	76.3 (380)	53.0 (97)	59.4 (549)	39.1 (204)
Child characteristics						
Age, (y)	6.0 (5.6, 7.8)	NA	6.0 (5.6, 8.1)	NA	6.1 (5.6, 8.1)	NA
Height, (cm)	119.4 (5.9)	NA	119.8 (6.2)	NA	119.5 (6.4)	NA
Weight, (kg)	23.1 (4.1)	NA	23.3 (4.2)	NA	24.0 (5.1)	NA
Body mass index, (kg/m^2)	16.1 (1.8)	NA	16.1 (1.9)	NA	16.7 (2.2)	NA
Body surface area, (m^2)	0.87 (0.09)	NA	0.88 (0.09)	NA	0.89 (0.11)	NA
Smoke at home, (%)						
No, seldom or never	91.0 (3821)	NA	80.5 (401)	NA	46.8 (433)	NA
Yes, but less than once a week	3.0 (127)	NA	6.2 (31)	NA	9.7 (90)	NA
Yes, more than once a week	6.0 (251)	NA	13.3 (66)	NA	43.5 (402)	NA
Combined kidney volume, (cm^3)	120.1 (23.2)	NA	119.8 (21.4)	NA	119.9 (25.0)	NA

Table S3. Continued

Creatinine, (µmol/l)	37.3 (5.3)	NA	37.5 (5.5)	NA	38.5 (5.7)	NA
eGFR, ml/min 1.73 m^2	119.3 (16.0)	NA	119.3 (15.3)	NA	116.0 (16.3)	NA
Microalbuminuria[‡], %)	7.2 (291)	NA	9.9 (48)	NA	7.1 (63)	NA

Values are means (SD), medians (95% range), or % (numbers). Participants were compared using independent samples t-test for continuous variables and chi-square test for categorical variables.
[‡]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls).

eGFR, estimated glomerular filtration rate

*P<0.05, [†]P<0.01.

Chapter 3.2

Protein intake in infancy and kidney size and function at the age of 6 years: The Generation R Study

Adapted from Pediatr Nephrol 2015;30(10):1825-33

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Abstract

Background High protein intake has been linked to kidney growth and function. Whether protein intake is related to kidney outcomes in healthy children is unclear.

Methods We examined the associations of protein intake in infancy with kidney outcomes at 6 years in 2,908 children participating in a population-based cohort study. Protein intake at 1 year was assessed with a food-frequency questionnaire and was adjusted for energy intake. At the children's age of 6 years we measured kidney volume and urinary albumin/creatinine ratio (ACR), and we estimated glomerular filtration rate (eGFR) using serum creatinine and cystatin C levels.

Results In models adjusted for age, sex, body surface area, and sociodemographic factors, a higher protein intake was associated with a lower ACR and a higher eGFR but not consistently with kidney volume. However, after further adjustment for children's other dietary and lifestyle factors, such as sodium intake, diet quality, and television watching, higher protein intake was no longer associated with kidney function. No differences in associations were observed between animal and vegetable protein intake.

Conclusions Protein intake in early childhood is not associated kidney size or function at the age of 6 years. Further study is needed on other early life predictors of later kidney size and function.

Introduction

Kidney function has been shown to track from childhood into adulthood.(1) Subclinical variations in kidney function are already present in childhood and have been shown to relate to kidney disease in later life.(2) This implicates that it is important to study determinants of kidney function already in childhood. We have recently observed that reduced infant weight growth is associated with smaller kidney volume in childhood,(3) and that longer breastfeeding duration is associated with larger kidney volume and an increased estimated glomerular filtration rate (eGFR).(4) These observations suggest that exposures in infancy are important for later kidney development.

Dietary protein intake during infancy is a key factor for growth and development and may be associated to kidney growth and function.(5) In animal studies, increased protein intake leads to increased kidney growth and function.(6-8) and early postnatal dietary protein affects kidney function.(8,9) Also in healthy adults, a higher protein intake has been associated with increased GFR.(10-12) In patients with chronic kidney disease, high protein intake may further decline kidney function, because the kidneys can no longer handle the excretion of protein metabolites.(13-16) However, randomized controlled trials with low-protein diets in adults or children with renal disease have not consistently been able to slow progression of kidney disease.(17-19)

Not much is known on the effects of protein intake on kidney function in children with a normal kidney function. Trials suggest that infants who receive additional dietary protein have larger kidneys(5) and a higher eGFR(20) in infancy than those who received no additional protein. Whether protein intake in infancy is associated with kidney size and function in later childhood is unknown.

Therefore, we examined the associations between protein intake at the age of 1 year and kidney outcomes at the age of 6 years in 2,908 children participating in a population-based prospective cohort study. Kidney measures included combined kidney volume, creatinine-based eGFR ($eGFR_{Creat}$), cystatin C-based eGFR ($eGFR_{CysC}$), and urinary albumin/creatinine ratio. In addition, we examined the association between protein intake at 2 years with kidney outcomes at 6 years in a subgroup of the children; and we aimed to evaluate whether the associations between protein intake and kidney health differed by protein source, child sex, birth weight, gestational age at birth, kidney volume, or ethnicity.

Methods

Study design and population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onward in Rotterdam, the Netherlands.(21) All children were born between April 2002 and January 2006. *The study was conducted according to the guidelines of the Helsinki Declaration and approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam (MEC 198.782/2001/31)*. Written informed consent was

given by all parents. A total of 7,893 children were available for follow-up studies in early childhood.(21) A questionnaire on child diet around the age of 1 year was sent to 5,088 mothers who provided consent for follow-up and had sufficient mastery of the Dutch language (Figure 1). In total 3,650 (72%) of these mothers returned the questionnaire. (22) After exclusion of subjects with invalid dietary data and withdrawn consent, information on infant diet was available for 3,629 children. From these 3,629 children, we excluded children with congenital kidney abnormalities or an albumin-creatinine ratio >25 mg/mmol (N=8).(23) Of the remaining children, 2,965 had one or more kidney measurements available at the age of 6 years (Figure 1).

Dietary assessment

Dietary intake was assessed at a median age of 12.9 months (95% range 12.2 to 18.9) using a semi-quantitative 211-item food frequency questionnaire (FFQ), as described previously in detail.(22,24) The FFQ was validated against three 24h-recalls in a representative sample of 32 Dutch children around the age of 1 year living in Rotterdam. The intraclass correlation coefficient was 0.7 for total protein intake.(22) Mothers of a subgroup of 899 Dutch children received an additional FFQ at their child's median age of 24.9 months (95% range 24.3 to 27.6).(24) Of these children, 715 had kidney measures at the age of 6 years available (Supplementary Figure S1).

Kidney outcome assessments

Children's kidney outcomes were assessed at a median age of 5.9 years (95% range 5.6 to 6.6) in a dedicated research center in the Sophia Children's Hospital in Rotterdam by well-trained staff.(23) Kidney volume was measured with ultrasound, using an ATL-Philips HDI 5000 instrument (Seattle, WA, USA), equipped with a 2.0-5.0 MHz curved array transducer, as described previously in detail.(25,23) Kidney volume was calculated using the equation for a prolate ellipsoid: volume (cm^3) = 0.523 x length (cm) x width (cm) x depth (cm).(25) Combined kidney volume was calculated by summing right and left kidney volume. We previously reported good intra-observer and inter-observer correlation coefficients.(26)

Non-fasting blood samples were drawn by antecubital venipuncture. Creatinine concentrations were measured with enzymatic methods, and cystatin C levels were measured with a particle-enhanced immunoturbidimetric assay (using Cobas 8000 analyzers, Roche, Almere, the Netherlands). Quality control samples demonstrated intra-assay coefficients of variation of 0.51% for creatinine and 1.65% for cystatin C, and inter-assay coefficients of 1.37% for creatinine and 1.13% for cystatin C.(23) Creatinine-based estimated glomerular filtration rate (eGFR) was calculated according to the revised Schwartz 2009 formula, the most common pediatric equation: $eGFR_{Creat}$ = 36.5 x (height (cm)/ creatinine (µmol/L)).(27) Additionally, we evaluated eGFR calculated using a cystatin C-based and a combined creatinine and cystatin C formula as proposed by Zappitelli in 2006: $eGFR_{CysC}$ = 75.94 / (cystatin C $(mg/L)^{1.17}$) and $eGFR_{Combined}$ =507.76 x $e^{0.003x\ height\ (cm)}$/ (cystatin C $(mg/L)^{0.635}$ x creatinine $(\mu mol/L)^{0.547}$).(28)

Urinary creatinine (mmol/L) and albumin (mg/L) levels were measured with a Beckman Coulter AU analyzer, and creatinine levels were determined using the Jaffe reaction. We calculated the urinary albumin/creatinine ratio (ACR). In addition to the continuous ACR, we defined microalbuminuria as an ACR ≥ 2.5 mg/mmol for boys, and ≥ 3.5 mg/mmol for girls.(29)

Covariates

Information on maternal age, educational level, and folic acid supplement use was obtained with a questionnaire at enrollment in the study. Maternal height and weight were measured at the research center at enrollment and body mass index (BMI, kg/m^2) was calculated. Maternal smoking during pregnancy was assessed using questionnaires in each trimester and was categorized into never; until pregnancy was known; or continued during pregnancy. Information on child's sex, birth weight and gestational age was available from medical records and hospital registries. Sex and gestational age specific SD scores for birth weight were calculated using Swedish reference data.(30) Child's ethnicity was defined according to Statistics Netherlands(31) and classified into eight categories (Western, Cape Verdean, Moroccan, Netherlands Antillean, Turkish, Surinamese Creole, Surinamese Hindustani, and other non-Western).

Information on breastfeeding was obtained from delivery reports and postnatal questionnaires, and breastfeeding was categorized as never; partial in the first 4 months; or exclusively in the first 4 months of life.(22) Total energy, fat and sodium intake from foods were estimated using the previously mentioned FFQs, and were adjusted for energy intake using the residual method.(32) A previously defined diet score was used to quantify overall diet quality using data obtained with the FFQ.(24) Information on child's television watching around the age of 2 years was obtained using a questionnaire. At the child's age of 6 years, we measured height and weight at the research center and calculated BMI (kg/m^2) and body surface area (BSA) (using the Du Bois formula: BSA (cm^2) = weight (kg)$^{0.425}$x height (cm)$^{0.725}$x 0.007184).(33) Lean body mass was measured using whole body dual-energy X-ray absorptiometry scans (iDXA, GE-Lunar, 2008, Madison, WI, USA).

Statistical analysis

We were interested in the effect of protein independent of its energy content and therefore we adjusted protein intake for total energy intake using the residual method.(32) Briefly, we used the residuals of a linear regression model with energy intake as independent variable and protein intake as dependent variable. These residuals provide a measure of protein intake uncorrelated with total energy intake. To enhance interpretability, predicted protein intake for the mean energy intake (1,311 kcal/d) was added to the residuals as a constant.(32) In line with recommendations for dietary exposures, protein intake was analyzed both as a continuous and as a categorical variable.(32) For the latter purpose we categorized protein intake into tertiles and used the lowest tertile as the reference category.

We used multivariable linear regression models to assess the associations of protein intake with combined kidney volume, eGFR, and ACR. We natural log-transformed ACR to obtain a normal distribution. For clinical interpretation, we also assessed the associations of protein intake with the risk of microalbuminuria, using multivariable logistic regression models. Model 1 was adjusted for child's sex, age and BSA at kidney measurement. Model 2 was further controlled for the following prenatal and sociodemographic factors: maternal age, educational level, BMI, smoking during pregnancy, folic acid supplement use, and for child's ethnicity and birth weight Z-score. The final model was additionally adjusted for childhood lifestyle factors: breastfeeding, children's television watching, total energy, fat and sodium intake, and diet quality score (model 3). Covariates were included in the regression models based on previously shown associations with kidney outcomes(23,34,4) or a significant change (>5%) in effect estimates. Because both protein intake and kidney volume are strongly related to body size(35) and because creatinine levels are associated with muscle mass,(36) we adjusted all models for BSA and we performed sensitivity analyses in which we replaced BSA by height and weight, by BMI, or by lean body mass. In addition, we examined the association between protein intake and the ratio of kidney volume with either body weight, BMI, or BSA.

To assess whether the associations were different by sex, ethnicity, birth weight, gestational age at birth, or kidney volume at 6 years we evaluated statistical interactions by adding the product term of the covariate and protein intake to model 2. Stratified analyses were conducted in case the interaction term was significant ($P < 0.05$). Because the FFQ was developed and validated for Dutch children, we performed a sensitivity analysis in Dutch children only. Furthermore, since kidney size and function are different in low birth weight children,(25) we performed a sensitivity analysis in children born with a normal birth weight (≥ 2,500g) and among children born at term (≥ 37 weeks).

Missing values of covariates were multiple imputed (N=5 imputations) according to the Fully Conditional Specification method (predictive mean matching), assuming no monotone missing pattern.(37) We present results as pooled effect estimates after the multiple imputation procedure. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics

Characteristics of the children and their mothers, stratified by tertiles of protein intake, are presented in Table 1. Mean (±SD) total protein intake at the age of 1 year was 41.2 g (±12.9), corresponding to 12.9% of total energy intake (E%). This is higher than recommended for this age group,(38) but similar to intakes observed in the general Dutch and other Western pediatric populations.(39,40) Children in the highest tertile of protein intake had more often been breastfed and were slightly heavier at the age of 6 years. Protein intake at the age of 2 years (13.9 E%) was slightly higher than intake at

the age of 1 years (Supplementary Table S1). At the age of 6 years, mean (SD) combined kidney volume was 121 cm^3 (±21) and mean $eGFR_{Creat}$ was 119 ml/min per 1.73m^2 (±16). Mean $eGFR_{CysC}$ was lower at 102 ml/min per 1.73m^2 (±13). Many children (34%) had urine albumin levels at or below the detection limit (≤ 2 mg/L) and microalbuminuria was present in 7.1% of the children.

Protein intake in early childhood and kidney outcomes at school age

Table 2 presents the associations between protein intake and kidney outcomes. In model 1, adjusted for age, sex, and BSA, a higher protein intake at the age of 1 year was associated with a higher eGFR and lower ACR at the age of 6 years. Protein intake was not consistently associated with kidney volume. Results for $eGFR_{CysC}$ were similar to those for $eGFR_{Creat}$ (Table 2) and for $eGFR_{Combined}$ (Supplementary Table S2). After further adjustment for sociodemographic variables and maternal factors (model 2), the effect estimates hardly changed and a higher protein intake remained significantly associated with a higher eGFR and a lower ACR. However, after further adjustment for child lifestyle factor (model 3), all associations attenuated towards null (Table 2). Important lifestyle confounders in the associations with kidney outcomes were child's television watching, overall diet quality, and sodium intake.

Additional analyses

No clear differences were observed for the associations of animal versus vegetable protein intake on kidney outcomes (Supplementary Table S3). Replacement of BSA for either height and weight, BMI, or lean body mass; or replacement of absolute kidney volume by the ratio of kidney volume with weight, BMI, or BSA did not change the effect estimates (data not shown). Protein intake was not associated with urinary albumin or creatinine levels (data not shown). No significant interactions were observed of total protein intake with sex, birth weight, gestational age, ethnicity, or kidney volume on any of the kidney outcomes. Sensitivity analyses in Dutch children only (N=1,994) showed similar patterns of associations as in the whole group, but with slightly larger effect estimates and smaller *P*-values (Supplementary Table S4). Among children born with a normal birth weight (≥ 2,500g, N=2,802) and among children born at term (≥ 37 weeks, N= 2,781), effect estimates were similar to those observed in the whole group (data not shown). In line with the results for protein intake at the age of 1 year, higher total protein intake at the age of 2 years was associated with a higher $eGFR_{Creat}$ and a trend towards a lower ACR in crude models, and the associations attenuated to null after adjustment for other lifestyle factors (Supplementary Table S5). In contrast to protein intake at the age of 1 year, protein intake at 2 years was in crude models not associated with $eGFR_{cysC}$ and was associated with a higher kidney volume, which was explained by other lifestyle factors (Supplementary Table S5).

Table 1. Subject characteristics [a]

	Tertiles of energy-adjusted total protein intake at 1 y				
	All (N=2,968)	**Tertile 1 (<37.5 g/d) (N=989)**	**Tertile 2 (37.5-43.9 g/d) (N=990)**	**Tertile 3 (>43.9 g/d) (N=989)**	**P-value [b]**
Maternal characteristics					
Maternal age (y)	31.5 (21.7-39.9)	31.8 (22.5-41.4)	31.9 (22.4-39.6)	31.6 (20.6-39.4)	*0.01*
Maternal body mass index at enrolment (kg/m^2)	23.4 (18.7-35.2)	23.4 (18.8-34.7)	23.6 (18.9-35.6)	24.5 (18.5-37.1)	*0.50*
Nulliparous (%)	60.4	59.5	59.8	61.6	*0.09*
Education level (%)					0.42
Primary	3.5	3.7	3.5	3.2	
Secondary	33.9	33.2	32.8	35.6	
Higher	62.7	63.1	63.7	61.2	
Folic acid supplement use (%)					0.74
Never	15.8	17.0	14.8	15.4	
In the first 10 weeks of pregnancy	30.2	28.5	30.9	31.3	
Periconceptional	54.0	54.4	54.3	53.2	
Smoking during pregnancy (%)					0.49
Never	78.1	78.7	78.6	77.0	
Until pregnancy was known	10.0	9.2	9.4	11.5	
Continued	11.8	12.1	11.9	11.5	
Child characteristics					
Girls (%)	50.9	51.6	52.6	48.5	*0.17*
Ethnicity (%)					0.24
Western	76.9	75.5	78.1	77.0	
Cape Verdean	2.0	1.8	1.8	2.3	
Moroccan	3.2	2.8	2.8	4.1	
Netherlands Antillean	1.7	2.3	1.9	1.1	
Turkish	4.5	4.5	4.0	4.9	
Surinamese Creoles	2.3	3.0	2.5	1.3	
Surinamese Hindustani	2.2	2.6	1.9	2.2	
Other non-western	7.1	7.5	6.9	7.0	
Gestational age at birth (wk)	40.0 (1.7)	39.9 (1.9)	39.9 (1.7)	40.0 (1.6)	*0.79*
Birth weight (g)	3,472 (551)	3,462 (562)	3,466 (557)	3,489 (532)	*0.51*
Breastfeeding (%)					<0.01
Exclusive ≥ 4 months	29.5	34.8	26.9	27.2	
Partial ≥ 4 months	62.5	58.1	65.5	63.7	
Never or ≤ 4 months	8.0	7.1	7.5	9.1	
Child characteristics at dietary measurement					
Age at FFQ (mo)	12.9 (12.2-18.9)	12.8 (12.2-18.6)	12.9 (12.2-18.7)	13.0 (12.2-19.4)	*<0.01*
Total energy intake (kcal/d)	1,266 (678-2212)	1,297 (619-2264)	1,238 (650-2093)	1,253 (765-2237)	0.02

Table 1. Continued

Protein intake (g/d) [c]					
Total protein	41.2 (12.9)	34.9 (10.8)	39.7 (10.1)	48.0 (12.1)	*<0.01*
Animal protein	25.7 (10.3)	20.8 (8.7)	25.0 (8.1)	33.1 (9.5)	*<0.01*
Vegetable protein	14.9 (5.7)	13.5 (5.4)	14.8 (5.2)	16.6 (5.9)	*<0.01*
Protein intake (E%)					
Total protein	12.9 (2.4)	10.5 (1.1)	12.9 (1.0)	15.4 (1.7)	*<0.01*
Animal protein	8.1 (2.4)	6.2 (1.7)	8.0 (1.6)	10.2 (2.1)	*<0.01*
Vegetable protein	4.6 (1.4)	4.1 (1.3)	4.7 (1.3)	5.1 (1.3)	*<0.01*
Total fat intake (g/d)[c]	42.3 (17.5)	43.1 (18.8)	40.8 (16.0)	42.7 (16.7)	*<0.01*
Sodium intake from foods (g/d) [c]	1.02 (0.35)	0.88 (0.32)	0.98 (0.30)	1.17 (0.35)	*<0.01*
Television watching (h/d)	0.9 (0.5)	0.9 (0.5)	0.9 (0.5)	0.9 (0.5)	*0.19*
Diet score	4.2 (1.3)	3.3 (1.1)	4.1 (1.1)	5.1 (1.2)	*<0.01*
Child characteristics at 6 y visit					
Age (y)	5.9 (5.6-6.6)	5.9 (5.6-6.5)	5.9 (5.6-6.6)	5.9 (5.6-6.6)	*0.03*
Height (cm)	118.2 (5.2)	117.8 (4.9)	118.2 (5.4)	118.5 (5.2)	*0.02*
Weight (kg)	22.4 (3.4)	22.1 (3.1)	22.4 (3.6)	22.7 (3.5)	*<0.01*
Body mass index (kg/m^2)	16.0 (1.6)	15.9 (1.5)	16.0 (1.6)	16.1 (1.7)	*<0.01*
Body surface area (kg/m^2)	0.86 (0.08)	0.85 (0.07)	0.86 (0.08)	0.86 (0.08)	*<0.01*
Combined kidney volume (cm^3)	121 (21)	119 (21)	122 (23)	122 (21)	*<0.01*
Creatinine (µmol/l)	37.0 (5.2)	37.2 (5.0)	36.8 (5.4)	36.8 (5.2)	*0.32*
Cystatin C (mg/L)	0.79 (0.08)	0.79 (0.08)	0.78 (0.08)	0.78 (0.08)	*0.02*
$eGFR_{Creat}$ (Schwartz) (ml/min per 1.73m^2)	119 (16)	118 (15)	120 (17)	120 (16)	*0.06*
$eGFR_{CysC}$ (Zappitelli) (ml/min per 1.73m^2)	102 (13)	100 (13)	102 (14)	102 (13)	*<0.01*
Urinary albumin/creatinine ratio	0.79 (0.20-5.70)	0.83 (0.20-7.33)	0.77 (0.190-5.56)	0.77 (0.20-5.00)	*0.01*
Microalbuminuria (%)	7.1	7.6	7.5	6.3	*0.49*

[a] 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.

[b] P-values for differences of means between the tertiles of protein intake, assessed using ANOVA for continuous variables with a normal distribution, Kruskal-Wallis test for continuous variables with a skewed distribution, and chi-square tests for categorical variables.

[c] Not adjusted for energy intake.

E%, energy percentage; eGFR, estimated glomerular filtration rate; FFQ, food frequency questionnaire

Table 2. Associations of protein intake at 1 y with childhood kidney volume and function at 6 y (N=2,968)[a]

	Kidney volume	$eGFR_{Creat}$ (Schwartz 2009)	$eGFR_{CysC}$ (Zappitelli 2006)	ACR
	(mm^3)	(ml/min per $1.73m^2$)	(ml/min per $1.73m^2$)	(% change)[c]
Protein intake	**N=2,755**	**N=2,006**	**N=2,007**	**N=2,868**
Model 1[b]				
Tertile 1	*Reference*	*Reference*	*Reference*	*Reference*
Tertile 2	**2.31 (0.61, 4.02)**	**1.90 (0.17, 3.63)**	**1.84 (0.44, 3.25)**	-6.8 (-14.6, 1.0)
Tertile 3	1.16 (-0.54, 2.87)	**2.46 (0.73, 4.19)**	**1.75 (0.35, 3.15)**	**-7.8 (-15.7, -0.1)**
P_{trend}[d]	*0.17*	***<0.01***	***0.01***	***0.04***
Per 10 g	0.29 (-0.67, 1.25)	**1.03 (0.04, 1.99)**	0.66 (-0.12, 1.44)	**-5.4 (-9.8, -1.1)**
Model 2[b]				
Tertile 1	*Reference*	*Reference*	*Reference*	*Reference*
Tertile 2	**2.33 (0.64, 4.03)**	**1.85 (0.12, 3.58)**	**1.84 (0.43, 3.25)**	-6.7 (-14.5, 1.1)
Tertile 3	1.21 (-0.50, 2.91)	**2.28 (0.56, 4.00)**	**1.70 (0.30, 3.11)**	**-6.9 (-14.8, 0.0)**
P_{trend}[d]	*0.16*	***<0.01***	***0.02***	*0.08*
Per 10 g	0.31 (-0.65, 1.27)	0.91 (-0.05, 1.86)	0.64 (-0.14, 1.43)	**-4.9 (-9.3, -0.01)**
Model 3[b]				
Tertile 1	*Reference*	*Reference*	*Reference*	*Reference*
Tertile 2	**1.96 (0.10, 3.82)**	1.21 (-0.69, 3.11)	1.58 (-0.21, 3.33)	-3.1 (-11.7, 5.4)
Tertile 3	0.36 (-1.91, 2.63)	1.11 (-1.20, 3.42)	1.60 (-0.28, 3.49)	-0.4 (-10.9, 10.0)
P_{trend}[d]	*0.74*	*0.35*	*0.10*	*0.93*
Per 10 g	-0.55 (-1.86, 0.76)	-0.17 (-1.49, 1.15)	0.37 (-0.71, 1.45)	-2.0 (-8.0, 4.0)

[a] Values are based on multivariable linear regression models and reflect differences or percentage change (95%CI) in kidney outcomes for tertiles of protein intake compared to the lowest tertile, and per 10 g of protein intake per day. Bold numbers indicate statistically significant results (P<0.05)

[b]Protein intake is energy-adjusted using the nutrient residual method.

Model 1 is adjusted for child's sex, age and body surface area at 6 y visit.

Model 2 is additionally adjusted for and maternal age, educational level, and BMI at enrolment, for smoking and folic acid supplement use during pregnancy, and for children's ethnicity, and gestational-age adjusted birth weight.

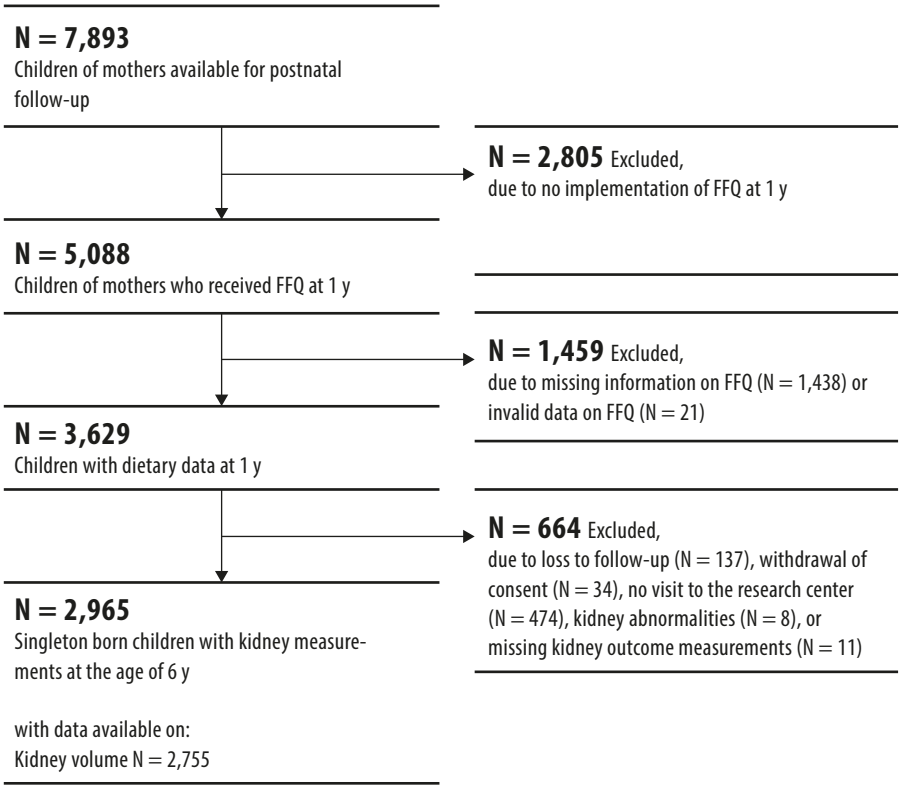
Model 3 is additionally adjusted for breastfeeding in the first four months of life, children's television watching, total energy intake, energy-adjusted total fat intake, energy-adjusted sodium intake, and diet quality score.

[c] Albumin/creatinine ratio is log-transformed, therefore the regression coefficients reflect the percentage change rather than the absolute difference.

[d] P_{trend} is obtained by including the number of the tertiles of protein intake as continuous variable in the model

ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate

Figure 1. Flow chart of study participants included in the main analysis.



ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate; FFQ, food frequency questionnaire

Discussion

We examined the associations between protein intake in early childhood with kidney size and function at school age in a large prospective population-based cohort study. We observed that associations between higher protein intake in infancy with higher eGFR and lower ACR at the age of 6 years were explained by other dietary and lifestyle factors of the children, such as sodium intake and television watching. Furthermore, protein intake was not associated with kidney size and no differences in associations were observed for animal versus vegetable protein intake.

Interpretation and comparison with previous studies

Contrary to findings of previous studies in infants and adults, protein intake in infancy was not consistently associated with combined kidney volume in our population-based sample of school-age children. In a multi-center trial in several European countries, healthy infants who received higher protein infant formula had higher kidney volumes at the age of 6 months than infants receiving the lower protein formula.(5) Whether this difference in kidney volume persisted until later age was not studied. A previous observational study in 631 healthy infants in Denmark reported a larger kidney size in 3-month-old infants who received formula feeding than in infants who received breastfeeding and the authors hypothesized that the effect might be attributable to the higher protein content in infant formula.(41) The difference was however no longer present at 18 months of age.(41) In line with this, in a study in young rats that received isocaloric high or low protein diets after weaning, a higher protein intake increased kidney size.(6) However, a month after discontinuation of the high protein diet, kidney size was comparable to that of the rats fed low protein diets. These studies suggest that the effect of protein intake on kidney growth could be reversible. In our study, kidney outcomes were measured a few years after the assessment of dietary protein intake. Therefore, we could speculate that a potential effect of protein intake in early life on kidney size might be no longer apparent in the children at the age of 6 years. Kidney hypertrophy in response to high protein intake could be a compensatory response to higher levels of nitrogenous protein metabolites (such as urea), and may be temporary response.(15) Alternatively, hypertrophy of the kidney in response to protein intake may occur via increased insulin-like growth factor I secretion, which may lead to permanent changes in kidney size.(42,43)

We observed a higher eGFR in relation to higher protein intake, but this association was explained by other lifestyle factors. Important confounding factors were television watching, overall diet quality, and breastfeeding in early infancy. This is in contrast to finding from a small trial in preterm born infants, which report a higher eGFR with additional dietary protein(20) and short-term trials in adults.(12,44,11) However, in line with our results, the previously mentioned large trial in healthy infants did not report an effect of a higher protein infant formula on eGFR at the age of 6 months,(5) and an observational study in healthy infants reported no associations between intake of

infant formula and eGFR.(41) Similar to kidney growth, a higher GFR in response to high protein intake is considered to be an adaptive responses to high levels of circulating protein metabolites. This will increase the workload of the kidneys, and may lead to hyperfiltration.(15) Like for kidney growth, this response may be reversible.(6)

In our population, a higher protein intake was associated with a lower albumin/creatinine ratio in crude models, but this was no longer significant after adjustment for other dietary factors, such as sodium intake. This is in contrast to results from a trial in healthy adults which showed that increased protein increases urinary albumin levels (12). However, in line with our results, a few other trials reported no associations between protein intake and urinary albumin excretion.(11,44)

In contrast to previous observational studies in adults,(45-47) we did not observe clear differences in associations for animal and vegetable protein intake. In contrast to studies in animals,(7,43) we also did not observe significant interactions between child sex and protein intake on kidney health. Furthermore, we observed no interaction between protein intake and birth weight or gestational age at birth. The results of our study do not indicate that changes in dietary recommendations for healthy infants are required with respect to later kidney health, however, further studies are needed to assess whether protein intake may specifically affect kidney outcomes in preterm or small-for-gestational age born children.

Strengths and limitations

An important strength of our study is its prospective design within a large population-based cohort. We had information available on protein intake and kidney outcomes for almost 3,000 children and we had information on many potential maternal and child confounders, which were not always considered in previous observational studies.

A limitation of our dietary assessment methods is that an FFQ relies on memory and reported food intakes are subject to measurement error.(48) However, validation of our FFQ against three 24h recalls showed a good intraclass correlation coefficient for protein intake. Another limitation of our FFQ is that it was only validated for Dutch children.(22,24) However, sensitivity analyses in Dutch children only, showed similar results. Strengths of our dietary assessment are that an FFQ measures habitual diet rather than dietary intake at just one or a few days, and that we calculated not only total protein, but also from animal and vegetable sources. A limitation of our study is that we did not have dietary data at the age of 6 years available, therefore we could not assess the association of current diet with kidney health.

We performed detailed kidney measurements, using ultrasound to measure kidney volume, and we had blood and urine samples to estimate kidney function. Unfortunately, we did not measure inulin clearance to calculate actual GFR. To estimate GFR we used a creatinine-based formula that has been validated and is widely used in pediatric populations.(27) A limitation of serum creatinine as marker of kidney function is its strong relationship with muscle mass.(36) Cystatin C is a more sensitive marker for kidney function in pediatric populations than serum creatinine, since it is not affected by child

age, height, or weight(49,50) and we therefore also evaluated eGFR based on cystatin C levels.(28) This formula has been evaluated against inulin clearance and compared with other eGFR formulas, and was found to be accurate and precise in estimating GFR in addition to the Schwartz 2009 formula.(36)

Conclusions

In this prospective cohort study, associations between protein intake in early childhood and kidney function at the age of 6 years were explained by other dietary and lifestyle factors of the children. Furthermore, protein intake was not associated with kidney size and no differences in associations were observed for animal versus vegetable protein intake.

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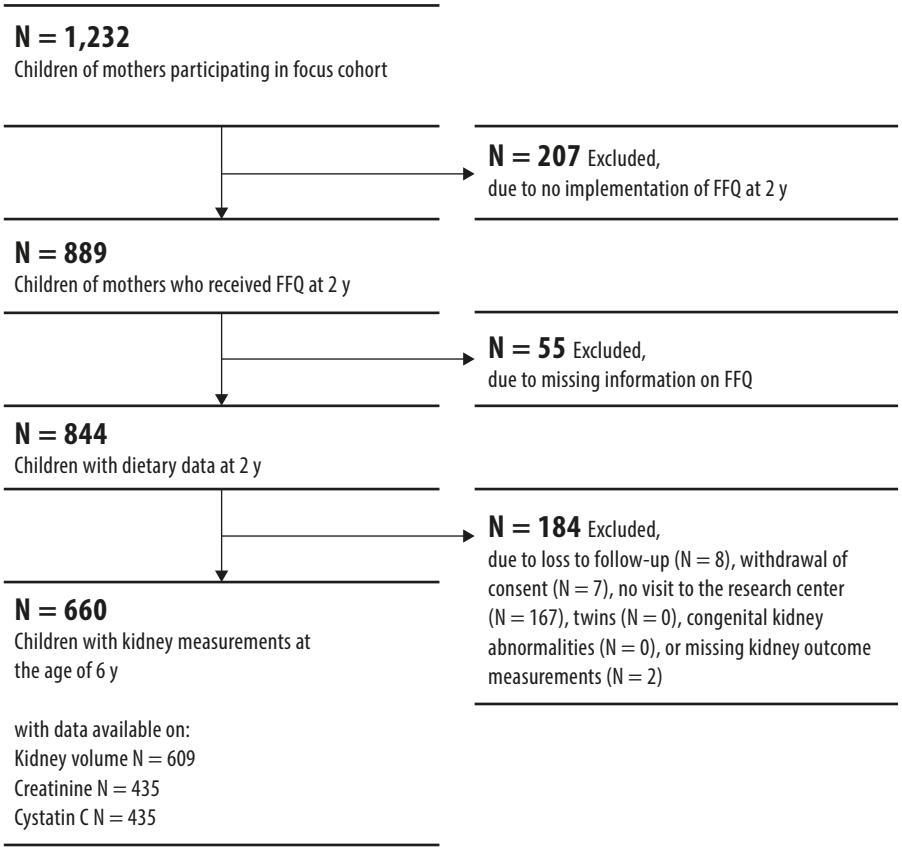
Supplementary Figure S1. Population for analysis for children with dietary data at the age of 2 y.



Supplementary Table S1. Dietary characteristics for children with dietary data at 2 y.

	Tertiles of energy-adjusted total protein intake at 2 y				
	All (N=660)	**Tertile 1 (<43.0 g/d) (N=220)**	**Tertile 2 (43.0-48.2 g/d) (N=220)**	**Tertile 3 (>48.2 g/d) (N=220)**	**P value†**
Age at FFQ (mo)	24.9 (24.3-27.5)	24.9 (24.3-27.7)	24.9 (24.3-27.5)	24.9 (24.3-27.2)	0.71
Total energy intake (kcal/d)	1301 (841-1967)	1336 (842-2049)	1244 (802-1896)	1310 (936-1996)	<0.01
Protein intake (g/d)*					
Total protein	46.0 (10.8)	40.0 (9.3)	44.3 (8.7)	52.4 (9.8)	<0.01
Animal protein	27.8 (8.8)	22.0 (6.8)	26.7 (6.4)	34.7 (8.0)	<0.01
Vegetable protein	18.1 (5.2)	17.7 (5.0)	17.6 (5.3)	18.9 (5.4)	<0.01
Total fat intake (g/d) *	46.5 (13.4)	44.7 (14.4)	44.4 (12.5)	50.4 (12.5)	<0.01
Sodium intake from foods (g/d) *	1.27 (0.33)	1.12 (0.30)	1.21 (0.29)	1.43 (0.33)	<0.01
Protein intake (E%)					
Total protein	13.9 (1.9)	11.8 (1.1)	14.0 (0.7)	15.9 (1.1)	<0.01
Animal protein	8.4 (2.1)	6.5 (1.4)	8.5 (1.4)	10.3 (1.7)	<0.01
Vegetable protein	5.5 (1.2)	5.1 (1.1)	5.5 (1.2)	5.6 (1.3)	<0.01

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

† p-values for differences of means between the tertiles of protein intake, assessed using ANOVA for continuous variables with a normal distribution and Kruskal-Wallis test for continuous variables with a skewed distribution.

* Unadjusted for energy intake

E%, energy percentage, FFQ, food frequency questionnaire

Supplementary Table S2. Associations of protein intake at 1 y with serum creatinine and cystatin C levels, and microalbuminuria at 6 y.

	Serum creatinine (µmol/l)	Serum cystatin C (µg/l)	$eGFR_{Ccombined}$ (Zappitelli 2006) (ml/min per 1.73m^2)	Microalbuminuria (OR)
	N=1,962	N=1,963	N=1,962	N=2,800
Crude model[§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	-0.42 (-0.97, 0.13)	**-11.6 (-15.9, -7.2)****	**2.23 (0.73, 3.74)***	0.97 (0.82, 1.16)
Tertile 3	**-0.60 (-0.88, 0.32)***	**-10.1 (-14.5, -5.8)***	**2.04 (1.27, 2.81)***	0.80 (0.56, 1.15)
P_{trend} [#]	***0.03***	***0.02***	***<0.01***	*0.28*
Per 10 g	**-0.20 (-0.34, -0.06)***	**-3.0 (-5.2, -0.9)***	**1.02 (0.63, 1.40)****	0.92 (0.84, 1.01)
Adjusted model[§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	-0.41 (-0.98, 0.16)	**-12.1 (-21.0, -3.3)****	**2.32 (0.81, 3.83)****	0.99 (0.69,1.43)
Tertile 3	-0.49 (-1.12, 0.15)	**-9.8 (-19.5, 0.0)***	**1.96 (0.46, 3.47)***	0.82 (0.54, 1.24)
P_{trend} [#]	*0.13*	***0.05***	***0.01***	*0.35*
Per 10 g	-0.11 (-0.42, 0.21)	*-2.4 (-7.4, 2.6)*	*0.61 (-0.14, 1.36)*	0.94 (0.77, 1.15)

Values are based on multivariable linear regression models and reflect differences or odds ratios (95%CI) in kidney outcomes for tertiles of protein intake compared to the lowest tertile, and per 10 g of protein intake per day.

Protein intake is energy-adjusted using the nutrient residual method.

[§]Crude models are adjusted for child's sex, age and body surface area at 6 y visit. Fully adjusted models are additionally adjusted for child's ethnicity, gestational-age adjusted birth weight, breastfeeding, total energy intake at 1 y, energy-adjusted total fat intake at 1 y, and energy-adjusted sodium intake at 1 y; and maternal age, and smoking and folic acid supplement use during pregnancy.

[#] P_{trend} is obtained by including the number of the tertiles of protein intake as continuous variable in the model

eGFR, estimated glomerular filtration rate; OR, odds ratio

* $p < 0.05$ and ** $p < 0.01$

Supplementary Table S3. Associations of animal and vegetable protein intake at 1 y with kidney volume and function at 6 y (N=2,908).

	Kidney volume	**$eGFR_{Creat}$ (Schwartz 2009)**	**$eGFR_{CysC}$ (Zappitelli 2006)**	**ACR**
	(mm^3)	**($ml/min/1.73m^2$)**	**($ml/min/1.73m^2$)**	**(% change) †**
	N=2,699	**N=1,961**	**N=1,961**	**N=2,800**
Animal protein intake				
Fully adjusted model [§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	0.74 (-1.05, 2.54)	0.12 (-1.69, 1.94)	-0.27 (-1.77, 1.23)	1.0 (-7.3, 9.3)
Tertile 3	0.19 (-1.54, 1.92)	1.60 (-0.08, 3.28)	0.56 (-0.83, 1.94)	2.8 (-5.1, 10.8)
P_{trend} [#]	0.69	0.06	0.12	0.60
Per 10 g	0.24 (-0.73, 1.20)	0.60 (-0.45, 1.54)	0.54 (-0.21, 1.30)	-2.0 (-6.6, 2.5)
Vegetable protein intake				
Fully adjusted model[§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.41 (-0.48, 3.31)	0.96 (-0.85, 2.77)	**1.77 (0.27, 3.26)***	-4.9 (-13.6, 3.9)
Tertile 3	-0.38 (-2.48, 1.73)	1.65 (-0.17, 3.47)	**1.52 (0.01, 3.02)***	-1.9 (-11.7, 7.8)
P_{trend} [#]	0.75	0.06	***0.04***	*0.74*
Per 10 g	-0.08 (-2.15, 1.20)	1.95 (-0.13, 4.03)	1.15 (-0.28, 2.59)	-7.2 (-16.7, 2.2)

Values are based on multivariable linear regression models and reflect differences or percentage change (95%CI) in kidney outcomes for tertiles of protein intake compared to the lowest tertile, and per 10 g of protein intake per day.
Protein intake is energy-adjusted using the nutrient residual method.
[§] Fully adjusted models are adjusted for child's sex, age, ethnicity, gestational-age adjusted birth weight, breastfeeding, total energy intake at 1 y, energy-adjusted total fat intake at 1 y, and energy-adjusted sodium intake at 1 y; and maternal age, and smoking and folic acid supplement use during pregnancy.
[†] Albumin/creatinine ratio is log-transformed, therefore the regression coefficients reflect the percentage change rather than the absolute difference.
[#] P_{trend} is obtained by including the number of the tertiles of protein intake as continuous variable in the model

ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate

* $p < 0.05$ and ** $p < 0.01$

Supplementary Table S4. Associations of protein intake at the age of 1 y with kidney volume and function at 6 y in Dutch children only (N=1,994).

	Combined kidney volume (mm^3)	$eGFR_{Creat}$ (Schwartz 2009) ($ml/min/1.73m^2$)	$eGFR_{CysC}$ (Zappitelli 2006) ($ml/min/1.73m^2$)	ACR (% change)
	N=1,843	**N=1,356**	**N=1,357**	**N=1,920**
Crude model[§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	1.70 (-0.37, 3.77)	**2.13 (0.09, 4.16)***	**2.50 (1.59, 3.41)****	**-10.9 (-20.5, -1.3)***
Tertile 3	-0.16 (-0.88, 1.95)	1.56 (-0.49, 3.62)	**1.97 (1.06, 2.89)***	**-15.2 (-25.0, -5.5)****
P_{trend}[#]	*0.88*	*0.21*	***0.03***	***<0.01***
Per 10 g	*-0.46 (-1.52, 0.61)*	*0.48 (-0.58, 1.55)*	*0.89 (-0.05, 1.82)*	***-8.3 (-10.9, -5.8)****
Adjusted model[§]				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	**2.55 (0.41, 4.69)***	**2.30 (0.19, 4.42)***	**2.53 (0.73, 4.33)****	-7.5 (-15.5, 0.03)
Tertile 3	1.35 (-1.05, 3.76)	1.91 (-0.47, 4.29)	**2.08 (0.27, 3.89)***	**-7.8 (-18.8, 0.00)***
P_{trend}[#]	*0.26*	*0.11*	***0.03***	*0.19*
Per 10 g	*0.31 (-0.93, 1.56)*	*0.76 (-0.50, 2.02)*	***1.05 (0.10, 2.00)****	*-4.9 (-10.8, 1.1)*

Values are based on multivariable linear regression models and reflect differences or percentage change (95%CI) in kidney outcomes for tertiles of protein intake compared to the lowest tertile, and per 10 g of protein intake per day. Tertiles of protein intake are defined for the whole group (N=2,908), the distribution of children over the tertiles was similar for the subgroup of Dutch children.

Protein intake is energy-adjusted using the nutrient residual method.

[§]Crude models are adjusted for child's sex, age and body surface area at 6 y visit. Fully adjusted models are additionally adjusted for child's ethnicity, gestational-age adjusted birth weight, breastfeeding, total energy intake at 1 y, energy-adjusted total fat intake at 1 y, and energy-adjusted sodium intake at 1 y; and maternal age, and smoking and folic acid supplement use during pregnancy.

[†] Albumin/creatinine ratio is log-transformed, therefore the regression coefficients reflect the percentage change rather than the absolute difference.

[#] P_{trend} is obtained by including the number of the tertiles of protein intake as continuous variable in the model

* $p < 0.05$ and ** $p < 0.01$

Supplementary Table S5. Associations of protein intake at 2 y with childhood kidney volume and function at 6 y (N=660).

	Combined kidney volume (mm^3) N=609	**$eGFR_{Creat}$ (Schwartz 2009) (ml/min/1.73m^2) N=435**	**$eGFR_{CysC}$ (Zappitelli 2006) (ml/min/1.73m^2) N=435**	**ACR (% change)† N=628**
Crude model§				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	-0.28 (-4.16, 3.60)	2.72 (-0.90, 6.35)	-2.47 (-5.63, 0.69)	-12.5 (-29.8, 4.9)
Tertile 3	2.55 (-1.25, 6.36)	**4.57 (0.96, 8.18)***	-0.27 (-3.41, 2.88)	**-19.6 (-6.9, -2.3)***
P_{trend}#	*0.19*	***0.01***	*0.86*	***0.03***
Per 10 g	***2.63 (0.25, 5.02)****	***3.34 (1.01, 5.68)****	*0.21 (-1.84, 2.26)*	***-12.2 (-23.3, -1.1)****
Adjusted model§				
Tertile 1	Reference	Reference	Reference	Reference
Tertile 2	-1.28 (-5.30, 2.74)	1.82 (-1.98, 5.63)	-2.76 (-6.08, 0.57)	-9.9 (-28.1 8.3)
Tertile 3	0.78 (-3.71, 5.27)	**3.86 (0.34, 8.08)***	-1.51 (-5.10, 2.09)	-15.6 (-35.7, 4.6)
P_{trend}#	0.75	0.07	0.40	0.13
Per 10 g	*1.89 (-1.06, 4.85)*	***3.02 (0.14, 5.90)****	*-0.61 (-3.00, 1.79)*	*-9.6 (-23.3, 4.2)*

Values are based on multivariable linear regression models and reflect differences or percentage change (95%CI) in kidney outcomes for tertiles of protein intake compared to the lowest tertile, and per 10 g of protein intake per day.
Protein intake is energy-adjusted using the nutrient residual method.
§ Crude models are adjusted for child's sex, age and body surface area at 6 y visit. Fully adjusted models are additionally adjusted for child's ethnicity, gestational-age adjusted birth weight, breastfeeding, total energy intake at 2 y, energy-adjusted total fat intake at 2 y, and energy-adjusted sodium intake at 2 y; and maternal age, and smoking and folic acid supplement use during pregnancy.
† Albumin/creatinine ratio is log-transformed, therefore the regression coefficients reflect the percentage change rather than the absolute difference.
# P_{trend} is obtained by including the number of the tertiles of protein intake as continuous variable in the model

ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate

* $p < 0.05$ and ** $p < 0.01$.

Supplementary Table S6. Correlations between measures of body composition and kidney function.

	Serum creatinine	Serum cystatin C	$eGFR_{Creat}$ Schwartz	$eGFR_{CysC}$ Zappitelli
	N=1,962	N=1,963	N=1,961	N=1,963
Age	0.147**	0.013	-0.048*	-0.004
Height	0.196**	0.017	0.105*	0.000
Weight	0.204**	0.030	0.016	-0.014
Body mass index	0.13**	0.029	-0.067**	-0.021
Body surface area	0.217**	0.027	0.049*	-0.009
Total fat mass	0.050*	0.008	0.077*	0.003
Lean body mass	0.281**	0.063**	-0.046*	-0.044

Values are Pearson correlation coefficients

* $p<0.05$, ** $p< 0.01$

Chapter 3.3

Childhood body composition and estimates of glomerular filtration rate based on creatinine and cystatin C concentrations

Adapted from Am J Nephrol 2017;45(4):320-326

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Abstract

Background: Creatinine and cystatin C concentrations are commonly used to estimate glomerular filtration rate (eGFR) in clinical practice and epidemiological studies. To estimate the influence of different body composition measures on eGFR from creatinine and cystatin C blood concentrations, we compared the associations of different anthropometric and body composition measures with eGFR derived from creatinine ($eGFR_{creat}$) and cystatin C ($eGFR_{cystC}$) blood concentrations.

Study design: Population-based cohort study.

Settings & participants: This study was performed among 4,305 children.

Predictors: At the age of 6.0 years (95% range 5.7 - 8.0), we measured weight and height and calculated body mass index and body surface area, and lean and fat mass by Dual-energy X-ray Absorptiometry.

Outcomes & measurements: At the same age, we measured creatinine and cystatin C blood concentrations and estimated the GFR.

Results: Correlation between eGFR based on creatinine and cystatin C concentrations was r = 0.40 (p-value <0.01). Higher body mass index was associated with lower $eGFR_{cystC}$ but not with $eGFR_{creat}$. Higher body surface area was associated with higher $eGFR_{creat}$ and lower $eGFR_{cystC}$ (p-values <0.05). Lean and fat mass percentages were associated with $eGFR_{creat}$ but not with $eGFR_{cystC}$.

Limitations: Lack of actual measurements of GFR.

Conclusions: Our findings suggest that both $eGFR_{creat}$ and $eGFR_{cystC}$ are influenced by body mass index and body surface area. $eGFR_{creat}$ is stronger influenced by body composition than $eGFR_{cystC}$. Further studies are needed to assess whether using $eGFR_{cystC}$ instead of $eGFR_{creat}$ leads to better care for pediatric kidney patients.

Introduction

Glomerular filtration rate (GFR) plays a key role in the management of kidney disease. Ideally, measuring GFR should be based on renal clearances of exogenous markers such as inulin, but this approach is complex, invasive and expensive.(1) Therefore, GFR is commonly estimated based on creatinine blood concentrations.(2) Using creatinine as marker of renal function has some limitations. Creatinine, is actively secreted by the proximal tubule, and is related to muscle mass, age, sex, ethnicity and dietary factors.(1,3) Creatinine concentrations can be higher in individuals with an increased muscle mass, independent of kidney function, leading to an underestimation of eGFR.(4) Studies in adults suggest that eGFR based on creatinine concentrations can be improved if lean mass percentage could be incorporated in the formula.(4,5) Next to creatinine, cystatin C blood concentrations can be used to estimate GFR.(6,7) The major advantage of cystatin C is that it is considered less related to body weight and height in children.(8,9) Some authors report a superior sensitivity of cystatin C for detecting impaired GFR in pediatric patients seems superior to that of creatinine, especially in children with low muscle mass.(9) However, studies in kidney disease patients suggest that lean mass affects cystatin C concentrations.(10,11) Many studies have explored the associations between body mass index (BMI) and eGFR, using BMI as a proxy for body composition.(12-14) Studies comparing the correlations and associations of detailed body composition measures and the eGFR among healthy pediatric populations are lacking.

To estimate the influence of different body composition measures on eGFR from creatinine and cystatin C blood concentrations, we compared the associations of different anthropometric and body composition measures with eGFR derived from creatinine ($eGFR_{creat}$) and cystatin C ($eGFR_{cystC}$) blood concentrations in a population-based prospective cohort study among 4,305, 6 year old children.

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands, which has been described in detail previously.(15) The study has been approved by Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam. All children were born between April 2002 and January 2006 and form a largely prenatally enrolled birth cohort that is currently being followed until young adulthood. Written consent was obtained for all children. A total of 8,305 children participated in the follow-up measurements at 6 years of age (median age 6.0 years; 95% range 5.7, 8.0). Of these children, 6,509 (78%) visited the research center for body composition follow-up measurements. For this study, we excluded children with congenital kidney abnormalities (N=12). The present analyses were performed among 4,305 children with body composition and kidney function measures available (**Supplementary Figure 3.3.1**).

Body composition measurements

Children's anthropometrics and body composition were measured at a median age of 6.0 years (95% range 5.7 to 8.0).(15) Height (m) was determined in standing position to the nearest millimeter without shoes using a Harpenden stadiometer (Holtain Limited, Dyfed, U.K.). Weight was measured using a mechanical personal scale (SECA, Almere, The Netherlands). We calculated BMI (kg/m^2) and body surface area (BSA) (m^2). For BSA, we used the DuBois formula: BSA = weight (kg)$^{0.425}$ x height (cm)$^{0.725}$ x 0.007184.(16) Whole body dual-energy X-ray absorptiometry (DXA) scans (iDXA, GE-Lunar, 2008, Madison, WI, USA) were performed to estimate fat and lean mass. We calculated lean mass percentage as (lean mass (kg) /weight (kg)) and fat mass percentage as (fat mass (kg) /weight (kg)).(17)

Kidney function measurements

Non-fasting blood samples were drawn by antecubital venipuncture and centrifuged for 10 minutes and stored at -80 °C at one location in the STAR-MDC laboratory. As previously described, creatinine concentrations were measured with enzymatic methods and cystatin C concentrations with a particle enhanced immunoturbidimetric assay (using Cobas 8000 analyzers, Roche, Almere, the Netherlands). Quality control samples demonstrated intra-assay and inter-assay coefficients of variation of 0.51% for creatinine and 1.65% for cystatin C, and 1.37% for creatinine and 1.13% for cystatin C, respectively. (18) We calculated the eGFR based on creatinine concentrations according to the revised Schwartz 2009 formula: $eGFR_{creat}$ = 36.5 * (height (cm)/ serum creatinine (µmol/l)),(19) and eGFR based on cystatin C concentrations using Zappitelli's formula: $eGFR_{cystC}$ = 75.94 / [CysC (mg/L)$^{1.17}$].(7)

Statistical analysis

We performed a non-response analysis by comparing subject characteristics between children with and without kidney function measurements using T-tests, Chi-square tests and Mann-Whitney tests. We created standard deviations scores (SDS) for all body composition measures to enable comparison between effect estimates. Next, we examined the Pearson rank correlation coefficients between childhood anthropometrics, body composition and eGFR measures. Third, we used multiple linear regression analyses to examine the associations of anthropometric and body composition measures with creatinine, cystatin C, $eGFR_{creat}$ and $eGFR_{cystC}$. These models were adjusted for child sex, age at measurements, and ethnicity. Additionally, we explored the associations of childhood BMI clinical cut-offs with creatinine, cystatin C and eGFRs. Because of the already reported associations of ethnicity with kidney function markers, we performed a sensitivity analysis in children of European ethnicity, the largest ethnic subgroup.(20) Based on previous literature we assessed whether the explored association differed by sex, which was not the case in this study.(21, 22) All analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS IBM, Chicago, IL, USA).

Results

Participant characteristics

Table 3.3.1 shows the characteristics of the participants. In the full group the mean (SD) $eGFR_{creat}$ and $eGFR_{cystC}$ were 118.89ml/min/1.73m^2 (15.87) and 101.59ml/min/1.73m^2 (11.24), respectively. The histograms of creatinine, cystatin C, $eGFR_{creat}$ and $eGFR_{cystC}$ are provided in **Supplementary Figure 3.3.2**. Results from the non-response analyses are given in the **Supplementary Table 3.3.1**. Children with kidney function measurements had higher lean mass percentage and lower fat mass percentage compared to children who did not have kidney function measurements.

Correlations of childhood anthropometrics and body composition measures with eGFR

The correlation coefficient between $eGFR_{creat}$ and $eGFR_{cystC}$ was r = 0.40 (p-value <0.01) (**Table 3.3.2**). Childhood height, weight, BMI and BSA were positively correlated with creatinine and cystatin C concentrations, with stronger coefficients for creatinine concentrations (p-values <0.01). Lean mass percentage was positively correlated with creatinine concentrations and negatively correlated with $eGFR_{creat}$ (r = 0.13, p-value < 0.01). Similar results, but in opposite directions were observed for fat mass percentage. Lean mass percentage and fat mass percentage were not correlated with cystatin C concentrations or $eGFR_{cystC}$.

Associations of childhood body composition measures with eGFR

Table 3.3.3 shows that childhood height was associated with creatinine concentrations (p-value< 0.05), but not with cystatin C concentrations. Higher childhood weight was associated with both higher creatinine and cystatin C concentrations (p-value <0.01). Higher childhood height was associated with higher $eGFR_{creat}$ (p-value <0.01), but not with $eGFR_{cystC}$ whereas higher childhood weight was associated with higher $eGFR_{creat}$ and lower $eGFR_{cystC}$ (p-value <0.01).

Each 1-SD increase in BSA was associated with 1.81 ml/min/1.73m^2 (95% confidence interval (CI) 1.24, 2.37) higher $eGFR_{creat}$ and 0.57 ml/min/1.73m^2 (95% CI -0.98, -0.17) lower $eGFR_{cystC}$. We observed tendencies for similar effect estimates when we restricted the analyses to Europeans only (N=2,727) (**Supplementary Table 3.3.2**). BMI was negatively associated with $eGFR_{cystC}$ (p-value <0.05) but not with $eGFR_{creat}$. Among Europeans, higher childhood BMI was associated with higher creatinine and cystatin C concentrations and lower $eGFR_{creat}$ and $eGFR_{cystC}$ (**Supplementary Table 3.3.2**). The associations of BMI clinical cut-offs with creatinine, cystatin C and the eGFR are given in **Supplementary Table 3.3.3.**

Higher lean mass percentage was associated with higher creatinine concentrations and with lower cystatin C concentrations (**Table 3.3.3**) (p-values <0.05). A 1-SD increase in lean mass percentage was associated with 2.74 ml/min/1.73m^2 (95% CI -3.27, -2.20) lower childhood $eGFR_{creat}$. No association was observed of lean mass percentage with $eGFR_{cystC}$.

Higher fat mass percentage was associated with lower creatinine concentrations and with higher cystatin C concentrations (**Table 3.3.3**, p-values <0.05). A 1-SD increase in fat mass percentage was associated with 2.68 ml/min/1.73m^2 (95% CI 2.14, 3.21) higher childhood $eGFR_{creat}$. No associations were observed of fat mass percentage with $eGFR_{cystC}$. Similar effect estimates were observed when we restricted our analyses to European subjects only, but not all associations were significant (**Supplementary Table 3.3.2**).

Table 3.3.1. Subjects characteristics (N=4,305)

Age at measurements (y)	**6.02 (5.68, 7.98)**
Sex, Girls (%)	48.30
Ethnicity (%)	
Dutch or European	65.10
Non-European	34.90
Height (cm)	119.72 (6.00)
Weight (kg)	23.37 (4.23)
Body mass index (kg/m^2)	16.21 (1.84)
Body surface area (m^2)	0.88 (0.09)
Lean mass percentage (%)	71.73 (5.36)
Fat mass percentage (%)	24.66 (5.56)
Creatinine (µmol/l)	37.40 (5.27)
Cystatin C (µg/l)	787.30 (74.40)
$eGFR_{creat}$ (ml/min/1.73m^2)	118.89 (15.87)
$eGFR_{cystC}$ (ml/min/1.73m^2)	101.59 (11.24)

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

$eGFR_{creat}$ estimated glomerular filtration rate calculated based on creatinine blood concentrations; $eGFR_{creat}$ = 36.5 * (height (cm)/ serum creatinine (µmol/l)); $eGFR_{cystC}$ estimated glomerular filtration rate calculated based on cystatin C blood concentrations $eGFR_{cystC}$ = 75.94 / [CysC(mg/L)$^{1.17}$]

Table 3.3.2. Correlation coefficients of the investigated variables

	Height	Weight	BMI	BSA	FMP	LMP	Creat	Cyst C	$eGFR_{creat}$	$eGFR_{cystC}$
Height	1.00									
Weight	0.77**	1.00								
BMI	0.31**	0.83**	1.00							
BSA	0.90**	0.97**	0.69**	1,00						
FMP	0.18**	0.57**	0.69**	0.46**	1,00					
LMP	-0.19**	-0.56**	-0.68**	-0.45**	-0.999**	1,00				
Creat	0.30**	0.28**	0.16**	0.30**	-0.05**	0.05**	1,00			
Cyst C	0.05**	0.06**	0.05**	0.06**	-0.001	0.003	0.40**	1,00		
$eGFR_{creat}$	0.06**	0.01	-0.04**	0.03	0.12**	-0.13**	-0.92**	-0.40**	1,00	
$eGFR_{cystC}$	-0.05**	-0.06**	-0.05**	-0.06**	0.01	-0.01	-0.39**	-0.99**	0.40**	1,00

** Correlation is significant at the 0.01 level (2-tailed).

BMI- body mass index, BSA- body surface area, FMP- fat mass percentage, LMP- lean mass percentage, Creat- creatinine, Cyst C- cystatin C, $eGFR_{creat}$ estimated glomerular filtration creatinine-based, $eGFR_{cystC}$ estimated glomerular filtration cystatin C-based.

Table 3.3.3. Associations of anthropometric and body composition measures with creatinine, cystatin C and eGFR (N=4,305)

	Difference (95% Confidence Interval)			
	Creatinine (µmol/l)	**Cystatin C (µg/l)**	**$eGFR_{creat}$ (ml/min/1.73m²)**	**$eGFR_{cystC}$ (ml/min/1.73m²)**
Anthropometrics and body composition (SDS)				
Height	0.97 (0.79, 1.15)***	1.99 (-0.70, 4.70)	2.78 (2.22, 3.35)***	-0.29 (-0.70, 0.12)
Weight	0.91 (0.73, 1.08)***	4.49 (1.91, 7.07)***	1.16 (0.61, 1.71)***	-0.66 (-1.05, -0.27)**
Body mass index	0.52 (0.36, 0.68)***	4.15 (1.80, 6.51)***	-0.37 (-0.87, 0.13)	-0.61 (-0.96, -0.26)**
Body surface area	1.02 (0.84, 1.20)***	3.93 (1.27, 6.59)**	1.81 (1.24, 2.37)***	-0.57 (-0.98, -0.17)**
Lean mass percentage	0.30 (0.07, 0.54)*	-2.73 (-5.28, -0.18)*	-2.74 (-3.27, -2.20)***	0.36 (-0.03, 0.74)
Fat mass percentage	-0.48 (-0.65, -0.31)***	2.86 (0.32, 5.40)*	2.68 (2.14, 3.21)***	-0.38 (-0.76, 0.01)

Values are beta coefficients and 95% Confidence Intervals, from linear regression models adjusted for child age, sex and ethnicity.
$eGFR_{creat}$ estimated glomerular filtration rate calculated based on creatinine blood concentrations; $eGFR_{cystC}$ estimated glomerular filtration rate calculated based on cystatin C blood concentrations
P value for the associations * < 0.05 ** <0.01 *** <0.001

Discussion

Results of this cross-sectional study in healthy 6 year old children suggest that BMI and BSA are associated with creatinine-based eGFR and cystatin C-based eGFR. Lean mass percentage and fat mass percentage are associated with creatinine-based eGFR, but not with cystatin C-based eGFR.

Interpretation of main findings

To our knowledge, this is the first study comparing the association of detailed measures of body composition with estimates of GFR based on creatinine and cystatin C concentrations in a population of healthy children. Both creatinine and cystatin C concentrations can be influenced by different factors. Creatinine is produced in active muscle and is reported to be determined by muscle mass and dietary intake, which may account for the variations in the concentrations of serum creatinine observed among different age and ethnic groups.(1,3,23) However, we have previously reported that childhood protein intake does not influence the eGFR.[24] Cystatin C is another marker to evaluate renal function, although is not used as commonly as creatinine.(6,25) Cystatin C is produced by all nucleated cells and is reported to be less strongly related to body weight and height in children compared to creatinine.(8,9) Besides, adult studies suggest that cystatin C concentrations are related to age, sex, height and weight and influenced by corticosteroid use.(26,27)

In children, the Schwartz formula is widely used to estimate GFR from creatinine concentrations.(19) The Schwartz formula is known to overestimate eGFR compared to inulin clearance GFR.(6,28) Schwartz formula estimates GFR using creatinine concentrations and child height.(19) Next to Schwartz's formula we estimated GFR using the Zappitelli's formula.(7) This formula is not dependent of any anthropometric measures. It estimates GFR by using only the cystatin C concentrations.(7) In a study among 42 healthy adults, eGFR using creatinine and cystatin C concentrations was compared with measured GFR. This study suggested that eGFR based on cystatin C concentrations was a better marker then eGFR based on creatinine concentrations for estimating kidney function.(29)

It has previously been reported that lean body mass, indicating muscular mass, is an important determinant of the GFR.(30) So far results from studies comparing the effects of body composition measures on creatinine and cystatin C concentrations and their derived eGFR are contradictory.(10,31,32) A number of studies have explored the associations of BMI with eGFR, using BMI as a proxy of body composition.(12-14) The associations of BMI with creatinine and cystatin C differed between populations studied. We observed that higher BMI was associated with higher creatinine and cystatin C concentrations and with lower $eGFR_{cystC}$, but not with $eGFR_{creat}$. In the subgroup of Europeans, we observed that BMI was associated with creatinine, cystatin C concentrations, and their derived eGFRs. In line with our findings in the European subgroup, in the general Japanese population, BMI was associated with lower $eGFR_{creat}$.(14) Similar to what we observe,

studies among both healthy and kidney diseased adults suggested that eGFR based on cystatin C concentrations is not independent of BMI.(10,33) These findings appear to be different among children with various kidney diseases, where BMI does not have a clinically relevant effect on $eGFR_{cystC}$.(13) Next to BMI, we observed that higher BSA was associated with higher creatinine, cystatin C concentrations, $eGFR_{creat}$ and lower $eGFR_{cystC}$. In the general adult population no associations have been reported of BSA with eGFR based on creatinine concentrations.(4) Studying detailed measures of body composition will therefore likely add to the understanding of the associations of body composition and kidney function measures.

Studies among healthy adults have shown lean mass to be associated with serum creatinine but not with cystatin C concentrations.(31) Also, a study among 1,630 randomly selected individuals from the general population has shown that lean mass percentage, but not fat mass percentage, was associated with eGFR based on creatinine concentrations.(4) Another study among 67 healthy individuals of ages between 18 and 52 years has shown that creatinine concentrations were highly affected by muscle mass, whereas cystatin C concentrations were affected by fat mass.(32) In children after renal or heart transplantation both creatinine or cystatin C-based equations tend to overestimate renal function at lower inulin clearance GFR values and underestimate renal function at higher inulin clearance GFR values.(34) The associations between lean mass and cystatin C are reported to be different among kidney disease patients compared to adults.(10) Among 77 chronic kidney disease patients lean mass affected cystatin C concentrations and GFR estimation based on cystatin C concentrations improved when lean mass was included in the formula, especially in patients with extreme body composition.(10) In severely obese children lean mass percentage has been reported to correlate with both creatinine and cystatin C concentrations.(35) In the current study, we observed that lean and fat mass percentage correlate with creatinine concentrations and $eGFR_{creat}$. Higher lean mass percentage and lower fat mass percentage were associated with higher creatinine concentrations and lower $eGFR_{creat}$. We did not observe a significant correlation of lean mass percentage or fat mass percentage with cystatin C concentrations. Our study shows that eGFR based on cystatin C concentrations is independent of lean mass percentage and fat mass percentage.

Our results suggest that BMI and BSA influence creatinine and cystatin C concentrations and their derived eGFRs. Furthermore, body composition measures are strongly related to creatinine derived eGFR. Therefore, increased lean mass percentage leads to a rise in creatinine concentration and subsequently underestimates GFR. We hypothesized that an increased fat mass is associated with a higher number of nucleated cells and therefore could increase serum cystatin C. However, eGFR based on cystatin C concentrations appeared to be independent of body composition measures. Meta-analyses have reported that cystatin C can be a better marker of kidney function compared to creatinine concentrations.(36-38) Comparing the effect estimates on $eGFR_{creat}$ and $eGFR_{cystC}$ we observe stronger estimates on $eGFR_{creat}$ suggesting that body composition measures are stronger associated with creatinine concentrations than with $eGFR_{cystC}$. As the revised

Schwartz formula ($eGFR_{creat}$) is the most widely used formula both in epidemiological studies and clinical practice, our findings suggest that body composition measures should be considered when eGFR is based on creatinine concentrations. Ideally, body composition measures would have been incorporated in the $eGFR_{creat}$ equations and compared with GFR based on renal clearances of exogenous markers, but unfortunately this is not possible in our study. Considering the feasibility and costs of performing DXA scans in school-aged children, whether and to what extent detailed body composition measures should be used in the clinical practice when eGFR can be argued. However, our findings suggest that $eGFR_{creat}$ is stronger influenced by body composition than $eGFR_{cystC}$. Other studies are needed to assess whether using $eGFR_{cystC}$ instead of $eGFR_{creat}$ leads to better care for pediatric kidney patients.

Strengths and limitations

To the best of our knowledge, this is the first and largest cross-sectional multiethnic study in a healthy pediatric population-based cohort examining the associations of body composition with estimates of GFR.GFR was estimated based on creatinine and cystatin C concentrations. Except height and weight to calculate BMI and BSA, we also measured fat mass and lean mass with DXA. Of all children 61% provided blood samples for measuring creatinine and cystatin C concentrations. Children without data on kidney function measures were shorter, had a higher fat mass percentage and lower lean mass percentage, but no difference in BMI as compared to children with available kidney function measures. We observed tendencies for similar effect estimates among Europeans only, although not all associations were significant in this subgroup. This might be due to the smaller study group, but may also reflect an effect of ethnicity. A limitation of our study is that creatinine and cystatin C concentrations were measured only once. Ideally, we would have been able to compare the explored associations with the measured GFR and validate our findings. Unfortunately, we do not have the urinary or plasma clearance of an ideal filtration marker, such as inulin, iothalamate or iohexol, as the gold standard for the measurement of GFR.[39] Our findings are based on a healthy pediatric population of a narrow age category and may not be generalizable to older, younger, or diseased populations.

Conclusion

Our results suggest that eGFR based on both creatinine and cystatin C concentrations are influenced by BMI and BSA, whereas only eGFR based on creatinine concentrations is influenced by lean mass percentage and fat mass percentage. Beside anthropometric measurements, body composition measures should be considered when estimating GFR in children. Further studies to compare these results with measured GFR are needed.

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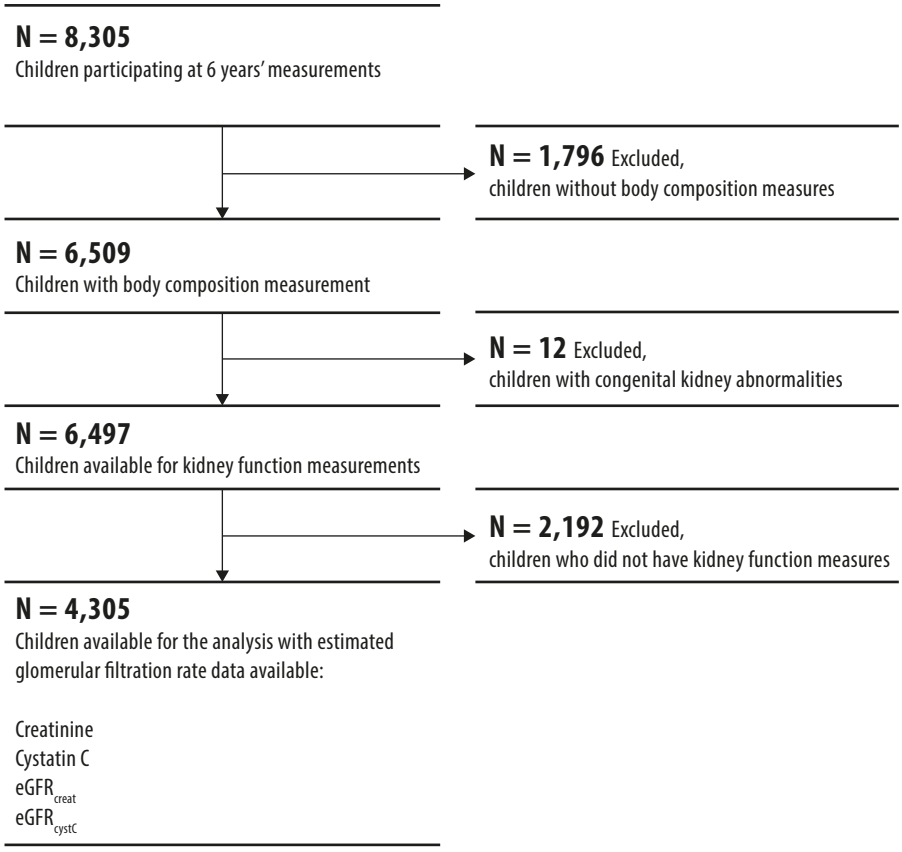
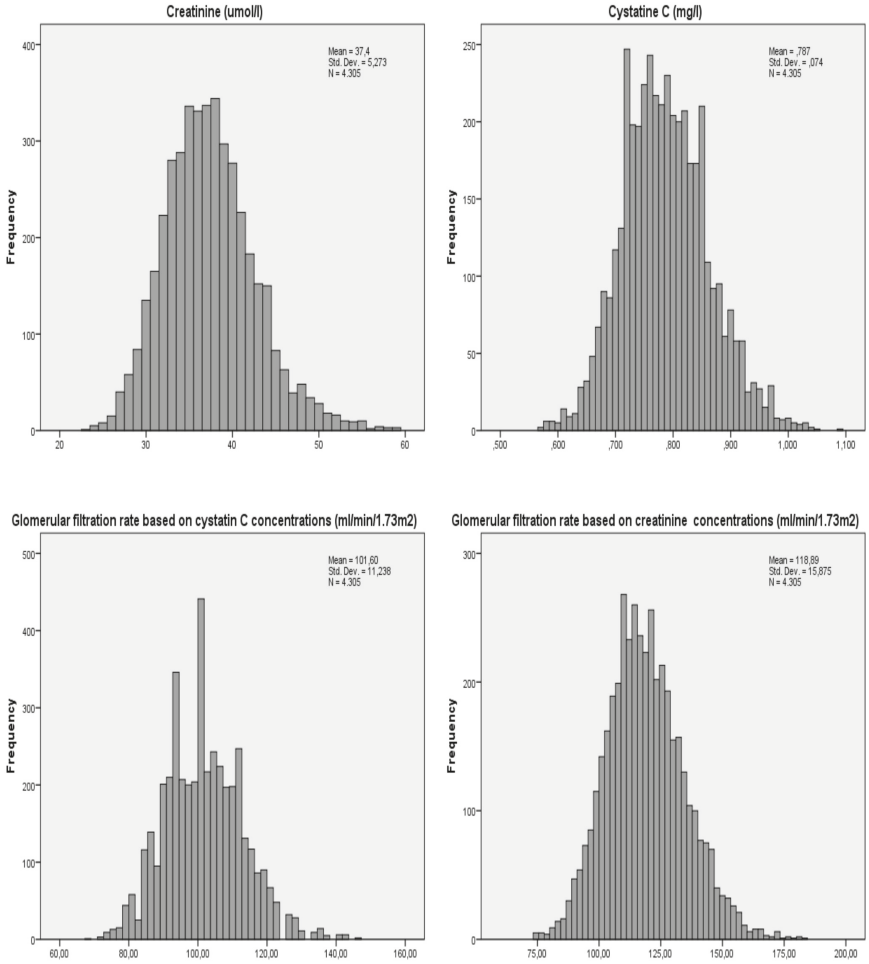
Supplementary Figure 3.3.1. Flow chart of the study participants



Supplementary Figure 3.3.2. Histograms of kidney function measures



Supplementary Table 3.3.1. Subject characteristics between children with and without kidney function measurements (N=6,509)

Subjects characteristics	With eGFR data (N=4,305)	Without eGFR data (N=2,204)	P-value
Age at measurements (y)	6.0 (5.7, 8.0)	6.0 (5.7, 7.6)	0.20
Sex, Girls (%)	48.3	53.4	0.07
Ethnicity (%)			0.26
Dutch or European	65.1	63.8	
Non-European	34.9	36.2	
Height (m)	119.7 (6.0)	119.0 (6.0)	**<0.01**
Weight (kg)	23.4 (4.2)	23.1 (4.3)	**0.01**
Body mass index (kg/m^2)	16.2 (1.8)	16.2 (1.9)	0.74
Body surface area (m^2)	0.88 (0.09)	0.87 (0.09)	**<0.01**
Fat mass percentage (%)	24.7 (5.6)	25.3 (5.9)	**<0.01**
Lean mass percentage (%)	71.7 (5.4)	71.1 (5.7)	**<0.01**

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

Chapter 4

General discussion

Discussion

Introduction

Chronic kidney disease (CKD) is a major public health problem worldwide. CKD by itself, and by its increasing risk of hypertension and cardiovascular disease, has a high impact on morbidity and mortality.(1,2) Early recognition of persons at risk for impaired kidney function would be helpful in developing prevention strategies for CKD.

The last decades, multiple studies suggest that fetal and childhood development are important factors in the risk of cardiovascular and renal disease in later life.(3,4) Previous studies show increased risks of CKD in low birth weight populations.(5,6) The specific roles of preterm birth and fetal growth restriction in these associations are not clear.(7-9) Also, adverse exposures in early life might influence fetal kidney development. Maternal smoking has been associated with low nephron number.(10) Early childhood dietary factors might influence kidney volume and function.(11,12)

The glomerular hyperfiltration hypothesis proposed by Brenner postulates that adverse factors in utero lead to a lower nephron number. A kidney with fewer nephrons might in response cause hyperfiltration, which can cause glomerulosclerosis and increase the risk of CKD in adulthood.(13,14)

The main aim of the studies presented in this thesis was to identify specific growth periods and growth patterns of body growth and kidney volume which are crucial for kidney function in childhood. Also, the associations of specific modifiable maternal and child factors with kidney function in childhood were evaluated. Since variations in kidney function and blood pressure track into adulthood,(15,16) it is important to identify individuals at risk for impaired kidney at young age. Subclinical differences in kidney function may already be present in childhood, and predict kidney disease in later life. This chapter discusses the overall findings presented in the studies in this thesis, addresses general methodological issues and gives perspective for future research.

Interpretation of the main findings

Fetal and infant growth

Background

Nephrogenesis starts in first trimester of pregnancy around the 8th week of gestation and ceases around 36 weeks of gestation.(17) Since nephrogenesis does not proceed after birth, life in utero might be crucial for nephron endowment. In population studies, birth weight is often used as a reflection of the outcome of life in utero. The relationship between birth weight and kidney development has been studied extensively. However, birth weight is a result of multiple fetal and maternal factors, and does not fully reflect kidney growth. A previous study from the same cohort as the studies in this thesis, showed that impaired fetal growth in late pregnancy is associated with fetal kidney volume. Raised placental resistance and fetal blood redistribution at expense of the

abdominal organs, were associated with kidney volume in utero.(18) In addition, fetal growth was positively associated with kidney volume at the age of 2 years.(19) Smaller kidney volume in fetal life tracks into early childhood.(19) Not much is known about kidney growth and the risk of impaired kidney function in a healthy population. A retrospective cohort study shows that kidney size in children with renal hypoplasia was associated with the risk of end-stage renal disease at the age of 9 years.(20)

Main findings

Results from studies in this thesis show that fetal blood flow redistribution at expense of the abdominal organs is associated with decreased kidney volume and function in childhood (**Chapter 2.1**). Furthermore, impaired fetal blood flow combined with smaller fetal kidney volume is associated with smaller kidney volume and lower kidney function in childhood as compared to normal fetal blood flow and fetal kidney volume (**Chapter 2.1**). The study focused on fetal first trimester growth did not show consistent associations with kidney volume and function in childhood (**Chapter 2.2**). Additional growth analyses did show positive associations of second and third trimester fetal growth, birth weight and weight at 6 months with kidney volume in childhood (**Chapter 2.3**). Only second trimester fetal weight was associated with kidney function at the age of 6 years (**Chapter 2.3**). Body growth is often used as a proxy for kidney volume. However, kidney volume itself can also be measured directly by ultrasound. The studies in this thesis show that both fetal and infant kidney growth are associated with childhood kidney function (**Chapter 2.4**). We did not observe consistent associations between early growth and microalbuminuria.

Thus, the studies presented in this thesis suggest that impaired body and kidney growth, in second half of pregnancy and infancy, is associated with smaller kidney volume and lower kidney function in childhood.

Underlying mechanisms

The studies presented in this thesis show no associations of fetal first trimester growth with kidney function in childhood. An explanation might be that kidney volume growth rate reaches its maximum after first trimester, between 26 and 34 weeks of gestation.(21) Also, it is not known whether renal compensatory hypertrophy in utero occurs affecting these association.(22) However, further studies are needed to get more insight in these mechanisms. Second and third trimester body and kidney growth are positively associated with kidney measures in childhood. Kidney development rates increase during pregnancy, third trimester is specifically important since approximately 60% of total nephron number develops in third trimester.(23) Impaired blood flow to the abdominal organs is associated with smaller kidney volume in childhood. This is in line with animal studies showing reduced nephron number as result of placental insufficiency.(24,25)

Various other factors, influencing early growth and possibly through direct pathways, are suggested to be associated with impaired nephron endowment. For example, animal studies show that increased cortisol levels during pregnancy can lead to low

nephron number even if birth weight is normal.(26,27) Also, maternal hypertensive disorders during pregnancy, maternal diabetes and early life drug exposure might influence nephron endowment.(14) Underlying pathways are not fully known. Placental insufficiency may cause changes in the renin-angiotensin-system and thereby increase the risk of impaired kidney function.(28,29) Placental insufficiency might, next to impaired growth, lead to adaptations in DNA-methylation of pro-apoptotic genes which are related to kidney development.(30) Several genetic and epigenetic mechanisms are hypothesized to be crucial in nephron endowment and fetal and childhood kidney volume. Common variants in the PAX2(31) or RET(32,33) genes are associated with reduced kidney volumes at birth. DNA methylation might also play an important role in kidney development.(34)

Maternal and child factors

Maternal smoking

Maternal smoking is a well-known, modifiable risk factor for adverse pregnancy outcomes. Fetal smoke exposure is associated with increased risks of preterm birth and low birth weight.(35-38) Previous studies suggest that maternal smoking is associated with altered organ development. (39,40) A previous study in the same cohort as the studies presented in this thesis, shows that maternal smoking during pregnancy is negatively associated with third trimester fetal kidney development.(41) We observed that continued maternal smoking during pregnancy is associated with smaller kidney volume and lower eGFR at the age of 6 years. Multiple pathways might underlie these associations. Since maternal smoking is related with low birth weight, impaired fetal growth might lead to low nephron endowment in infants who are exposed to smoke during fetal life. However, also after adjustment for birth weight, maternal smoking was associated with kidney volume and function. This suggests that other pathways, apart from fetal growth impairment, might be involved in the observed associations. Several teratogenic and toxic factors in cigarettes, such as nicotine, carbon monoxide and cadmium, might influence placental and fetal vascular development and thereby kidney growth and function.(42,43) Effects of maternal smoking during pregnancy on childhood kidney volume and function were much stronger than effects of paternal smoking. Therefore, an intrauterine effect of smoking on childhood kidney volume and function seems more likely than a general environmental effect. Recent studies on maternal smoking and CKD suggests an effect of maternal smoking on DNA methylation, and fetal and renal mitochondrial function.(44) An animal study shows renal morphological and tubular renal functional changes in offspring after fetal smoke exposure.(45) The specific pathways are not fully known.

Infant protein intake

Fetal and infant growth and nutrition are important for kidney development. Previous studies from the same cohort show that higher total and vegetable, but not animal, maternal protein intake during the first trimester of pregnancy is associated with a higher eGFR at in childhood.(46) In addition, longer breastfeeding duration is associated with larger kidney volume and higher eGFR in childhood.(12) However, other studies show higher kidney volume and increased kidney function in formula fed infants as compared to breastfed children. But, at longer follow-up at the age of 18 months these effect had disappeared.(11,47) Studies in animal models show that early life protein intake may stimulate kidney growth and function, but these associations might be reversible.(48,49) A few previous studies observed associations of early life protein intake with adult kidney function and the risk of hypertension.(50,51) The mechanisms underlying the associations between protein intake and kidney function are not fully understood. It is hypothesized that higher protein intake increases kidney volume in order to be able to excrete in increased levels of nitrogen metabolites. Furthermore, higher protein intake stimulates Insulin-Like Growth Factor (IGF) I secretion, which stimulates kidney volume growth. Finally, increased total protein intake might cause total body growth and thereby also kidney volume growth.(52) We studied the associations of protein intake at the age of 1 year and kidney volume and function at the age of 6 years. We observed a positive association between protein intake and kidney function in basic models. However, after adjustment for dietary and lifestyle factors protein intake was no longer associated with kidney function in childhood. Thus, our findings do not support a strong effect of protein intake in early life on kidney function in childhood. The differences in results between our study and previous studies may be explained by differences is study subjects, populations and age of outcome measurements.

Childhood body composition and eGFR estimations based on creatinine and cystatin C concentrations

Estimations of GFR are important in screening, diagnosing and management of kidney disease. Direct measurement of GFR is most often based on renal clearances of exogenous markers such as inulin and iohexol.(53) However, these measurements are difficult to use for screening and clinical practice since they are complex, invasive and have high costs.(53) Thus, creatinine concentrations are widely used in estimating GFR in screening and clinical practice. However, creatinine use in GFR estimations has some limitations. Creatinine is actively secreted by the proximal tubule and its concentrated might be related with age, sex, ethnicity and dietary factors.(53,54) Moreover, creatinine concentrations are associated with muscle mass. In individuals with higher muscle mass, creatinine concentrations might be higher and therefore eGFR can be underestimated.(55) Cystatin C is proposed as a possible better marker for GFR estimation. Cystatin C is produced at a constant rate and is secreted by the tubule. Also, cystatin C concentrations seem to be less influenced by weight and height in children.(56)

The study presented in this thesis focused on the associations of detailed measures of body composition and estimations of GFR based on creatinine and cystatin C concentrations. Lean mass and fat mass were evaluated by Dual-energy X-ray Absorptiometry (DXA). We observed associations of childhood lean and fat mass percentages with both creatinine and cystatin C based GFR estimations. GFR estimations based on creatinine concentrations tended to be more strongly associated with childhood lean and fat mass percentages than GFR estimations based on cystatin C levels. Results of this study show that incorporating childhood body composition measures in GFR estimations might provide more accurate estimations of GFR. However, in screening programs and in clinical practice it is difficult and expensive to perform DXA scanning to obtain this information.

Methodological considerations

Specific methodological issues of the studies presented in this thesis have been discussed in the specific chapters of this thesis. The studies performed in this thesis have been conducted within the Generation R Study. This is a population-based, prospective cohort study which provides detailed data collection and enables the opportunity to assess temporal associations.

Selection bias

Selection bias can appear if the association between the determinant and outcome of interest is different in subjects who participate in the study and those who did not participate in the study, but were eligible for the study. Of all eligible children at birth, 61% participated in the Generation R Study. Participation of mothers belonging to ethnic minority groups and with low socioeconomic status was lower than expected from overall population in Rotterdam.(57) Furthermore, mothers who were included in the study had less medical complications during pregnancy and unfavorable pregnancy outcomes, for example hypertensive disorders and low birth weight, as compared to eligible mothers who were not included. These differences suggest that the study population is more healthy and affluent then the general population. Specifically the focus cohort, the group of mothers in which more frequent and more detailed follow up measures were conducted, tended to be healthier. Since we do not expect that the associations of the exposures and outcomes presented in this these are different between participating and not participating mothers, we do not assume that the differences between those groups have influenced our results. This selection will probably affect the prevalence rates and thereby reduce statistical power and generalizability of our results.

Selection bias may arise from non-response at baseline, and from loss to follow-up. Loss to follow-up can occur if the studied associations differ between those included in the study population and those lost to follow-up. In the studies presented in this thesis participation in the follow-up measurements at the age of 6 years was around 70%. Furthermore, response rate for blood samples was around 67% of the included

participants at the age of 6 years. This lower percentage for serum creatinine and cystatin C measurements was mainly due to non-consent for venous puncture. Mothers of children who did not participate in the follow-up measurements at the age of 6 years, were more often less educated and had more unhealthy lifestyle as compared to the total study population. Also, children of mothers who were not participating at the age of 6 years had lower birth weight, but similar kidney size as compared to the included children.

Information bias

Information on the determinants and outcomes in the studies described in this thesis are obtained by physical examinations, ultrasound examinations, blood and urine analyses and parental questionnaires. Differential misclassification may occur when misclassification of the determinant is related to the outcome, and vice versa. Differential misclassification can lead to underestimation or overestimation of the results.

Data of exposures in our study were collected prospectively and before measurement of the kidney outcome measures. The parents and data collectors were not aware of specific research questions. Therefore, differential misclassification of the exposures and the outcomes seems unlikely. However, underreporting or overreporting of specific lifestyle habits may have occurred and lead to underestimation of overestimation of effect of the studied association. For example, parental smoking data was obtained by questionnaires. Since most parents are aware of the possible negative effect of parental smoking on the health status of their children, they might have underreported their smoking habits. Also, information about infant protein intake was obtained by the food frequency questionnaire (FFQ) around the age of 1 year. Although this is a validated questionnaire,(58) we cannot exclude misclassification. Measurement error might occur in self-reported retrospective dietary assessment methods. We assume that this measurement error, misclassification of the exposure, will mainly be non-differential which means it is unrelated to the outcome. Non-differential misclassification may have resulted in attenuation of the results. However, differential misclassification might also occur, for example if people with unhealthy dietary patterns underreport their unhealthy habits as compared to the healthy aspects of their diet. Not much is known about misclassification of parental reporting of childhood dietary habits. Since parents were unaware of specific research questions on dietary habits, differential misclassification seems unlikely. In the studies presented in this thesis, outcome measures were hands-on assessments of kidney growth and laboratory analyses of kidney function. Observers of these outcomes were unaware of exposures status, which makes differential misclassification of the outcomes less likely.

Confounding

The Generation R Study is an observational study which provides a comprehensive amount of data about variables related to lifestyle, growth and development. A limitation of observational studies is the risk of confounding. A confounder is a specific variable which

is associated with the exposure and the outcome, and is not an intermediate in the causal pathway of the association between the exposure and the outcome. If a confounding variable is not taken into account, results might be biased because the observed effect estimates might be attributed to the exposure whilst the effect estimates are caused by the confounding variable. In this thesis, we used several approaches to explore the role of confounding in the studied associations. First, we adjusted all analyses for multiple confounders. Confounders were included in the analyses based on previous studies, their obvious relation with exposure and outcome or a change in effect estimate over 10%. Although information about many confounders is available within the Generation R Study, residual confounding might still be present. Furthermore, information about multiple confounding factors was obtained by questionnaires and this might have led to measurement error. Second, in the studies where it was possible, we used information about maternal and paternal exposures during pregnancy, for example in the study on parental smoking and kidney outcomes. Stronger effect estimates for maternal smoking as compared to effect estimates of paternal smoking suggests that a direct intra-uterine mechanism is more likely to explain the found associations, rather than unmeasured environmental factors.

Causality

The studies presented in this thesis were observational. The longitudinal design of the study with repeated measurements provides the possibility to study temporal relationships between multiple exposures and outcomes. A disadvantage from a cohort study as compared to a randomized controlled trial (RCT), is that it is not possible to prove causal relationships between exposures and outcomes. A widely accepted method to assess causality in cohort studies is the use of Bradford Hill's criteria. These criteria were developed as a guideline to interpretation of results.(59) In the textbox below the criteria with respect to the findings in this thesis are given.

Clinical implications

In this thesis, several growth periods and patterns and maternal and child factors associated with kidney volume and function in childhood are identified. In several of the studies in this thesis, the effect estimates are small. The studies were conducted in a healthy, population-based cohort. However, these results are important from an etiological perspective. These results will be helpful in increasing awareness of clinicians and researchers which individuals might be at risk of impaired kidney function.

A recent review on CKD emphasizes that CKD is a disorder with high prevalence and is associated with increased risks of mortality and morbidity, but it's prevalence differs among populations.(60) People with CKD are often a symptomatic or have nonspecific symptoms and diagnosis often is made in a late phase. Diagnosis can be made earlier by screening tools. Therefore, it is important to identify risk factors for impaired kidney function at young age.

The associations between fetal and early childhood kidney and body growth and kidney function in later life seem to be quite convincing. It is suggested that individuals with impaired growth and impaired nephron endowment are at risk for CKD in later life. Longitudinally growth development in young children must be monitored, and can be used for screening for impaired kidney function at young age. Recently, it is hypothesized that various factors might contribute to development of CKD in these individuals. For example, maternal and child nutrition, childhood catch-up growth and childhood obesity.(14) Preventive strategies and education for parents and children specifically in populations at risk will be helpful in preventing CKD in later life. Further studies with long follow up duration are needed to develop guidelines for screening tools in populations at risk for impaired kidney function.

Future research

Development during fetal life and early childhood is crucial for nephron endowment.(14) Identification of individuals at risk with low nephron endowment, during pregnancy and in early childhood, is crucial. Since nephron number cannot be measured *in vivo*, in studies and clinical practice kidney volume is used as a proxy for nephron endowment.(61) However, ultrasound kidney volume does not take into account size, shape and distribution and function of separate glomeruli. Glomerular enlargement due to hyperfiltration may increase kidney volume when there is actually low nephron number. Therefore, improvement of counting nephron number in living individuals should be studied further. Studies in animals show that it might be possible to count glomeruli with cationic ferritin using MRI. This method shows that it is possible to count glomeruli of different sizes, which implies the possibility to count nephron number in individuals with glomerular hypertrophy.(62) This MRI based glomerular imaging method provides new potential to quantify nephron number in humans. Next to improving measurement of nephron endowment, more precise estimation of childhood kidney function might help identifying individuals at risk for impaired kidney function. Further studies need to focus on which formula gives the best estimation of glomerular filtration rate in childhood, independent from other individual characteristics, for example body composition. Identification possible risk factors, for example lifestyle factors and dietary habits, may give more insight in the biological pathways underlying renal disease. Also, identification of these modifiable risk factors may help in development of prevention programs targeting these risk factors. We observed multiple associations between early growth and maternal and child factors and kidney function in childhood. In this observational study design, we cannot be exact sure that these assocations reflect causal relationships between exposures and outcomes.(63) As mentioned previously, besides impaired kidney volume, other pathways might be underlying the associations between adverse fetal exposures and smaller kidney volume and lower kidney function in childhood. Further studies focused on the role of alterations in the renin-angiotensin-aldosteron system (RAAS) in reduced

nephron number are needed.(29) Furthermore, the effect of maternal corticosteroid use during pregnancy and changes in tubular function and its transporters should be further investigated.(64) It is hypothesized that adverse fetal and early childhood exposures might have stronger effect in individuals with altered levels of gene expression, however additional studies are needed to provide more evidence.(14) Identification of potentially modifiable epigenetic mechanisms might provide the opportunity to develop strategies to extend nephrogenesis in newborns with impaired growth.

Conclusion

Results from this thesis suggest that growth in fetal life and early childhood is associated with kidney volume and function in childhood. Fetal smoke exposure is associated with lower childhood kidney function. Further research on dietary factors and most accurate estimations of glomerular filtration rate is needed. As early life growth is crucial for childhood kidney development, future preventive strategies should focus on optimizing factors influencing growth.

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Chapter 5

Summary
Samenvatting

Summary

In **Chapter 1.1**, the background and hypothesis for the studies presented in this thesis are described. Chronic kidney disease (CKD) is a major public health problem worldwide and is associated with increased risk of mortality. A few decades ago, epidemiological studies showed associations between early life exposures and risk of disease in later life. Multiple studies showed associations between low birth weight and increased risk of non-communicable diseases in adulthood. These findings support the hypothesis that adverse exposures in utero and early childhood may cause adaptations that might be beneficial on the short term, but are unfavourable on the long term. In low birth weight children, impaired fetal growth might lead to permanent adaptations in kidney development which might be the underlying mechanism of the increased risk of CKD in adulthood in this population. Subclinical differences in kidney volume and function, which predispose individuals for CKD in later life, might already be present in early childhood. Differences in kidney function at younger age tend to track into later life. Therefore, we consider it important to identify specific developmental periods and influencing factors of kidney function in childhood. More insight in factors influencing kidney development may give direction for prevention programmes to decrease the prevalence of CKD. In this thesis, we present studies which evaluate specific kidney and body growth periods, and specific early life factors which are relevant for kidney volume and function in childhood.

In **Chapter 1.2**, we present the characteristics of the study population. Descriptive values of kidney volume and function in the study population are given. Differences in kidney volume and function between boys and girls, and between individuals of different ethnic background are evaluated. Furthermore, the relationship between kidney volume and kidney function at the age of 6 years is presented.

In **Chapter 2,** we studied specific body and kidney growth periods and patters, and their associations with kidney function in childhood. In **Chapter 2.1**, we examined childhood kidney volume and function in relation to fetal blood flow and kidney volume. We found that children with a preferential fetal blood flow to the upper parts of the body combined with small fetal kidney volume had a lower estimated glomerular filtration rate (eGFR) as compared to children with average fetal blood flow distributions and average fetal kidney volume. The results of this study show that maternal and fetal vascular and growth characteristics in pregnancy are associated with subclinical differences in kidney function in childhood.

Chapter 2.2 describes the relationship between fetal first trimester growth and kidney volume and function at the age of 6 years. We found no consistent associations between fetal first trimester crown-rump length and kidney volume and function in childhood. First trimester growth measurements around 12 weeks gestational might be too early in kidney development to be associated with kidney outcomes in later life.

In **Chapter 2.3**, we evaluated the associations of longitudinal fetal and infant body growth patterns and kidney volume and function in childhood. We observed that fetal

weight in second and third trimester, birth weight and infant weight at 6 months was positively associated with higher kidney volume at the age of 6 years. Only higher second trimester fetal weight was associated with higher eGFR. These associations were independent from growth measures at other ages. Fetal and childhood length were not consistently associated with kidney volume and function. The results of this study indicate that lower fetal and infant growth might be associated with subclinical lower kidney outcomes in childhood. These differences might lead to an increased risk of CKD in later life.

In **Chapter 2.4**, we focused on the longitudinal fetal and infant kidney growth patterns and kidney function at the age of 6 years. We showed that small kidney volume in fetal life which persisted to be small at the age of 6 years, is associated with lower eGFR as compared to persistent large kidney volume from fetal life into childhood. Also, children at the age of 6 years with lower eGFR had smaller combined kidney volume growth patterns as compared to children with high eGFR. Furthermore, fetal and early childhood kidney growth are, independently from kidney growth at other time points, positively associated with eGFR based on creatinine levels. These associations were fully explained by kidney volume at the age of 6 years. Results from this study suggest that both fetal and childhood kidney growth are positively associated with kidney function in childhood.

Studies focused on the identification of maternal and child, modifiable, factors which might be associated with childhood kidney outcomes are described in **Chapter 3.**

In **Chapter 3.1** we evaluated the associations of fetal smoke exposure and kidney volume and function in childhood. We found that continued maternal smoking during pregnancy is associated with smaller kidney volume and lower eGFR in children at the age of 6 years. Effect estimates for maternal smoking are stronger than effect estimates for paternal smoking, this suggests that intrauterine adaptations may play a role in the biological pathway underlying these associations.

In **Chapter 3.2**, we studied the relationship between dietary protein intake in infancy and kidney volume and function in childhood. We found associations between higher protein intake and better kidney function. However, these associations are fully explained by dietary and life style factors of the children. No associations are observed with kidney volume, and no differences in associations between animal and vegetable protein intake are found. This study does not support the hypothesis that high protein intake is associated with kidney volume and function.

Chapter 3.3 evaluates the associations of different body composition measurements and eGFR based on creatinine and cystatin C levels. Results of this study show that both creatinine based and cystatin C based GFR estimations are associated with body mass index and body surface area. Only eGFR based on creatinine levels is associated with lean mass percentage and fat mass percentage. It is concluded that implications of these findings for clinical practice need to be studied further.

In **Chapter 4**, we discuss the results presented in this thesis in a broader perspective. Implications for clinical practice are given, and methodological considerations and suggestions for future research are discussed.

In conclusion, results from this thesis suggest that growth in fetal life and early childhood is associated with kidney volume and function in childhood. Fetal smoke exposure is associated with lower childhood kidney function. Further research on dietary factors and most accurate estimations of glomerular filtration rate is needed. Early life growth is crucial for childhood kidney development, future preventive strategies should focus on optimizing factors influencing growth.

Samenvatting

In **Hoofdstuk 1.1** beschrijven we de achtergrond en hypothese voor de studies beschreven in dit proefschrift. Chronische nierziekten zijn groot probleem voor de volksgezondheid in de hele wereld en is geassocieerd met een toegenomen risico op mortaliteit. Enkele decennia geleden toonden de eerste epidemiologische onderzoeken een verband aan tussen specifieke blootstellingen in het jonge leven and het risico op ziekte in het latere leven. Meerdere studies hebben associaties aangetoond tussen een laag geboortegewicht en een toename van het risico op chronische, niet overdraagbare ziekte in het latere leven. Deze resultaten onderschrijven de hypothese dat blootstelling aan ongunstige factoren tijdens de zwangerschap en de jonge kinderleeftijd gunstig kan zijn op de korte termijn, maar ongunstige zijn op de langere termijn. In kinderen met een laag geboortegewicht, veroorzaakt de groeivertraging in het foetale leven mogelijk permanente veranderingen in de nierontwikkeling en verhoogt daardoor het risico op chronische nierziekten op de volwassen leeftijd. Verschillen in nier grootte en nierfunctie, die nog niet leiden tot ziekte op de jonge leeftijd maar mogelijk wel het risico op chronische nierziekte op latere leeftijd verhogen, zijn mogelijk al op de kinderleeftijd aanwezig. Verschillen in nierfunctie op jongere leeftijd persisteren op latere leeftijd. Meer inzicht in factoren die de vroege ontwikkeling van de nieren beïnvloeden kunnen van belang zijn bij de ontwikkeling van preventie programma's om de prevalentie van chronische nierziekten te verlagen. In dit proefschrift beschrijven we studies welke specifieke periodes van niergroei en lichaamsgroei van belang voor nierfunctie op de kinderleeftijd onderzoeken. Daarnaast presenteren we studies die gericht zijn op het verband van specifieke, deels beïnvloedbare, factoren in het vroege leven en nierfunctie op de kinderleeftijd.

In **Hoofdstuk 1.2**, worden kenmerken van de onderzoeksgroep waarin de studies in dit proefschrift zijn uitgevoerd vermeld. Beschrijvende waardes van niergrootte en nierfunctie worden gegeven. Verschillen in nier grootte en nierfunctie tussen jongens en meisjes, en tussen individuen van verschillende etnische afkomst worden onderzocht. Daarnaast wordt de relatie tussen nier grootte en nierfunctie op de leeftijd van 6 jaar beschreven.

In **Hoofdstuk 2**, onderzochten we specifieke periodes en patronen van niergroei en lichaamsgroei en de associatie daarvan met nierfunctie op de kinderleeftijd. In **Hoofdstuk 2.1**, hebben we de relatie tussen foetale bloed voorziening en foetale nier grootte met grootte en functie van de nier op de kinderleeftijd onderzocht. We toonden aan dat foetale bloedvoorziening met voorkeur voor de bovenste lichaamshelft in combinatie met een kleine foetale nier grootte geassocieerd was met een lagere geschatte glomerulaire filtratie snelheid (estimated glomerular filtration rate; eGFR) in vergelijking met een gemiddelde foetale bloedvoorziening en gemiddelde foetale nier grootte. Deze resultaten laten zien dat maternale en foetale vasculaire en groeikenmerken zijn geassocieerd met kleine verschillen in nierfunctie op de kinderleeftijd.

Hoofdstuk 2.2 beschrijft de relatie tussen foetale groei in het eerste trimester van de zwangerschap en nier grootte en nierfunctie op de leeftijd van 6 jaar. We vonden

geen consistent verband tussen foetale kruin-romp lengte in het eerste trimester en nier grootte en nierfunctie op de kinderleeftijd. Het is mogelijk dat groei metingen rond de 12e week van de zwangerschap te vroeg zijn in de nierontwikkeling en vandaar niet geassocieerd zijn met nierfunctie op de kinderleeftijd.

In **Hoofdstuk 2.3** onderzochten we het verband tussen patronen van herhaalde groeimetingen op de foetale en vroege kinderleeftijd de grootte en functie van de nier op de kinderleeftijd. We toonden aan dat foetaal gewicht in het tweede en derde trimester van de zwangerschap, geboortegewicht en gewicht op de leeftijd van 6 maanden een positieve associatie heeft met de grootte van de nier op de leeftijd van 6 jaar. Alleen hoger foetaal gewicht in het tweede trimester van de zwangerschap was geassocieerd met een hoge eGFR op de kinderleeftijd. Deze associaties waren onafhankelijk van groeimetingen op andere leeftijden. Lengte in het foetale leven en op de kinderleeftijd toonde geen consistent verband met de grootte en functie van de nier. De uitkomsten van deze studie impliceren dat en verminderde groei gedurende het foetale leven en de vroege kinderleeftijd mogelijk verband houdt met kleine verschillen in de grootte en functie van de nier op de kinderleeftijd. Deze verschillen verhogen mogelijk het risico op chronische nierziekte op latere leeftijd.

Hoofdstuk 2.4 is gericht op de mogelijke associatie tussen patronen van herhaalde niergroei metingen op de foetale en vroege kinderleeftijd en de nierfunctie op de kinderleeftijd. We vonden dat kinderen met kleinere nieren in het foetale leven, die klein bleven op de leeftijd van 6 jaar, een lagere eGFR hadden dan kinderen met persisterende grote nieren. Daarnaast toonden we aan kinderen met een lagere eGFR op de leeftijd van 6 jaar een patroon van kleinere niergroei hadden in vergelijking met kinderen met een hogere eGFR. Gedetailleerde analyses lieten zien dat niergroei in het foetale leven en op de vroege kinderleeftijd, onafhankelijke van niergroei metingen op de andere leeftijden, positief is geassocieerd met eGFR gebaseerd creatinine waarden. Deze resultaten werden verklaard door de grootte van de nier op de leeftijd van 6 jaar. Uitkomsten van deze studie suggereren dat groei op de foetale en op de vroege kinderleeftijd positief verband houdt met nierfunctie op de leeftijd van 6 jaar.

Studies gericht op het identificeren van, gedeeltelijk beïnvloedbare, maternale en kind factoren die mogelijk zijn geassocieerd met de grootte en functie van de nieren op de kinderleeftijd worden besproken in **Hoofdstuk 3.** In **Hoofdstuk 3.1** hebben we mogelijk verbanden tussen foetale blootstelling aan roken en grootte en functie van de nieren onderzocht. Deze studie toonde aan roken tijdens de gehele zwangerschap is geassocieerd met kleinere nieren en een lagere eGFR op de leeftijd van 6 jaar. De gevonden effecten waren groter voor roken van de moeder tijdens de zwangerschap dan roken door vader tijdens de zwangerschap, dit suggereert dat aanpassingen in de baarmoeder een rol kunnen spelen in het onderliggende biologische mechanisme van deze associaties.

In **Hoofdstuk 3.2** onderzochten we het verband tussen eiwit inname via de voeding op de peuterleeftijd en de grootte en functie van de nieren op de kinderleeftijd. We toonden een relatie aan tussen hogere eiwit inname en betere nierfunctie. Deze relatie werd echter volledig verklaard door andere voedingsfactoren en leefstijl kenmerken van kinderen. Er weden geen associaties gevonden met de grootte van de nieren, en er waren geen verschillen tussen dierlijke en plantaardige eiwitten. De resultaten van deze studie ondersteunen de hypothese dat eiwit inname is geassocieerd met de grootte en functie van de nieren niet.

In **Hoofdstuk 3.3** bestuderen we de relatie tussen verschillende lichaamssamenstelling metingen en eGFR gebaseerd op creatinine en cystatine C waardes. De resultaten tonen aan dat schattingen van GFR gebaseerd op creatinine en cystatine C waardes zijn geassocieerd met body mass index en body surface area. Alleen schattingen van GFR gebaseerd op creatinine waardes zijn geassocieerd met lean mass percentage en fat mass percentage. We stellen vast dat verdere onderzoeken nodig zijn om de praktische gevolgen voor de klinische praktijk van deze verbanden te beoordelen.

In **Hoofdstuk 4** bespreken we de uitkomsten van de studies in dit proefschrift in een breder perspectief. Aanwijzingen voor de klinische praktijk worden gegeven, en methodologische overwegingen worden besproken en suggesties voor vervolgonderzoek worden gegeven.

Concluderend, suggereren de resultaten van dit proefschrift dat groei in het foetale leven en op de vroege kinderleeftijd zijn geassocieerd met de grootte en de functie van nieren op de kinderleeftijd. Foetale blootstelling aan rook heeft een relatie met verminderde grootte en functie van de nieren op de kinderleeftijd. Aanvullend onderzoek naar voedingsfactoren en de meest nauwkeurige schattingen van GFR is nodig. Groei in het vroege leven is cruciaal voor de ontwikkeling van de nieren, toekomstige preventie programma's moeten gericht zijn op het optimaliseren van factoren die van invloed zijn op groei.

Chapter 6

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Publication list

Bakker H, Miliku K, Dorresteijn EM, Cransberg K, Steegers EAP, Jaddoe VW. Early longitudinal kidney growth patterns and glomerular filtration rate at school-age. *Submitted*

Bakker H, Gaillard R, Hofman A, Reiss IK, Steegers EA, Jaddoe VW. Fetal first trimester growth is not associated with kidney outcomes in childhood. *Pediatr Nephrol. 2017;32(4):651-658*

Miliku K, **Bakker H,** Dorresteijn EM, Cransberg K, Franco OH, Felix JF, Jaddoe VW. Childhood body composition and estimates of glomerular filtration rate based on creatinine and cystatin C concentrations. *Am J Nephrol. 2017;45(4):320-326*

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About the author

Hanneke Bakker was born on June 13th 1982 in Kampen, the Netherlands. She passed secondary school (Atheneum) at the 'Wessel Gansfort College' in Groningen in 2001. In the same year, she started studying movement sciences at the Rijksuniversiteit Groningen. In the fourth year she started medical education at the Rijksuniversiteit Groningen. In 2008 she obtained her Master of Science (MSc) in movements sciences after research projects focussed on development of postural control in reaching in infants in the Beatrix Children's Hospital in the University Medical Centre Groningen (Prof.dr. M. Hadders-Algra). In 2010, Hanneke moved to Rotterdam to finish her last internships in Erasmus MC-Sophia's Childrens hospital. After obtaining her medical degree in 2011, she started working as a resident in pediatrics (ANIOS) in Erasmus MC-Sophia's Childrens hospital. She applied succesfully for a combination of pediatric training and a PhD project (AGIKO) in 2011. In 2012 she started her PhD project focussed on early life factors influencing kidney function in childhood under supervision of Prof.dr. V.W.V. Jaddoe. Her pediatric training (Prof.dr. M. de Hoog) started in 2013. During her AGIKO period, Hanneke obtained her Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES) in Rotterdam (2016).

Hanneke lives in Voorburg, together with Bas, and their two sons Casper en Pim.

PhD Portfolio

Summary PhD training and teaching activities

Name PhD student:	Johanna Bakker
Erasmus MC Department:	Epidemiology
Research School:	Netherlands Institute for Health Sciences
PhD period:	April 2012 – December 2016
Promotors:	Prof.dr. V.W.V. Jaddoe, Prof.dr. E.A.P. Steegers

PhD training		
	Year	**Workload (ECTS)**
Master of science clinical epidemiology	2012-2016	40

Study Design
Biostatistical Methods I: Basic Principles
Development Research Proposal
Biostatistical Methods II: Classical Regression Models
Research period PIN Health Sciences
Oral Research Presentation
English language
Introduction to medical writing

Clinical epidemiology
Methodological Topics in Epidemiologica Research
Principles of Research in Medicine and Epidemiology
Methods of Public Health Research
The Practice of Epidemiologic Analysis
Clinical Decision Analysis
Pharmaco-epidemiology
Markers and Prognostic Research

Repeated Measurements in Clinical Studies
Missing Values in Clinical Research
Women's Health
Methods of Clinical Research
Conceptual Foundation of Epidemiologic Study Design
History of Epidemioloc Ideas
Causal Mediation Analysis
Preventing Failed Interventions in Behavorial Research
Health Economics
Fundamentals of Medical Decision Making

	Year	Workload (ECTS)
General academic skills		
Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC, the Netherlands	2012	0.7
Veiligheidstraining MRI, Erasmus MC the Netherlands	2012	0.7
Research Integrity, Erasmus MC the Netherlands	2016	2.0
Seminars and workshops		
Annual PhD-day Sophia's Children's Hospital, Rotterdam	2012	0.4
Generation R research meetings, Erasmus MC, The Netherlands	2012-2016	1.5
Seminars at the department of Epidemiology, Erasmus MC, the Netherlands	2012-2013	1.0
Effect van leefstijl tijdens de zwangerschap op gezondheid van het kind, ministerie van VWS, Den Haag	2016	0.2
Expertmeeting FAS, ministerie van VWS, Den Haag	2016	0.2
(Inter)national congresses and presentations		
Invited speaker		
Kidney Week, American Society of Nephrology, San Diego, United States of America	2015	1.4
IPNA Workshop on Clinical and Epidemiological Research, Heidelberg, Germany	2017	1.4
Congres of the Dutch Society of Pediatrics, Arnhem, the Netherlands	2017	1.4
Oral presentation		
DOHaD 2017, Rotterdam, the Netherlands	2017	1.4
Other		
Generation R Research Meeting, Erasmus MC, the Netherlands	2016	1.0
Other acitivities		
Participation in organizing DOHaD conference, Rotterdam, the Netherlands		

Teaching		
Supervising master's thesis		
Amra Sabic, medical student, university of Belgrade, Serbia. Project title: *Associations of fetal kidney dimensions and kidney function and blood pressure at the age of 6 years.*	2012	2.0
Supervising data analysis students	2012-2013	2.0

Dankwoord

Op het allerlaatste moment schrijf ik nu met veel plezier het dankwoord van mijn proefschrift. Vele mensen hebben geholpen bij het onderzoek en de totstandkoming van mijn proefschrift. Graag wil ik een aantal van hen in het bijzonder bedanken.

Allereerst wil ik ouders en kinderen die deelnemen aan Generation R hartelijk bedanken. Ik heb bewondering voor de inzet van alle kinderen tijdens de bezoeken aan het onderzoekscentrum en grote waardering voor de ouders die tijd investeren in de bezoeken en vragenlijsten. Zonder jullie betrokkenheid was het niet mogelijk dit longitudinale onderzoek uit te voeren.

Mijn promotoren, Prof.dr. Jaddoe en Prof.dr. Steegers. Beste Vincent, jaren geleden kwam ik tijdens mijn oudste co-schap bij jou op de afdeling terecht. Dit was het begin van het (zeer lange) AGIKO traject. Bedankt dat je met mij die uitdaging bent aangegaan. In de afgelopen jaren heb ik op wetenschappelijk gebied, maar ook in de kliniek, veel van je geleerd. Bedankt voor de prettige samenwerking, de kritische blik en het overbrengen van het enthousiasme voor de wetenschap! Tijdens dit traject ben ik vaak aan "echt hele leuke dingen die maar heel weinig werk" waren begonnen, en heb dit met plezier gedaan. In de samenwerking heb ik erg gewaardeerd dat je me ruimte hebt gegeven wanneer ik dat nodig had. Prof.dr. Eric Steegers, hartelijk dank voor uw bijdrage aan dit promotie traject. Uw commentaar en ideeën vanuit de gynaecologie hebben een waardevolle bijdrage geleverd aan de inhoud van dit proefschrift.

Prof.dr. Reiss, Prof.dr. Franco en Prof.dr. Roseboom, bedankt voor het beoordelen van het manuscript. Prof.dr. de Hoog, dr. Cransberg en dr. Schreuder hartelijk dank voor het plaats nemen in de commissie. Prof.dr. Reiss bedankt dat u de secretaris van de commissie wilt zijn.

Alle co-auteurs wil ik graag bedanken voor de prettige samenwerking. Alle collega's van Generation R wil ik bedanken voor de bijdrage aan deze onvergetelijke tijd. In al die jaren zijn er te veel mensen die positief hebben bijgedragen om iedereen persoonlijk te bedanken. De onderzoeksmedewerkers en bureaumedewerkers, bedankt voor alle hulp, zonder jullie was dit proefschrift er niet gekomen. Alle kamergenoten, bedankt voor de gezelligheid en fijne gesprekken. Collega's van het allereerste begin en opnieuw in de laatste periode, bedankt voor de goede samenwerking en ook voor alle koffiemomenten en ontspanning.

Ank, Claudia, Marieke, Nienke en Rolieke, bedankt voor alle relativering en humor in de afgelopen jaren. Ank, het was een warm welkom om bij jou als kamergenoot terecht te komen. Claudia, ik heb bewondering voor jouw harde werken, en bedankt dat we ook tijdens jouw tijd in het Bronovo even met jou mochten sparren. Marieke, ik heb zelden met iemand zo vaak tranen over mijn wangen van het lachen als met jou, knap hoe jij alle ballen in de

lucht houdt. Rolieke, bijzonder om te zien hoe gefocust jij bezig bent met je toekomst. Ik koester onze vriendschap en hoop dat er nog vele etentjes met vooral veel wijntjes volgen!

Irene en Marjolein, jaren geleden als ANIOS gestart en straks allemaal kinderarts, bedankt voor jullie support.

Mede Agiko's Nienke, Evelien en Esther. Bedankt voor het delen van hetzelfde-schuitje gevoel in de afgelopen jaren. Uiteindelijk is het gelukt: allemaal gepromoveerd!

Mentorclubje Alike, Annemarie en Nienke. Fijn en gezellig om af en toe even stoom af te blazen en te discussiëren over de toekomst, ik hoop dat we dat blijven doen.

Alle A(N)IOS in het Sophia, ondanks dat het niet altijd duidelijk was hoe mijn opleiding in elkaar zat, was er elke keer weer een warm welkom terug in de kliniek, bedankt daarvoor.

Lieve vrienden en vriendinnen in Groningen en Den Haag, bedankt dat jullie er zijn en voor jullie begrip voor mijn drukke werkschema en dat het soms lastig is om momenten te vinden om elkaar te zien. Ik heb al het meeleven erg gewaardeerd.

Lieve Dazzels, wat is het fijn dat het na al die jaren altijd als vanouds blijft als we elkaar zien. We hebben een mooie tijd gehad in Groningen en daarna. Jammer genoeg kon ik de afgelopen jaren niet altijd overal bij zijn, ik hoop dat het in de toekomst vaker lukt. Bedankt voor jullie support in de afgelopen jaren bij mijn onderzoek en opleiding maar vooral daarbuiten. Mooi om in jullie gezelschap letterlijk de laatste woorden van mijn proefschrift te schrijven.

Mijn paranimfen, Nienke en Ilse. Mijn dank aan jullie is groot. Lieve Nien, wat is het prettig om samen de ups en downs van zo'n lang promotietraject te delen. Het is heerlijk om samen even te spuien en te relativeren zodra het kan. Ik waardeer je vriendschap tijdens alle life events in deze jaren. Ik ben trots dat je naast me staat vandaag, en vol vertrouwen dat jouw dag snel volgt! Lieve Ilse, buuf, wat ben ik blij met jouw vriendschap. Ik koester jouw humor, talent voor wijn drinken, luisterend oor, en ons gezamenlijke Doutzen project. Jullie steun in de laatste jaren bleek onmisbaar, daar ben ik jullie enorm dankbaar voor. Ik ben blij dat je vandaag naast me wilt staan.

Mijn familie. Wietse en Monique, Marjolein en Mattias, en alle kinderen, bedankt dat jullie er waren in de afgelopen jaren. Ton en Marinka, bedankt voor de continue interesse en betrokkenheid met het onderzoek en mijn opleiding. Lieve oma, mijn naamgenoot. Wat bijzonder en fijn dat je er bij bent. Lieve papa, wat was je er graag bij geweest vandaag, ik vind het onbeschrijfelijk jammer dat het niet zo is. Lieve mama, ik heb enorm veel bewondering voor jou. Ik ben je dankbaar voor je steun en interesse, ook in lastige tijden. Het is fijn dat we er altijd voor elkaar zijn!

Lieve Bas, wat ben ik blij met jou. Jij tempert mijn drang om continue teveel dingen tegelijk te doen. Jouw steun in deze jaren was onontbeerlijk. Ik geniet van ons leven nu en zie uit naar onze plannen voor de toekomst! Casper en Pim, jullie vrolijke onbevangenheid relativeert al het andere. Ik hou van jullie!