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Fetal kidney volume and its association with growth and blood flow in fetal life: The Generation R Study

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An adverse fetal environment may lead to smaller kidneys and subsequent hypertension with renal disease in adult life. The aim of our study was to examine whether maternal characteristics, fetal growth, fetal blood flow redistribution, or inadequate placental perfusion in different periods of fetal life affect kidney volume in late fetal life. We also determined if fetal kidney volume was linked to the amount of amniotic fluid. In a population-based prospective study from early fetal life, fetal growth characteristics and fetal blood flow parameters were assessed by ultrasound and Doppler examinations in 1215 women in mid- and late-pregnancy. Kidney volume was measured in late pregnancy. Maternal height and pre-pregnancy weight were associated with kidney volume. After adjustment for the same characteristics in late pregnancy, fetal growth and blood flow in mid-pregnancy were not associated with kidney volume in late pregnancy. In late pregnancy, however, all fetal growth parameters were positively linked with kidney volume. The largest effect on kidney volume was found for abdominal circumference. Signs of fetal blood flow redistribution and increased placental resistance were associated with decreased kidney volume in late pregnancy. Amniotic fluid volume was positively associated with kidney volume. Our study shows that maternal anthropometrics, fetal growth, fetal blood flow redistribution, and raised placental resistance all correlate with kidney volume.

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Epidemiological studies have demonstrated low birth weight and fetal growth restriction to be risk factors contributing to renal disease and hypertension in adult life.[1–3] It has been hypothesized that an adverse fetal environment leads to fetal growth restriction and smaller kidneys with a reduced number of nephrons.[4,5] Because nephrogenesis continues until 36 weeks of gestation and the induction of nephron number ceases thereafter, suboptimal kidney growth and development in fetal life may have lifelong consequences.[6,7] A permanently reduced number of nephrons would lead to compensatory higher glomerular pressure, and progressive glomerular sclerosis, and would subsequently predispose the individual to impaired kidney function and hypertension.[4] This hypothesis is supported by studies in animals and humans. Animal studies have shown that low protein intake and reduced placental perfusion lead to fetal growth restriction and a permanent nephron deficit.[8,9] Human studies demonstrated that low birth weight infants and hypertensive subjects have lower kidney weight with a reduced number of nephrons in adult life.[10–13] Thus, an adverse environment *in utero* may lead to fetal growth restriction and impaired kidney development with a nephron deficit, eventually leading to hypertension.[4,14] Fetal kidney weight cannot be measured *in utero*. Renal volume measured by ultrasound is a valid substitute.[14,15]

The cause of fetal growth restriction and low birth weight is multifactorial. Nutritional deficiencies, smoking, and placental insufficiency are causes that might provoke fetal growth restriction and low birth weight infants. Placental insufficiency is the most common and is associated with raised placental blood flow resistance.[16] In response to general fetal malnutrition there is a preferential fetal blood flow to the brain and heart, depriving other organs, including the kidneys, from oxygen and nutrients. The increased blood flow to the brain is caused by vasodilatation in the brain resulting in lower peripheral resistance ('brain sparing effect').[17] This is part of the phenomenon known as fetal redistribution, which may be related to disturbed development of the kidneys.

Amniotic fluid is known to represent fetal well-being.[18] An adverse fetal environment as shown by raised placental

resistance often results in decreased amniotic fluid indices as well.[19] The main component of amniotic fluid is fetal urinary production, which may therefore be related to kidney volume and reflect kidney function.

The first aim of this population-based prospective cohort study was to evaluate the associations of maternal characteristics and fetal growth with kidney volume during pregnancy. The second aim was to examine the associations of placental resistance indices (RIs) and fetal blood flow redistribution, as a measure of adverse fetal environment, with kidney volume. Finally, we assessed the relation of fetal kidney volume with amniotic fluid as a measure of fetal urine production. If associations of maternal and fetal growth characteristics with kidney volume exist, further studies designed to identify the causal genetic and environmental mechanisms underlying these associations would be needed.

RESULTS

Characteristics of the subjects who participated in the study and their mothers for boys and girls in mid- and late-pregnancy are presented in Table 1. The percentage of boys was 51%. Median maternal age was 31.9 (95% range 21.5, 39.0) years. The median gestational age for the mid-pregnancy visit was 20.5 (95% range 18.7, 22.8) weeks and for the late pregnancy visit 30.4 (95% range 28.4, 32.6) weeks. Head circumference and abdominal circumference were larger and umbilical artery flow pulsatility index (PI) was lower in boys than in girls at both measurements. Estimated fetal weight was higher for boys in late pregnancy only. No gender differences were observed for femur length and uterine artery flow at both visits. At birth, weight was higher in boys than in girls.

Table 2 presents kidney characteristics in late pregnancy for boys and girls. The size of all kidney measurements was larger in boys than in girls. Left and right kidney did not differ in length, but the right kidney had a larger width difference 0.67 mm (95% confidence interval (CI): 0.53, 0.82, depth difference 0.80 mm (95% CI: 0.68, 0.93), and volume difference 0.72 cm^3 (95% CI: 0.60, 0.83) (not shown in Table 2).

Figure 1 shows measurements for kidney structures in late pregnancy with the 5th and 95th percentiles. Formulas for normal ranges for mean fetal kidney size and volume between 28 and 34 weeks of gestational age are listed beneath the figures.

Table 3 gives the associations of maternal characteristics with combined (left plus right) kidney volume. Maternal pre-pregnancy weight and height were positively associated with kidney volume. Other maternal characteristics such as obesity, blood pressure, pre-eclampsia, diabetes, or smoking were not associated with kidney volume.

Table 4 presents the associations of fetal growth characteristics and placental RIs in mid-pregnancy with combined kidney volume measured in late pregnancy. In model A, adjusted for gestational age and fetal gender only, all fetal growth characteristics were positively associated with combined kidney volume and umbilical artery PI negatively associated (model A). After additional adjustment for the same fetal growth characteristic or blood flow parameter in

Table 1 | Subject characteristics (*n*=1215)

	Boys (*n*=629)	Girls (*n*=586)
Maternal characteristics		
Age (years)	31.8 (21.1–39.2)	32.0 (22.7–39.0)
Height (cm)	170.8 (5.9)	170.9 (6.0)
Pre-pregnancy weight (kg)	68.2 (13.0)	69.2 (12.3)
Pre-pregnancy BMI (kg/m^2)	23.3 (4.2)	23.7 (4.0)
Weight gain until late pregnancy (kg)	8.5 (3.7)	8.4 (3.6)
Systolic blood pressure in late pregnancy (mm Hg)	120.4 (11.3)	121 (11.0)
Diastolic blood pressure in late pregnancy (mm Hg)	69.8 (9.3)	70.0 (9.5)
Hypertension (%)	6.9%	8.7%
Pre-existent or pregnancy-induced diabetes (%)	1.4%	1.3%
Pre-eclampsia (%)	1.2%	2.1%
Smoking during pregnancy (%)	15.0%	12.6%
Mid-pregnancy characteristics		
Gestational age (weeks)	20.6 (18.8–22.8)	20.5 (18.7–22.8)
Head circumference (cm)	18.0 (1.4)	17.7 (1.3)*
Abdominal circumference (cm)	15.8 (1.3)	15.6 (1.3)*
Femur length (cm)	3.3 (0.3)	3.3 (0.3)
Estimated fetal weight (g)	377 (84)	370 (80)
Umbilical artery, PI	1.18 (0.19)	1.21 (0.17)*
Uterine artery, RI	0.54 (0.09)	0.54 (0.09)
Late pregnancy characteristics		
Gestational age (weeks)	30.5 (28.6–32.9)	30.3 (28.3–32.5)
Head circumference (cm)	28.8 (1.2)	28.3 (1.2)*
Abdominal circumference (cm)	26.7 (1.7)	26.5 (1.7)*
Femur length (cm)	5.70 (0.3)	5.73 (0.3)
Estimated fetal weight (kg)	1631 (259)	1599 (259)**
Umbilical artery, PI	0.96 (0.16)	0.99 (0.17)*
Uterine artery, RI	0.49 (0.08)	0.49 (0.08)
Birth characteristics		
Gestational age (weeks)	40.3 (35.9–42.4)	40.1 (35.6–42.4)
Birth weight (g)	3549 (547)	3460 (557)*

BMI, body mass index; PI, pulsatility index; RI, resistance index.
Values are means (s.d.) or medians (95% range).
Differences between boys and girls were compared using independent sample *t*-tests.
*$P<0.05$, **$P<0.01$.

Table 2 | Fetal kidney characteristics in late pregnancy

	Boys (*n*=629)	Girls (*n*=586)
Left kidney structures		
Length (mm)	39.5 (3.8)	38.4 (3.5)*
Width (mm)	22.7 (2.9)	22.1 (2.5)*
Depth (mm)	21.6 (2.8)	21.1 (2.5)*
Volume (cm^3)	10.3 (3.0)	9.5 (2.5)*
Right kidney structures		
Length (mm)	39.6 (3.8)	38.5 (3.5)*
Width (mm)	23.2 (3.0)	22.9 (2.7)**
Depth (mm)	22.4 (2.9)	22.0 (2.7)**
Volume (cm^3)	11.0 (3.3)	10.3 (2.8)*
Combined kidney volume (cm^3)	21.3 (5.9)	19.9 (5.0)*

Values are means (s.d.).
Differences between boys and girls were compared using independent samples *t*-tests.
*$P<0.05$, **$P<0.01$.

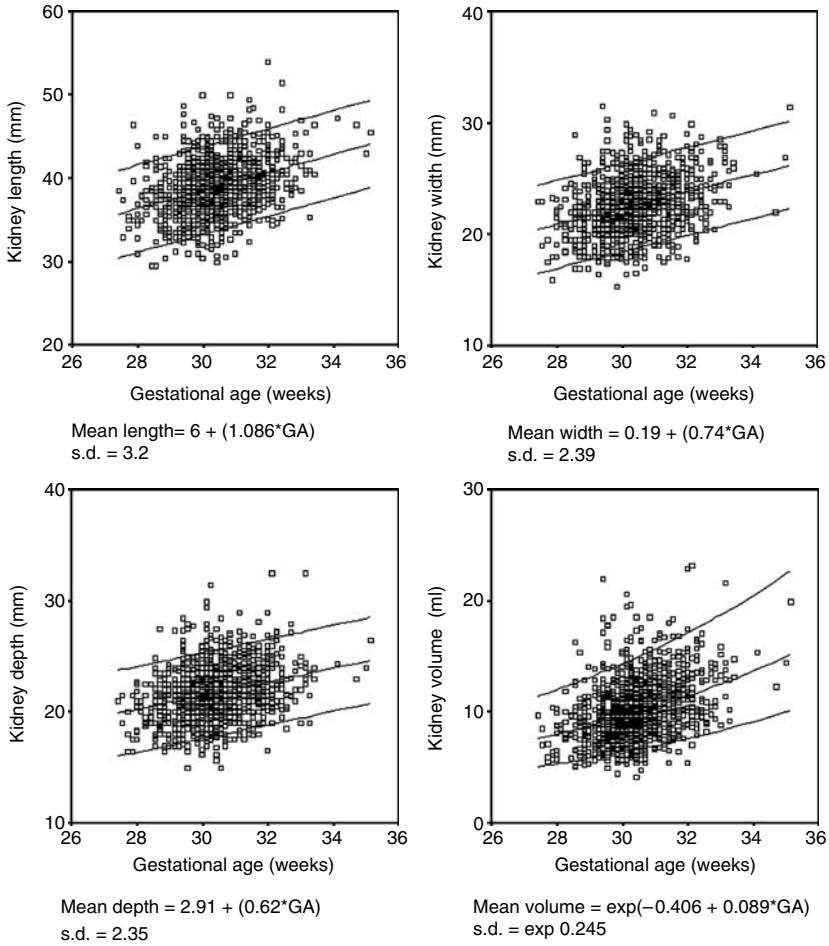

Figure 1 | Individual measurements of mean fetal kidney length, width, depth, and volume with fitted median, 5th and 95th percentiles with gestational age and formulas for normal values. GA, gestational age in exact weeks between 28 and 34 weeks; s.d., standard deviation.

Table 3 | Associations of maternal characteristics with combined fetal kidney volume in late pregnancy

Maternal characteristics	Difference in total kidney volume (cm^3) (95% CI)
Age (years)	−0.01 (−0.07, 0.06)
Height (cm)	0.07 (0.02, 0.11)**
Pre-pregnancy weight (kg)	0.03 (0.01, 0.05)*
Pre-pregnancy BMI (kg/m^2)	0.04 (−0.03, 0.11)
Weight gain during pregnancy (kg)	0.05 (−0.04, 0.13)
Systolic blood pressure in late pregnancy (mm Hg)	−0.01 (−0.03, 0.02)
Diastolic blood pressure in late pregnancy (mm Hg)	−0.01 (−0.04, 0.02)
Hypertension (yes vs no)	−0.03 (−1.28, 1.33)
Pre-existent or pregnancy induced diabetes (yes vs no)	−0.60 (−3.49, 2.30)
Pre-eclampsia (yes vs no)	2.14 (−0.25, 4.53)
Smoking during pregnancy (yes vs no)	−0.06 (−0.88, 0.77)

BMI, body mass index; CI, confidence interval.
Values are regression coefficients (95% CI) and reflect the difference in kidney volume per unit increase in maternal characteristics or lifestyle measure.
Models adjusted for fetal abdominal circumference in late pregnancy, gestational age, and fetal gender.
*$P<0.05$, **$P<0.01$.

late pregnancy, measured at the same time as kidney volume, associations were no longer significant (model B). These results suggest that the associations of mid-pregnancy growth characteristics and placental RIs with late pregnancy kidney volume are largely explained by the same characteristics in late pregnancy.

Table 5 shows that in late pregnancy, all growth characteristics were positively associated with combined kidney volume. The largest effects on combined kidney volume were found for estimated fetal weight and abdominal circumference (combined kidney volume increased 1.77 (95% CI: 1.46, 2.08) cm^3 and 1.76 (95% CI: 1.47, 2.05) cm^3 for each standard deviation score (SDS) increase in estimated fetal weight and abdominal circumference, respectively). Placental RIs were inversely associated with combined kidney volume, indicating that signs of increased placental resistance reduced kidney volume. Signs of fetal redistribution as quantified by the cerebro-umbilical ratio were associated with reduced kidney volume. No associations were found for middle cerebral artery PI.

Table 4 | Associations of fetal growth characteristics and placental RIs in mid-pregnancy with combined fetal kidney volume in late pregnancy

Measurements in mid-pregnancy	Difference in combined kidney volume (cm^3) (95% CI)	
	Model A	Model B
Growth characteristic		
Head circumference (SDS)	0.49 (0.16, 0.82)**	0.30 (−0.03, 0.64)
Abdominal circumference (SDS)	0.80 (0.46, 1.14)**	0.20 (−0.13, 0.53)
Femur length (SDS)	0.48 (0.14, 0.82)**	0.34 (−0.02, 0.69)
Ratio abdominal circumference/head circumference (SDS)	0.59 (0.18, 1.00)**	0.18 (−0.22, 0.58)
Estimated fetal weight (SDS)	0.84 (0.50, 1.19)**	0.04 (−0.14, 0.56)
Placental resistance indices		
Umbilical artery, PI	−2.23 (−4.09, −0.37)*	−1.38 (−3.22, 0.53)
Uterine artery, RI	−2.24 (−6.52, 2.03)	−1.30 (−5.91, 3.31)

CI, confidence interval; PI, pulsatility index; RI, resistance index; SDS, gestational age-adjusted standard deviation score.
Values are regression coefficients (95% CI) and reflect the difference in kidney volume per unit increase in fetal growth and placental perfusion blood flow characteristic.
Model A: adjusted for gestational age and fetal gender.
Model B: additionally adjusted for the same parameter and gestational age in late pregnancy.
*$P<0.05$, **$P<0.01$

Table 5 | Associations of fetal growth and blood flow characteristics with combined fetal kidney volume in late pregnancy

Measurements in late pregnancy	Difference in combined kidney volume (cm^3) (95% CI)
Growth characteristic	
Head circumference (SDS)	0.91 (0.57, 1.23)*
Abdominal circumference (SDS)	1.76 (1.47, 2.05)*
Femur length (SDS)	1.03 (0.71, 1.35)*
Ratio head circumference/ abdominal circumference (SDS)	1.71 (1.34, 2.09)*
Estimated fetal weight (SDS)	1.77 (1.46, 2.08)*
Placental resistance indices	
Umbilical artery, PI	−2.74 (−4.55, −0.92)*
Uterine artery, RI	−6.40 (−10.4, −2.43)*
Redistribution parameters	
Middle cerebral artery (PI)	0.46 (−0.41, 1.33)
Cerebro-umbilical (C/U) ratio	0.87 (0.22, 1.51)*

PI, pulsatility index; RI, resistance index; SDS, standard deviation score.
Values are regression coefficients (95% CI) and reflect the difference in kidney volume per unit increase in fetal growth and fetal blood flow characteristic.
Models adjusted for gestational age and fetal gender. Blood flow parameters additionally adjusted for abdominal circumference.
*$P<0.05$.

Figure 2 shows that in late pregnancy, there is a tendency toward larger relative kidney volume in subjects in the smallest tertile of SDS abdominal circumference. This suggests that small for gestational age fetuses have a larger kidney volume per kg fetal weight.

Figure 3 shows that in late pregnancy, kidney volume is positively associated with amniotic fluid deepest pocket.

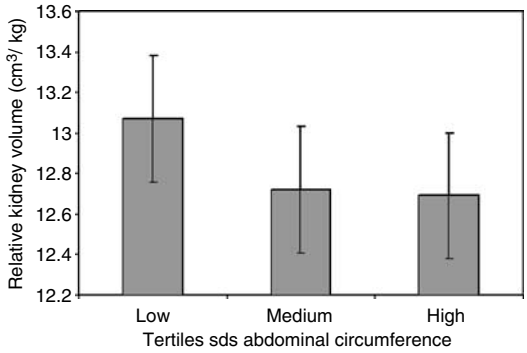

Figure 2 | The association between fetal abdominal circumference and fetal weight-adjusted combined fetal kidney volume in late pregnancy. SDS gestational age-adjusted SDS $P=0.07$ (*P*-value for trend using linear regression models).

DISCUSSION

This population-based prospective cohort study from early fetal life showed that maternal pre-pregnancy anthropometrics, fetal growth characteristics, and indices of placental resistance as well as fetal blood flow redistribution parameters were associated with kidney volume. Larger kidneys yielded a deeper amniotic fluid pocket.

The main strength of our study is the prospective design from fetal life with serial growth measurements within a large population-based cohort. Of all mothers who were approached for the detailed subgroup, 80% participated in the focus study. Non-participation was mainly due to lack of time. No differences in offspring birth weight were found between mothers participating and not participating in this study. Thus, we do not assume major health-related differences between these groups. To our knowledge, this is the largest population-based cohort in which kidney size in late pregnancy was studied. The population-based setting enabled us to assess kidney size and volume over the whole range of normal fetal size rather than in fetuses with growth restriction or other complications only.

Both environmental and genetic factors are important determinants of fetal growth.[20–22] We examined the association of maternal characteristics with fetal kidney volume in late pregnancy. Maternal weight and height were positively associated with kidney volume. This association may be explained by both environmental (maternal nutritional status) and common genetic factors that are important in the determination of kidney volume during pregnancy. Maternal smoking, obesity, blood pressure, and diabetes did not considerably influence fetal kidney size in this study. Even though these factors have a known influence on overall fetal size.[20,21]

We found positive associations of fetal growth characteristics in mid-pregnancy with kidney volume in late pregnancy. But after adjustment for the same growth parameter in late pregnancy these effects are no longer present, suggesting that the main influence of fetal growth on kidney volume in late pregnancy exerts after mid-pregnancy.

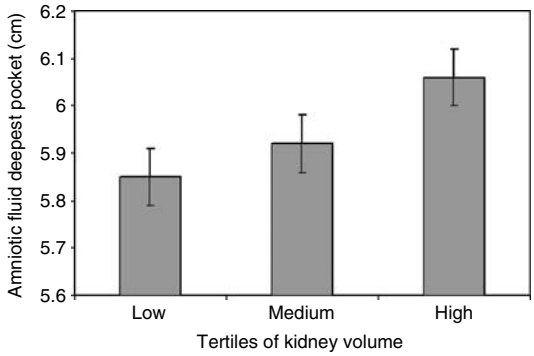

Figure 3 | Relation of fetal kidney volume with amniotic fluid deepest pocket. Model adjusted for gestational age, abdominal circumference, and fetal gender $P < 0.05$ (*P*-value for trend using linear regression models).

In late pregnancy, all fetal growth characteristics were positively associated with kidney volume. Abdominal circumference and the characteristics that included abdominal circumference were most strongly associated with kidney volume. The positive association for the ratio of abdominal circumference/head circumference suggests that asymmetrical fetal growth restriction reduced kidney volume more than symmetrical growth restriction although this effect might be partially explained by abdominal circumference only.

Our results showed the largest effect of fetal growth on kidney volume in late pregnancy. This is in line with previous studies that found the period of maximum kidney growth to occur between 26 and 34 weeks of gestation.[14] Growth restriction in this period most probably affects kidney size and volume considerably.

Inadequate placental perfusion leads to an adverse fetal environment by decreased supply of nutrients and oxygen and is one of the most important causes of fetal growth retardation in Western countries.[16] Increased placental vascular resistance and signs of blood flow redistribution with decreased cerebral resistance is known to be associated with reduced fetal growth. In our study, measures of placental vascular resistance were associated with estimated fetal weight (decrease in estimated fetal weight per unit increase in umbilical artery PI: 151 (95% CI: 90, 212) g and per unit increase in uterine artery RI: 273 (95% CI: 141, 405) g). Also, signs of fetal blood flow redistribution were associated with estimated fetal weight (estimated fetal weight decrease per unit decrease in middle cerebral artery PI: 16 (95% CI: −5, 57) g and per unit decrease in cerebro-umbilical artery ratio: 45 (95% CI: 24, 67) g). We showed that in late pregnancy, adverse blood flow resistance patterns of the umbilical and uterine artery were associated with reduced kidney volume, independent of fetal abdominal circumference at the time of the kidney measurement. This implies that kidney volume did not solely depend on abdominal circumference and overall fetal size but to some extent directly on placental vascular resistance or blood flow redistribution. Thus signs of increased placental resistance and fetal blood flow redistribution to protect the developing central nervous system are sufficiently deleterious to reduce fetal kidney volume as well.

A hypothesis for a decrease in renal size in growth-restricted fetuses is alteration in renal perfusion caused by a preferential blood flow to the brain.[23] In our study, we did not find any relation with middle cerebral artery PI and kidney volume. Another parameter is the cerebro-umbilical ratio, which did show a relation with kidney size. It is not unlikely that redistribution with decrease in middle cerebral artery PI is a later sign in fetal growth restriction that is not yet eminent in this population-based study. A direct measure of renal blood flow would be renal artery PI, which we did not measure. A previous study showed that renal artery blood flow was not altered in growth-restricted fetuses.[5] However, abnormal renal artery Doppler flow velocity waveforms were demonstrated in hypoxic growth-restricted fetuses in another study.[23] Altered renal artery flow velocity seems to be a late effect that is not present in growth-restricted fetuses that do not yet show signs of redistribution. We think that the reduced kidney volume in our study is not solely explained by redistribution because it is already present in smaller fetuses when signs of redistribution are absent.

This study showed that fetuses in the lowest tertile of gestational age-adjusted abdominal circumference had a tendency toward larger relative kidney volume, suggesting an organ or kidney sparing effect in small for gestational age fetuses. Thus, smaller fetal body size is associated with smaller kidneys, but these kidneys are relatively large for that body size. Previous studies suggested that the ratio of kidney volume with estimated fetal weight or abdominal circumference is constant in fetuses with different size and age.[15,24] This inconsistency with our results may be due to different and smaller study populations. Therefore, further studies are needed to focus on the effects of various fetal growth characteristics on relative kidney volume.

The main component of amniotic fluid is fetal urinary production. In our study, the amniotic fluid deepest pool was decreased in fetuses with smaller kidneys, after adjustment for abdominal circumference and gestational age. This indicates that the reduction of amniotic fluid is not solely attributable to growth restriction but to kidney volume as well. It is possible that the association we found between kidney volume and amniotic fluid is a reflection of the kidney function.

Our study underlines the importance of fetal growth and growth characteristics for determination of kidney size. Because we know that the number of nephrons is largely determined in prenatal life, suboptimal kidney growth, and development in fetal life may have lifelong consequences.[10–13]

In conclusion, our findings suggest that reduced fetal growth, signs of raised placental resistance, and fetal blood flow redistribution result in a decreased kidney volume in late fetal life. Further research to disentangle the causal mechanisms underlying the demonstrated associations is needed. Impaired fetal development results in smaller kidneys

and may result in increased risk of hypertension and renal disease in later life. Follow-up studies in our children are currently performed to examine whether and to what extent changes in fetal kidney size persist during childhood and whether they are related to renal function and blood pressure development in postnatal life.

MATERIALS AND METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development, and health from fetal life until young adulthood and has been described previously in detail.[25,26] In total, the cohort includes 9778 mothers and their children living in Rotterdam, The Netherlands. A vast majority (69%) of all mothers were enrolled in the first trimester of pregnancy.[26] Gestational age was determined by ultrasound during the first visit in early pregnancy. Assessments in pregnancy included physical examinations, fetal ultrasounds, biological samples and questionnaires, and were planned in early- (gestational age <18 weeks), mid- (gestational age 18–25 weeks), and late-pregnancy (gestational age >25 weeks) to collect information about fetal growth and its main determinants. The children were born between April 2002 and January 2006 and form a prenatally recruited birth cohort that is currently followed until young adulthood. Of all eligible children born in the study area, 61% participated at birth in the study.[26] Additional more detailed assessments of fetal growth and development were conducted in a subgroup of 1232 Dutch children and their parents, referred to as the Generation R Focus cohort. For this study, kidney size was assessed at the fetal ultrasound examination in late pregnancy in this subgroup. This subgroup is ethnic homogeneous to exclude possible confounding or effect modification by ethnicity. Of all approached women, 80% were enrolled in this subgroup study in the third trimester of pregnancy (gestational age of 30 weeks). Written informed consent was obtained from all participants. The Medical Ethics Committee of the Erasmus Medical Center (Rotterdam) has approved the study.

Population for analysis

In total, 1232 women were enrolled in the Generation R Focus Study at a gestational age of 30 weeks. Twin pregnancies ($n=15$) and pregnancies leading to perinatal death ($n=2$) were excluded from the analysis. No renal or uterovesical anomalies other then mild pyelectasis over 10 mm ($n=3$) were present in our study population. Renal ultrasounds were only partially performed in six subjects because of unfavorable fetal position or maternal adipositas. The present analysis was performed in a total of 1215 subjects.

Ultrasound measurements

Fetal biometry. Ultrasound exams were carried out in a research setting at a regional health facility in the center of Rotterdam in early-, mid-, and late-pregnancy. These fetal ultrasound procedures were used for both establishing gestational age and assessing fetal growth characteristics. Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks and 5 days (crown-rump length smaller than 65 mm), and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter larger than 23 mm). Fetal biometry including head circumference, abdominal circumference, and femur length was measured during each ultrasound examination using a transabdominal probe. Standard ultrasound planes for fetal measurements were used as described previously.[27–29] Briefly, head circumference was measured in a transverse section of the head with a central mid-line echo, interrupted in the anterior third by the cavity of the septum pellucidum with the anterior and posterior horns of the lateral ventricles in view. An ellipse was drawn around the outline of the skull. Abdominal circumference was measured in a symmetrical, transverse, round section through the abdomen, with visualization of the vertebrae on a lateral position in alignment with the ribs. The measurement was taken in a plane with the stomach and the bifurcation of the umbilical and hepatic veins. Femur length was measured with the full length of the bone in view. Gestational age-adjusted SDSs were constructed for these fetal growth measurements. These were based on reference growth curves from the whole-study population. Estimated fetal weight was calculated using the formula by Hadlock using head circumference, abdominal circumference, and femur length.[30]

Placental and fetal blood flow profiles. Placental resistance as a proxy of placental function was assessed using recorded flow velocity waveforms from the umbilical and uterine arteries, in mid- and late-pregnancy. Raised umbilical artery PI and uterine artery RI indicate increased placental resistance.[16] Umbilical artery PI was measured in a free-floating loop of the umbilical cord. Uterine artery RI was measured in the uterine arteries near the crossover with the external iliac artery. The redistribution of blood flow in favor of the fetal brain was quantified by the middle cerebral artery PI and the cerebro RI/umbilical RI ratio, in late pregnancy. A reduction in middle cerebral artery PI and a decreasing cerebro-umbilical ratio are valid indicators of 'brain sparing effect' due to fetal redistribution.[17,31] The middle cerebral artery Doppler was performed with color Doppler visualization of the circle of Willis in the fetal brain and the flow velocity wave forms were obtained in the proximal part of the middle cerebral artery.

Kidney measurements. Assessment of fetal kidney size and volume was performed at the scan in late pregnancy. The left and right kidney was measured. In a sagittal plane, the maximum longitudinal kidney length was measured placing the calipers on the outer edges of the caudal and cranial side. Antero-posterior and transverse kidney diameter were measured perpendicular to each other, outer to outer, in an axial plane. The cross-sectional area in which the kidney appeared symmetrically round and at its maximum width was used. The images were sufficiently magnified to ensure optimal measurements.[32] Kidney volume was calculated using the approximation of an ellipsoid: Volume = length $\times$ width $\times$ thickness $\times$ 0.523.[15] Left and right kidney volume were added for the combined kidney volume (cm^3).[33] Another frequently used measure of the kidney in fetal life is the relative kidney volume. This is the ratio of kidney volume/estimated fetal weight.[15,24]

Amniotic fluid. Amniotic fluid was assessed using single deepest pocket measurements, the preferable method to give an indication about the quantity of amniotic fluid in clinical practice.[34] All the ultrasound exams were performed using an ATL-Philips® Model HDI 5000 (Seattle, WA, USA) equipped with a 5.0 MHz, high frequency curved array transducer.

Intra- and inter-observer reproducibility. Three well-trained, experienced sonographers performed all measurements. Quality

checks were frequently carried out and feedback was provided to minimize interoperator differences. To assess intra- and inter-observer reproducibility of the fetal ultrasound measurements, the intraclass correlation coefficient and coefficient of variation between and among observers were calculated in 21 subjects for various ultrasound measurements and Doppler parameters.[35] For fetal ultrasound measurements, the intraclass correlation coefficient was higher than 0.98 and the corresponding coefficient of variation lower than 6%. Bland and Altman plots to test agreement of measurements for fetal ultrasound, demonstrated 95% limits of agreement in proportions to be within 10% difference from the mean of the measurements, indicating good reproducibility.[35] Furthermore, for Doppler parameters, the results show high intraclass correlation coefficient (>0.80) with corresponding low coefficient of variation ($<10\%$) values as well, indicating adequate reproducibility for all Doppler measurements.

Data analysis

To establish normal ranges for renal growth parameters with gestation, we created scatter plots of the individual measurements and applied the best fitting formula.

The associations of maternal characteristics with combined kidney volume were assessed using multiple linear regression models. The models were adjusted for fetal abdominal circumference in late pregnancy, gestational age, and fetal gender. The associations of fetal growth characteristics (head circumference, abdominal circumference, and femur length), placental RIs, and fetal redistribution parameters in mid- and late-pregnancy with combined kidney volume measured in late pregnancy were also assessed using multiple linear regression models. Gestational age-adjusted SDSs for the fetal growth measurements were used to compare effect sizes. All models were adjusted for fetal gender. The Doppler measurements were additionally adjusted for gestational age and abdominal circumference. Because fetal size in mid-pregnancy is strongly related to fetal size in late pregnancy, we adjusted the mid-pregnancy models (model A) for the same growth and Doppler characteristic in late pregnancy (model B) to estimate the effect size on kidney volume that is explained by fetal growth in mid-pregnancy only.

Furthermore, we examined the effect of gestational age-adjusted abdominal circumference in late pregnancy on relative kidney volume (kidney volume/estimated fetal weight).

Finally, the effect of kidney volume on amniotic fluid was assessed, adjusted for gestational age, gender, and abdominal circumference, using linear regression models.

All statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

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