The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development

Thesis submitted by

Sonja Brennan

Diploma of Applied Science Diagnostic Radiography (QUT)

Graduate Diploma in Ultrasonography (RMIT)

For the degree of Doctor of Philosophy

in the College of Public Health, Medical and Veterinary Sciences

James Cook University

August 2020

Acknowledgements

Foremost, I would like to thank my research mentor Prof Yogavijayan Kandasamy, a dedicated clinician and remarkable and inspirational person, for opening my mind to research. I will always be grateful to him.

I am indebted forever to my four amazing PhD advisors, Prof Yogavijayan Kandasamy, Dr David Watson, A/Prof Donna Rudd and Prof Michal Schneider. I could not have had a better advisory team. They are some of the most intelligent and talented professionals I know; also, the most modest. I am honoured they generously gave up their time to assist and guide me. I have learnt an enormous amount from them, and I hope that at least a small amount of their awesomeness rubs off on me.

I would also like to thank Emeritus Prof Rhondda Jones for her patience and expertise in steering me through the complexities of mixed effects modelling.

A special thank you must go to the participants who agreed to be a part of this study – without whom this thesis would not be possible. I acknowledge the support of my colleagues at Townsville University Hospital. In particular, Ms Sue Bloomfield and Ms Kate Gerard for their help in conducting the many ultrasound examinations. Also, the generous assistance of Ms Nicole Clapham in organising the participants and collating and inputting data.

Thank you to the Cohort Doctoral Studies Program and mentors, Dr Melissa Crowe and Dr Diana Mendez, and my fellow PhD colleagues. In their fellowship I gained a much broader research understanding, along with some fun, motivation and friendship.

To my parents, who taught me the value of hard work, I credit my past, present and future success. To my sisters who have always been there for me, I am forever thankful.

Finally, but most importantly, I thank my two beautiful sons, Morgan and Sheldon, who are the light of my life and my ultimate inspiration; and my husband Daryl for his kind heart, silly sense of humour and unwavering love and support for me.

This thesis is dedicated to my sister Ingrid,

who was fierce and courageous in the face of adversity

…. wish you could still be with us.

Statement on the Contribution of Others

Nature of assistance	Contribution	Name and affiliation
Intellectual	Advisor - Primary	Prof Yogavijayan Kandasamy Townsville University Hospital (TUH)/James Cook University (JCU)
	Advisors - Secondary	Dr David Watson TUH/JCU A/Prof Donna Rudd JCU Prof Michal Schneider Monash Uni
	Research proposal	Prof Yogavijayan Kandasamy TUH/JCU Dr David Watson TUH/JCU A/Prof Donna Rudd JCU Prof Michal Schneider Monash Uni
	Data analysis	Prof Yogavijayan Kandasamy TUH/JCU Dr David Watson TUH/JCU A/Prof Donna Rudd JCU Prof Michal Schneider Monash Uni
	Statistical advice	Prof Rhondda Jones JCU provided support for the mixed effects modelling. Hunter Medical Research Institute assisted in the development of the standard ranges charts. The primary and secondary advisors provided support for other statistical analyses.

Nature of assistance	Contribution	Name and affiliation
	Editorial assistance	Prof Yogavijayan Kandasamy TUH/JCU Dr David Watson TUH/JCU A/Prof Donna Rudd JCU Prof Michal Schneider Monash Uni Prof Rhondda Jones JCU
	Professional development, mentoring and peer support	JCU Division of Tropical Medicine, Cohort Doctoral Studies Program
Financial support	Research fellowship	I received a Queensland Advancing Clinical Research Fellowship (2019) to cover 0.3FTE to conduct research
	ASUM Grant	Australasian Society for Ultrasound in Medicine (ASUM) research grant 2018 for $20,000 to pay for a research assistant part-time
	THHS SERTA Grant	Townsville Hospital and Health Service (THHS) Study, Education and Research Trust Account (SERTA) grant for $20,000 to assist in the expenses of conducting the research
	JCU Travel Grant	College of Public Health, Medical and Veterinary Sciences Higher Degree Research Enhancement Scheme – travel grant to attend and present a poster at Perinatal Society of Australia and New Zealand (PSANZ) 2019
Ultrasound examinations	Conducting ultrasound examinations	TUH sonographers, Ms Sue Bloomfield and Ms Kate Gerard for assisting in performing the ultrasound examinations for the study

Nature of assistance	Contribution	Name and affiliation
	Use of TUH ultrasound machines and ancillary equipment	TUH, Medical Imaging, Ultrasound department. Some of the service costs were covered by the SERTA grant and the remainder was given in kind from Medical Imaging
Administrative & data support	Research assistant to assist in tracking and booking participants and inputting data	Ms Nicole Clapham TUH was employed as a part-time research assistant using the ASUM grant
Editorial assistance	Formatting and layout of thesis	Ms Katharine Fowler - professional editor
Illustrations	Design and creation of some illustrations in the thesis	Mr Daryl Brennan

The Contribution of Authors to the Publications

Thesis chapter	Publication	List of authors: SB – Sonja Brennan YK – Yogavijayan Kandasamy DW – David Watson DR – Donna Rudd MS – Michal Schneider RJ – Rhondda Jones
Chapter 2	Brennan S, Watson D, Rudd D, Schneider M, Kandasamy Y. Evaluation of fetal kidney growth using ultrasound: A systematic review. Eur J Radiol. 2017;96:55-64.	The protocol for the systematic review was designed by SB under the guidance of YK, DW, DR and MS. Independent screening of papers was done by SB and YK. SB wrote the manuscript and DW, DR, MS & YK contributed to the critical revision, editing and approved the final manuscript.
Chapter 3	Brennan S, Schneider M, Watson D, Kandasamy Y, Rudd D. The renal parenchyma—evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study. BMJ Open. 2017;7(12).	SB conceived the study and drafted the study design under supervision of YK, DW, DR and MS. All authors contributed to the conception, design and development of the study protocol. MS provided her expertise for the study design, ethics and critically revising the manuscript. DW provided his expertise for the ultrasound protocol, recruitment of participants and revising the protocol. YK provided his expertise for ethics, statistics and drafting the study protocol. DR provided her expertise for the study design, data management and analysis. All authors approved the final version.
Chapter 4	Brennan S, Kandasamy Y, Rudd D, Schneider M, Watson D. Fetal kidney charts of a novel measurement of the renal parenchymal thickness to evaluate fetal kidney growth and potential function. Prenat Diagn. 2020;40(7):860-9.	SB designed the study with assistance of YK, DW, DR and MS. SB and DW recruited patients and SB performed some of the ultrasound examinations. The statistical analysis and interpretation were done by SB with the support of Hunter Medical Research Institute and other assistance from YK, DW, DR and MS. SB wrote the manuscript and YK, DW, DR and MS contributed to the critical revision, editing and approved the final manuscript.

Thesis chapter	Publication	List of authors: SB – Sonja Brennan YK – Yogavijayan Kandasamy DW – David Watson DR – Donna Rudd MS – Michal Schneider RJ – Rhondda Jones
Chapter 5	Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Fetal renal artery blood flow charts and correlation with amniotic fluid: a prospective, longitudinal, cohort study. **Under review** BMC Pregnancy Childbirth	SB designed the study with assistance of YK, DW, DR and MS. SB and DW recruited patients and SB performed some of the ultrasound examinations. The statistical analysis and interpretation were done by SB with the support of Hunter Medical Research Institute and other assistance from YK, DW, DR and MS. SB wrote the manuscript and YK, DW, DR and MS contributed to the critical revision, editing and approved the final manuscript.
Chapter 6	Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number? J Dev Orig Health Dis. 2020. DOI: https://doi.org/10.1017/S204017442000015X	SB designed the study with assistance of YK, DW, DR and MS. SB and DW recruited patients and SB performed some of the ultrasound examinations. The statistical analysis and interpretation were done by SB with the support of RJ and other assistance from YK, DW, DR and MS. SB wrote the manuscript and YK, DW, DR and MS contributed to the critical revision, editing and approved the final manuscript.
Chapter 8	Brennan S, Kandasamy Y, Rudd D, Schneider M, Jones R, Watson D. The effect of diabetes during pregnancy on fetal renal parenchymal growth. J Nephrol. In press.	SB designed the study with assistance of YK, DW, DR and MS. SB and DW recruited patients and SB performed some of the ultrasound examinations. The statistical analysis and interpretation were done by SB with the support of RJ and other assistance from YK, DW, DR and MS. SB wrote the manuscript and YK, DW, DR and MS contributed to the critical revision, editing and approved the final manuscript.

Copyright Declaration

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7 August, 2020

Date

Signature

Sonja Brennan

Name

Abstract

Introduction

There are well established links between abnormal fetal kidney development and increased risk of developing chronic kidney disease (CKD), hypertension and cardiovascular disease later in life. Abnormal fetal growth may adversely impact kidney development and in particular, the number and quality of the functional units of the kidney known as nephrons. We need a better understanding of how an adverse intrauterine environment may alter nephrogenesis. Currently there is no sensitive, non-invasive, in-vivo method for assessing fetal kidney growth to assess the number and quality of fetal nephrons. This thesis used a novel ultrasound measurement of the fetal renal parenchyma along with fetal renal artery Doppler measurements to evaluate fetal renal growth and provide an indirect estimate of nephron number. The effects of growth restriction, overgrowth and diabetes on the developing kidneys was explored. The main aim of this thesis was to assess fetal renal parenchymal development and fetal renal blood flow through serial ultrasound measurements of pregnant women demonstrating either normal or abnormal fetal growth and determine if abnormal fetal growth impacts fetal renal parenchymal growth.

Methods

A prospective, longitudinal, observational study was conducted among a cohort of mixed-risk pregnant women at the Townsville University Hospital, Australia. Serial ultrasound measurements were performed approximately every four weeks between 16 to 40 weeks gestational age and multiple novel fetal renal measurements were taken as well as routine fetal measurements. Using mixed effects modelling, the normal range of fetal renal parenchymal thickness and fetal renal artery blood flow were established. Subsequently, the fetal renal parenchymal thickness and fetal renal artery Doppler flow were compared between different groups of pregnant women to analyse the effects of growth restriction, overgrowth and diabetes on the developing kidneys using mixed

effects modelling. An ANOVA model was used to evaluate intra and interobserver reliability of the fetal renal measurements and fetal renal Doppler traces.

Results

In all, 155 participants were recruited. Seven participants were excluded, six for fetal or chromosomal abnormality and one who did not attend any ultrasound appointments. This resulted in 148 participants remaining in the study. Standard charts of the normal ranges of fetal renal parenchymal thickness, kidney length and kidney volume, and fetal renal artery resistivity index (RI) and pulsatility index (PI) were developed from a group of low-risk pregnant women (N = 72). The intraclass correlation coefficient (ICC) for the fetal renal parenchymal measurements was excellent for both intraobserver reliability (ICC = 0.97) and interobserver reliability (ICC = 0.96). For the fetal renal artery RI and PI, the reliability was moderate for the intraobserver (RI = 0.66, PI = 0.88) and poor for the interobserver reliability (RI = 0.11, PI = -0.56).

Thirty fetal growth restricted (FGR) fetuses were compared to 102 appropriate for gestational age (AGA) fetuses. The fetal renal parenchymal thickness was seen to be thinner in the growth restricted fetuses (likelihood ratio (LR) = 21.06, p =<0.0001) and their renal parenchyma had a slower growth trajectory. In fetuses with the same head circumference, a growth restricted fetus was more likely to have a thinner renal parenchyma than an AGA fetus. No significant difference was seen in the RI (p = 0.182) or PI (p = 0.554) of the fetal renal arteries between the FGR and AGA groups.

Sixteen pregnancies resulted in infants who were large for gestational age (LGA). The fetal renal parenchymal thickness for these fetuses were compared to 102 AGA fetuses. The LGA group had fetal renal parenchyma that was significantly thicker than the AGA group (LR = 6.1, p = 0.013), however, the renal parenchymal thickness was proportional to the overall size of the fetus. No

significant difference was seen in the RI (p = 0.403) or PI (p = 0.956) of the fetal renal arteries between the LGA and AGA groups.

Finally, the renal parenchymal thickness of a group of 55 women with diabetes in pregnancy (46 with gestational diabetes and 9 with pregestational diabetes) were compared to a control group of low-risk pregnant women (N = 72). The fetal renal parenchyma was significantly thicker in the fetuses of mothers with gestational diabetes than the fetuses in the control group (LR = 4.8, p = 0.029), however, the renal parenchymal thickness was proportionate to the overall size of the fetus. In the pregestational diabetes group the fetal renal parenchyma was not significantly thicker than those in the control group even though these fetuses were significantly larger. Therefore, in contrast to the fetuses of mothers with gestational diabetes, the renal parenchyma of fetuses of mothers with pregestational diabetes was thinner than expected considering the overall size of the fetus.

Conclusions

Measurement of the fetal renal parenchyma is an easy to perform, non-invasive single measurement to evaluate changes in fetal kidney growth and indirectly estimate nephron endowment. The charts of the normal ranges of fetal renal parenchymal thickness can be utilised in clinical practice together with established measurements of renal size. Alterations from these normal ranges may be utilised to improve the identification of infants at a higher risk of future kidney disease and hypertension. The fetal renal parenchymal thickness could be combined with other markers to assist in the diagnosis of renal parenchymal pathologies. Monitoring and support of these infants could then be implemented early to try and reduce the future risk of CKD and hypertension.

List of Abbreviations

Abbreviation	Name
2D	Two-dimensional
3D	Three-dimensional
AC	Abdominal circumference
ADIPS	Australasian Diabetes in Pregnancy Society
AEDF	Absent end diastolic flow
AGA	Appropriate for gestational age
AIC	Akaike's Information Criteria
AP	Antero-posterior
ASUM	Australasian Society for Ultrasound in Medicine
BMI	Body mass index
BPD	Biparietal diameter
Btw	Between
BW	Birth weight
CKD	Chronic kidney disease
CPR	Cerebroplacental ratio
CRL	Crown rump length
CS	Cross-sectional
CT	Cortical thickness
EFW	Estimated fetal weight
FGR	Fetal growth restriction
FI	Flow index
FL	Femur length
GA	Gestational age
GFR	Glomerular filtration rate
GTT	Glucose tolerance test
H	Height
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
HbA1c	Haemoglobin A1c
HC	Head circumference

IADPSG	International Association of the Diabetes and Pregnancy Study Groups
ICC	Intraclass correlation coefficient
IGF	Insulin-like growth factor
IQR	Interquartile range
IUGR	Intrauterine growth restriction
JCU	James Cook University
L	Length
LGA	Large for gestational age
LNMP	Last normal menstrual period
Long	Longitudinal
LR	Likelihood ratio
KC	Kidney circumference
KL	Kidney length
M	Median
MCA	Middle cerebral artery
MRI	Magnetic resonance imaging
MT	Medullary thickness
NICU	Neonatal intensive care unit
NR	Not recorded
P	Prospective
PI	Pulsatility index
Preg	Pregnancy
PSANZ	Perinatal Society of Australia and New Zealand
R	Retrospective
RI	Resistivity index
RPT	Renal parenchymal thickness
RV	Renal volume
SCN	Special care nursery
SD	Standard deviation
SDP	Single deepest pool
SE	Standard error
SERTA	Study Education Research Trust Account

SGA	Small for gestational age
THHS	Townsville Hospital and Health Service
TS	Transverse
TUH	Townsville University Hospital
TV	Transvaginal
UA	Umbilical artery
US	Ultrasound
VFI	Vascularisation flow index
VI	Vascular index
VOCAL	Virtual Organ Computer-Aided AnaLysis
W	Width
Wks	Weeks

Publications Arising from Thesis

Brennan S, Watson D, Rudd D, Schneider M, Kandasamy Y. Evaluation of fetal kidney growth using ultrasound: A systematic review. Eur J Radiol. 2017;96:55-64.

Brennan S, Schneider M, Watson D, Kandasamy Y, Rudd D. The renal parenchyma - evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study. BMJ Open. 2017;7(12). http://dx.doi.org/10.1136/bmjopen-2017-019369.

Brennan S, Kandasamy Y, Rudd D, Schneider M, Watson D. Fetal kidney charts of a novel measurement of the renal parenchymal thickness to evaluate fetal kidney growth and potential function. Prenat Diagn. 2020;40(7):860-9.

Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number? J Dev Orig Health Dis. 2020:1-9.

Brennan S, Kandasamy Y, Rudd D, Schneider M, Jones R, Watson D. The effect of diabetes during pregnancy on fetal renal parenchymal growth. J Nephrol. In press.

Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Fetal renal artery blood flow charts and correlation with amniotic fluid: a prospective, longitudinal, cohort study. **Under review**.

Publications During Candidature Not Included in Thesis

Brennan S, Kandasamy Y. Ultrasound imaging of the renal parenchyma of premature neonates for the assessment of renal growth and glomerulomegaly. Ultrasound Med Biol. 2017;43(11):2546-9.

Lalzad A, Wong FY, Singh N, Coombs P, Brockley C, **Brennan S**, Ditchfield M, Rao P, Watkins A, Saxton V, Schneider S. Surveillance practice for sonographic detection of intracranial abnormalities in premature neonates: a snapshot of current neonatal cranial ultrasound practice in Australia. Ultrasound Med Biol. In press. doi.org/10.1016/j.ultrasmedbio.2020.06.002.

Lalzad A, Wong F, Singh N, Coombs P, Brockley P, **Brennan S**, Ditchfield M, Rao P, Watkins A, Saxton V, Schneider M. Knowledge of safety, training and practice of neonatal cranial ultrasound: a survey of operators. J Ultrasound Med 2018;37(6):1411-1421. doi:10.1002/jum.14481.

Awards and Presentations During Candidature

Research Fellowship

Successfully obtained a two-year Queensland Advancing Clinical Research Fellowship (2019).

Awards

James Cook University (JCU) My Research Rules 31/10/2019 – Winner Late candidature presentations.

James Cook University (JCU) Science Research Festival 7/9/2017 – Winner Early candidature presentations.

Townsville Health Research Showcase - Best oral presentation 5th September 2017 presentation - "A non-invasive method to detect glomerulomegaly in premature neonates".

Presentations

James Cook University My Research Rules Science Festival 2019. "The fetal renal parenchyma: Evaluation of a novel ultrasound measurement to assess kidney development". Townsville October 2019.

Invited speaker - 17th World Federation of Ultrasound in Medicine and Biology (WFUMB) Congress 2019. "Fetal renal parenchyma: Evaluation of a novel ultrasound measurement to assess kidney development". Melbourne September 2019.

Australasian Socitey of Medical Imaging and Radiation Therapy, Technology in the Tropics. "Fetal renal parenchyma – A good kidney recipe". Townsville 20-21 October 2018.

Three Minute Thesis (3MT) 2018, College of Public Health, Medical and Veterinary Sciences JCU. "Good Kidney Recipe". 2nd August 2018.

Fetal and Neonatal Workshop of Australia and New Zealand 32nd Annual Meeting. "The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development". Queenstown, New Zealand 22-23 March 2018.

Three Minute Thesis (3MT) 2017, College of Public Health, Medical and Veterinary Sciences JCU. "How do fetal kidneys grow?" August 2017.

James Cook University (JCU) Science Research Festival. "The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development". Townsville, 7th September 2017.

Townsville Health Research Showcase. "The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development". Townsville 4-8 September 2017.

Townsville Health Research Showcase. "A non-invasive method to detect glomerulomegaly in premature neonates". Townsville 4-8 September 2017.

Queensland Perinatal Consortium (QPaCT) 2017 Conference Hot Topics in Perinatal Research. "Ultrasound imaging of the renal parenchyma of premature neonates for the assessment of renal growth and glomerulomegaly". Brisbane 20 July 2017.

Australian Sonographers Association (ASA) 24th Annual International Conference. "Evaluation of fetal kidney growth using ultrasound: a systematic review". Brisbane 2-4 June 2017.

Fetal and Neonatal Workshop of Australia and New Zealand 31st Annual Meeting. "The renal parenchyma – Evaluation of a novel ultrasound measurement to assess fetal renal development". Canberra 30-31 March 2017.

Poster

Perinatal Society of Australia and New Zealand (PSANZ) 2019 Congress. "Fetal kidneys – A new method to assess kidney growth". Gold Coast 17-20 March 2019.

Table of Contents

List of Tables

List of Figures

Chapter. 1 Introduction and Background

1.1 Chronic Kidney Disease

Globally, chronic kidney disease (CKD) is an important, under recognised and neglected chronic disease.[1-3] Additionally, CKD has strong associations, if not causative pathogenesis with other major diseases, such as hypertension, cardiovascular disease and diabetes, which is often underestimated.[1, 4] Deaths from kidney disease continue to escalate globally with an increase in deaths of 41% from 1990 to 2017.[3] In recent Australian data, 1 in 9 deaths have CKD as a primary and/or associated cause of death.[5] CKD is a condition of the kidney which is diagnosed when abnormal biomarkers of kidney function or damage that persists for three or more months.[5] CKD arises from a multitude of pathological processes that adversely impact the kidney. A reduction in the functional units of the kidney (nephrons) results in hyperfiltration of the remaining nephrons followed in time by glomerular hypertension and hypertrophy, proteinuria and the progressive advancement of CKD.[6] Patients with CKD typically present with hypertension, proteinuria and a progressive decline in glomerular filtration rates (GFR). The pathological insults that give rise to CKD may also occur *in-utero*, in infancy or childhood, and acute kidney injury in childhood has been shown to precede the chronic sequelae of CKD.[7, 8]

Abnormalities in normal fetal growth, such as fetal growth restriction and preterm birth, have been shown to have a profound effect on the development of the fetal kidney.[9, 10] An adverse intrauterine environment may therefore result in a reduced nephron number and an increased risk of developing CKD later in life.[11-13] Consequently, the normal development of the fetal kidneys is crucial to an individual's long-term health outcomes. Expert advice suggests acting early to optimise fetal and child health to prevent the development of CKD and hypertension.[2, 14, 15] This firstly requires understanding and recognition of CKD risk factors so that preventative strategies can be implemented to reduce these risks.

1.2 Kidney Anatomy and Physiology

The kidneys play an essential role in keeping a human body functioning with over 180 litres of blood filtered by the kidneys per day.[16] The two bean-shaped organs extract waste from the blood, balance body fluids, form urine and maintain a stable extracellular environment to support the function of all body cells.[17] Kidneys maintain the water and electrolyte balance by controlling the excretion of substances such as water, sodium, potassium, chloride, calcium, magnesium, phosphate and by managing acid-base status.[17] Via the renin-angiotensin-aldosterone system, the kidneys also modulate blood pressure.[16] Each kidney is normally supplied by one main renal artery, which arises from the lower aorta.[18] The three main internal parts of the kidney are the renal cortex, medulla and pelvis[17] (Fig 1.1).

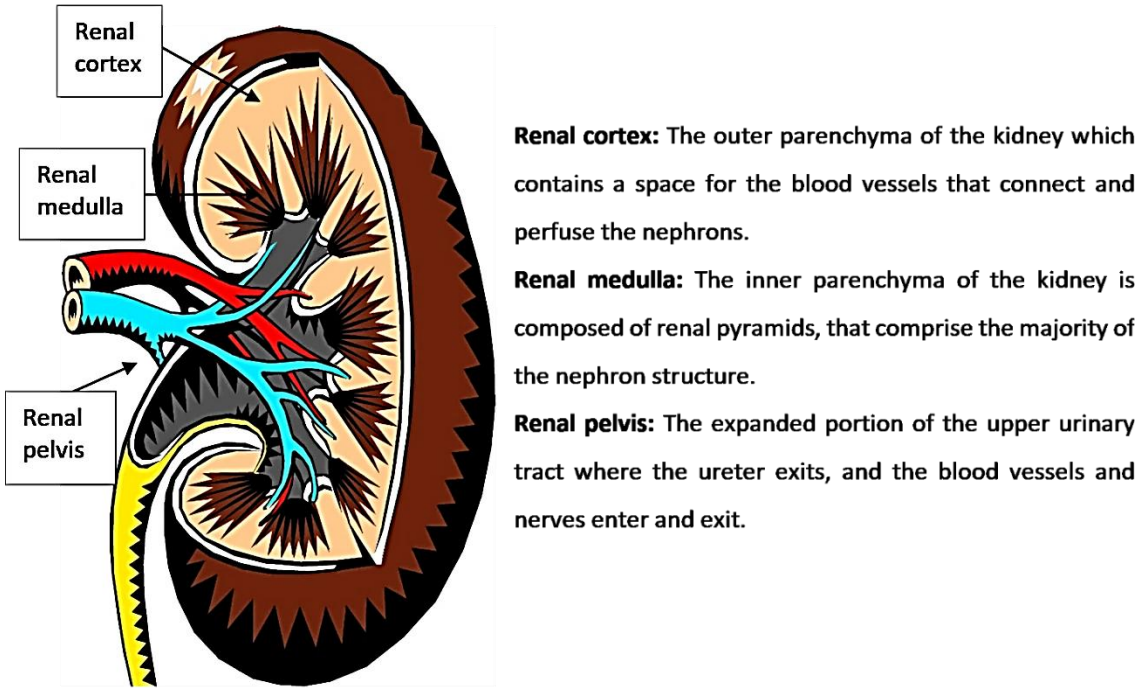


Figure 1.1 The three main internal parts of the kidney – renal cortex, renal medulla and renal pelvis[17]. The renal parenchyma refers to the combined cortex and medulla. Source: Adapted from Image by Balik from Pixabay https://pixabay.com/illustrations/kidney-cross-section-medical-organ-2183443/.

1.3 Kidney Development and Nephrogenesis

The human kidney develops through three successive embryonic stages. Transient development and regression of the primary (pronephros) and secondary (mesonephros) occurs between day 23 and day 112 of life.[19] These primitive fetal kidneys serve as temporary excretory organs. The definitive, tertiary fetal kidney is the metanephros and this matures into the fully functional permanent kidney. The metanephros begins developing in the fifth week and leads to the formation of nephrons through a process known as nephrogenesis.[19, 20]

Nephrons are the functional units within the kidney. Each nephron is comprised of a glomerulus, proximal tubule, loop of Henle and distal tube. Their role is to filter blood, reabsorb water, excrete waste and perform some endocrine functions.[20, 21] New nephrons are formed through successive branching of the ureteral bud, with concentric layers of nephrons proceeding outwards. Hence, the newest nephrons are in the outer layer and any disruption to nephrogenesis results in fewer layers of nephrons.[12, 22] Fetal kidneys are unlike most other fetal organs in that the maximum cell proliferation occurs in the third trimester. Nephrogenesis continues up until 34 to 36 weeks gestation with approximately 60% of nephrons formed in the third trimester.[23] An autopsy study of a small number of term infants, demonstrated there were four to five glomerular layers around 22 weeks gestational age, with the formation of another new glowerular layer every four weeks, layering out towards the outer cortex.[24] Once nephrogenesis is complete, no new nephrons are formed, providing the maximum number of nephrons an individual will have at this point in life.[23,24] It is therefore essential that appropriate nephrogenesis is supported *in-utero*, as the number and quality of nephrons directly influences kidney function throughout life.[25]

A normal healthy kidney contains around one million nephrons, however, autopsy studies of human kidneys demonstrate a wide variation of between 200,000 to over 2.5 million nephrons per kidney.[10, 26, 27] This wide range in nephron number is attributed primarily to uterine environmental factors during nephrogenesis, as well as genetic factors.[2] After birth, exposure to a variety of

different stressors, such as renal infections, smoking, drugs and poor nutrition along with the loss of renal function that occurs with increasing age lead to a further reduction in nephron endowment.[28] These secondary insults on those individuals with an already depleted nephron number is what exacerbates the occurrence of renal disease (Fig 1.2).[14, 28, 29] The main determinant of life-long nephron number and kidney function is nephrogenesis *in-utero*. Insults during intrauterine life and the first few weeks of life can induce lasting structural and functional changes not just to the kidneys but, to many aspects of the developing fetus. This process is termed developmental origins of health and disease.[30]

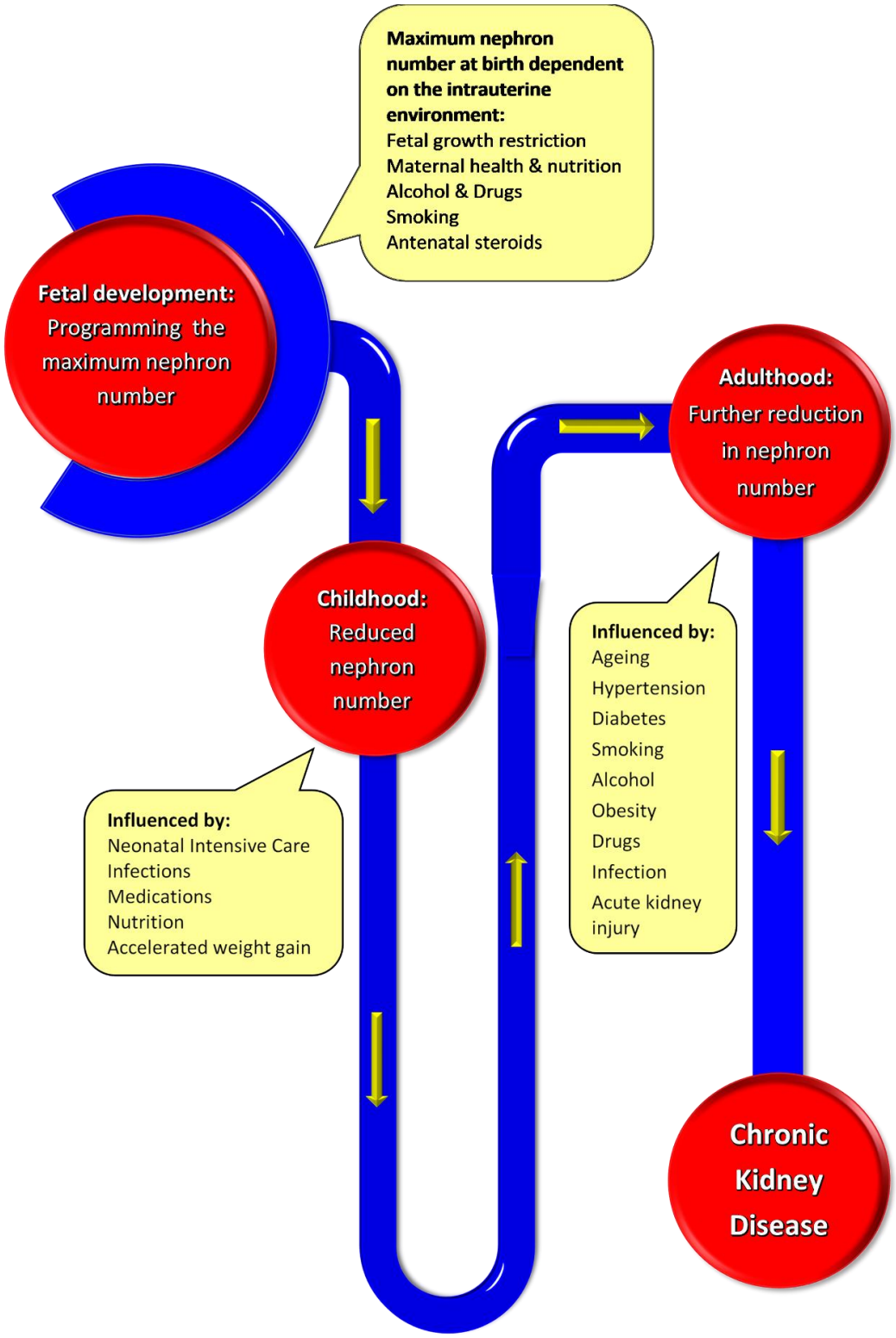


Figure 1.2 Demonstration of how the intrauterine environment can influence nephron number at birth which is then followed by insults to the kidney over the life course that can lead to further reductions in nephron number and possibly chronic kidney disease. Source: D. Brennan.

1.4 Developmental Origins of Health and Disease

Barker and colleagues, in the late 80's, first proposed the hypothesis that adult diseases could have origins in an adverse environment *in-utero* and in early life.[31] This concept later became known as developmental origins of health and disease and is now well established and accepted.[32, 33] The basis of the concept is that during the human body's development, its structure and function are vulnerable and permanently programmed by intra-uterine and early post-natal environment.[30, 31] Barker et al. initially identified that increased rates of cardiovascular disease and chronic bronchitis were associated with a low birth weight.[31] Since then an adverse intrauterine environment has been associated with many other chronic conditions such as hypertension, obesity, type 2 diabetes and CKD.[34, 35] Barker suggested that the early environment can be thought of as the trigger for branching pathways for the development of diseases. These pathways in turn determine each individual's susceptibility to future health consequences.[34]

Studies have now demonstrated that an adverse intrauterine environment is associated with CKD and hypertension.[11, 36] Compromised intrauterine conditions are thought to effect fetal kidney evolution resulting in a reduced nephron endowment which, can later in life, result in essential hypertension and reduced kidney function.[14, 37, 38] There is strong evidence that fetal growth restriction can impact on kidney development, however less evidence is available to demonstrate the relationship between fetal overgrowth or diabetes during pregnancy and kidney growth and development.[9, 10, 15, 38-40]

1.5 Fetal Growth

The growth of a fetus is determined by genetic factors and the uterine environment.[41] Maternal health status prior to and during pregnancy is also an important determinant of fetal growth and well-being.[42, 43] Nutrition and oxygen are delivered to the fetus via the placenta.[44] Consequently, anything that affects the placental function or interferes with this supply may impact on fetal

growth. Abnormal growth may be restrictive, such as is seen in placental insufficiency which results in a slowing of fetal growth, or may result in an acceleration of fetal growth as a result of an increase in placental transfer, such as occurs in pregnancies complicated by diabetes and the associated pathologically increased glucose availability.[41]

1.6 Fetal Growth Restriction

Fetal growth restriction (FGR) is defined as growth of a fetus below its genetically determined growth potential.[45, 46] FGR is a major cause of morbidity and mortality and is also believed to lead to a predisposition for a range of diseases later in life.[34, 45, 46] The pathogenesis of FGR is multifactorial and includes placental, maternal and fetal causes (Fig 1.3). FGR is usually due to utero-placental causes with a restriction in oxygen and nutrient supply to the fetus, resulting in chronic hypoxia and under nutrition.[46, 47] The fetus compensates for hypoxia by centralising the fetal circulation via preferentially shunting blood, rich in nutrients and oxygen, away from organs such as the kidneys and bowel towards more essential organs such as the heart, brain and adrenals.[47] The difficulty in studies involving FGR is based on the lack of a gold standard for its identification – it is unknown what the genetically determined growth potential of a fetus will be.[48,49]

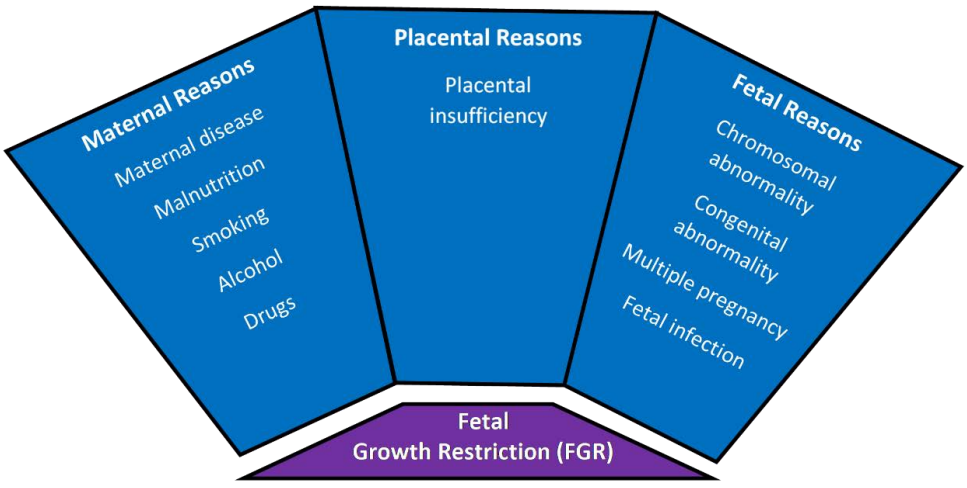


Figure 1.3 A variety of placental, maternal and fetal factors can individually, or in combination, cause fetal growth restriction[46, 47]. Source: D. Brennan.

1.7 Defining Fetal Growth Restriction and Small for Gestational Age

Previously, fetal weight centile cut-offs, such as an estimated fetal weight below the 10th centile or 5th centile were commonly used to identify FGR, however this simplistic approach is not accurate or ideal.[49] The term small for gestational age (SGA) is used to refer to a constitutionally small, but healthy fetus and FGR for a fetus that is pathologically small. Difficultly arises when differentiating between these two groups. There is also a group of fetuses that will be in the normal weight range at birth but are, in fact, growth restricted because they did not reach their potential size. The single largest risk of stillbirth is FGR; however, 50% of stillbirths are within the normal weight range.[50] In addition to measuring fetal biometry, Doppler assessment of maternal and fetal blood flow is being utilised to identify pregnancies at risk of FGR, affected by FGR or to identify fetal compensation or decompensation in the presence of FGR and placental insufficiency.[45, 49, 51, 52] Presently, there is no definitive measure to distinguish FGR from SGA fetuses.[45, 48]

In an attempt to obtain an international consensus on the identification of FGR, a Delphi survey was conducted internationally among 45 experts in fetal growth disorders and was published in 2016.[53] Early FGR was defined as growth restriction identified prior to 32 weeks gestation and late FGR as identified from 32 weeks and greater. For this thesis the criteria for classification of FGR will be based on this consensus paper (Table 1.1).[53]

Table 1.1
Classification of fetal growth restriction (FGR)

Early FGR: Gestational Age < 32 weeks	**Late FGR: Gestational Age ≥ 32 weeks**
• AC or EFW < 3rd centile or UA - AEDF	• AC or EFW < 3rd centile
Or	**Or at least two of the following**
• AC or EFW < 10th centile combined with	• AC or EFW < 10th centile
• Uterine artery - PI > 95th centile and/or	• AC or EFW crossing centiles > 2 quartiles
• UA-PI > 95th centile	• CPR < 5th centile or UA-PI > 95th centile

Note: AC, abdominal circumference; AEDF, absent end diastolic flow; CPR, cerebroplacental ratio; EFW, estimated fetal weight; FGR, fetal growth restriction; PI, pulsatility index; UA, umbilical artery. (based on Gordijn et al.[53]).

1.8 Fetal Growth Restriction and Kidney Development

Low birth weight, preterm birth and FGR are all intricately linked.[54, 55] Low birth weight may be due to FGR or preterm birth or a combination of both and FGR is associated with both spontaneous and iatrogenic preterm birth.[55, 56] Both FGR and preterm birth can adversely impact kidney development and often both occur together, increasing the risk of renal insults.[38] There is strong evidence that a low birth weight is associated with an increased risk of CKD and hypertension later in life and these increased risks are assumed to be due to depleted layers of nephrons. [9, 34, 37, 57, 58] True nephron number, however, can currently only be determined at autopsy.

Human autopsy studies have demonstrated reduced nephron numbers in those born with a low birth weight compared to a normal birth weight, with one study reporting a 20% reduction in nephron numbers in low birth weight infants.[9, 10, 26, 28] An autopsy study investigating preterm infants, some of which were also growth restricted, observed that up to 13% of the glomeruli within the nephrons were abnormally formed.[25] Animal models have also established other factors such as low protein intake, maternal chronic diseases, relative vitamin A deficiency and administration of steroids late in the pregnancy can result in FGR and impaired nephrogenesis,

resulting in a reduction in nephron numbers.[59-62] A low birth weight has been demonstrated to be a significant factor in final nephron number and quality, however, this has not yet been confirmed *in vivo* as presently we do not have an accurate non-invasive method to estimate nephron numbers during development.

Most studies on the effect of low birth weight on kidney development and future CKD and hypertension have not separately analysed low birth weight due to either FGR or to preterm birth.[37, 38] Studies usually use the standard definition of low birth weight as newborns weighing less than 2,500 grams.[63] Many neonates may not reach the standard definition of low birth weight, but they did however, experience FGR and they would not be included in the studies. Further studies are required to define specifically the role FGR and preterm birth has on nephrogenesis, as one occurs *in-utero* and one occurs after birth.

1.9 Fetal Overgrowth

Large for gestational age (LGA) is most commonly defined as having an estimated fetal weight (EFW) greater than the 90th centile and macrosomia as a birth weight greater than 4,000 grams.[64] The use of the term LGA is preferred over macrosomia as macrosomia is only concerned with birth weight and does not factor in gestational age.[41] For this thesis neonates were classified as LGA if their birth weight was greater than the 90th centile using the Hadlock et al. fetal weight charts.[65] The main risk factors for fetal overgrowth are obesity, maternal weight gain and pregestational and gestational diabetes mellitus.[41, 66] Almost half the women in Australia who gave birth in 2018 were overweight or obese; 21% were obese (BMI ≥30), 26% were overweight (BMI of 25.5 – 29.9), 49.5% were within the normal weight range (BMI of 18.5 – 24.9), whilst the remaining women were underweight (BMI <18.5).[67] Fetal overgrowth is a serious condition that is associated with increased risks to the mother and infant, such as instrumental delivery, emergency caesarean section, shoulder dystocia and trauma to the birth canal.[64, 68]

1.10 Fetal Overgrowth and Kidney Development

There is mounting evidence of the long-term health risks for infants born LGA, such as obesity and metabolic syndromes.[69-71] These conditions are both strong risk factors for CKD and hypertension.[72, 73] Furthermore, there are emerging indications that a high birth weight may be associated with CKD and hypertension.[40, 71] The findings are more equivocal than those studies investigating low birth weight and kidney disease. In one study a high birth weight was associated with an increased risk of hypertension in children, but a decreased risk in adulthood.[40] Another study found no significant increase in kidney disease in children less than 21 years of age who were born LGA[74] Considering, however, that the causes of a high birth weight are thought to have programming effects on the fetus and that there is an increased likelihood of obesity and metabolic syndromes for these infants, further research is warranted to attempt to understand the variations in the data. Additionally, fetal overgrowth, when related to maternal hyperglycaemia, appears to be associated with an increased risk of proteinuria and kidney disease later in life and this requires further investigation.[40, 71]

1.11 Diabetes in Pregnancy

Diabetes in pregnancy is a significant and increasingly common complication of pregnancy which is exacerbated by an increasing prevalence of obesity and increasing maternal age of pregnant women.[75, 76] Gestational diabetes mellitus is diabetes that is first identified during pregnancy and pregestational diabetes denotes diabetes mellitus (usually type 1 or type 2 diabetes) that exists prior to pregnancy.[76] In Australia, in 2018, around 40,000 (13.5%) of pregnant women had gestational diabetes and around 2,500 (0.8%) had pregestational diabetes.[67]

During pregnancy, increased maternal insulin resistance facilitates adequate transfer of the essential nutrient glucose to the fetus.[76, 77] However, this does predispose the mother to maternal hyperglycaemia which results in abnormally high circulating levels of glucose.[75, 76] Maternal

hyperglycaemia is associated with increased adverse maternal and neonatal outcomes, such as a birth weight above the 90th centile, preterm delivery and birth trauma.[75, 78] In 2008, the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study reported that even minor degrees of hyperglycaemia during pregnancy were associated with adverse outcomes.[78] These findings resulted in reclassification of the criteria for the diagnosis of gestational diabetes which were adopted by the Australasian Diabetes in Pregnancy Society (ADIPS) in late 2014.[79, 80] For this thesis, the diagnosis of gestational diabetes was based on the ADIPS guidelines which are consistent with the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) guidelines and are outlined in Table 1.2.[79, 80]

Table 1.2
Criteria for the diagnosis of gestational diabetes based on ADIPS guidelines[79]

	Glucose measure	**Blood glucose level**
Diagnosis of gestational diabetes if one or more criteria are met	Fasting overnight	≥ 5.1mmol/L
	1 hr - following 75g oral glucose load	≥ 10.0mmol/L
	2hr – following 75g oral glucose load	≥ 8.5mmol/L

Maternal glucose can easily cross the placenta, however, insulin does not. Therefore, if the mother is hyperglycaemic, the fetus must secrete additional insulin.[81] Fetal hyperglycaemia and hyperinsulinaemia can result in:

- Increased urine production, which may lead to increased liquor (polyhydramnios);
- Increased fat deposition, which may lead to a LGA fetus and increased birth weight;
- Polycythaemia; and
- Neonatal hypoglycaemia, jaundice, hypocalcaemia and hypomagnesemia.[76]

Complications are directly related to circulating glucose levels, with higher glucose levels associated with higher risks of adverse outcomes.[77, 78] Consequently, pregnant women with gestational diabetes have less complications than those who are affected with pregestational diabetes.[77] Adverse effects can be reduced by treatment - the better controlled the hyperglycaemia is, the lower the risk of adverse outcomes.[78, 82]

1.12 Diabetes in Pregnancy and Kidneys

Hyperglycaemia can also be teratogenic to the fetus, particularly at conception and during embryogenesis.[81] With regards to the developing kidneys, one study demonstrated a three-fold increase in renal dysgenesis and agenesis in fetuses of mothers with diabetes.[83] However, this study did not elucidate the type, duration, or treatment of diabetes. Another study found infants of mothers with pregestational diabetes were 7.5 times more likely to be born with renal dysgenesis or agenesis and infants of mothers with gestational diabetes had a 1.3 times increased risk of obstructive renal pathology.[74] Animal models have also demonstrated that maternal hyperglycaemia may be associated with a reduced nephron number, diminished glomerular filtration, microalbuminuria and hypertension in later life.[84, 85] Again, the mechanisms and etiology of maternal hyperglycaemia and any increased risks in kidney disease and hypertension in humans remained to be elucidated. However, there is consensus that maternal hyperglycaemia affects nephron endowment and quality.[39]

1.13 Antenatal Ultrasound Imaging

Antenatal ultrasound uses high frequency sound waves to image the developing fetus and the surrounding maternal structures.[86] Ultrasound is a non-invasive, safe, relatively inexpensive and widely available imaging modality.[87] Images are produced in real-time which enables assessment of fetal movement, heart motion and blood flow. The last decade has seen dramatic advances in ultrasound resolution, computer processing power, volumetric imaging and extended and

improved colour and spectral Doppler applications.[86-88] Ultrasound is the principal imaging modality in pregnancy, which enables prenatal diagnosis of many fetal abnormalities and facilitates ideal prenatal management.[86, 89]

Ultrasound is the primary imaging modality for evaluating fetal kidneys. Kidney size can be measured non-invasively and efficiently with ultrasound.[86] We initially conducted a systematic review of the evaluation of fetal kidney growth using ultrasound.[90] This full systematic review is presented in Chapter 2. In summary, after reviewing the studies it was found that there were some normal ranges of kidney length, transverse (TS) and antero-posterior (AP) diameters, and 2D and 3D kidney volumes. There were, however, few large, good quality, longitudinal studies. Fetal kidney length was demonstrated to not be a good indicator of kidney growth with no significant difference in kidney length seen between growth restricted fetuses and appropriately grown fetuses. In contrast, the TS and AP diameters of the fetal kidneys were reduced in growth restricted fetuses, indicating the kidney maintained its length however was "thinner". There are few studies investigating abnormal fetal growth and associated kidney growth. A few small studies assessed FGR on fetal kidney growth, but, no single study was found on the effect of fetal overgrowth on kidney growth and only one study on the effect of diabetes in pregnancies on fetal kidney volumes.[90]

Antenatal ultrasound reliably diagnoses obstructive renal abnormalities such as hydronephrosis, hydroureter and bladder outlet obstruction, whereas it is much less reliable at diagnosing parenchymal pathologies such as renal dysplasia, polycystic kidney disease, and tuberous sclerosis.[91, 92] In part this is due to a lack of effective criteria to aid diagnosis. Reviewing the literature highlighted the need to discover more accurate *in-vivo* methods to estimate nephron number and assess fetal kidney growth which could be used to detect changes in kidney development and function under altered fetal conditions.

1.14 Estimation of Nephron Number

In order to further examine the mechanisms and timing of effects that influence future kidney health and blood pressure, an accurate, *in-vivo*, non-invasive method to estimate nephron number is required.[2, 15] At present, the only accurate method of calculating human nephron number is during an autopsy.[14] It is essential to have a good understanding of fetal kidney development under normal and abnormal fetal conditions and what can effect the number and quality of nephrons. This led to the investigations in this thesis measuring the functional renal mass which contains the layers of nephrons and is known as the renal parenchyma.

1.15 Renal Parenchymal Thickness

Measuring the renal parenchyma with ultrasound is a novel method to assess fetal kidney development and possibly provide an indirect estimate of nephron number. There are currently no studies in the literature to date that have utilised this measurement to assess growth of the fetal kidneys under compromised intrauterine conditions. The renal parenchyma consists of the renal cortex and medullary pyramids (Fig 1.4).[93] Ultrasound technology has improved tremendously over the last ten years and current machines can produce very high-quality images of the fetal kidneys.[86] Ultrasound assessment and measurement of the fetal renal parenchyma could potentially be a more reliable criteria of kidney development and estimate of nephron number than what is currently available. We have previously conducted studies on term neonates and the findings indicated that renal parenchymal thickness may be a more reliable investigative method to define normal and abnormal kidney development.[94]

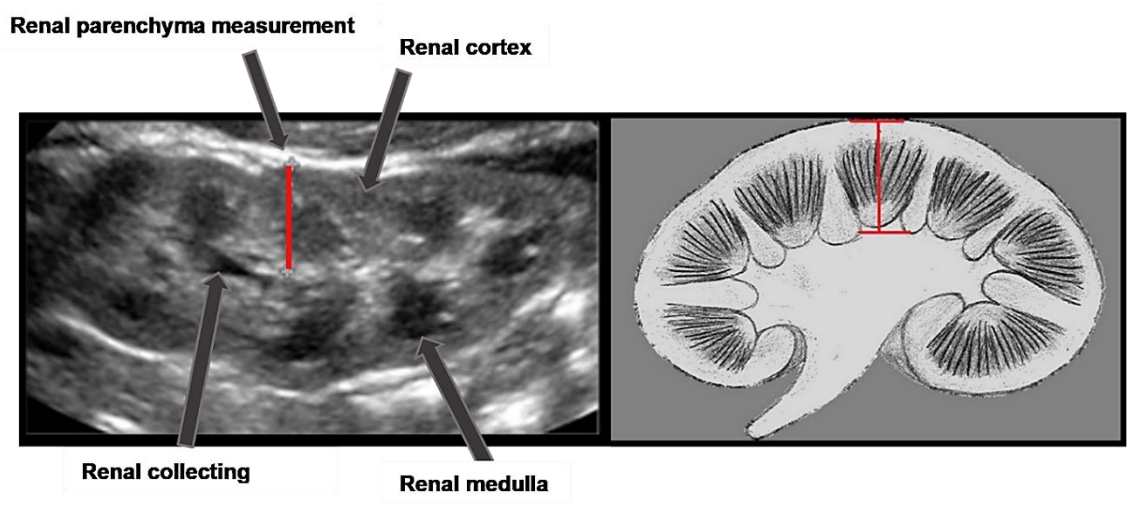


Figure 1.4 Ultrasound image of kidney (left) with measurement of the renal parenchymal thickness (red line). Line drawing of kidney (right) demonstrating the renal parenchymal thickness measurement (red line). Source: Ultrasound image -Townsville University Hospital, line drawing – D. Brennan.

By measuring the renal parenchyma, instead of the entire kidney volume or size, only the important functional part of the kidney containing the nephrons is analysed. The collecting system of the kidney is not included. For example, in the setting of fetal renal dilatation of the collecting system (hydronephrosis), a kidney length or a 2D or 3D kidney volume would result in a significant overestimation of the volume of kidney tissue, due to the enlargement of the collecting system with fluid.[95] The renal parenchyma itself may be thinner than normal and the kidney could have impaired function (Fig 1.5). Further investigations are therefore needed into the potential of renal parenchymal thickness as a marker of nephron endowment and its role in improving the diagnosis of pathologies that specifically affect the parenchyma, such as, fetal glomerulopathies.[92, 95, 96]

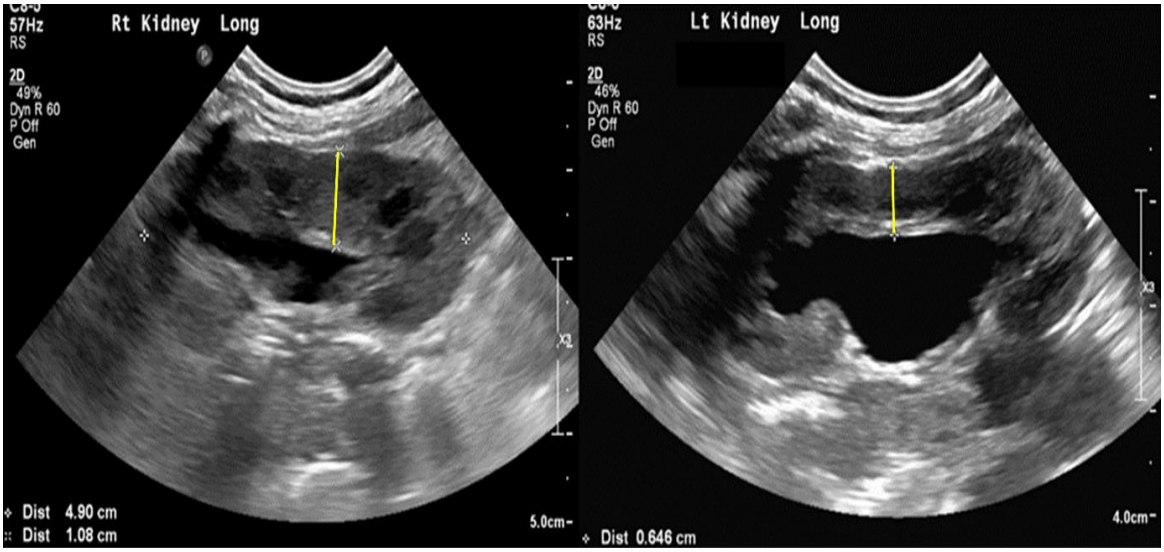


Figure 1.5 One-week old neonate with normal right kidney without hydronephrosis and a parenchymal thickness of 10.8mm and left kidney with hydronephrosis and parenchymal thickness of 6.5mm. Source: Townsville University Hospital.

1.16 Fetal Kidney Function

Fetal kidney function is difficult to assess non-invasively in-utero. FGR, caused by placental insufficiency, can result in chronic hypoxaemia with redistribution of oxygenated blood away from organs, such as the kidneys, to more essential organs.[46, 47] By analysing the fetal renal arteries with Doppler mode, a few studies have demonstrated an elevation in the resistance of blood flow to the kidneys of growth restricted fetuses.[97-99] Changes in kidney perfusion may influence urine output as urine production rate has been shown to be reduced in FGR fetuses in observational human studies and in animal models.[59, 100-102] Amniotic fluid volume does have some relationship with fetal kidney function, however, there is currently no well-established non-invasive method to assess fetal kidney function.[44, 103, 104] Analysing renal parenchymal thickness together with fetal renal artery Dopplers and amniotic fluid levels may provide more accurate information on fetal kidney growth and function.

1.17 Purpose of This Thesis

The purpose of this thesis is to evaluate the use of antenatal ultrasound to measure the fetal renal parenchyma and to investigate if this measurement can be used as an indirect estimate of nephron endowment. The fetal artery blood flow will also be explored. These measurements will then be applied to gain a better appreciation of kidney development and function in the normally and abnormally grown fetus and in diabetes in pregnancy. The results could help identify factors that adversely affect kidney development. Subsequently, public health interventions and education programs could be implemented to try and address those factors that might be modifiable. A low nephron number at birth does not necessarily lead to hypertension and chronic kidney disease but, renders the kidney vulnerable to disease later in life.[11, 39] It is necessary to optimise fetal nephron number and quality at birth to try and reduce susceptibility to CKD and hypertension in later life. To achieve this, we firstly need a non-invasive method to estimate nephron number.

1.17.1 Overall aim

The overall aim of this thesis was to assess the fetal renal parenchymal growth through serial ultrasound measurements of a pregnant population and determine if abnormal fetal growth or diabetes in pregnancy impacts fetal renal parenchymal thickness.

1.17.2 Hypothesis

Abnormal fetal growth adversely affects fetal renal parenchymal growth.

Aim 1

Conduct a systematic review of the use of antenatal ultrasound to evaluate fetal kidney growth. This aim is addressed in Chapter 2.

Aim 2

Determine the normal ranges for fetal renal parenchymal thickness, kidney length and kidney volume between 16- to 38-weeks gestational age from a group of low-risk pregnancies and to assess the reliability of these measurements. This aim is addressed in Chapter 4.

Aim 3

Determine the normal ranges for fetal renal artery resistivity index (RI) and pulsatility index (PI) between 16- to 38-weeks gestational age from a group of low-risk pregnancies and to assess the reliability of these measurements. This aim is addressed in Chapter 5.

Aim 4

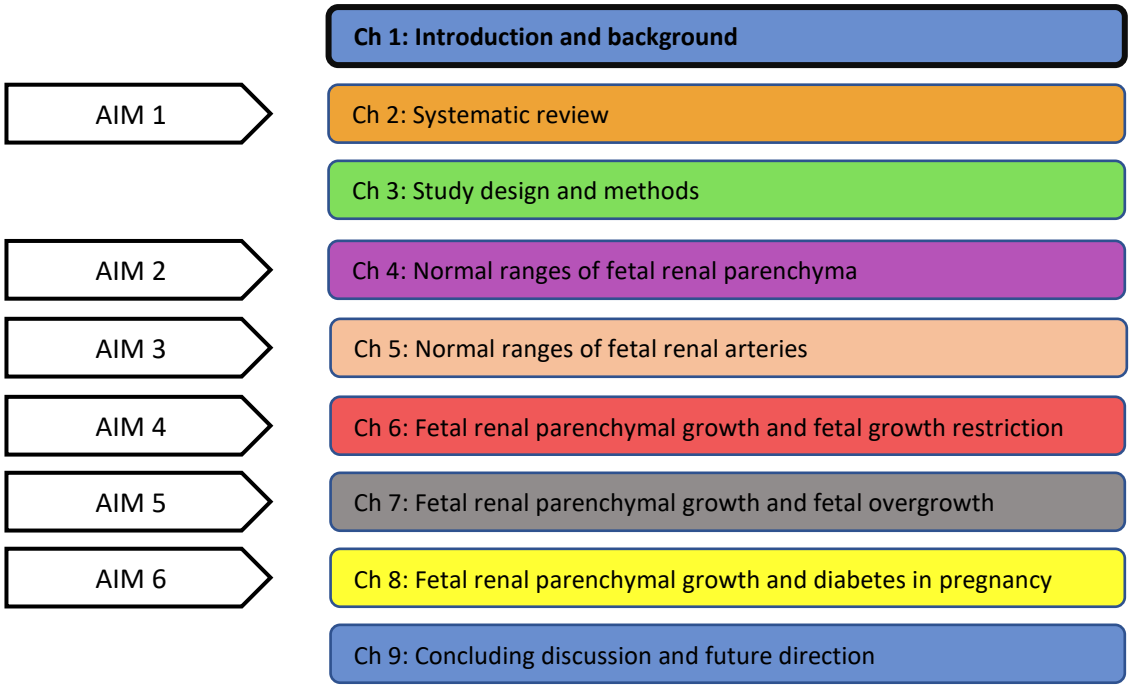
Evaluate the effects of FGR on the growth of the fetal renal parenchyma and renal artery blood flow. This aim is addressed in Chapter 6.

Aim 5

Evaluate the effects of fetal overgrowth on the growth of the fetal renal parenchyma and renal artery blood flow. This aim is addressed in Chapter 7.

Aim 6

Evaluate the effects of diabetes in pregnancy on the growth of the fetal renal parenchyma. This aim is addressed in Chapter 8.



This thesis contains published papers, and as such, the different papers may contain similar concepts and methods. The original papers contained different styles, spelling and referencing depending on the journal. Generally, the style, spelling and referencing has been reformatted to fit the overall style of this thesis.

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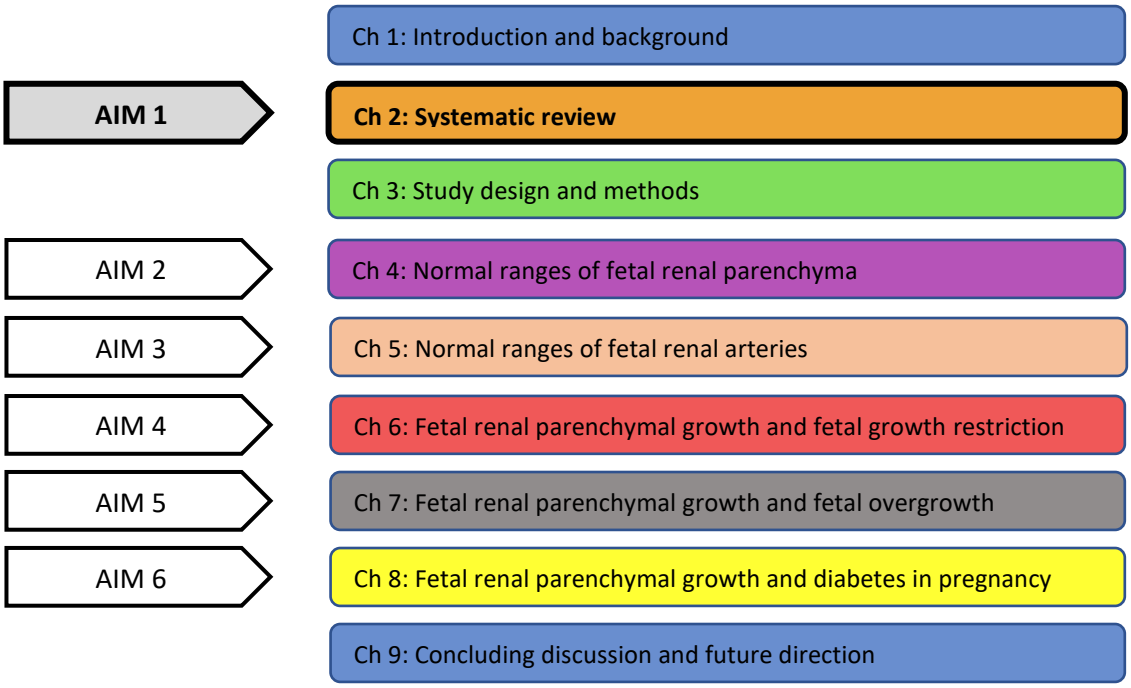
Chapter. 2 Evaluation of Fetal Kidney Growth Using Ultrasound: A Systematic Review

Brennan S, Watson D, Rudd D, Schneider M, Kandasamy Y. Evaluation of fetal kidney growth using ultrasound: A systematic review. Eur J Radiol. 2017; 96:55-64.



This chapter includes an exact copy of the journal paper, referenced above and attached in Appendix D, except the formatting of section sub-headings and the figure, table and reference numbers have been modified for the purpose of this thesis. An update to this systematic review based on literature published between 2017 and June 2020 is included after the paper in Section 2.7 of this chapter.

2.1 Abstract

Purpose

To determine the role of ultrasound imaging in evaluating fetal kidney growth.

Methods

MEDLINE, CINAHL and EMBASE databases were electronically searched for studies between 1996 and January 2017 and limited to English language. Studies were included if they reported on an ultrasound technique to assess fetal kidney growth and they were not a case report or case series. There was independent selection of studies by two reviewers in consensus with one other reviewer. Data were extracted by one reviewer in consensus with two other reviewers.

Results

A total of 1,785 articles were identified. The full text of 39 of these were assessed for eligibility for inclusion. Twenty-eight studies were then included in the review. Standard two-dimensional fetal renal (2D) measurements are easy to perform, however, this review identified that most studies had some methodological limitations. The disadvantage with 2D and three dimensional (3D) fetal renal volumes are that they include the entire kidney and good reproducibility of 3D volumes has not yet been demonstrated. Currently there is limited research on fetal kidney growth in the setting of abnormal fetal growth. Research focusing directly on fetal kidney parenchyma and blood flow is scarce.

Conclusions

Some nomograms of 2D and 3D fetal kidney size and volume have been developed. Kidney length is the most popular single fetal kidney measurement; however, it does not seem to be a good indicator of growth. In IUGR fetuses, kidney length remained similar to appropriately grown fetuses whereas AP and TS dimensions were significantly decreased. New ultrasound techniques focusing on the parenchyma of the kidney and perfusion to the kidney should be explored as they

may provide more meaningful information on kidney development in the fetus and future kidney function.

2.2 Introduction

It is well established that an adverse intrauterine environment can affect fetal kidney development resulting in possible hypertension and chronic kidney disease later in life.[1, 2] Intrauterine growth restriction (IUGR) can result in significant reductions in nephron number[3], which may ultimately result in decreased renal function.[4] Although most studies concentrate on IUGR and low birth weight infants, overgrowth or large for gestational age (LGA) are also emerging as factors that can disrupt normal fetal kidney development and increase risks for hypertension and chronic kidney disease.[5] The normal development of the fetal kidneys can be crucial to an individual's long-term health outcomes.

The human kidney develops through three successive embryonic stages. Transient development and regression of the primary (pronephros) and secondary (mesonephros) fetal kidneys occurs between day 23 and day 112.[6] These primitive fetal kidneys have no impact on fetal renal function. The definitive, tertiary fetal kidney is the metanephros and this is the permanent functional kidney. It begins developing on day 30 leading to the formation of nephrons – the functional units within the kidney.[6, 7] Fetal kidneys are unlike most other organs in that the maximum cell proliferation occurs in the third trimester. Nephrogenesis continues up until 34 to 36 weeks gestation with approximately 60% of nephrons formed in the third trimester.[8]

Assessment of the fetal kidneys is an essential part of an obstetric ultrasound. Accurate information regarding kidney size is crucial to identifying kidney abnormalities and detecting changes in fetal kidney growth. Ultrasound imaging is safe, cost effective and widely available to evaluate fetal kidney size, echotexture and perfusion. A variety of two and three-dimensional ultrasound techniques have emerged and advanced to evaluate kidney development. The aim of this review

was to systematically review the literature to determine what role ultrasound plays in evaluating fetal kidney growth. Current ultrasound imaging techniques and accuracy will be reviewed.

2.3 Method

A systematic review of observational studies was conducted using a protocol designed *a priori* and following the PRISMA guidelines for systematic reviews. Author SB developed and conducted the search strategy using medical subject headings (MeSH) and keywords and this was reviewed by author YK (Appendix 1). MEDLINE (Ovid), CINAHL and EMBASE electronic databases were electronically searched in August 2016 and again in January 2017, for publications from the year 1996 onwards. The literature search was limited to the English language. The reference lists of relevant articles were hand-searched for additional relevant studies.

Human observational ultrasound studies that were not a case report or case series were included. Only studies reporting on an ultrasound technique assessing fetal kidney growth were included. Studies that only assessed fetal pelvic renal dilatation were excluded.

Only studies published from the last 20 years (from 1996) were included as it was felt that the significant advances in ultrasound techniques and improvements in diagnosis and definition in prenatal imaging made these older studies less relevant. Also excluded were unpublished studies, non-peer-reviewed, conference abstracts, letters to the editor and opinion articles. If data from a single study population was reported more than once, the publication containing the most complete information was included.

Study selection was performed in two sequential steps, firstly assessing articles by title and abstract and secondly by full text of the article. Two reviewers (SB and YK) independently screened the titles and abstracts of all identified citations and potentially eligible studies were selected. The full text of these potentially eligible studies was screened by the same two reviewers. Any discrepancies between the reviewers were resolved by consultation with a third reviewer (DW).

A data extraction sheet was developed. Only pre-specified outcomes of interest in the review were collected. Review author SB extracted the data from the studies and the second review author YK checked the extracted data. Any disagreements were resolved by discussion with a third reviewer (DW). Figure 2.1 outlines study selection process. A narrative synthesis, including tables, was done on the extracted data to explain and summarise the characteristics and findings of the included studies.

2.4 Results

A total of 1,785 articles were identified and after review of the title and abstract, the full text of 39 of these were assessed for eligibility for inclusion (Figure 2.1). Four papers from the Generation R study reported the same renal data[9-12] and therefore these data were only considered once using the paper by Verburg et al.[9] as it contained the most relevant and complete data assessing fetal kidney measurements. Finally, 28 studies were reviewed.

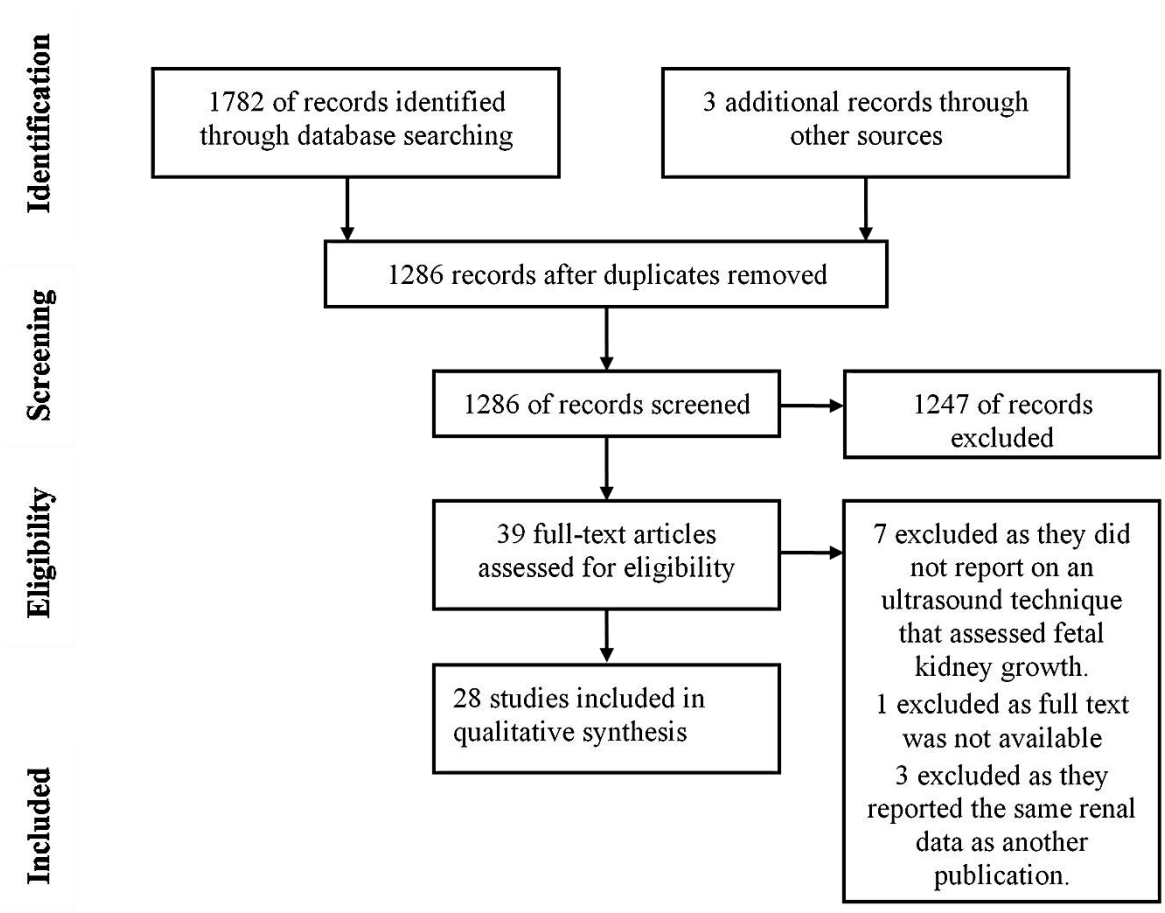


Figure 2.1 Study selection process.

Relevant characteristics of these included studies are presented in Tables 2.1, 2.2 and 2.3. Most studies were prospective in design with only 2 of the 28 studies being retrospective.[13, 14] A cross-sectional design was utilised by 21 studies while 7 studies had a longitudinal design. Selected studies were divided into three groups depending on the ultrasound technique used to assess the kidneys. Some studies reported on more than one ultrasound technique (Tables 2.1, 2.2, and 2.3).

Generally, the study time and duration, how participants were recruited, and missing participants and data was poorly reported. Calculation of estimated gestational age (GA) was most commonly achieved using the last normal menstrual period (LNMP) correlated with a first or early second trimester ultrasound (18 studies).[13-30] Six studies used ultrasound dating only[9, 31-35], one used only an accurate LNMP[36] and three did not report how GA was determined.[37-39]

Table 2.1
Main characteristics of studies included for standard 2D whole kidney measurements

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Konje et al. 1996[37]	UK	P/Long	Singleton preg AGA & SGA	AGA = 50 SGA = 37	NR	20 – 38 wks	Every 2 weeks after recruitment	TA, 2D, N° operators NR KL, TS, AP & circumference	SGA different growth to AGA. AGA acceleration of growth 26-34 weeks (critical period) not seen in SGA. No difference in KL.
Rosati et al. 1996[15]	Italy	P/CS	Normal AGA singleton preg high risk population	489	LNMP & US	11 – 16 wks	Once	TV, 2D, 2 operators KL, TS, AP, circumference & KC/AC	Normal fetal kidney size in early pregnancy. There was linear growth of all kidney measurements throughout early pregnancy.
Konje et al. 1997[31]	UK	P/CS	Singleton preg AGA & SGA	AGA=129 SGA = 90	10-12 wks US	22 – 38 wks	Once	TA, 2D, 1 operator KL, TS, AP & circumference	Between AGA & SGA – no difference in KL, significant difference in TS & AP from 26 weeks. SGA resulted in long thin kidneys = “sausage shaped” kidneys.
Gloor et al. 1997[38]	USA	P/CS	Normal AGA singleton preg	100	NR	18 – 39 wks	Once	TA, 2D, 4 operators KL, TS, AP, 2D volume, EFW	Provides normal fetal kidney size relative to both GA & EFW. Constant ratio between KL to weight. Ratio of KL to weight declines.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Ansari et al. 1997[16]	Bangla-desh	P/CS	Normal AGA singleton preg	793	LNMP & BPD & FL	16 – 40 wks	Once	TA, 2D, N° operators NR KL, BPD & FL	Good correlation between KL, BPD & FL. GA can be assessed by BPD, FL & KL.
Guariglia et al. 1998[36]	Italy	P/CS	Normal AGA singleton preg	807	LNMP	11 – 16 wks	Once	TV, 2D, 2 operators KL, BPD, BPD/KL	BPD/KL plotted against GA for early pregnancy & appears constant through 11-16wks.
Zalel et al. 2002[17]	Israel	P/CS	Normal AGA singleton preg	269	LNMP & CRL	13 – 22 wks	Once	TV, TA, 2D, 1 operator KL	Normal range of KL in early pregnancy (13 -22weeks).
Lampl et al. 2002[18]	Belgium	P/CS	Normal AGA singleton preg	25	LNMP & CRL 8-10 weeks	23 & 32 wks	Twice (23 weeks & 32 weeks)	TA, 2D, 1 operator KL, TS, AP & 2D volume	Thinner neonates at birth have smaller kidneys relative to body size. Mean KV/EFW ratio did not significantly change between 23 and 32 weeks. KL not related to birthweight & ponderal index, however, TS & AP related positively to both.
Konje et al. 2002[19]	UK	P/Long	Normal AGA singleton preg	73	LNMP & CRL	24 – 38 wks	Every 2 weeks from 24 weeks	TA, 2D, 2 operators KL, BPD, HC, AC & FL	KL predicted GA better than AC & FL.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Chitty et al. 2003[20]	UK	P/CS	Normal AGA singleton preg	661	LNMP & US 18-20weeks	14 – 42 wks	Once	TA, 2D, N° operators NR KL, TS, AP, 2D volume	Normal ranges of KL, TS & AP for 14 to 42 weeks.
Silver et al. 2003[21]	USA	P/CS	Singleton preg IUGR & SGA	AGA = 43 IUGR = 34	LNMP & CRL or 2nd US	27 – 41 wks	Once	TA, 2D, 2 operators KL, TS, AP, circumference & 2D volume Renal artery Doppler	Kidney volume in IUGR fetuses was 31% less than in AGA fetuses & 15% less when adjusted for fetal weight. No difference in renal artery blood flow AGA vs SGA.
Lampl et al. 2005[22]	Belgium	P/Long	Singleton preg smoking exposure & none	Smokers = 10 Non-smokers = 24	LNMP & CRL	19 - NR	Every week from 19 weeks	TA, 2D, 1 operator KL, TS, AP, 2D volume	Recorded birth outcomes for 6 smokers & 21 non-smokers this was insufficient for meaningful results. Smoke exposed fetal kidneys were thicker in 2nd trimester and then became thinner in 3rd trimester than non-exposed kidneys.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Verburg et al. (Gen R) 2007[9]	Nether-lands	P/CS	Singleton preg, population-based cohort	1215	CRL or BPD if >12 weeks	28.4 – 32.6 wks	Once	TA, 2D, 3 operators KL, TS, AP, 2D volume, EFW, UA, MCA & UA/MCA ratio	Normal ranges of KL, TS, AP & 2D volume for 28 to 34wks. Maternal weight & height positively associated with kidney volume. Kidney volume is positively associated with all growth characteristics & amniotic fluid. Umbilical artery RI's & CPR negatively associated with kidney volume.
Van Vuuren et al. 2012[32]	Nether-lands	P/Long	Normal AGA singleton preg	96	CRL	16 – 42 wks	Every 4 weeks, 2 groups staggered by 2 weeks	TA, 2D, 1 operator KL, TS, AP, 2D volume	Normal ranges of KL, TS, AP & 2D volume for 16 to 42 weeks.
Neves et al. 2013[33]	Brazil	P/Long	Singleton preg hyperglycaemic & normoglycaemic	Hyper-glycaemic = 92 normo-glycaemic = 339	US 11-14 weeks	22 – 38 wks	Every 2 weeks, not all women attended all	TA, 2D, 1 operator KL, TS, AP, 2D volume	Significant difference between groups. Median kidney volume of hyperglycaemic group > 75th percentile for normoglycaemic group. Maternal hyperglycaemia is associated with fetal kidney growth modification.
Seilanian Toosi et al. 2013[23]	Iran	P/CS	Normal AGA singleton preg	89	LNMP & US 8-10weeks	NR ?3rd trim	Once	TA, 2D, 2 operators KL, BPD, HC, AC, FL	KL, AC, FL & HC had a linear & strong correlation with GA. Best GA predictor combined HC, BPD, FL & KL (±14.2 days).

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Roderick et al. 2016[34]	UK	P/CS	Normal AGA singleton preg South Asian & White British	South Asian = 715 White British = 872	US 8-14 wks	32 + 3 days to 34 + 4 days	Once	TA, 2D, 4 sonographers KL, TS, AP, 2D volume, kidney circumference in TS	After adjusting for GA, all renal dimensions for South Asians were significantly smaller than for White British. Proportion reduction greatest for TS the AP. Kidney volume reduced with adjusted BW, kidney volume still higher in White British. Findings may partly explain the increased risk of adult chronic kidney disease in South Asians.

Note: AC, abdominal circumference; AGA, appropriate-for-gestational age; AP, anterio-posterior; BPD, biparietal diameter; Btw, between; BW, birth weight; CPR, cerebroplacental ratio; CS, cross-sectional; CRL, crown rump length; EFW, estimated fetal weight; FL, femur length; GA, gestational age; HC, head circumference; IUGR, intrauterine growth restriction; KC, kidney circumference; KL, kidney length; LNMP, last normal menstrual period; Long, longitudinal; NR, not recorded; P, prospective; Preg, pregnancy; R, retrospective; RI, resistive index; SGA, small for gestational age; TA, transabdominal; TS, transverse; TV, transvaginal; US, ultrasound.

Table 2.2
Main characteristics of studies included for 3D techniques

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Yu et al. 2000[24]	Taiwan	P/CS	Normal AGA singleton preg	152	LNMP & CRL	20 -40 wks	Once	TA, 3D, N° operators NR 3D kidney multiplanar volume	Good correlation between GA and kidney volume. Normal ranges of 3D kidney volumes.
Hsieh et al. 2000[25]	Taiwan	P/CS	Normal AGA singleton preg	112	LNMP & CRL	15 -40 wks	Once	TA, 2D & 3D, 1 operator 2D calculated volume & 3D kidney multiplanar volume	Normal ranges of 3D kidney volumes. Due to limitations of 3D volumes this study found the constant for volume to be calculated using 2D measures for right & left kidneys.
Kuno et al. 2006[26]	Japan	P/Long	Normal AGA singleton preg	13	LNMP & CRL or early 2nd US	20 wks - birth	Every 2-3 wks from 20 wks to birth	TA, 3D, N° operators of acquisition NR 1 operator analysed 3D data. 3D kidney multiplanar volume	Normal ranges of 3D kidney volumes. Significant difference found between their study and two others (24, 25). They were doubtful about reproducibility of 3D intrauterine volumes.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Chang et al. 2008[27]	Taiwan	P/CS	Singleton preg AGA & IUGR	AGA = 221 IUGR = 28	LNMP & CRL or early 2nd US	20 -40 wks	Once	TA, 3D, 1 operator but unclear if they did acquisition as well 3D kidney multiplanar volume	Using 3D kidney volumes, the best screening criteria for detection of a growth restricted fetus is the 10th percentile – sensitivity = 96.4%, specificity = 95.9%, PPV = 75%, NPV = 99.5%, accuracy = 96%.
Tedesco et al. 2009[28]	Brazil	P/Long	Normal AGA singleton preg	57	LNMP & CRL or early 2nd US	24 -34 wks	Every 2 wks – some started 24 wks others 25 wks until 34 wks	TA, 3D, 1 operator 3D kidney VOCAL volume	Strong correlation between GA and kidney volume. Normal ranges of 3D kidney volumes using VOCAL. Kidney volume highly correlated with other fetal biometry and EFW.
Yoshizaki et al. 2013[35]	Brazil	P/CS	Normal AGA singleton pregnancy	213	CRL	20 -40 wks	Once	TA, 3D, 1 operator 3D kidney VOCAL volume	Normal ranges of 3D kidney volumes using VOCAL.

Note: AGA, appropriate-for-gestational age; CS, cross-sectional; CRL, crown rump length; GA, gestational age; IUGR, intrauterine growth restriction; LNMP, last normal menstrual period; Long, longitudinal; NR, not recorded; P, prospective; TA, transabdominal; TS, transverse; VOCAL, virtual organ computer-aided analysis; Wks, weeks.

Table 2.3
Main characteristics of studies included for other novel ultrasound measurements

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Suranyi et al. 2003[29]	Hungary	P/CS	AGA & IUGR Singleton preg	IUGR with hyper-echoic kidney = 28 IUGR without hyper-echoic kidneys = 62 AGA N^{o} NR	LNMP & CRL	Unclear – states 3 stages 1st, 2nd & 3rd trimesters	Once	TA, 2D, N^{o} operators NR Assess for renal medullary hyperechogenicity KL & BPD /KL ratio	IUGR fetuses divided into those with hyperechoic kidneys and those without. Normal nomogram BPD/KL correlated with GA. Significant difference btw KL of normal fetuses and IUGR fetuses with hyperechoic kidneys, but no difference btw KL of normal fetuses and IUGR fetuses without hyperechoic kidneys.
Kennedy et al. 2003[14]	USA	R/CS	Normal AGA singleton preg	123	LNMP & 2nd trimester ultrasound	16 – 38 wks	Once	TA, 2D, N^{o} readers NR Reviewed US images to find max KL image then measured area of kidney	Normal ranges of total fetal renal parenchymal area. Combined fetal nomogram with their previously devised birth to adolescent nomogram of total parenchymal area.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Shin et al. 2007[13]	Korea	R/CS	Normal AGA singleton preg	216	LNMP or CRL	16 – 41 wks	Once	TA, 2D, 1 reader Reviewed US images to find max KL image & TS image then measured length, TS & area of kidney in long & TS	Normal ranges of total fetal renal parenchymal area in long and TS and normal ranges of KL. KL of Korean fetuses are similar to other races.
Hadar et al. 2012[30]	Israel	P/ CS	Normal AGA singleton preg	128	LNMP & CRL	20 – 40 wks	Once	TA, 2D, 2 operators Anterior & posterior parenchymal thickness measured in long & TS planes	Normal ranges of ant & post parenchymal thickness in long & TS. Gradual linear growth of parenchyma throughout pregnancy. Findings indicate parenchymal thickness measured on long section are more reliable & reproducible than those made on transverse section.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Ranges (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Devriendt et al. 2013[39]	Belgium / France	P/CS	Normal AGA singleton preg	156	"Known GA" NR how GA determined	21 – 37 wks	Once	TA, 2D, 2 readers did offline measurements, operators that obtained images NR Length, cortical thickness (CT) & medullary thickness (MT) both in long, assessed cortical echogenicity	KL increased with GA. Cortical echogenicity (CE) evolved with GA. After 32 weeks GA, the CE should not be higher than the liver or spleen. Normal ranges of CT and MT according to GA. CT/MT ratio calculated and decreased with increasing GA.

AGA, appropriate-for-gestational age; BPD, biparietal diameter; Btw, between; CRL, crown rump length; CS, cross-sectional; CT, cortical thickness; GA, gestational age; IUGR, intrauterine growth restriction; KL, kidney length; LNMP, last normal menstrual period; Long, longitudinal; MT. medullary thickness; NR, not recorded; P, prospective; R, retrospective; TA, transabdominal; TS, transverse.

Overall ultrasound features of the studies and measurement methods were well described. Most studies focused on the mid-second trimester to third trimester[9, 13, 14, 16, 18-31, 33-35, 37-39], as imaging the fetal kidneys well under 20 weeks can be difficult.[33, 40] The three studies that reported data below 14 weeks GA used transvaginal scanning.[15, 17, 36] The GA range assessed was very variable between studies. Two studies showed only a snap shot in time with a GA range of 15 days (around 34 weeks)[34] and 4 weeks (28 to 32 weeks).[9] One study measured the fetal kidneys at 23 weeks and again at 32 weeks.[18] Studies covering the longest GA ranges were Chitty and Altman[20] 16 to 42 weeks, van Vuuren et al.[32] 16 to 42 weeks and Hsieh et al.[25] 15 to 40 weeks. The GA range was unclear in one study.[23]

2.4.1 Differences between right and left kidneys and gender

Overall, the evidence strongly supported no significant difference between right and left fetal kidney size (17 of the 18 studies) for all ultrasound measurements regardless of the technique used.[13, 15-17, 19, 23-26, 28-33, 35, 38] Six of the seven studies that examined gender differences found no significant difference between fetal kidney measurements.[17-19, 23, 29, 30] Only one study demonstrated a difference in size between right and left kidneys and males and females.[9] This was a large study; however, it had a small four-week gestational window (28.4 to 32.6 weeks) when each fetus was measured once. The study revealed right kidneys had a larger transverse and antero-posterior dimension when compared to left kidneys, resulting in larger calculated renal volumes. No difference, however, was found between kidney lengths. All kidney measurements were smaller in females than males.[9]

2.4.2 Standard two-dimensional (2D) measurements

Nineteen studies reported on a standard two-dimensional (2D) ultrasound measurement.[9, 13, 15-23, 29, 31, 32, 34, 36-39] Standard 2D measurements of the fetal kidneys were the earliest and simplest method utilised to assess kidney size at different gestational ages. [41, 42] Most reviewed

studies involved a low risk, uncomplicated pregnancy to obtain normal fetal kidney nomograms.[16-20, 23, 32, 36, 38]

A longitudinal design was used by four studies that reported on 2D measurements[19, 22, 32, 37], with the best quality longitudinal study being Van Vuuren et al.[32] This study of 96 participants measured all three dimensions of the kidney and clearly defined how GA was determined. Good longitudinal data was obtained every two weeks. Participants were divided into two groups. Each group came every four weeks, however, one group started at 16 weeks and the other at 18 weeks. Reference charts were constructed for kidney length, antero-posterior diameter, transverse diameter and volume.[32] In the other longitudinal studies, one did not report how GA was determined[37], another only measured kidney length[19] and the third study had a small sample size of ten normal pregnancies.[22]

Overall, evidence from the selected studies using standard 2D ultrasound measurements suggest fetal kidney growth correlates positively with GA. The velocity of kidney growth is highest between 26 and 34 weeks in appropriately grown fetuses. This was termed the "critical period" for fetal kidney growth[37] and was supported by other studies. [9, 34] Kidney size was linked positively with fetal weight and size.[9, 18, 38]

Few studies used standard 2D methods to investigate fetal kidney growth in abnormal fetal growth. Compared to appropriately grown fetuses, kidneys of IUGR fetuses demonstrated significant reductions in transverse and antero-posterior dimensions.[31] This is particularly marked during the critical kidney growth period (26 to 34 weeks). No significant difference was demonstrated between the renal lengths of IUGR fetuses compared to appropriately grown fetuses.

2.4.3 Renal volumes – Calculated from two dimensional (2D) measurements

Kidney volume calculations should technically be a better estimate of overall kidney size and shape than single linear measurements.[43, 44] Traditionally fetal renal volumes were calculated using 2D

ultrasound measures. Three orthogonal kidney diameters are applied to the volume formula of an ellipsoid shape to obtain a volume estimate (length x transverse x antero-posterior x 0.523).[45] The ellipsoid formula was used to calculate renal volumes in nine studies.[9, 18, 20-22, 32-34, 38] Findings from the studies of appropriately grown fetuses in normal pregnancies demonstrated fetal kidney volume increases exponentially until birth[32] or with some slowing of growth velocity after 36 weeks.[20]

Studies assessing fetal renal 2D volumes during abnormal fetal growth were scarce. One such study was a large study, however was cross-sectional in design and examined a very narrow GA window of 28.4 weeks to 32.6 weeks (median age 30.4 weeks).[9] Their findings suggested that IUGR, placental insufficiency and fetal redistribution of blood flow result in decreased fetal kidney volumes, around the critical kidney growth period of 26 to 34 weeks. Smaller kidney volumes were also associated with reduced amniotic fluid suggesting an association with fetal kidney function.[9] Another cross-sectional study of IUGR fetuses reported considerable reductions in the kidney volumes of IUGR fetuses.[21] When corrected for fetal weight, there was a 15% reduction of kidney volume for IUGR fetuses compared to appropriately grown fetuses.[21]

No studies were found evaluating fetal kidney growth in large for gestational age (LGA) fetuses. Fetal kidney growth in hyperglycaemic pregnancies was investigated by one study.[33] This longitudinal study compared 2D kidney volumes of normoglycaemic pregnancies with hyperglycaemic pregnancies. Often fetuses of hyperglycaemic mothers are LGA due to organomegaly and increased fat deposition from the increased glycogen to the fetus[33], however, the relationship between fetal size and kidney size was not reported. This study demonstrated maternal hyperglycaemia was associated with alterations in fetal kidney volume. The median fetal kidney volumes of hyperglycaemic pregnancies were significantly larger than the 75th percentile for normoglycaemic pregnancies.[33] This finding warrants further examination.

2.4.4 Renal volumes – Three dimensional (3D)

Three dimensional (3D) techniques were used to calculate fetal kidney volumes in six studies. One of two methods were used to obtain 3D volumes: the multiple parallel plane method (multiplanar) or the rotational Virtual Organ Computer-aided AnaLysis (VOCAL) method. The older multiplanar technique is utilised in the four earlier studies, between 2000 and 2008[24-27] and involves manually tracing multiple adjacent sequential planes of the organ. VOCAL is the newer, more automated volumetric tool employed by the two later 3D studies (2009 and 2013).[28-35]

Most of the included 3D studies focused on constructing reference curves for 3D fetal kidney volumes correlating to GA. Only one of these studies was longitudinal in design and able to truly assess growth, unfortunately this study had the narrowest gestational range of between 24 to 34 weeks.[26] All studies reporting on 3D kidney volumes demonstrated substantial differences of reported "normal" values. For example, two similarly designed studies published the same year, from the same country, using the same equipment reported at 35 weeks a kidney volume of 7.9mL as the 50th centile in one study[24] and below the 5th centile for the other.[25] Even when reviewing two studies using the newer VOCAL method, one study reported the 50th centile of the right kidney at 34 weeks as 21.8cm^3 [35] and the other reported 11.7cm^3 as their 50th centile at 34 weeks.[28] These considerable variations are highly unlikely to be due to different population characteristics. In clinical practice, it would be impossible to know which reference curves to use and these results should therefore be used with caution.

3D ultrasound assessment of abnormal fetal kidney growth was evaluated in only one study. This study compared 3D fetal renal volumes between IUGR fetuses and appropriately grown fetuses.[27] Volumes in IUGR fetuses were significantly smaller when compared to appropriately grown fetuses.[27] This evidence is not strong as this one cross-sectional study measured volumes only once in 28 IUGR fetuses and the considerable variation in normal 3D fetal kidney volumes is likely to also be an issue in measurements of abnormally grown fetuses.

2.4.5 Other ultrasound techniques

Five studies investigated other ultrasound techniques to assess fetal renal growth or size, with a focus on the kidney parenchyma.[13, 14, 29, 30, 39] The kidney parenchyma has more recently become an emphasis of investigation as it contains the nephrons. In a hydronephrotic kidney, the length, transverse and antero-posterior dimensions and kidney volume may be normal or above normal. However, the parenchyma may be thinner than normal and the kidney may have impaired function.[46]

The study by Devriendt et al.[39] measured cortical thickness (from the outer renal capsule to the external limit of the pyramids) and medullary thickness (the pyramid from the papilla to its base) of the parenchyma separately and demonstrated that these measures increased with GA. There was poor reproducibility with an inter-observer variability of 16.5% for cortical thickness and 28.6% for medullary thickness[39]. This was likely due to the various and changing shape of the renal pyramids, making accurate and reproducible placement of the calipers difficult.

In contrast, Hadar et al.[30] conducted a study measuring the entire kidney parenchyma from the renal capsule to the sinus-pyramidal interface and indicated there was a gradual linear growth of the parenchyma with increasing GA.[30] Both the anterior and posterior parenchyma thicknesses were measured in transverse and longitudinal planes. Their findings demonstrated that measuring parenchymal thickness on longitudinal sections was more reliable and reproducible than transverse measurements and had significantly better intra and inter-observer variability than Devriendt et al.[39] at 0.6% and 8.8% for anterior parenchyma and 3.5% and 2.4% for posterior parenchyma respectively.[30] Supporting the consistency of this study[30] is that measurements were completed while performing the ultrasound rather than measured offline, by a reader, from archived images, as was done in the study by Devriendt et al.[39]

The echogenicity of the parenchyma was subjectively evaluated and compared to kidney size by one study.[29] This study reported a significantly higher biparietal diameter/kidney length ratio in those fetuses with hyperechogenicity of the kidney parenchyma and proposed this parenchymal hyperechogenicity is an indicator of depression of fetal renal perfusion. These results should be interpreted with caution as their standard deviation for fetal kidney length was large at 5.4mm and the study had other technical issues, including a non-validated technique to assess fetal hypoxia.[29]

Kidney parenchymal area was measured in two other studies.[13, 14] Unfortunately, they were retrospective in design and the entire area of the kidney in transverse and longitudinal was measured. These two studies in fact did not measure renal parenchyma and actually measured kidney area. Both used their data to develop nomograms of kidney area.

2.5 Discussion

After reviewing 28 studies using ultrasound to assess fetal kidney development, this systematic review revealed several ultrasound techniques to evaluate fetal kidney growth. These techniques had a wide variety of sensitivity and reproducibility. Identification of abnormal kidney morphology or growth is aided by availability of normal fetal kidney biometry charts. Unfortunately, this review identified most studies had some methodological limitations.

There is limited data on actual kidney growth. Some studies reported kidney growth as an outcome, however, they were cross-sectional in design.[13, 14, 24, 25, 30, 38] It is common for size and growth to be confused and used interchangeably. A limitation of cross-sectional studies is they are not appropriate to evaluate growth and can only be used to produce kidney size reference curves.[47] Only one cross-sectional study recognised this limitation.[20] Longitudinal studies can overcome this limitation as the same fetal kidneys are measured at multiple time periods during the pregnancy. A large, longitudinal study of kidney size and volume would provide reference curves for kidney size and growth.[47]

In IUGR fetuses, AP and TS dimensions were significantly decreased, but kidney length remained similar to appropriately grown fetuses. The kidney shape changes to long and skinny or "sausage-shaped" as described by Konje et al.[31] and suggests the thinning of the kidney could be due to fewer layers of nephrons. Lampl et al.[22] investigated effects of maternal smoking during pregnancy on fetal kidney growth, proposing maternal smoking affected growth patterns, resulting in long thin "sausage-shaped" kidneys late in the third trimester. Unfortunately, the number of participants with recorded birth outcomes was small (6 smokers and 21 non-smokers) and thus the results deemed insufficient to suggest any meaningful outcomes.[22]

Kidney length is the most popular single fetal kidney 2D measurement used in current clinical practice and in the selected studies. It was not, however, seen to be a good indicator of growth. This may make kidney length more useful for estimating GA, where dates are uncertain[16, 19, 23] and highlights its lack of sensitivity in assessing alterations in fetal kidney growth.

Kidney volume calculations estimate overall kidney size and shape. 2D volumes are simpler and quicker than 3D volume calculations and can be done with any basic ultrasound equipment. 3D volume calculations require higher-level ultrasound equipment with additional 3D-specific transducers and proprietary software. The disadvantage of calculating fetal kidney volumes from 2D measurements is it erroneously assumes that the kidney is an ellipsoid shape. Compared to the gold standard of fluid displacement, an *in vitro* study demonstrated using the ellipsoid formula underestimated actual renal volumes by 24%.[48]

3D ultrasound volume calculations are not dependent on an assumed geometric shape and therefore are thought to more precisely estimate volumes of irregular shaped organs.[49] Large variations between reported results for these 3D studies is likely due to methodological inconsistencies. It is apparent from the selected studies that there is substantial variation in data collection, analysis and presentation of 3D volumes. This is mostly due to ultrasound machines having proprietary file formats that prevent viewing and analysis of data sets outside the specific

machine brand. Universal standardisation of 3D file formats and software, regardless of equipment used, is overdue.

All studies describe the technique utilised to acquire 3D data sets and subsequent 3D volume calculations; however, the studies lack explicit image landmarks for volume acquisition and calliper placement for volume measurement. This is necessary for any ultrasound measurement to provide accurate and reproducible results. This is even more crucial when calculating 3D volumes as several planes need to be consistently demarcated and any error in calliper placement is multiplied over the volume.[50]

By far the biggest issue compromising the evidence from all the reviewed 3D volume studies is the lack of acceptable reproducibility data. Two studies did not report any intra or inter-observer variability.[24, 25] The other four reported either only intra-observer variability, when there was one operator[26, 27], or both intra and inter-observability, if there was more than one operator.[28, 35] Surprisingly all four studies only assessed reproducibility on the analysis and measurement of the already obtained 3D data set. Errors introduced during acquisition of the 3D data set can be a considerable source of error. Factors such as the depth of the kidney, the number and orientation of the slices and movement of the patient, the fetus or the probe all affect spatial accuracy and can significantly influence the 3D kidney volume obtained.[26] It is important to assess the variability of the post-processed images; however, it is illogical for all studies to ignore the variability associated with 3D data acquisition.

Additionally, it was not clear in most studies how many operators had obtained the 3D data sets and no information on their qualifications and skill level. It was also unclear if the same person acquiring the data was analysing the data. These omissions may considerably influence the quality of the results. In most clinical settings ultrasound scans are performed by multiple operators and variability is unavoidable. This needs to be accounted for, or at least, it needs to be reported along

with what quality assurance steps were taken to try and maintain some consistency and standardisation of measurements.

3D ultrasound equipment and software are expensive and not as readily available as 2D ultrasound equipment. 3D imaging has a higher workload than 2D measurements: longer acquisition times, followed by time for post processing the volume of interest. The quality of the acquired image significantly affects the 3D outcome. All studies report that performing the 3D acquisition requires a "quiet" fetus which can take substantial time to achieve.[24] In summary, the reviewed 3D ultrasound studies had no acceptable evidence of true intra or inter-observer variability and therefore good reliability and reproducibility of 3D volumes has not yet been demonstrated.

Hyperechogenicity of the fetal kidneys may be associated with disruption of kidney growth and changes in kidney function. Suranyi et al.[29] investigated the echogenicity of the parenchyma and fetal kidney size to establish a correlation with fetal hypoxia. Their findings, however, are questionable for several reasons. Hyperechogenicity of the kidney medullae was subjectively established by comparing the echogenicity of the kidney to the liver or spleen. The authors stated that kidney hyperechogenicity was a sensitive sign of fetal hypoxia and they appear to use this to determine severity of fetal hypoxia.[29] Renal parenchymal echogenicity and corticomedullary differentiation are reported in the literature, however, are yet to be validated.[39] In 2003, as it is today, fetal medullary hyperechogenicity is not a validated or clinically used method to establish the presence or absence of fetal hypoxia.

During fetal hypoxia, blood flow is redistributed away from the kidneys to more essential organs.[51] Suranyi et al.[29] propose that this reduced kidney perfusion possibly delays fetal kidney development resulting in hypoplasia of the kidney and a hyperechogenic appearance. The role of the kidney parenchyma and blood flow in assessing fetal kidney growth and future renal function has not yet been well evaluated. Preliminary data from term neonates[52] and children[53] indicates parenchymal

thickness may be a more reliable investigative method to define normal and abnormal kidney development. More research is needed to validate these propositions.

Magnetic resonance imaging (MRI) is being increasingly utilised to image the fetal kidneys and provides excellent delineation of anatomy.[54] Fetal kidney measurements and volumes have been described with MRI.[55, 56] There are still some safety concerns around MRI for the fetus, such as biological effects and acoustic noise.[54] MRI is more expensive than ultrasound, is limited by fetal movement, has several contraindications and has similar problems to ultrasound with regards to maternal obesity. Ultrasound imaging provides real-time images, is non-invasive and relatively inexpensive and is still the modality of choice for routine evaluation of fetal kidney growth.[54, 57]

2.5.1 Limitations

The search was restricted to English language and studies published from the last 20 years (from 1996) as it was felt that the significant advances in ultrasound imaging made these older studies less relevant. The major limitation of this systematic review is most of the included studies were of a cross-sectional design rather than longitudinal. Widely variable gestational age ranges were analysed. Most studies involved low-risk, uncomplicated pregnancies as most studies were establishing normal ranges.

2.6 Conclusions

Following a review of 28 studies investigating fetal kidney growth using ultrasound, we can conclude that the collective results provide some normal ranges of 2D and 3D fetal kidney size and volume. However, there are few large, good quality, longitudinal studies. There is also a paucity of research into the effects of abnormal fetal growth, particularly overgrowth, on fetal kidney development. Kidney length is the most popular single fetal kidney measurement; however, it does not seem to be a good indicator of growth. In IUGR fetuses, kidney length remained similar to appropriately grown fetuses whereas antero-posterior and transverse dimensions were significantly

decreased. Currently there is no easily reproducible, sensitive method for measuring changes in fetal kidney growth. New ultrasound techniques concentrating on the parenchyma of the kidney and the perfusion to the kidney may provide improved information on fetal kidney development.

2.7 Update: Recent Progress in The Evaluation of Fetal Kidney Growth Using Ultrasound (Jan 2017 – June 2020)

2.7.1 Introduction

This section summarises recent progress in the literature that was published after January 2017 as this was the period the above published systematic review included up until. During this time, the studies for this thesis were being conducted.

2.7.2 Method

A systematic review of observational studies was conducted using the same protocol designed for the above paper "Evaluation of fetal kidney growth using ultrasound: a systematic review" which was published in the European Journal of Radiology. The protocol follows the PRISMA guidelines. The same databases and search strategy were used other than the search time was between January 2017 to June 2020. Only studies reporting on an ultrasound technique assessing fetal kidney growth were included.

2.7.3 Results

A total of 177 articles were identified. After review of the title and abstract and removal of duplicates, the full text of seven of these were assessed for eligibility for inclusion. All seven were eligible for inclusion and a summary of the main characteristics of these studies are presented in Table 2.1. All studies were prospective with four cross-sectional in design[58-61] and three longitudinal.[62-64] Two studies were both from the ongoing Gomeroi Gaaynggal study, which is a prospective longitudinal cohort study involving mothers and their Indigenous Australian fetus into childhood.[59, 63] These two studies used a sample from the same cohort of participants, however,

they reported on different renal measurements and analysed them with different variables. For example, one study used only one time point from the 3rd trimester[59] and the other used measurements from each of the three trimesters.[63] Consequently, both studies were kept in the review.

Participant selection methods, the number of participants eligible, included, excluded, and lost to follow-up was poorly reported, as was the number of operators performing the ultrasound examinations and missing data points. No study clearly outlined all of these items as recommended in the STROBE guidelines.[65] GA was most commonly estimated using LNMP or first trimester ultrasound or both. Only one study did not state how GA was estimated.[64]

Table 2.4
Main characteristics of studies from update of systematic review (2017 – 2020)

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Barbosa et al. 2019 [62]	Brazil	P/Long	Normal AGA singleton preg	115	LNMP & 1st trimester US	14 – 40 wks	Approximately every 4 weeks from 14 weeks	TA, 2D, 3 operators KL, TS, AP & 2D volume	References ranges of KL, TS, AP & 2D volume 14 to 39wks with 10th, 50th & 90th centiles. No significant difference between males and females and right and left kidneys.
Osho et al. 2019 [58]	Nigeria	P/CS	Normal AGA singleton preg	470 (not clear if this is the total analysed)	LNMP or 1st trimester US	28 – 42 wks but not clear	Once	TA, 2D, 1 operator KL, TS, AP & 2D volume	Kidney length most accurate single biometric measurement for GA comparing it to FL, BPD, HC and AC. No significant difference between right and left kidneys.
Lee et al. 2019 [59]	Australia	P/CS	Indigenous Australian Singleton preg. Kidney development and maternal adiposity	147 – (not clear)	1st trimester US	>28 wks	Once	TA, 2D, Nº operators NR KL, TS, AP, 2D volume, EFW	In late preg, fetal kidney volume relative to EFW is negatively associated with maternal adiposity. No association between infant kidney function and maternal adiposity measures.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Diehm et al. 2018 [63]	Australia	P/Long	Indigenous Australian Singleton preg. Smoking exposure and none	158	1st trimester US	20 – 40 wks but not clear	Each trimester (1st, 2nd, 3rd) might have more but no clear	TA, 2D, N° operators NR KL, TS, AP, 2D volume	Strong relationship between EFW and combined kidney volume. Kidney volume of fetuses from mothers who smoke are small than mothers that don't however were in proportion to birth weight and disappears when gender is considered.
Edevbie et al. 2018 [61]	Nigeria	P/CS	Normal AGA singleton preg (not clear)	400	LNMP or 1st trimester US	20 – 41 wks	Once	TA, 2D, N° operators NR KL	Kidney length most accurate single biometric measurement for GA comparing it to FL, BPD, HC and AC. Left kidney was significantly longer than the right.
Nagar et al. 2018 [60]	Israel	P/CS	Normal AGA singleton preg group & fetal renal abnormality group	AGA = 210 Abnormality = 9	LNMP & 1st trimester US	14 – 40 wks	Once	TA, 2D, N° operators NR Ratio of AP kidney: AP abdomen in transverse at level of renal pelvis	Renal AP to abdominal AP ratio was constant throughout pregnancy. Very good intraobserver and good interobserver reliability.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Hindryckx et al. 2017 [64]	Belgium	P/Long	Normal AGA singleton preg group & single functioning kidney (SFK) group	AGA = 58 SFK = 74	NR	20/24 – 36 wks	AGA – every 4 wks. SFK – every 4wks or more	TA, 2D, 3D, 2 operators KL, TS, AP, 2D volume, 3D VOCAL	2D & 3D volumes significantly higher in SFK group. 3D volumes greater than 2 D volumes. No correlation between prenatal or postnatal SFK volume and GFR at 2 years of age.

Note: AC, abdominal circumference; AGA, appropriate-for-gestational age; AP, anterio-posterior; BPD, biparietal diameter; CS, cross-sectional; EFW, estimated fetal weight; FL, femur length; GA, gestational age; HC, head circumference; KL, kidney length; LNMP, last normal menstrual period; Long, longitudinal; NR, not recorded; P, prospective; Preg, pregnancy; SFK, single functioning kidney; TA, transabdominal; TS, transverse; US, ultrasound.

Generally, the ultrasound measurements and methodology were well described. All seven studies investigated 2D ultrasound measurements of the kidney, with only one of these studies also obtaining a 3D volume of the kidneys using the VOCAL technique.[64] Most of the studies (5/7) measured all three linear dimensions of the kidney (length, AP, TS) and then used the ellipsoid formula to calculate a 2D volume.[58, 59, 62-64] Fetal kidney length was found by two studies, to be the most accurate single biometric measurement for estimating GA in the 2nd and 3rd trimester when comparing it to femur length, bi-parietal diameter (BPD), head circumference and abdominal circumference.[58, 61]

One longitudinal study involving a low-risk pregnancy cohort created reference charts for kidney length, AP and TS dimensions and kidney volume. The authors provide little information on the statistical analysis used to generate the charts other than stating they were built by quantile regression analysis for each measurement related to GA.[62] No table reporting the mean and SD of each measurement and number of participants at each gestational week, and no scatter diagram of raw data is provided.[62]

A novel ratio of the AP dimension of the kidney to the AP dimension of the abdomen in the transverse plane at the level of the renal pelvis was found to be constant throughout pregnancy. This ratio was then evaluated for its use in identification of alterations in normal kidney size.[60] The reliability of the ratio was also assessed and the intraobserver reliability was found to be excellent (ICC = 0.95) and the interobserver reliability as moderate (ICC = 0.72).[60] The authors suggest this ratio can be used as a marker for screening renal abnormalities, however so far, they have only used the marker to assess nine cases of renal abnormality that did not demonstrate any hydronephrosis.

The two reports from the Gomeroi Gaaynggal study were concerned with factors that may affect fetal kidney development. Maternal smoking was associated with significantly smaller

2D fetal kidney volumes than when compared with those from mothers who did not smoke. However, this effect disappeared when gender was taken into consideration.[63] This is likely due to fetal kidney volumes of males being higher than females and male kidney volume to body weight ratio also higher than in females.[63] Generally, fetal kidney measurements in the third trimester were not associated with maternal body fat or pre-pregnancy BMI other than the right AP diameter being positively associated with maternal body fat, however not strongly ($p = 0.03$, $r^2 = 0.2$).[59] The left fetal 2D kidney volume to EFW ratio and the combined fetal kidney volume to EFW ratio were negatively associated with maternal body fat in the third trimester, but again the effect was small ($p = 0.04$, $r^2 = 0.02$ / $p = 0.03$, $r^2 = 0.03$, respectively).[59]

2.7.4 Discussion

This update on the earlier systematic review revealed seven studies which have evaluated fetal kidney growth between January 2017 and June 2020. Remarkably, there were not any significantly new techniques or advances reported on since the systematic review was published in 2017.[66] Furthermore, it was disappointing to see that there continues to be vague reporting of some aspects of studies even when increasingly journals are insisting on following guidelines such as STROBE for reporting observational studies.[65]

Several studies investigated normal fetal kidney lengths and its accuracy to determine GA.[58, 61, 62] Reference ranges of fetal kidney measurements and the use of kidney length as an estimate for GA has now been well established in multiple different populations.[9, 15-17, 19, 20, 23, 32, 38] These new studies of normal kidney length ranges and their accuracy to estimate GA provide little additional information.[58, 61, 62] They do, however, continue to support the principle that fetal kidney length is not a sensitive method to assess alterations in kidney

growth, for example in cases of fetal growth restriction, as the kidney length appears to be maintained.[22, 31, 34, 37]

The reference charts for kidney length, AP, TS and volume by Barbosa et al.[62] were developed using a non-parametric method which is not commonly used to derive fetal charts.[47, 67] The selection of an appropriate statistical methodology for fetal charts is important for accuracy, particularly if clinical decisions are to be based on these charts. It is known that there is increasing variation in fetal measurements with increasing GA.[47, 68] Additionally, the between-subject variability also tends to increase with GA.[67] These charts do not appear to account for how variability (SD) is changing with increasing GA and this can have significant impact on the accuracy of the charts. The paper does not provide a summary table reporting the sample size, mean and SD of each measurement for each gestational week. There is also no verification that the centiles are a good fit to the data, for example, no scatter diagram of the raw data with the centiles superimposed. Currently, mixed effects (multilevel) modelling with fractional polynomials is suggested as the best method to model growth data.[47, 67, 69]

It was predicted that there would be more studies investigating the use of 3D volumes of kidneys since the earlier review, however this was not the case with only one study including 3D kidney volumes.[64] The difficulties previously highlighted, such as no universal standardisation 3D file formats and software, no standardised image landmarks, no established calliper placement for volume acquisition and measurement, and poor reproducibility, have not yet been overcome.[66] Obtaining a 2D kidney volume from linear measurements was more prevalent.[58, 59, 62-64] It is relatively easy to obtain a 2D volume from three orthogonal linear measurements of the kidney and it does not require additional equipment to the standard ultrasound machine.

The studies in this review utilised similar 2D measurements as was reported in our earlier review, however, there were some novel applications of these measurements, particularly to account for kidney size relative to body size. A ratio of the AP diameter of the kidney to AP diameter of the fetal abdomen was found to be constant throughout pregnancy in a low risk pregnancy group and was then tested on a group of nine pregnancies with suspected renal anomaly but no hydronephrosis.[60] This ratio may be sensitive to changes in kidney size, however, is not specific for diagnosis and has not been validated in cases of fetal growth restriction or large for gestational age fetuses. Furthermore, upper urinary tract dilatation (hydronephrosis) is one of the most diagnosed fetal abnormalities by ultrasound.[70-72] Therefore, if this ratio is unable to be used in cases of hydronephrosis its usability is limited. At this time, it is not possible to use it as a diagnostic criterion for renal abnormalities and will need to be further validated in larger cohorts of abnormal fetal growth.

Aside from congenital renal abnormalities, factors that might affect the development of the fetal kidneys in a high-risk population for kidney disease and hypertension were assessed by analysing 2D kidney volumes in fetuses.[59, 63] Both maternal body fat and maternal smoking were linked to alterations in fetal kidney development, particularly when the fetal kidney volume to overall fetal size was considered. The study by Lee et al., appears to be the only study so far to investigate any association between maternal adiposity measures and fetal kidney development.[59] Evidence from these studies has not yet demonstrated a strong association between maternal smoking or maternal obesity with altered kidney growth. The Gomeroi gaaynggal study is still ongoing and therefore, a larger sample collected over a longer time may present more definitive data. Obesity during pregnancy, as well as smoking and poor maternal health are all thought to have a negative effect on renal development and further research is required in this area.[73-76]

2.7.5 Future research

Overall, the disadvantage of 2D measurements and 3D volumes are that they include the entire kidney containing the parenchyma and the collecting system. As proposed in the earlier review, measuring only the renal parenchyma concentrates the analysis on the important functional part of the kidney containing the developing nephrons. Therefore, methods such as measuring renal parenchymal thickness, renal volume to parenchymal thickness ratio and renal perfusion may give us more meaningful information on nephron endowment and future renal function. Larger, longitudinal, rather than cross-sectional, studies are needed to assess fetal kidney growth in compromised intrauterine environments. These are encouraging parameters that should be investigated to see if they can provide improved diagnostic criteria for renal abnormalities and be a better predictor of future renal disease

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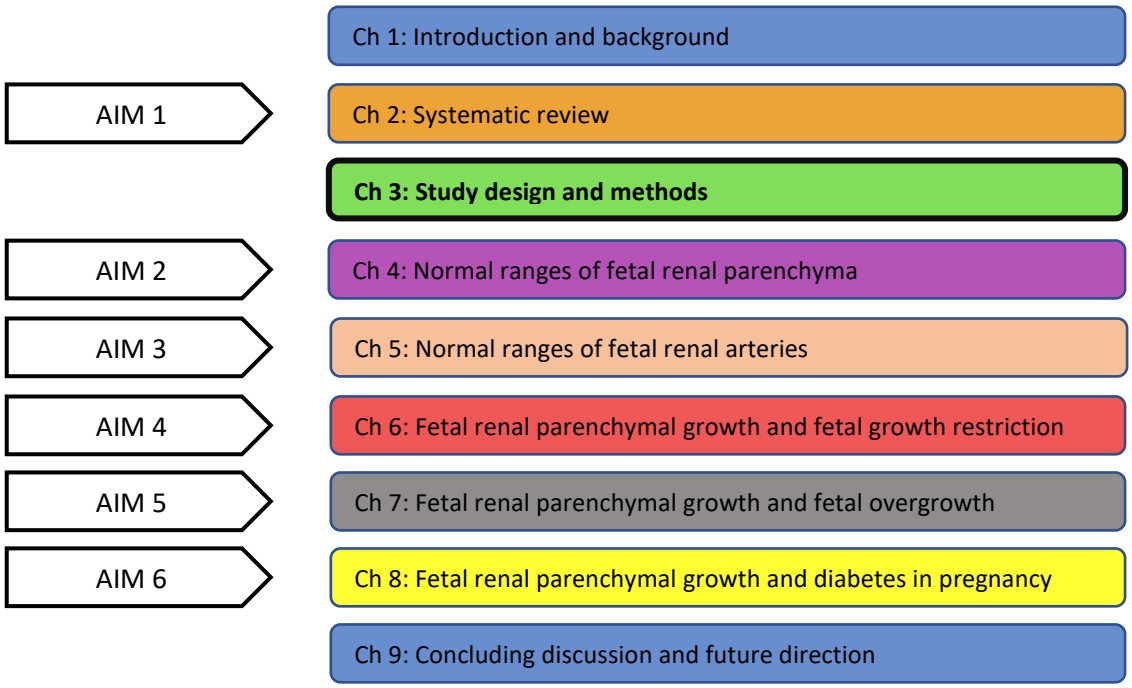
Chapter. 3 Study Design and Methods



At the beginning of this thesis the research protocol for the overall study was published. The reference for this paper is below and the paper is attached in Appendix D.

Brennan S, Schneider M, Watson D, Kandasamy Y, Rudd D. The renal parenchyma - evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study. BMJ Open. 2017; 7(12).

As the thesis evolved, portions of the research protocol changed. Therefore, the original published paper referenced above has been updated for this chapter so that the chapter accurately reflects how the study was performed. The formatting of section sub-headings and references, and numbering of figures and tables have been modified from the original publication to match the thesis format. The wording has also been changed to past tense. This chapter describes the study design and methods of the overall study. As the results

chapters are publications, included in chapters 4 to 8 is additional relevant methodology specific to the study for that chapter.

3.1 Introduction

Chronic kidney disease is an increasing contributor to the global burden of disease, with hypertension now the leading risk factor.[1] Recognition of the risk factors and implementation of preventive strategies is crucial to reducing hypertension and kidney disease. Abnormalities in fetal growth, such as fetal growth restriction (FGR), have a profound effect on the kidney development.[2, 3] The association between an adverse intrauterine environment and chronic kidney disease later in life is now compelling.[4-6]

During early fetal life, there is transient development and regression of the primary (pronephros) and secondary (mesonephros) fetal kidneys between day 23 and day 112 of embryonic life.[7] The permanent functional tertiary fetal kidney is the metanephros which begins developing on day 30.[7] Nephrogenesis involves the formation of the functional units of the kidney called nephrons and continues up to 36 weeks gestational age.[8, 9] It is essential that appropriate nephrogenesis is achieved *in-utero* as the number and quality of nephrons directly influences lifetime kidney function.[10]

FGR can result in a significant reduction in nephron number, however, being large for gestational age (LGA), particularly related to maternal hyperglycaemia, is also associated with abnormal fetal kidney development and an increased risk of hypertension and chronic kidney disease.[11] A better understanding of the relationship between abnormal fetal growth and nephrogenesis is needed. Presently, the only accurate method of calculating human nephron number is during an autopsy.[12] A non-invasive measure of nephron endowment is needed.

Ultrasound is the primary imaging modality for evaluating fetal kidneys. We conducted a systematic review into the evaluation of fetal kidney growth using ultrasound which revealed there are few good quality, longitudinal studies.[13] The most commonly reported ultrasound measurement was renal length; however, renal length alone was not found to be very sensitive to evaluate disruptions in fetal kidney growth in the presence of FGR.[13] Few studies analysed the effects of FGR on fetal kidney growth and no studies have, to date, analysed if LGA has an effect on fetal kidney growth. Results from 2D and 3D renal volume calculations were disappointing. Volumes calculated from 2D measurements underestimate renal volumes by as much as 24%.[14] Substantial variations were reported for "normal" 3D kidney volumes and good reliability and reproducibility has not yet been demonstrated. Currently there is no easily repeatable, sensitive method of measuring changes in fetal kidney growth.[13]

Measuring the renal parenchyma with ultrasound is a novel method to assess fetal kidney development and predict future renal function. Measuring just the renal parenchyma will measure only the important functional part of the kidney which contains the nephrons (Fig. 3.1). One small cross-sectional study measured fetal renal parenchyma in normally grown fetuses[15] however, no studies have evaluated the fetal renal parenchyma in abnormally grown fetuses. Preliminary data from term neonates[16] and children[17] indicates that the parenchymal thickness may be a more reliable investigative method to define normal and abnormal kidney development. Methods such as measuring the parenchymal thickness, renal volume to parenchymal thickness ratio, renal artery Dopplers are potential non-invasive methods to evaluate nephron endowment and future renal function.

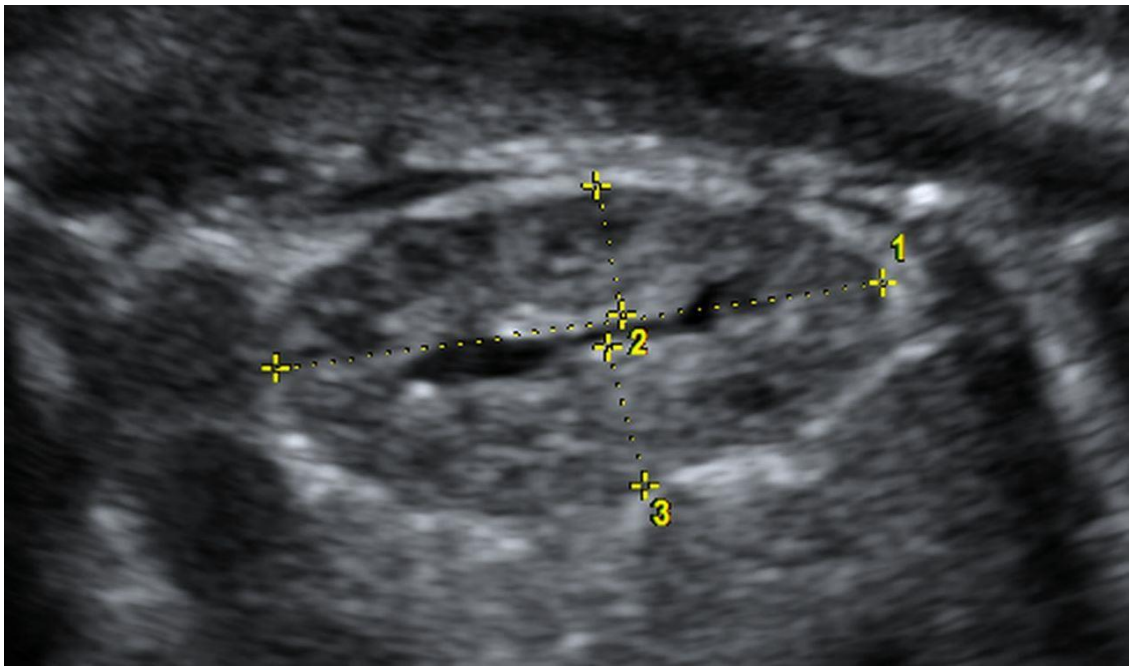


Figure 3.1 Measurement of kidney length [1] and the anterior [2] and posterior [3] fetal renal parenchymal thickness from the inner aspect of the renal capsule to the sinus-pyramidal apex interface. Source: Townsville University Hospital.

The aim of this study was to use ultrasound to assess the fetal renal parenchymal growth in a pregnant population demonstrating either normal or abnormal growth, or diabetes in pregnancy, and determine if these abnormal fetal conditions influence fetal renal parenchymal growth. Non-invasive ultrasound techniques were used. The results could help identify factors that adversely affect kidney development so that they could be modified by public health interventions and education programs. This may promote improved fetal nephron number and quality at birth and reduce susceptibility to chronic disease in later life.

3.2 Methods and Analysis

3.2.1 Study design and setting

This was a prospective, longitudinal, observational study conducted between May 2017 and February 2019, in the Ultrasound Department of the Townsville University Hospital,

Australia. Townsville Hospital and Health Service (THHS) provides tertiary perinatal services to North Queensland, which has a catchment area of around 700,000 with 10,000 births per year.[18]

3.2.2 Participants

Pregnant women of 18 years or older, with an accurately dated singleton pregnancy of 16 weeks gestation or more were included. Gestational age was based on the last normal menstrual period (LNMP) and first trimester ultrasound, that agreed within seven days, or on first trimester ultrasound if LNMP was uncertain. Pregnant women with uncertain dates, multiple pregnancy or any known major congenital fetal abnormality or chromosomal fetal abnormality were excluded. Explicitly, any fetal kidney abnormality, including dilatation of the renal pelvis of ≥ 4mm up to 28 weeks gestation or ≥ 7mm after 28 weeks gestation, was excluded from the study.

3.2.3 Recruitment and consent of participants

Between May 2017 and October 2018, pregnant patients who present to the Medical Imaging Department at the Townsville University Hospital for an obstetric ultrasound were invited to participate. In addition, mixed risk patients were also informed about the study by their treating obstetrician, midwife or sonographer. Detailed written information was given to the patient and written consent obtained. The participant information sheet and consent form are attached in Appendix B.

3.2.4 Study process

Figure 3.2 outlines the flow schedule of the participants. On commencement of the study, participants completed a questionnaire which included demographic, medical and obstetric data. The participant questionnaire form is attached in Appendix B. The first ultrasound scan

was typically performed between 16- to 26-weeks and then follow-up ultrasounds were performed at least every four weeks from the first ultrasound examination. Some women, particularly with high risk pregnancies, required more than one clinically indicated ultrasound. For example, some women with a growth restricted fetus needed to be monitored by ultrasound more frequently than monthly. Conversely, the control group of low-risk pregnant women may have only required one to two clinically indicated scans between 16 to 40 weeks gestation. Therefore, to obtain good longitudinal data, the women were asked to attend for additional research scans every 4 weeks until delivery.

3.2.5 Ultrasound examinations

Three Australian Accredited Medical Sonographers, with at least two years post ultrasound qualification experience, performed all ultrasound examinations. Prior to commencement of the study, sonographers were trained to use a standardised protocol for renal measurements by authors DW and SB. A follow-up audit, to ensure measurement consistency between sonographers, was conducted three months after commencement of the study. The sonographers were not blinded to all clinical and biometric information as most studies included a diagnostic ultrasound scan. A Voluson E8 (GE Healthcare Ultrasound, Milwaukee, WI, USA) or an Epiq 7 (Philips Ultrasound, Bothell, WA, USA) were used for the ultrasound examinations and the highest frequency transducer possible (1-5MHz), which matched the mother's body habitus was selected. When a woman attended for a clinically indicated scan, this was the priority and then the additional fetal renal measurements required for the research study were performed thereafter.

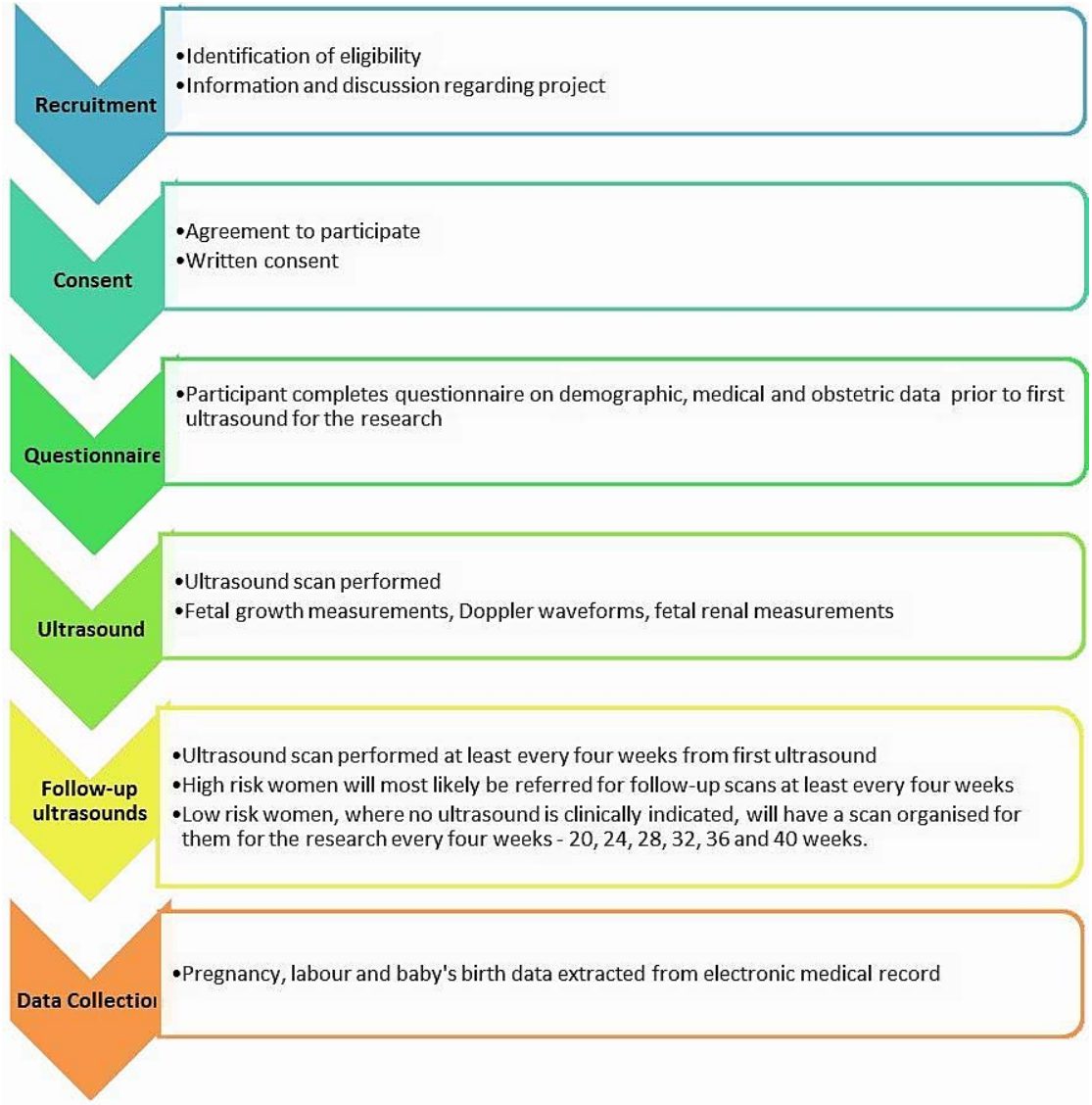


Figure 3.2 Study participants flow chart.

Where possible the fetal kidneys were measured with the fetal spine up (anterior) or as close as possible to this position. All measurements were performed on both kidneys. The image was magnified so that the kidney occupied most of the image. The renal parenchymal thickness measurement was obtained from a midsagittal plane of the kidney by measuring from the inner aspect of the renal capsule to the sinus-pyramidal apex in both the anterior and posterior aspects (Fig 3.1). The maximum kidney length (L) was also measured in this

midsagittal plane. In a transverse section of the fetal kidney, at the level of the renal pelvis, the maximum anteroposterior diameter (H) and transverse diameter (W) were measured. Every measurement was performed twice, with the mean measurement recorded.

Bilateral fetal renal artery Dopplers were obtained in a coronal view of the kidneys. Colour flow was utilised to identify the renal artery arising from the aorta and entering the kidney. A low wall filter of between 30 to 60 Hertz was used and a sample gate of size 2mm to 3mm was placed in the mid trunk of the renal artery. Using an angle as close to 0 degrees as possible, a pulse wave signal was obtained. The mean of at least three consistent, consecutive waveforms was used to calculate the resistivity index (RI) and pulsatility index (PI).

The following routinely performed obstetric measurements were also recorded for the study:

- Single deepest vertical pocket of amniotic fluid
- Umbilical artery (UA) Doppler
- Middle cerebral artery (MCA) Doppler (where clinically indicated or 30 weeks gestation and over)
- Ductus venosus (where clinically indicated)
- Biometry – head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL).

3.2.6 Birth data

3.2.6.1 Outcome measures

Perinatal data was collected from the mother and baby's electronic medical record (Table 3.1).

Table 3.1
After baby's birth, perinatal data was collected from the mother and baby's electronic medical record

	Birth Data to be collected	
Demographic, medical and obstetric history from participant questionnaire	Gestational age at birth	Antenatal steroids
	Birth weight	Other antenatal medications
	Gender	Maternal medical history: e.g.
	Apgar scores at 1 & 5mins	• Diabetes
Onset of labour	Umbilical artery cord PH	• Hypertension
Mode of delivery	Base Excess (BE)	• Renal disease
Admission to NICU or SCN	Lactate	

Note: NICU, neonatal intensive care unit; SCN, special care nursery.

3.2.6.1.1 Primary outcome measure

1. Renal parenchymal thickness – anterior and posterior thickness in the longitudinal plane.

3.2.6.1.2 Secondary outcome measures:

1. Renal volume (RV) - calculated using the formula RV = Length x Width x Height x 0.523
2. Fetal growth biometry – HC, BPD, AC and FL
3. Amniotic fluid – single deepest vertical pocket
4. UA Doppler flow – RI and PI calculated from the average of at least three consecutive waveforms
5. MCA Doppler flow – RI and PI calculated from the average of at least three consecutive waveforms
6. Cerebroplacental ratio (CPR) – calculated using the formula CPR = MCA PI/UA PI, with the last CPR recorded before birth used to assist in group classification

7. Renal artery Doppler flow – RI and PI calculated from the average of at least three consecutive waveforms.

3.2.7 Sample size

Optimal sample size was calculated based on a statistical power of 80% and a significance level of 0.05 (two-tailed). Data from our previously published study[16] has demonstrated that the renal parenchymal thickness was 9.4mm (± 1.1mm) for normal birth weight neonates and 8.3mm (± 1.0) mm for low birth weight neonates at term**.** Therefore, it was estimated that a sample size of 45 will be needed (15 FGR fetuses, 15 LGA fetuses and 15 appropriate-for-gestational-age). Allowing for the possibility of loss to follow-up, at least 20 participants would be recruited for each group resulting in a total of 60 participants, each having an ultrasound scan at least every four weeks.

3.2.8 Data analysis

After the ultrasound examination was completed, and the baby was born, all data was collated, and participants were assigned to different groups:

- Low-risk pregnant women
- Women with an infant born appropriate-for-gestational age (AGA)
- Women with a FGR infant
- Women with an infant born LGA
- Women with diabetes in pregnancy.

The different groups were decided *a priori* and were used for different analyses. Therefore, participants could be in more than one group e.g. a woman could be in the AGA group and the diabetes in pregnancy group. Criteria for the groups and the different analyses performed for each study is explained in detail in the relevant chapter for that study. For the different

analyses, the participant's data were independently analysed and assigned to groups by two authors (SB and DW). Any discrepancies between the authors were discussed and resolved. A third author was available (YK) if a consensus was not able to be reached, however, they were not required. Intraobserver and interobserver reliability were also assessed. Statistical analyses were performed using IBM SPSS Statistics 24 (Armonk, NY, USA), R statistical Language in R studio (v1.2.1335, Vienna, Austria) and Stata/MP (v14.2, Stata Corp LP, College Station, Texas, USA). The full details of the statistical analyses for the different studies is explained in the relevant chapters.

3.2.9 Data management

Data collection commenced in May 2017 and finished in June 2019. Participant data was de-identify and assigned a number code to ensure confidentiality for each woman and baby.

Electronic data was stored and saved on a password protected computer. Hard (paper) copies of the consent form, questionnaire and data sheets from the ultrasound examinations are stored in a locked filing cabinet in the principal researcher's office. This office is a secure room within the Townsville University Hospital. Only the principal researcher and members of the research team had access to the data.

3.3 Ethics

This study was approved by the Townsville Health District Human Research Ethics Committee (HREC/16/QTHS/216) (Appendix A).

3.4 Summary

This was the first study to use a novel ultrasound measurement of the fetal renal parenchyma and measurements of fetal renal blood flow to assess fetal kidney growth. The goal of the

study was to evaluate the measurement of the fetal renal parenchymal thickness and to increase our understanding of fetal kidney growth.

3.5 References

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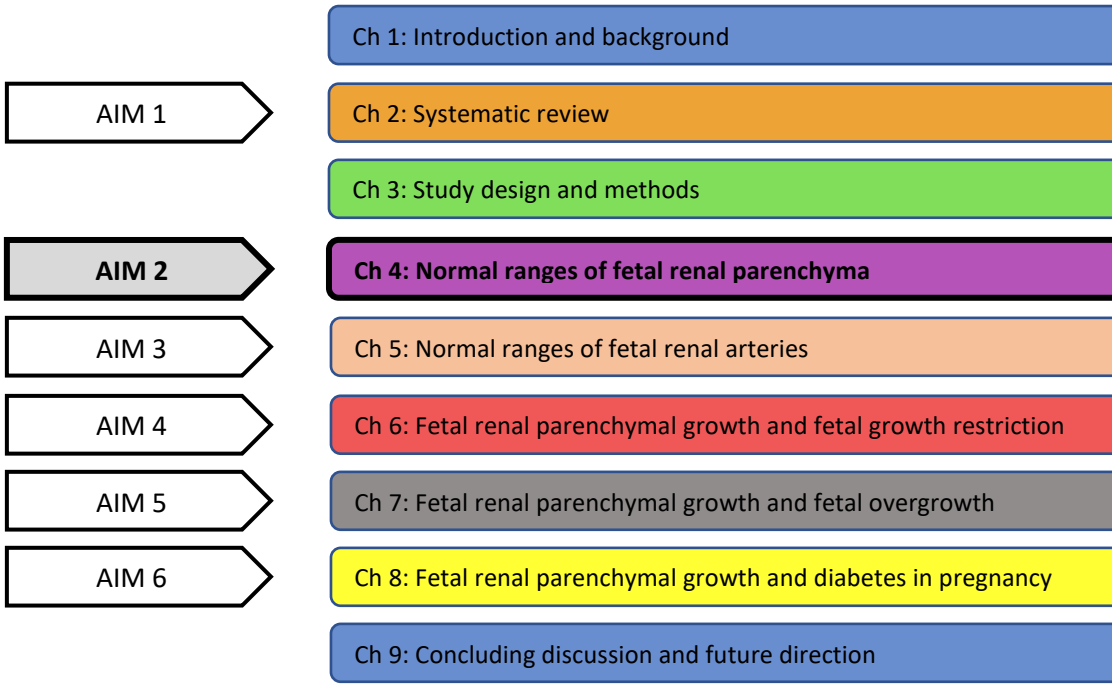
Chapter. 4 Fetal Kidney Charts of a Novel Measurement of the Renal Parenchymal Thickness to Evaluate Fetal Kidney Growth and Potential Function

Brennan S, Kandasamy Y, Rudd D, Schneider M, Watson D. Fetal kidney charts of a novel measurement of the renal parenchymal thickness to evaluate fetal kidney growth and potential function. Prenat. Diagn. 2020;40(7):860-9.



This chapter is a copy of the journal paper, referenced above and attached in Appendix D, except for minor textural modifications. The formatting of section sub-headings and references, and numbering of figures and tables have been modified from the original publication to match the thesis format. Supplementary figures and tables have also been included as part of the main text.

This study presents a novel evaluation of the fetal renal parenchyma to better assess kidney growth and estimate nephron number. The fetal renal parenchymal thickness chart may be useful to aid diagnosis of prenatal kidney disease and predict future kidney function.

4.1 Abstract

Objective

The objective of this study was to develop new standard charts for fetal renal parenchymal thickness, length and volume to define normal ranges for use in clinical practice and to assess the reliability of these measurements.

Methods

This was a prospective, longitudinal study of 72 low-risk singleton pregnancies undergoing serial ultrasound examinations at least every four weeks. Multiple renal measurements were performed on both kidneys at each scan. The renal parenchymal thickness was measured in the mid-sagittal plane. Standard charts were developed and the intra and interobserver reliability for the renal measurements was analysed.

Results

Standard charts were developed for fetal renal parenchymal thickness, length and volume.

Conclusion

We present novel charts which demonstrate the growth of the fetal renal parenchyma during pregnancy. They will be useful in clinical practice to identify any alterations from these normal ranges, which may be an important criterion for assisting prenatal diagnosis of renal pathologies and future studies in the prediction of kidney function.

4.2 Introduction

Normal development of the kidneys during pregnancy is vital for future kidney health. It is well established that abnormal early kidney development is associated with increased risks of hypertension, kidney disease and cardiovascular disease in post-natal life.[1-4] Found within the parenchyma, nephrons are the functional units of the kidneys. Abnormal fetal growth and/or prematurity is thought to impact nephron number and development.[5-7] Ultrasound is the primary imaging modality to visualise fetal kidneys as it is non-invasive, safe and able to accurately measure the size of the kidneys[8]. Obstructive fetal renal pathologies are commonly diagnosed with antenatal ultrasound; however, renal parenchymal pathologies are underdiagnosed due to the lack of objective criteria of the renal parenchyma.[9, 10]

Ultrasound charts of normative fetal kidney growth mostly include kidney length and volume.[11, 12] Kidney length has not proven to be a sensitive method of assessing kidney growth.[13, 14] Kidney volume measurements may appear to be a more accurate measure, however, tends to underestimate actual kidney volumes by around 20% due to inherent variance present in the three orthogonal measurements applied to the formula.[15-17] Furthermore, kidney volume measurements include the entire kidney and therefore, parts of the kidney not directly involved in filtration, such as the collecting system and the capsule are included. There is an unmet need for a more reliable method of detecting alterations in kidney growth.

Ultrasound measurement of the renal parenchymal thickness is a non-invasive, easily performed measurement that focusses analysis on the functional tissue of the kidney.[15, 18, 19] Measurement of the fetal renal parenchymal thickness may aid in the prenatal diagnosis of parenchymal pathologies and be an indirect measure of nephron number and future kidney function.

The aim of this study was to use serial ultrasound scans to develop standard charts for fetal renal parenchyma, as well as kidney length and volume from 16- to 38-weeks' gestation.

4.3 Methods

A prospective, longitudinal, observational study was conducted among a cohort of low-risk pregnancies between May 2017 and February 2019 in the Maternal Fetal Medicine Unit and Ultrasound Department of the Townsville University Hospital, Australia. This study was approved by the Townsville Hospital and Health Service Human Research Ethics Committee (HREC/16/QTHS/216).

4.3.1 Study population

Townsville Hospital and Health Service (THHS) provides tertiary perinatal services to North Queensland, which has a catchment population of around 700,000 with 10,000 births per year.[20] Between May 2017 and October 2018, pregnant patients aged 18 years or older, who presented to the Medical Imaging Department at the Townsville University Hospital for a second trimester obstetric ultrasound scan were invited to participate. Alternatively, patients were informed about the study by their treating obstetrician, midwife or sonographer.

Inclusion criteria were singleton pregnancy up to 28-weeks' gestation, accurate dating based on last normal menstrual period (LNMP) and 1st trimester ultrasound, that agreed within seven days, or on 1st trimester ultrasound if LNMP was uncertain. Exclusion criteria were uncertain dates, multiple pregnancy, congenital or chromosomal fetal abnormality, maternal disease that was likely to affect the growth of the fetus (diabetes mellitus, heart disease, hypertension requiring treatment, kidney disease, pre-eclampsia) and subsequent preterm birth less than 32-weeks.

4.3.2 Study process

Prior to the first ultrasound scan, the participant completed a questionnaire which included demographic, medical and obstetric data. The first ultrasound was planned to be performed between 16- to 24-weeks, however, 11 women had their first ultrasound between 26- and 29-weeks. Women were then asked to attend for ultrasound scans every four weeks from their first scan until delivery. Some women had additional clinically indicated ultrasounds, for example, for decreased fetal movements, and if this ultrasound was performed two or more weeks from the previous ultrasound, renal measurements were performed again for the study. After baby's birth, data relating to the delivery and condition of baby at birth was collected. The gestational ages (GA) of 16- to 38-weeks were used for creation of the standard charts.

4.3.3 Ultrasound measurements

Three Australian Accredited Medical Sonographers with at least two years post ultrasound qualification experience performed the examinations. A protocol clearly outlined the additional renal measurements and how they were to be performed. Training of the sonographers was conducted by the authors (DW and SB) prior to commencement of the study and an audit and follow-up was conducted with the sonographers three months after commencement of the study to confirm adherence to the study protocol. An Epiq 7 (Philips Ultrasound, Bothell, WA, USA) or Voluson E8 (GE Healthcare Ultrasound, Milwaukee, WI, USA) were used for the examinations. To obtain the highest image resolution, the highest frequency transducer possible, matching the mother's body habitus, was selected (1-5MHz).

Where possible the fetal kidneys were measured with the fetal spine anteriorly, or as close as possible to this fetal position. The image was magnified so that the kidney occupied most of the image and the right and left kidney were identified. A midsagittal scan of both kidneys

along their longest length was recorded and the longest length (L) of both kidneys was measured. The anterior and posterior renal parenchymal thickness was also measured in this midsagittal plane. It was measured from the inner aspect of the renal capsule to the sinus-pyramidal interface (Fig 4.1). A transverse section of the fetal kidneys at the level of each renal pelvis was obtained. The maximum anteroposterior diameter (H) and transverse diameter (W) was measured for both kidneys (Fig 4.1). Each measurement was performed twice and the mean of the two measurements recorded. Kidney volume was calculated using the ellipsoid formula: KV = 0.523 × L × W × AP (π/6 x L x W x AP).[21]

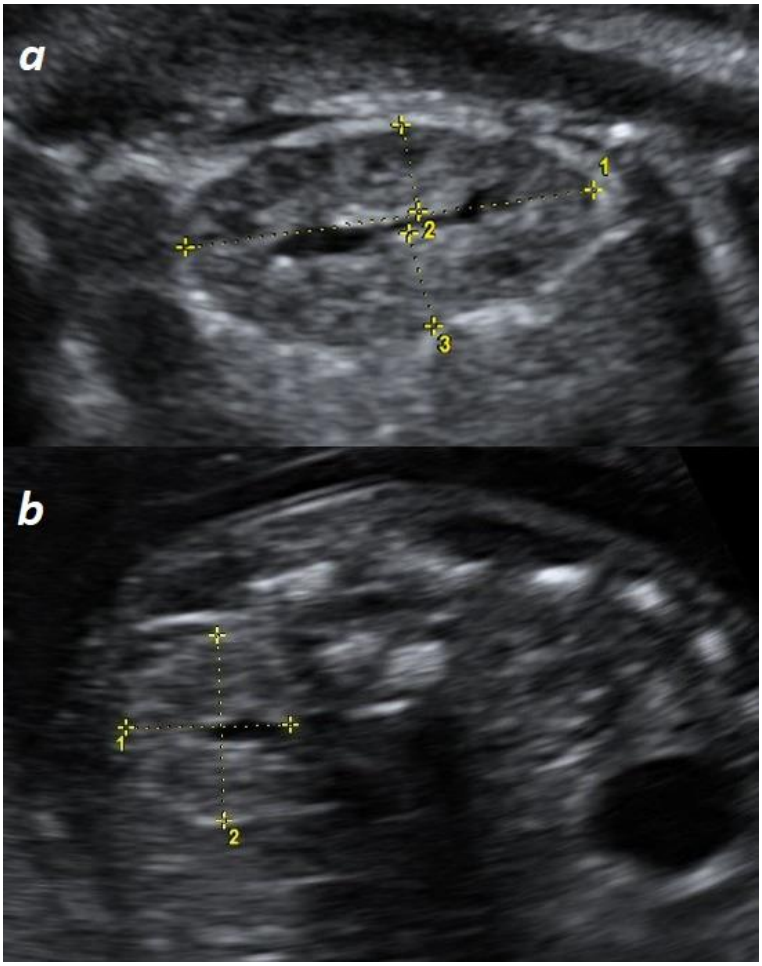


Figure 4.1 Fetal kidney measurements (a) Measurement of kidney length (1) the anterior (2) and posterior (3) fetal renal parenchymal thickness from the inner aspect of the renal capsule to the sinus-pyramidal apex interface at 24 weeks gestational age. (b) Measurement of kidney transverse (1) and antero-posterior (2) dimensions at 20 weeks gestational age. Source: Townsville University Hospital.

4.3.4 Measurement reliability

A sample of 15 pregnancies, some who were a part of the study and some additional pregnancies, were measured for analysis of intra and interobserver reliability. The gestational age range of the pregnancies was 19 to 36 weeks. The three sonographers involved in the study obtained real time measurements of each renal measurement twice. They were blinded to their own and each other's measurements by concealing the measurements on the monitor. The mean of each observer's two measurements was analysed.

4.3.5 Analysis

Maternal and birth characteristics and intra and interobserver variability were analysed using IBM SPSS version 25. Normality of the maternal and birth data was tested using Kolmogorov-Smirnov test and visually inspecting histograms. Normally distributed variables were reported as mean and standard deviation (SD) and non-normally distributed variables as a median and interquartile range. To assess intra and interobserver reliability an ANOVA model was used and SD, Cronbach's alpha (α) and the intraclass correction coefficient (ICC) with 95% confidence intervals were calculated.

All other statistical analyses were performed using Stata/MP v14.2 for windows (Stata Corp LP, College Station, TX, USA) and the graphics were created with ggplot2[22] in R Studio (version 1.2.1335).[23] Statistical significance was accepted as $p < 0.05$. Methods of the INTERGROWTH 21st Project for fetal growth were applied.[24] The Stata function fp was used to ascertain the best fitting model for the mean of each renal measurement as a function of a fractional polynomial in GA (rounded down to whole week). For kidney volume a cubed root transformation was required as models for kidney volume fit poorly (in terms of functional form, non-constant variance, skewness of data at later time points and predicted values less than zero). A two level, mixed effect regression model was then built for each

measurement, including fixed effects for the polynomial function of GA, and accounting for repeated measures on participants using random intercept and slope with unstructured covariance (i.e. allowing each individual to have different growth trajectories and starting values, which allows partitioning of the variation within and between participants).

Normality of the residuals was assessed using quantile plots and histograms. The Huber-White sandwich (robust) estimator was used for standard error estimation due to non-constant variance. Scaled (multiplied by $\sqrt{(\pi/2)}$) absolute residuals from this model were then regressed on GA to determine the optimal factional polynomial terms for the standard deviation function in a fixed effects model. Percentiles of the distribution of the renal measures by GA were assumed normal and calculated using the formula:

$$Mean + Z \times SD$$

Where Z is – 1.88, - 1.645, - 1.28, 0, 1.28, 1.645, 1.88 for the 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles respectively.

Differences between the renal measurements were investigated according to gender, side (right/left) or in the case of parenchymal thickness, if the anterior or posterior parenchyma was measured. This did reduce the sample size of the group, for these analyses, and therefore the models are likely to be underpowered and there is a risk of overfitting. Due to there being less data at some gestational weeks, two weekly classification of GA (rounded down to the nearest even number) was used. For each of the variables (gender, side (right/left) and with parenchymal thickness, anterior/posterior parenchyma) a two-level saturated mixed effects model was fitted with fixed effects for GA and the variable, and a term for the interaction of GA and exposure. A random intercept accounted for repeated measurements on participants and all models, except for the anterior/posterior interaction model used Huber-white (robust) standard errors estimated for non-constant variance.

4.4 Results

The study recruited 155 pregnant women. Eighty-three participants were excluded (Fig 4.2). For the remaining 72 low-risk pregnancies, the mothers' and babies' characteristics are summarised in Table 4.1. The median GA at delivery was 38.8 weeks with a range of 34.1 – 41.7 as this included 8 neonates that were born preterm (before 37 weeks but after 32 weeks). Induction of labour was often for a combination of reasons; however, the primary reasons were for reduced fetal movements 6 (25%), pre-labour rupture of members 5 (21%), concern around slowing of growth 5 (21%), post-dates 2 (1%) and 6 (25%) for other reasons. No labour, resulting in caesarean section, was due to repeat caesarean section 10 (63%), breech presentation 3 (19%) and 3 (19%) for other reasons. Measurements were obtained from both fetal kidneys with a total of 393 separate ultrasound examinations carried out between 16 to 39 weeks GA. The median number of ultrasound scans per pregnancy was five (range of three to nine scans).

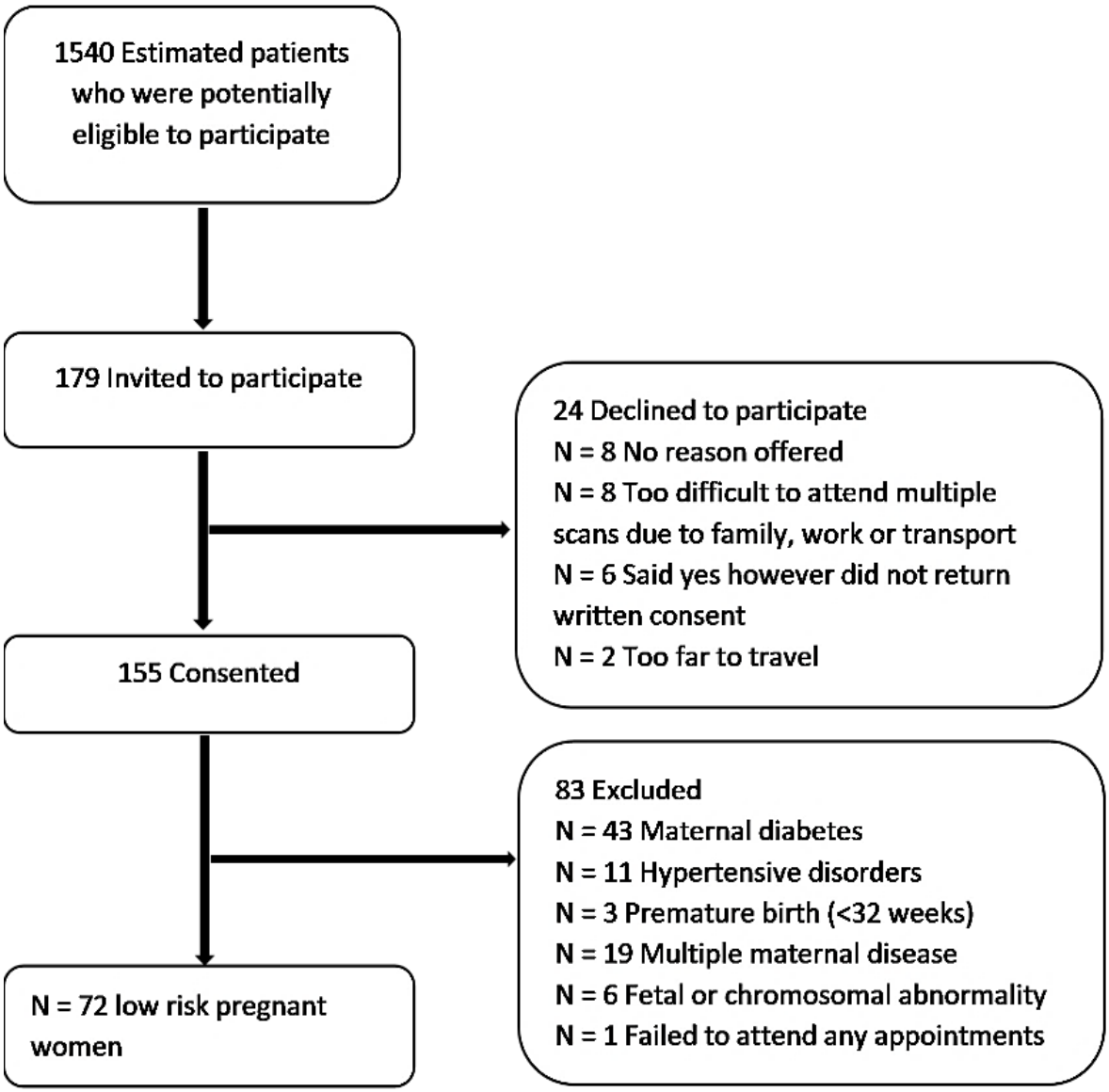


Figure 4.2 Flowchart of participant inclusion and exclusion.

Table 4.1
Characteristics of study population of 72 women and their newborns

Participant Characteristics	N = 72
MATERNAL	
Maternal age (years)	29.3 ± 5.2
Maternal height (cm)	164 ± 6.0
Maternal weight (kg)	66.0 (58.0–79.5)
Maternal BMI (kg/cm2)	24.9 (21.6 – 28.5)
Maternal race origin	N=56†
• Aboriginal/Torres Strait Islander	4 (5.6%)
• Asian	1 (1.4%)
• Caucasian	49 (68.0%)
• Other	3 (4.2%)
Parity	
• Nulliparous	39 (54.2%)
• Parous	33 (45.8%)
Conception	N=60‡
• Spontaneous	51 (70.8%)
• Assisted	9 (12.5%)
Onset of labour	
• Spontaneous labour	32 (44.5%)
• Induction of labour	24 (33.3%)
• No labour	16 (22.2%)
NEONATAL	
GA at birth (weeks)	38.8 (37.9–39.7)
Preterm - <37 weeks > 32 (weeks)§	8 (11.1%)
Birth weight (grams)	3143 (2850-3568)
Male	41 (56.9%)
Female	31 (43.1%)

Note: Data are given as means ± SD, median (interquartile range) or n (%). †16 (22.2%) participants declined to answer. ‡12 (16.7%) participants declined to answer. §Pregnancies resulting in a preterm birth before 32 weeks were excluded. GA, gestational age.

For renal parenchymal thickness, there were 1576 observations, as there were four measurements on each participant at each ultrasound examination measuring the anterior and posterior parenchyma for the right and left kidney. For the other measurements, there were two measurements (right and left) at each ultrasound examination. Some patients, however, only had one measurement at some visits, therefore there were 786 observations for kidney length and 785 for kidney volume in total. Table 4.2 shows the number of participants for each GA. In one participant's scan at 28-weeks the transverse and antero-posterior measurements of the left kidney were not performed, and the left kidney volume could not be calculated at this GA. In another participant at 36-weeks, the length of both kidneys was missed and therefore the kidney volume could also not be calculated at that particular GA. For all renal measurements there was a lack of evidence of any significant difference between right and left kidneys, gender or in the case of parenchymal thickness, between the anterior or posterior parenchymal thickness.

Table 4.2
Number of participants according to each gestational age (rounded down to whole week)

Gestational age	Number of Participants
16	16
17	3
18	1
19	4
20	31
21	8
22	8
23	7
24	42
25	6
26	14
27	12
28	41
29	11
30	21
31	16
32	34
33	20
34	18
35	15
36	41
37	11
38	11
39	3

4.4.1 Growth models for renal parenchymal thickness

The centiles for all renal measurements were calculated using the equation: mean +Z x SD, where Z is the Z score for the respective centile. Table 4.3 presents the calculated centiles for each completed gestational week and the fitted standard deviation. A standard chart of the 3rd, 10th, 50th, 90th and 97th smoothed centiles of the fetal renal parenchymal thickness measurements between 16- and 38-weeks GA with the raw data superimposed is presented in Figure 4.3.

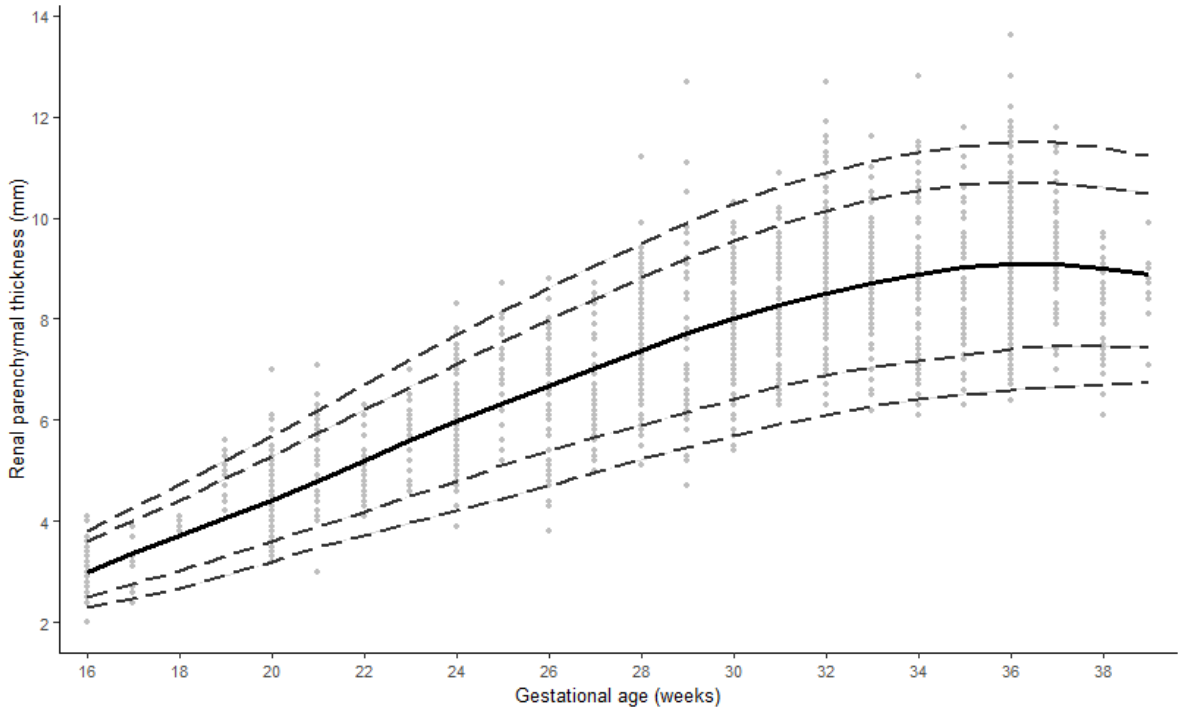


Figure 4.3 Standard chart of fetal renal parenchymal thickness showing all raw measures (dots) and the 3rd, 10th, 50th, 90th and 97th smoothed centiles calculated from the derived equations for the mean and SD according to gestational age.

The resulting equation for the mean renal parenchymal thickness was:

$$Mean = 0.6127533 + 0.0019942 \times GA^3 - 0.000506 \times GA^3 \times ln(GA).$$

The resulting equation for the standard deviation (SD) was:

$$SD = -0.0223467 + 0.0003774 \times GA^3 - 0.0000974 \times GA^3 \times \ln(GA).$$

Table 4.3
Fitted 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles calculated from the derived equations for the mean and SD of fetal renal parenchymal thickness (mm) for gestational age (GA) in weeks rounded down

GA	Nº of Obser-vations	Mean	SD	c3	c5	c10	c50	c90	c95	c97
16	64	3.03	0.42	2.30	2.30	2.50	3.00	3.60	3.70	3.80
17	12	3.37	0.48	2.50	2.60	2.80	3.40	4.00	4.10	4.30
18	4	3.71	0.54	2.70	2.80	3.00	3.70	4.40	4.60	4.70
19	16	4.07	0.60	2.90	3.10	3.30	4.10	4.80	5.10	5.20
20	124	4.44	0.66	3.20	3.40	3.60	4.40	5.30	5.50	5.70
21	32	4.81	0.73	3.50	3.60	3.90	4.80	5.70	6.00	6.20
22	32	5.19	0.79	3.70	3.90	4.20	5.20	6.20	6.50	6.70
23	28	5.57	0.85	4.00	4.20	4.50	5.60	6.70	7.00	7.20
24	168	5.95	0.91	4.20	4.40	4.80	6.00	7.10	7.50	7.70
25	24	6.32	0.97	4.50	4.70	5.10	6.30	7.60	7.90	8.20
26	56	6.69	1.03	4.70	5.00	5.40	6.70	8.00	8.40	8.60
27	48	7.04	1.09	5.00	5.30	5.70	7.00	8.40	8.80	9.10
28	164	7.38	1.14	5.20	5.50	5.90	7.40	8.80	9.20	9.50
29	44	7.69	1.18	5.50	5.80	6.20	7.70	9.20	9.60	9.90
30	84	7.99	1.22	5.70	6.00	6.40	8.00	9.60	10.00	10.30
31	64	8.26	1.25	5.90	6.20	6.70	8.30	9.90	10.30	10.60
32	136	8.50	1.28	6.10	6.40	6.90	8.50	10.10	10.60	10.90
33	80	8.70	1.30	6.30	6.60	7.00	8.70	10.40	10.80	11.10
34	72	8.86	1.31	6.40	6.70	7.20	8.90	10.50	11.00	11.30
35	60	8.98	1.31	6.50	6.80	7.30	9.00	10.70	11.10	11.40
36	164	9.06	1.30	6.60	6.90	7.40	9.10	10.70	11.20	11.50
37	44	9.08	1.27	6.70	7.00	7.40	9.10	10.70	11.20	11.50
38	44	9.04	1.24	6.70	7.00	7.50	9.00	10.60	11.10	11.40

Only one small study was identified that had measured the fetal renal parenchymal thickness (between 20- and 40-weeks) and had published raw centile data.[19] It was difficult to directly compare our results with their results due to technical differences in the studies. Their mean number of participants for a whole gestational week was 5.7 (min = 1, max = 14). Their exclusions included any maternal disease or pregnancy complication (however did not state what these might be), small for gestational age or fetal growth restriction (defined as fetal weight below the 10th centile), oligohydramnios or any kidneys that were smaller or larger than expected for GA. Excluding any smaller or larger than expected kidneys introduces bias into the chart.

4.4.2 Growth models for fetal kidney lengths and volumes

Centiles for the fetal kidney lengths and kidney volumes were calculated using the equation: mean +Z x SD, where Z is the Z score for the respective centile. The standard charts of the 3rd, 10th, 50th, 90th and 97th smoothed centiles of the measurements between 16- and 38-weeks GA for fetal kidney length and kidney volume, are presented in Figure 4.4. Tables 4.4 and 4.5 present the calculated centiles for each and below are the resulting equations of the mean and SD:

$$\textit{Mean kidney length} = 4.122128 + (0.0087023 \times GA^{3}) - (0.0022069 \times GA^{3} \times \ln(GA)).$$

$$\textit{SD kidney length} = 4.738434 - (53.57991 \times GA^{-1}) + (0.0000218 \times GA^{3}).$$

$$\textit{Mean kidney volume} = 2.422287 + (0.0045001 \times GA^{3}) - (0.0011408 \times GA^{3} \times \ln(GA)).$$

$$\textit{SD kidney volume} = -0.1589674 + (0.004974 \times GA^{2}) - (0.0000899 \times GA^{3}).$$

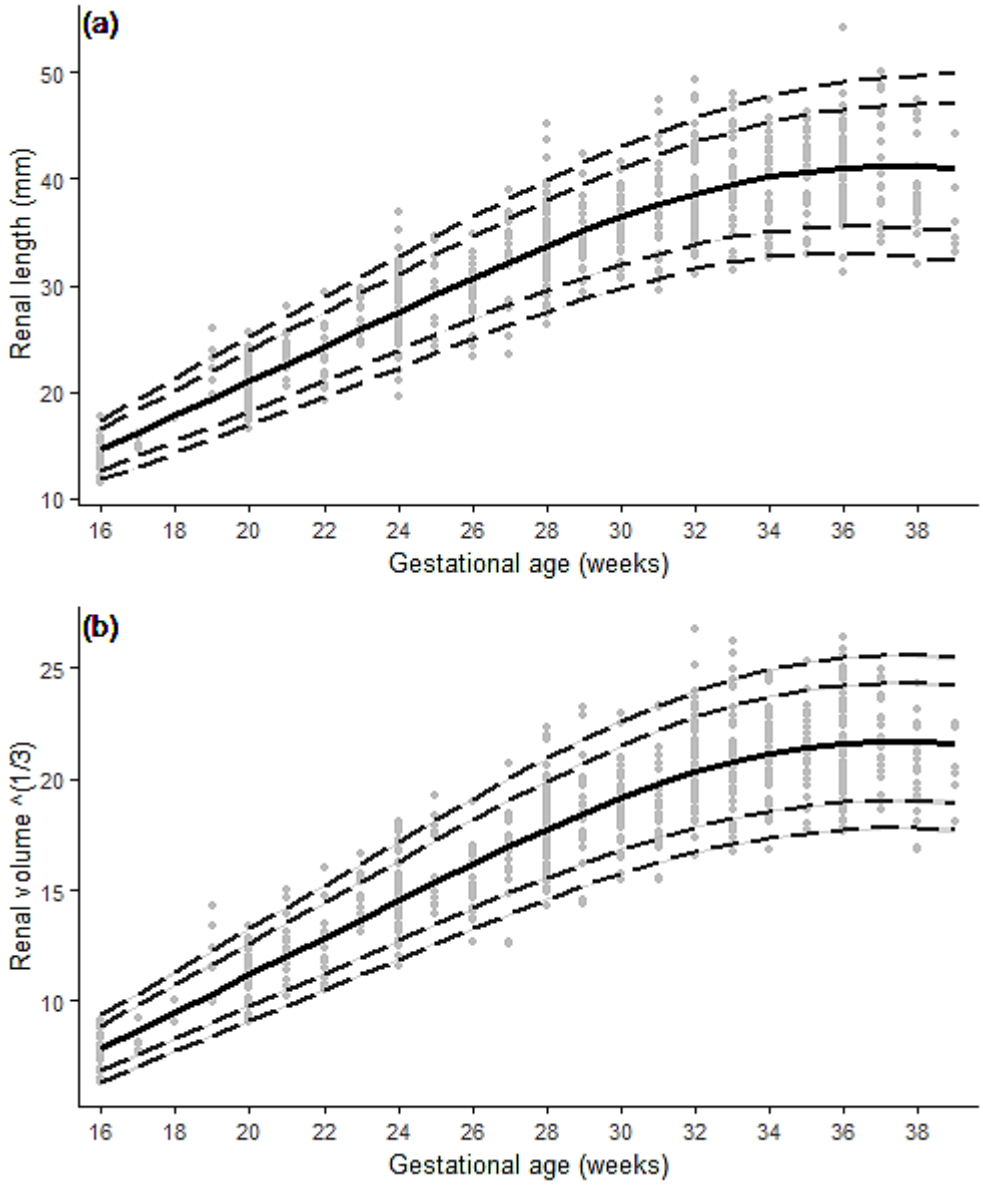


Figure 4.4 Standard fetal charts of (a) kidney length and (b) kidney volume showing all raw measures (dots) and the 3rd, 10th, 50th, 90th, and 97th smoothed centiles calculated from the derived equations for the mean and SD according to gestational age.

These new kidney length and volume charts were compared with charts previously published by Chitty and Altman (2003) as these charts had very similar inclusion and exclusion criteria to this study and published the 3rd 10th, 50th, 90th and 97th centiles for each measurement [12]. A comparison was made with our study by plotting equivalent centiles on their charts (Fig 4.5 & 4.6).

Table 4.4
Fitted 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles calculated from the derived equations for the mean and SD of fetal kidney length for gestational age (GA) in weeks rounded down

GA	Nº of Obser-vations	Mean	SD	c3	c5	c10	c50	c90	c95	c97
16	32	14.7	1.5	11.9	12.3	12.8	14.7	16.6	17.1	17.5
17	6	16.2	1.7	13.0	13.4	14.0	16.2	18.3	18.9	19.3
18	2	17.7	1.9	14.1	14.6	15.3	17.7	20.1	20.8	21.2
19	8	19.2	2.1	15.4	15.8	16.6	19.2	21.9	22.6	23.1
20	62	20.9	2.2	16.7	17.2	18.0	20.9	23.7	24.5	25.0
21	16	22.5	2.4	18.0	18.6	19.4	22.5	25.5	26.4	27.0
22	16	24.1	2.5	19.4	20.0	20.9	24.1	27.4	28.3	28.9
23	14	25.8	2.7	20.8	21.4	22.4	25.8	29.2	30.2	30.8
24	84	27.5	2.8	22.2	22.8	23.9	27.5	31.1	32.1	32.7
25	12	29.1	2.9	23.6	24.3	25.3	29.1	32.9	33.9	34.6
26	28	30.7	3.1	24.9	25.7	26.8	30.7	34.6	35.7	36.5
27	24	32.2	3.2	26.3	27.0	28.2	32.2	36.3	37.5	38.2
28	82	33.7	3.3	27.5	28.3	29.5	33.7	38.0	39.2	39.9
29	22	35.1	3.4	28.7	29.5	30.7	35.1	39.5	40.7	41.6
30	42	36.4	3.5	29.8	30.6	31.9	36.4	41.0	42.2	43.1
31	32	37.6	3.7	30.7	31.6	32.9	37.6	42.3	43.6	44.5
32	68	38.7	3.8	31.5	32.4	33.8	38.7	43.5	44.9	45.8
33	40	39.6	3.9	32.2	33.1	34.6	39.6	44.5	46.0	46.9
34	36	40.3	4.0	32.7	33.7	35.1	40.3	45.4	46.9	47.8
35	30	40.8	4.1	33.0	34.0	35.5	40.8	46.1	47.6	48.6
36	82	41.2	4.3	33.1	34.1	35.7	41.2	46.6	48.2	49.2
37	22	41.3	4.4	33.0	34.0	35.6	41.3	46.9	48.5	49.5
38	20	41.1	4.5	32.6	33.7	35.3	41.1	46.9	48.6	49.6

Table 4.5
Fitted 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles calculated from the derived equations for the mean and SD of fetal kidney volume for gestational age (GA) in weeks rounded down

GA	Nº of Obser-vations	mean	sd	c3	c5	c10	c50	c90	c95	c97
16	32	7.9	0.7	6.5	6.7	6.9	7.9	8.9	9.1	9.3
17	6	8.7	0.8	7.1	7.3	7.6	8.7	9.7	10.0	10.2
18	2	9.4	0.9	7.7	7.9	8.2	9.4	10.6	11.0	11.2
19	8	10.2	1.0	8.3	8.6	8.9	10.2	11.6	11.9	12.2
20	62	11.1	1.1	9.0	9.3	9.7	11.1	12.5	12.9	13.2
21	16	11.9	1.2	9.7	10.0	10.4	11.9	13.5	13.9	14.2
22	16	12.8	1.3	10.4	10.7	11.1	12.8	14.4	14.9	15.2
23	14	13.7	1.4	11.1	11.4	11.9	13.7	15.4	15.9	16.2
24	84	14.5	1.5	11.8	12.1	12.6	14.5	16.4	16.9	17.3
25	12	15.4	1.5	12.5	12.8	13.4	15.4	17.3	17.9	18.3
26	28	16.2	1.6	13.1	13.5	14.1	16.2	18.3	18.9	19.2
27	24	17.0	1.7	13.8	14.2	14.8	17.0	19.2	19.8	20.2
28	81	17.8	1.8	14.4	14.9	15.5	17.8	20.0	20.7	21.1
29	22	18.5	1.8	15.0	15.5	16.1	18.5	20.8	21.5	21.9
30	42	19.2	1.9	15.6	16.1	16.7	19.2	21.6	22.3	22.7
31	32	19.8	1.9	16.1	16.6	17.3	19.8	22.3	23.0	23.4
32	68	20.3	2.0	16.6	17.1	17.8	20.3	22.9	23.6	24.1
33	40	20.8	2.0	17.0	17.5	18.2	20.8	23.4	24.1	24.6
34	36	21.2	2.1	17.3	17.8	18.5	21.2	23.8	24.6	25.0
35	30	21.5	2.1	17.6	18.0	18.8	21.5	24.1	24.9	25.4
36	82	21.6	2.1	17.7	18.2	19.0	21.6	24.3	25.1	25.6
37	22	21.7	2.1	17.8	18.3	19.0	21.7	24.4	25.2	25.6
38	20	21.6	2.1	17.7	18.2	19.0	21.6	24.3	25.1	25.6

4.4.3 Measurement reliability

A summary of the analysis of the interobserver and intraobserver variation for all renal measurements are shown in Tables 7 and 8. The interobserver and intraobserver ICC was excellent for renal parenchymal thickness, length, transverse and antero-posterior dimensions being over 0.95.

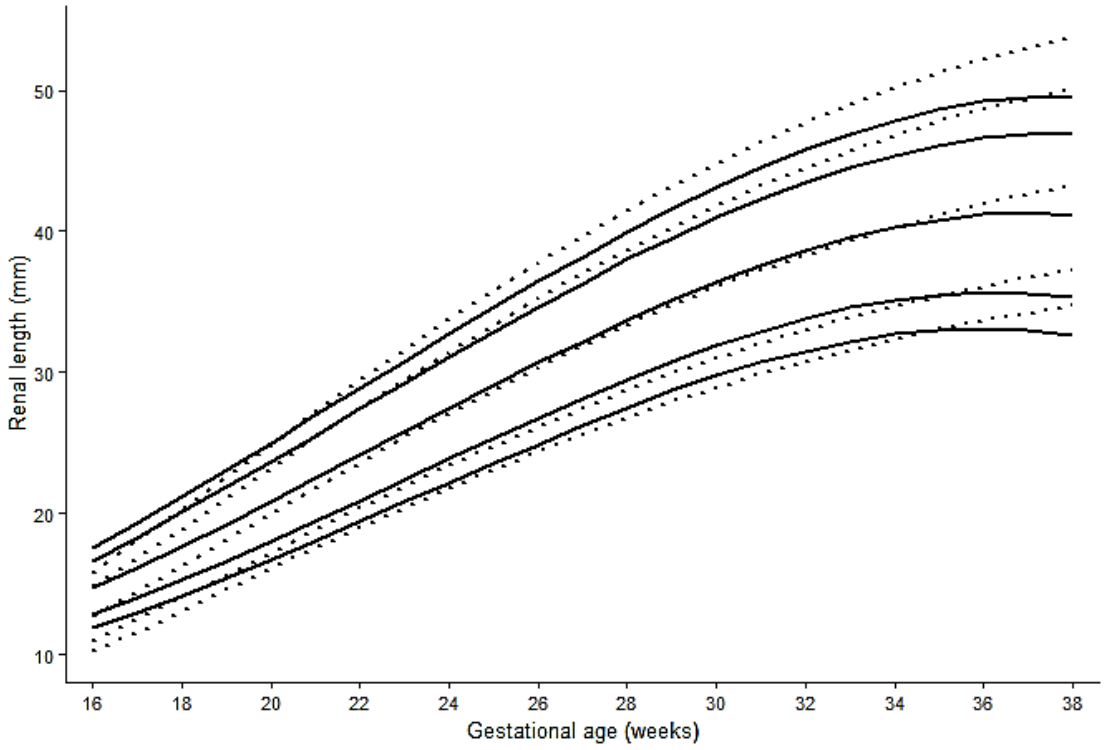


Figure 4.5 Comparison of 3rd, 10th, 50th, 90th and 97th centiles for fetal kidney length measurements according to gestational age obtained from this study (solid black lines) with Chitty and Altman (2003)[12] (dashed lines).

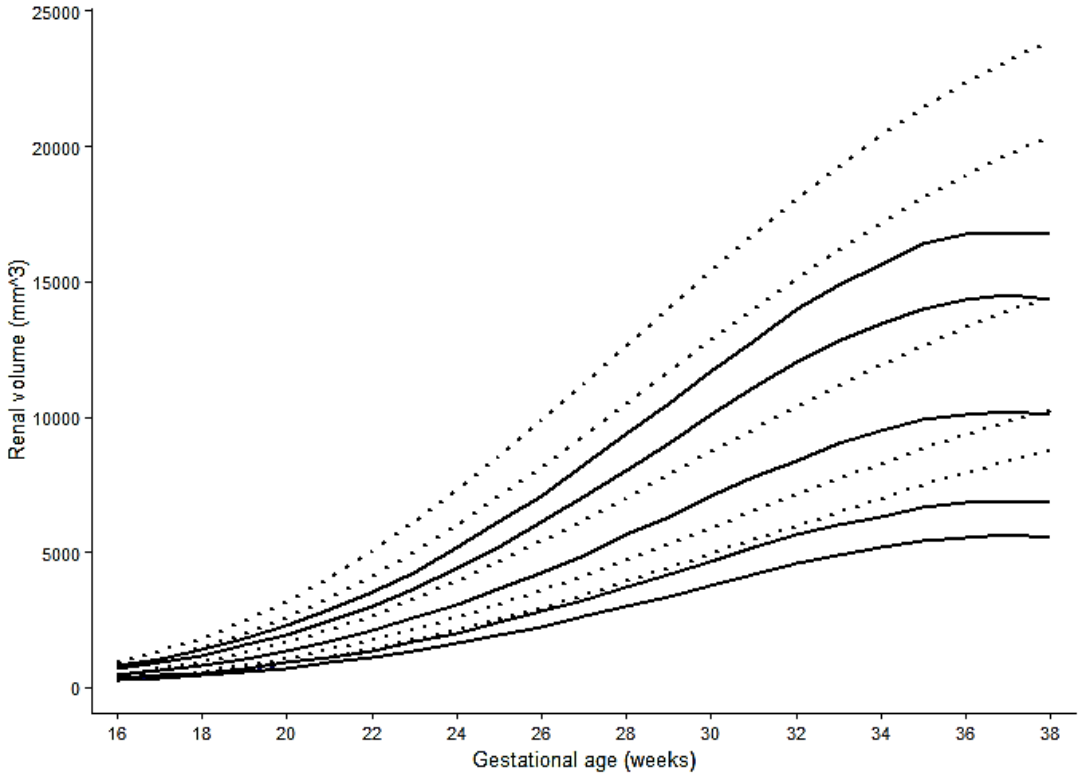


Figure 4.6 Comparison of 3rd, 10th, 50th, 90th and 97th centiles for fetal kidney volume according to gestational age obtained from this study (solid black lines) with Chitty and Altman (2003)[12] (dashed lines).

Table 4.6
Interobserver reliability for fetal renal measurements

Variable (N = 15)	Mean +/- SD (mm)			Cronbach's α	ICC (95% CI)
	Observer 1	Observer 2	Observer 3		
Anterior parenchymal thickness	7.60 +/- 1.85	7.53 +/- 1.97	7.19 +/- 1.79	0.98	0.98 (0.94-0.99)
Posterior parenchymal thickness	7.97 +/- 1.95	7.57 +/- 1.92	7.12 +/- 1.85	0.97	0.96 (0.86-0.99)
Kidney length	35.50 +/- 7.58	36.37 +/- 7.78	35.44 +/-7.62	0.99	0.99 (0.97-0.99)
Kidney transverse diameter	17.26 +/- 3.33	17.74 +/- 3.67	17.19 +/- 3.88	0.97	0.97 (0.92-0.99)
Kidney antero-posterior diameter	19.01 +/- 4.20	19.94 +/- 4.10	18.22 +/- 4.04	0.97	0.96 (0.87-0.99)

Table 4.7
Intraobserver reliability for fetal renal measurements

Variable	Mean +/- SD	Cronbach's α	ICC (95% CI)
Anterior parenchymal thickness	7.58 +/- 1.89	0.98	0.98 (0.92-0.99)
Posterior parenchymal thickness	7.95 +/- 1.97	0.97	0.97 (0.90-0.99)
Kidney length	35.65 +/- 7.76	0.98	0.98 (0.95-0.99)
Kidney transverse diameter	17.23 +/- 3.39	0.96	0.96 (0.89-0.99
Kidney antero-posterior diameter	19.00 +/- 4.25	0.97	0.97 (0.92-0.99)

4.5 Discussion

This study developed a standard chart of fetal renal parenchymal thickness, length and volume from 16- to 38-weeks' gestation. These charts can be utilised for both growth and size comparison as they are derived from longitudinal data using mixed effects modelling. Mixed effects modelling is a sophisticated and powerful method utilising every data point and can better account for the heterogeneity of the timing of the scans and missing data.[25] This chart of renal parenchymal thickness represents an innovative method that will provide more detailed information on fetal kidney growth and potentially function.

Antenatal ultrasound is a reliable technique for diagnosing fetal obstructive pathologies, such as hydronephrosis and lower urinary tract obstruction; however, it is less reliable at assessing parenchymal pathologies and determining renal reserve.[9] The parenchyma of the kidney comprises the cortex and medulla. Nephrons develop within the parenchyma and are the important filtration part of the kidney containing the glomeruli.[26] Nephrogenesis continues up until 34 to 36 weeks GA in a normal pregnancy with around 60% of nephrons formed in the third trimester.[27] The role of measuring the fetal renal parenchyma to assess kidney growth and estimate nephron number has not yet been well established.[13] We previously investigated the renal parenchymal thickness of neonates and demonstrated that it was a single measure which had less variance than kidney volume and could be used to assess neonatal kidney growth.[15]

Renal parenchymal thickness measurements offer a superior evaluation of fetal kidney growth and future kidney function as changes in the parenchymal thickness through the pregnancy should indirectly reflect nephron number. A dramatic improvement in ultrasound imaging over the last decade provides high-quality ultrasound images and allows non-invasive, accurate identification of the fetal renal parenchyma and presents a potentially

reliable technique to assess kidney development and indirectly estimate nephron number. This is not only important for prenatal diagnosis of renal parenchymal pathologies but also as a marker of future kidney function. Diminished nephron numbers at birth likely renders the kidney more vulnerable to damage which could substantially impact kidney function throughout later life.[28]

Our renal parenchymal thickness chart demonstrates that the parenchyma increases in thickness with increasing GA up until around 34-weeks. It then plateaus between 34- and 36-weeks which correlates with completion of nephrogenesis. This is consistent with an ultrasound study by Konje et al. (1996) which demonstrated that the period between 26- and 34-weeks' gestation underwent the most rapid fetal kidney growth.[29]

This standard chart of renal parenchymal thickness could be used in clinical practice to assist in the antenatal diagnosis of renal parenchymal pathologies and as an indirect estimate of nephron number. Deviation in the thickness of the renal parenchyma from these normal standards may indicate an alteration in normal kidney growth and could be employed as criteria for identifying infants with parenchymal pathologies or those who might be at future risk of kidney disease. Providing multiple centiles enables the chart to be used as a screening tool (using the 10^{th} and 90^{th} as lower and upper limits respectively) or more as a diagnostic tool (using the 3^{rd} and 97^{th} as lower and upper limits respectively).[30]

Our standard fetal kidney length and volume charts add to the knowledge of previously created charts. The most widely used kidney size charts are those by Chitty and Altman (2002) and we compared our charts to these.[12] Kidney lengths in our cohort were very similar, other than in earlier GA where our kidney measurements are longer. We had more participants at these early gestations and better imaging resolution due to the advancement of ultrasound equipment. The kidney length in our chart plateaus from around 34-weeks,

while their chart shows continued increases. However, the Chitty and Altman charts are based on cross-sectional data and we speculate that our longitudinal data may be more accurate with regards to kidney growth and the slowing of this growth towards the end of pregnancy as nephrogenesis is complete. There is some difference between our kidney volume charts and those published by Chitty and Altman.[12] Our chart appears to have narrower ranges. This is likely due to kidney volumes being based on a composite calculation and having more variance than a single linear measurement, as well as the longitudinal characteristics of our data measured using more modern equipment.

4.5.1 Limitations of the study

Due to the small sample size at GA prior to 20-weeks and after 36-weeks, estimates from the models may only be reliable in the GA range from 20 to 36 weeks. There may have been some potential measurement bias due to the lack of blinding of the sonographers. As most of the ultrasound examinations included a diagnostic scan, it was difficult to blind the sonographer to all clinical and biometric information. Sonographers are generally trained not to look at measurement as they are performing them and having multiple sonographers, rather than only one, perform the examinations reduces some of the possible bias.

4.6 Conclusion

This study highlights the need for an accurate method to assess fetal kidney growth and indirectly estimate nephron number as a surrogate for kidney function. The standard chart for renal parenchymal thickness developed by our group will be useful in clinical practice to identify alterations in kidney development. Deviations in the thickness of the renal parenchyma from these established normal standards presents a new criterion in the diagnosis of kidney anomalies, particularly in the diagnosis of fetuses with parenchymal

pathologies or reduced nephron numbers, which leaves them susceptible to future kidney disease. We demonstrated excellent intra and interobserver reliability for measurement of the parenchymal thickness. The charts of fetal kidney length and volume strengthens our knowledge of fetal kidney growth and will potentially alert clinicians to abnormal renal development.

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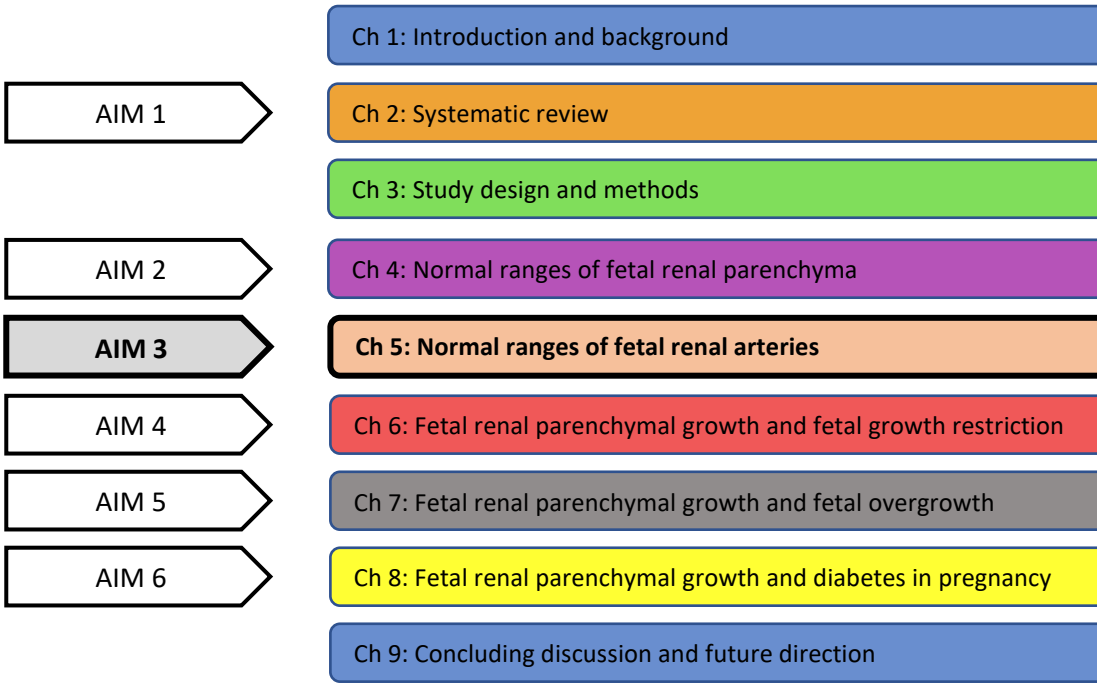
Chapter. 5 Fetal Renal Artery Blood Flow Charts and Correlation with Amniotic Fluid: A Prospective, Longitudinal, Cohort Study

Brennan S, Watson D, Schneider M, Rudd D, Kandasamy K. Fetal renal artery blood flow charts and correlation with amniotic fluid: a prospective, longitudinal, cohort study **Under review** BMC Pregnancy Childbirth.



This chapter is a copy of the journal paper referenced above which is under review. The formatting of section sub-headings and references, and numbering of figures and tables have been modified from the original paper to match the thesis format.

The purpose of this chapter was to establish the normal ranges of fetal renal artery resistivity index (RI) and pulsatility index (PI) during pregnancy. The relationship between fetal renal blood flow and amniotic fluid levels was also investigated.

5.1 Abstract

Objective

The objective of this study was to develop new standard charts for fetal renal artery blood flow to define normal ranges and to assess the reliability of these measurements. The correlation between amniotic fluid levels and fetal renal artery blood flow were also analysed.

Methods

This was a prospective, longitudinal study of 72 low-risk singleton pregnancies undergoing serial ultrasound examinations at least every four weeks. Pulse wave Doppler was used to obtain the resistivity and pulsatility indices of the fetal renal arteries. Standard charts of the fetal renal arteries were developed and the intra and interobserver reliability for the renal blood flow measurements were analysed.

Results

Standard charts of the normal ranges of the renal artery resistive index (RI) and pulsatility index (PI) of the fetal renal arteries were created. The 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles were calculated. No correlation was observed between the amniotic fluid levels and RI or PI. The intraclass correlation coefficient was acceptable for intraobserver reliability (RI = 0.66, PI = 0.88) and poor for interobserver reliability (RI = 0.11, PI = -0.56).

Conclusion

We present novel charts which demonstrate the change of the fetal renal artery blood flow during pregnancy. These may be useful in clinical practice to identify any variations from these normal ranges and for use in future studies in the prediction of kidney function.

5.2 Introduction

Appropriate vascularisation and blood flow to fetal organs is crucial for normal fetal development and organ growth.[1] To enable glomerular filtration and tubular reabsorption and secretion, the mature kidneys require a complex arterial and venous system.[2] It is therefore essential to understand what blood flow is expected for the normal development of the fetal kidneys.

Increasing advancements in antenatal ultrasound have allowed the investigation of many fetal vessels including the renal arteries.[3] Pulsed wave Doppler is the best non-invasive method to study the haemodynamic changes in fetal vessels. Quantification of the normal evolution of the fetal renal blood flow during pregnancy will enable identification of aberrations in blood flow, such as may be seen during fetal hypoxia when blood flow is preferentially shunted away from the kidneys to more vital organs.[4] Analysis of the resistivity index (RI) and pulsatility index (PI) of the fetal renal arteries may potentially provide a more sensitive method to assess renal haemodynamics and may be used to assist in the differential diagnosis of renal pathologies.

Antenatal assessment of renal function is challenging, and a non-invasive method has not yet been uncovered.[2, 5] Innovative methods need to be explored to try and find a solution. After 20 weeks gestation, the fetal kidneys provide over 90% of the amniotic fluid through urination.[6] We postulated that there may be a correlation between fetal renal blood flow and amniotic fluid levels which may contribute to a surrogate, non-invasive evaluation of fetal renal function.

The aim of this study was to use serial ultrasound examinations to develop standard charts of the normal ranges of RI and PI of the fetal renal arteries from 16- to 38-weeks' gestation

for use in clinical practice. This study also aimed to evaluate the reliability of RI and PI measurements and assess for any correlation between renal artery blood flow indices and amniotic fluid levels.

5.3 Materials and Methods

In this prospective, observational study, serial ultrasound examinations on a cohort of low-risk pregnancies were performed between May 2017 and February 2019. The study was conducted in the Maternal Fetal Medicine Unit and Ultrasound Department of the Townsville University Hospital, Australia. This study was approved by the Townsville Hospital and Health Service Human Research Ethics Committee (HREC/16/QTHS/216).

5.3.1 Study population

Townsville Hospital and Health Service (THHS) provides tertiary perinatal services to North Queensland and has a catchment population of around 700,000.[7] Pregnant women aged 18 years or older, who presented to the Medical Imaging Department at the Townsville University Hospital for a second trimester obstetric ultrasound scan between May 2017 and October 2018 were invited to participate. Patients were also informed about the study by their obstetrician, midwife or sonographer.

Accurately dated singleton pregnancies, based on last normal menstrual period (LNMP) and 1st trimester ultrasound, that agreed within seven days, or on 1st trimester ultrasound if LNMP was uncertain, up to 28-weeks' gestation were included in the study. Women with uncertain dates, multiple pregnancy, congenital or chromosomal fetal abnormality, maternal disease that was likely to affect the growth of the fetus (diabetes mellitus, heart disease, hypertension requiring treatment, kidney disease, pre-eclampsia) and subsequent preterm birth less than 32-weeks were excluded.

5.3.2 Study process

On commencement of the study, the participant completed a questionnaire which included demographic, medical and obstetric data. The first ultrasound was typically performed between 16- to 26-weeks, however, six women had their first ultrasound between 28- and 29-weeks. Women were asked to attend for ultrasound examinations every four weeks from their first scan until delivery. If participants had additional clinically indicated ultrasounds, such as for decreased fetal movements, fetal renal Dopplers were performed if this additional ultrasound was two or more weeks from the previous ultrasound. After birth, data were collected on mode of delivery, gestational age (GA) at birth, birth weight, gender and condition of infant at birth from the electronic medical record.

5.3.3 Ultrasound measurements

All examinations were performed by three Australian Accredited Medical Sonographers with at least two years post ultrasound qualification experience using a clearly defined protocol. Training of the sonographers was conducted by the authors (DLW and SB) and a follow-up audit was conducted with the sonographers three months after commencement of the study to verify adherence to the study protocol. An Epiq 7 (Philips Ultrasound, Bothell, WA, USA) or Voluson E8 (GE Healthcare Ultrasound, Milwaukee, WI, USA) were used for the examinations. A curved linear transducer of the highest frequency possible, which matched the mother's body habitus, was selected (1-5MHz) so that the maximum image resolution could be achieved.

A coronal view of the fetal kidneys was obtained, and colour flow Doppler was utilised to identify the renal artery arising from the aorta and entering the kidney. A sample gate of size 2 - 3mm was placed in the mid trunk of the main renal artery and the wall filter was set low at between 30 to 60 Hertz. A pulse wave signal was obtained from both fetal renal arteries

using an angle as close to 0 degrees as possible (Fig 5.1). The mean of at least three consistent, consecutive waveforms was used to calculate the RI and PI.

The amniotic fluid level at each ultrasound examination was assessed by using the standard measurement of single deepest vertical pocket (SDP).[8, 9] While the participant was lying supine, a single vertical measurement of the deepest pocket of amniotic fluid, that was at least 1cm wide and did not contain any fetal parts or umbilical cord, was measured.[8,9]

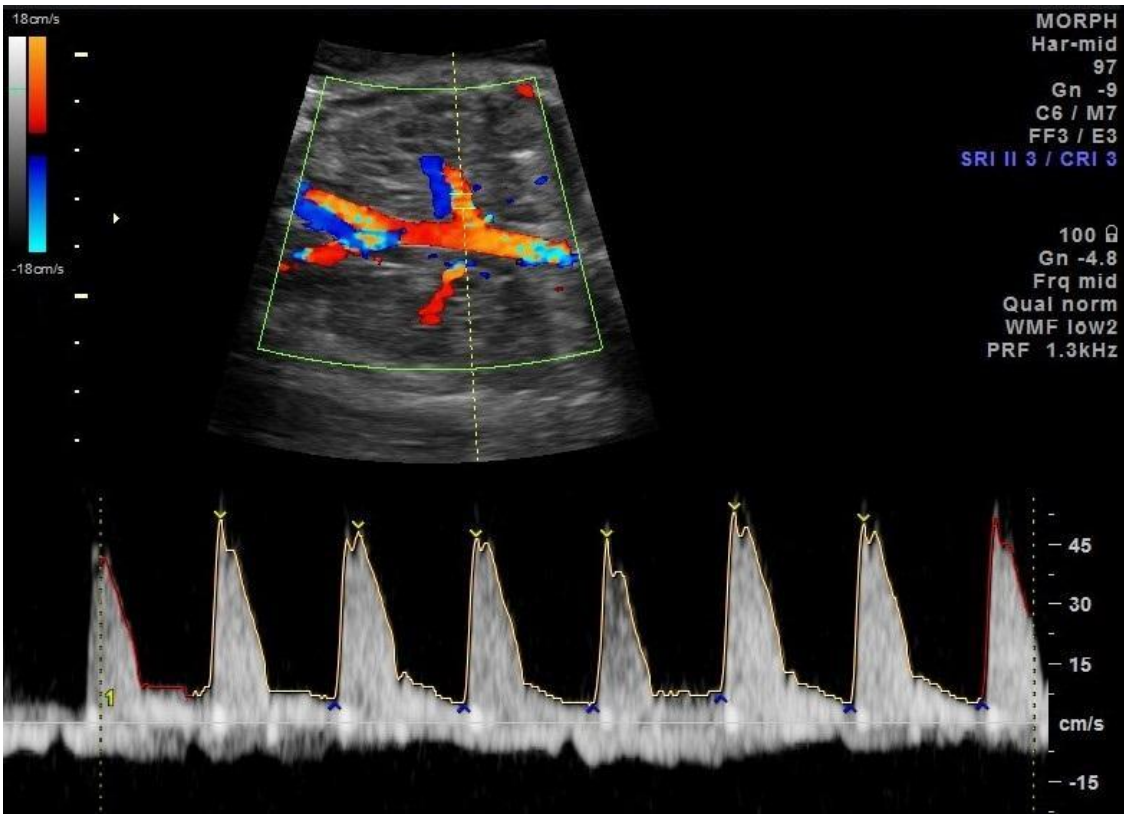


Figure 5.1 Colour and pulse wave Doppler from the mid-trunk of the left main renal artery at 28 weeks gestational age. Source: Townsville University Hospital.

5.3.4 Measurement reliability

For analysis of intra and interobserver reliability, a sample of 15 pregnant women across a range of gestational ages, some who were a part of the study and some additional pregnant women who were happy to have repeated measurements underwent these assessments. The

gestational age range of these pregnancies was 19 to 36 weeks. All three sonographers engaged in the study obtained real-time measurements of each renal artery Doppler twice. They were blinded to their own and each other's measurements by concealing the measurements on the monitor. The mean of each sonographer's two measurements was analysed.

5.3.5 Statistical analysis

The maternal and neonatal characteristics, and intra and interobserver reliability were analysed using IBM SPSS (Version 25, Armonk, NY, USA). Normality of the maternal and infant data was tested by visually inspecting histograms and a Kolmogorov-Smirnov test. Variables that were normally distributed were reported as mean and standard deviation (SD) and variables that were non-normally distributed as a median and interquartile range. An ANOVA model was used to analyse intra and interobserver reliability. The SD, Cronbach's alpha (α) and the intraclass correlation coefficient (ICC) with 95% confidence intervals were calculated.

All other statistical analyses were performed using Stata/MP v14.2 for windows (Stata Corp LP, College Station, TX, USA). The program ggplot2[10] in R Studio (version 1.2.1335)[11] was used to create the graphs. Statistical significance was taken as $p < 0.05$. Methods of the INTERGROWTH 21st Project for fetal growth were applied.[12] The Stata function fp was used to ascertain the best fitting model for the mean RI and PI of the renal artery as a function of a fractional polynomial in GA (rounded down to whole week). A two level, mixed effects regression model for RI and PI was then built, including fixed effects for the polynomial function of GA, and accounting for repeated measures on participants using random intercept and slope with unstructured covariance (i.e. allowing each individual to have different growth trajectories and starting values, which allows partitioning of the

variation within and between participants). The standard charts were developed for 16- to 38 weeks GA.

Quantile plots and histograms were used to evaluate normality of the residuals. The Huber-White sandwich (robust) estimator was applied for standard error estimation due to non-constant variance. To determine the ideal factional polynomial terms for the standard deviation function in the fixed effects model, scaled (multiplied by $\sqrt{(\pi/2)}$) absolute residuals from this model were regressed on GA. Percentiles of the distribution of RI and PI by GA were assumed normal and calculated using the formula:

$$Mean + Z \times SD$$

Where Z is – 1.88, - 1.645, - 1.28, 0, 1.28, 1.645, 1.88 for the 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles respectively.

Differences between the RI and PI of the renal arteries were investigated according to gender and kidney side (right/left). Dividing the cohort for this analysis did reduce the sample size of the groups and the models are likely to be underpowered, resulting in a risk of overfitting. Due to there being less data at some gestational weeks, for these analyses, two weekly classification of GA (rounded down to the nearest even number) was used. For each of the variables (gender and side) a two-level saturated mixed effects model was fitted with fixed effects for GA and the variable, and a term for the interaction of GA and exposure. A random intercept accounted for repeated measurements on participants and Huber-white (robust) estimator was used for standard errors estimated for non-constant variance. Pearson's correlation was used to compare the amniotic fluid level to the RI and PI of the fetal renal artery at each ultrasound examination.

5.4 Results

In total, 155 pregnant women were recruited into this study. Eighty-three participants were ultimately excluded, due to maternal disease likely to affect fetal growth, (73) fetal abnormality, (6) premature birth before 32 weeks GA (3) or failure to attend, (1) resulting in 72 low-risk pregnancies being included in the study (Fig 5.2). The characteristics of the mothers and infants are summarised in Table 5.1. Three hundred and ninety-three separate ultrasound examinations were performed between 16 to 39 weeks GA, with the median number of ultrasound scans per pregnancy being five (range of three to nine scans). There were 761 RI and PI measurements each. The renal artery Doppler was unable to be obtained for one kidney in twelve scans and for both renal arteries in four scans. This was due to the renal artery Doppler being technically difficult to obtain due to fetal position, movement and breathing. There was no significant difference between right and left kidneys or gender for RI or PI of the renal arteries.

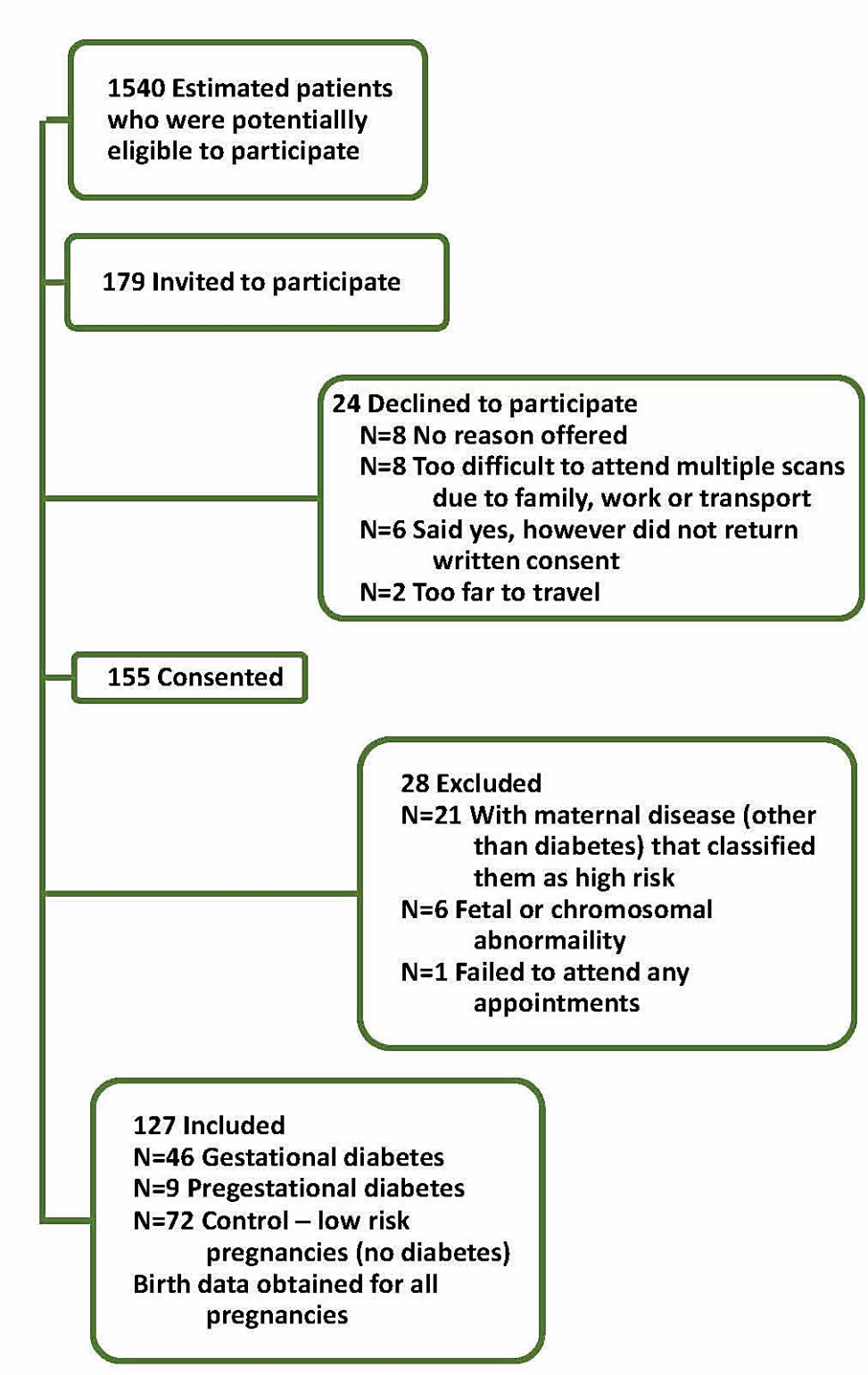


Figure 5.2 Flowchart of participant inclusion and exclusion.

Table 5.1
Characteristics of study population of women and their infants

Participant Characteristics	N = 72
MATERNAL	
Maternal age (years)	29.3 ± 5.2
Maternal height (cm)	164 ± 6.0
Maternal weight (kg)	66.0 (58.0–79.5)
Maternal BMI (kg/cm2)	24.9 (21.6 – 28.5)
Maternal race origin	N=56[a]
• Aboriginal/Torres Strait Islander	4 (5.6%)
• Asian	1 (1.4%)
• Caucasian	49 (68.0%)
• Other	3 (4.2%)
Parity	
• Nulliparous	39 (54.2%)
• Parous	33 (45.8%)
Conception	N=60[b]
• Spontaneous	51 (70.8%)
• Assisted	9 (12.5%)
NEONATAL	
GA at birth (weeks)	38.8 (37.9–39.7)
Preterm – (<37 weeks > 32 weeks)[c]	8 (11.1%)
Birth weight (grams)	3143 (2850-3568)
Male	41 (56.9%)
Female	31 (43.1%)

Note: Data are given as means ± SD, median (interquartile range) or n (%). [a]16 (22.2%) participants declined to answer. [b]12 (16.7%) participants declined to answer. [c]Pregnancies resulting in a preterm birth before 32 weeks were excluded. GA, gestational age.

5.4.1 Fetal renal artery RI and PI charts

Centiles for the RI and PI of the renal arteries were calculated using the equation:

mean +Z x SD, where Z is the Z score for the respective centile.

The standard charts of the 3rd, 10th, 50th, 90th and 97th smoothed centiles of the measurements between 16- and 39-weeks GA for fetal renal artery RI and PI are presented in Figure 5.3 and 4. Tables 5.2 and 5.3 present all the calculated centiles for RI and PI. The equations for RI and PI are:

- Mean RI = -0.2556362 – (0.043501 X GA) + (0.449057 X $GA^{0.5}$);
- SD RI = 0.0781253 – (0.0003515 X GA^2) + (0.0000903 X GA^2 X *ln*(GA));
- Mean PI = -0.7966501 – 176.19 X GA^{-1} + 79.45.009 X GA^{-1} X *ln*(GA); and
- SD PI = 0.3675488 – 0.0000423 X GA^3 + 0.0000119 X GA^3 X *ln*(GA).

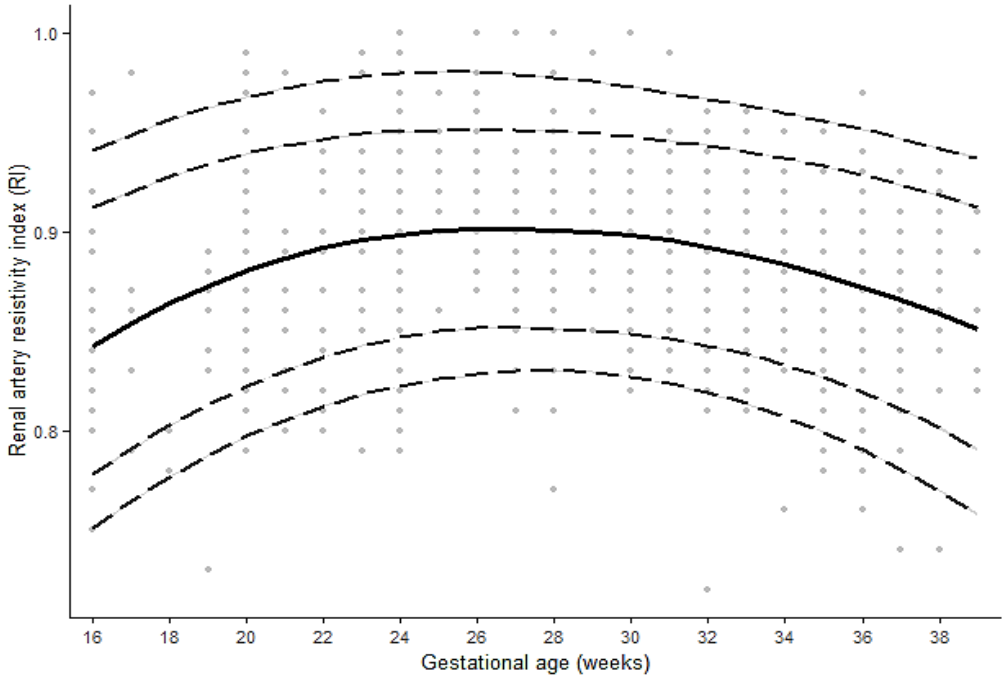


Figure 5.3 Standard fetal chart of fetal renal artery resistivity index (RI) showing all raw measures (dots) and the 3rd, 10th, 50th, 90th, and 97th smoothed centiles calculated from the derived equations for the mean and SD according to gestational age.

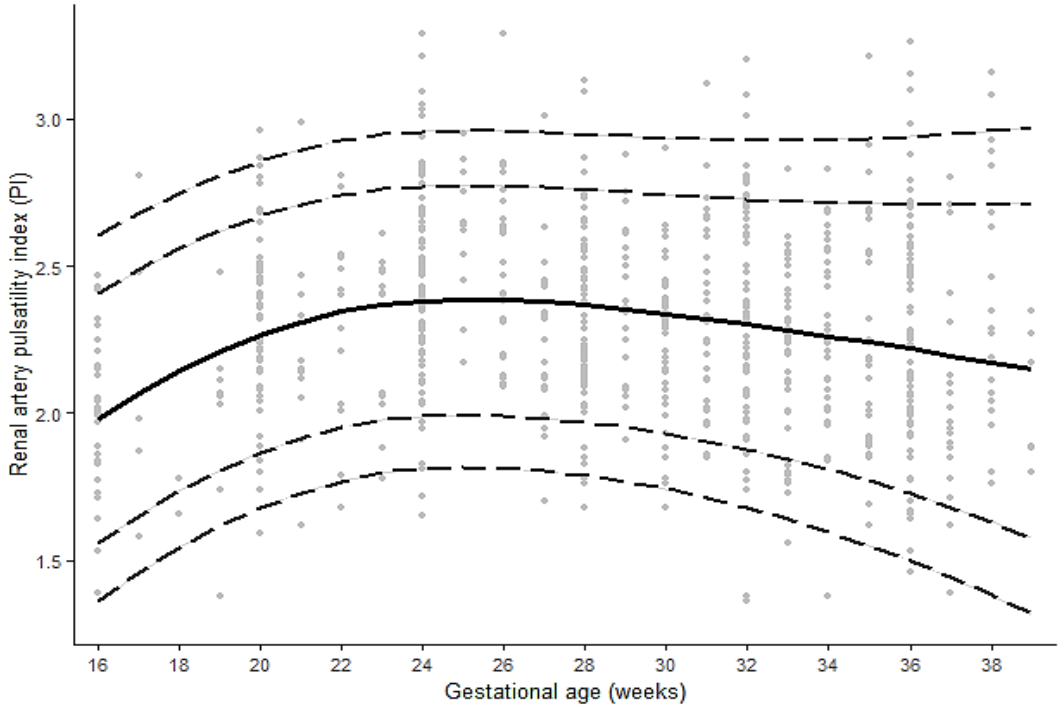


Figure 5.4 Standard fetal chart of fetal renal artery pulsatility index (PI) showing all raw measures (dots) and the 3rd, 10th, 50th, 90th, and 97th smoothed centiles calculated from the derived equations for the mean and SD according to gestational age.

Table 5.2
Fitted 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles calculated from the derived equations for the mean and SD of fetal renal artery resistivity index (RI) for gestational age (GA) in weeks rounded down

GA	Nº of Obser-vations	Mean	SD	c3	c5	c10	c50	c90	c95	c97
16	27	0.84	0.05	0.75	0.76	0.78	0.84	0.91	0.93	0.94
17	5	0.86	0.05	0.76	0.77	0.79	0.86	0.92	0.94	0.95
18	2	0.87	0.05	0.77	0.79	0.80	0.87	0.93	0.95	0.96
19	8	0.88	0.05	0.79	0.80	0.81	0.88	0.94	0.95	0.96
20	61	0.88	0.05	0.80	0.81	0.82	0.88	0.94	0.96	0.97
21	13	0.89	0.04	0.81	0.82	0.83	0.89	0.95	0.96	0.97
22	16	0.89	0.04	0.81	0.82	0.84	0.89	0.95	0.96	0.97
23	13	0.90	0.04	0.82	0.83	0.84	0.90	0.95	0.97	0.98
24	82	0.90	0.04	0.82	0.83	0.85	0.90	0.95	0.97	0.98
25	11	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.97	0.98
26	27	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.97	0.98
27	24	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.97	0.98
28	81	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.97	0.98
29	22	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.96	0.97
30	42	0.90	0.04	0.83	0.84	0.85	0.90	0.95	0.96	0.97
31	32	0.90	0.04	0.82	0.83	0.85	0.90	0.95	0.96	0.97
32	68	0.89	0.04	0.82	0.83	0.84	0.89	0.94	0.96	0.97
33	38	0.89	0.04	0.81	0.82	0.84	0.89	0.94	0.95	0.96
34	36	0.88	0.04	0.81	0.82	0.83	0.88	0.94	0.95	0.96
35	30	0.88	0.04	0.80	0.81	0.83	0.88	0.93	0.95	0.96
36	77	0.87	0.04	0.79	0.80	0.82	0.87	0.93	0.94	0.95
37	20	0.87	0.04	0.78	0.79	0.81	0.87	0.92	0.94	0.95
38	20	0.86	0.05	0.77	0.79	0.80	0.86	0.92	0.93	0.94

Table 5.3
Fitted 3rd, 5th, 10th, 50th, 90th, 95th and 97th centiles calculated from the derived equations for the mean and SD of fetal renal artery pulsatility index (PI) for gestational age (GA) in weeks rounded down

GA	Nº of Obser-vations	Mean	SD	c3	c5	c10	c50	c90	c95	c97
16	27	2.0	0.3	1.3	1.4	1.5	2.0	2.4	2.5	2.6
17	5	2.1	0.3	1.5	1.5	1.7	2.1	2.5	2.6	2.7
18	2	2.2	0.3	1.6	1.6	1.8	2.2	2.6	2.7	2.8
19	8	2.2	0.3	1.6	1.7	1.8	2.2	2.6	2.8	2.8
20	61	2.3	0.3	1.7	1.8	1.9	2.3	2.7	2.8	2.9
21	13	2.3	0.3	1.7	1.8	1.9	2.3	2.7	2.8	2.9
22	16	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	2.9
23	13	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	3.0
24	82	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	3.0
25	11	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	3.0
26	27	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	3.0
27	24	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	3.0
28	81	2.4	0.3	1.8	1.9	2.0	2.4	2.8	2.9	2.9
29	22	2.4	0.3	1.8	1.8	2.0	2.4	2.8	2.9	2.9
30	42	2.3	0.3	1.7	1.8	1.9	2.3	2.7	2.9	2.9
31	32	2.3	0.3	1.7	1.8	1.9	2.3	2.7	2.9	2.9
32	68	2.3	0.3	1.7	1.8	1.9	2.3	2.7	2.8	2.9
33	38	2.3	0.3	1.6	1.7	1.8	2.3	2.7	2.8	2.9
34	36	2.3	0.4	1.6	1.7	1.8	2.3	2.7	2.8	2.9
35	30	2.2	0.4	1.6	1.6	1.8	2.2	2.7	2.8	2.9
36	77	2.2	0.4	1.5	1.6	1.7	2.2	2.7	2.8	2.9
37	20	2.2	0.4	1.4	1.5	1.7	2.2	2.7	2.9	2.9
38	20	2.2	0.4	1.4	1.5	1.6	2.2	2.7	2.9	3.0

5.4.2 Measurement reliability

A summary of the analysis of the intraobserver and interobserver reliability for renal artery RI and PI are shown in Tables 5.4 and 5.5. The intraobserver ICC for measuring the renal artery was moderate at 0.66 for the RI and good for the PI at 0.88. However, the interobserver ICC for measuring the renal artery was poor at 0.11 for RI and -0.56 PI. Poor reliability was assessed as an ICC less than 0.50, moderate as between 0.50 and 0.75, good as between 0.75 and 0.90 and excellent as more than 0.90.[13]

Table 5.4
Intraobserver reliability for fetal renal artery Dopplers

Variable	Mean +/- SD	Cronbach's α	ICC (95% CI)
Renal artery RI	0.90 +/- 0.03	0.66	0.66 (-0.01-0.89)
Renal artery PI	2.40 +/- 0.29	0.88	0.88 (0.64-0.96)

Table 5.5
Interobserver reliability for fetal renal artery Dopplers

Variable (N = 15)	Mean +/- SD (mm)			Cronbach's α	ICC (95% CI)
	Observer 1	Observer 2	Observer 3		
Renal artery RI	0.90 +/-0.02	0.89 +/-0.04	0.87 +/-0.04	0.12	0.11 (-0.77-0.64)
Renal artery PI	2.41 +/- 0.27	2.38 +/- 0.38	2.12 +/-0.27	-0.71	-0.56 (-2.11-0.37)

5.4.3 Correlation between amniotic fluid level and renal artery RI and PI

There was no correlation between the single deepest pocket of amniotic fluid and the RI (cor = 0.035, p=0.179) or PI (cor = 0.027, p=0.296) of the renal arteries.

5.5 Discussion

This study developed standard charts of fetal renal artery RI and PI from 16- to 38-weeks' gestation to provide more detailed information on normal ranges of fetal renal artery blood flow during pregnancy. We showed that the RI and PI of the fetal renal arteries demonstrated little alteration during the pregnancy. These charts may be utilised to provide additional information in cases of high-risk pregnancy and possible fetal renal abnormalities. The strengths of this study are the wide range of gestational ages assessed and that these charts are derived from longitudinal data using mixed effects modelling which considers every data point and allows for variation between and within participants. This provides true change of renal haemodynamics over the duration of the pregnancy.

There are a limited number of studies investigating normal fetal renal artery blood flow, and most have small sample sizes, are cross sectional in design and have heterogeneous inclusion and exclusion criteria making direct comparison difficult.[14-19] Some earlier studies showed that the PI decreased with increasing GA.[14, 15] The study most similar to our study, in that it was a longitudinal design and used mixed effects modelling, also demonstrated that the RI and PI remained relatively unchanged throughout the pregnancy.[18] Other recent studies demonstrated similar findings; however, these were not longitudinal studies.[16, 17] A study in 2015 reported longitudinal reference intervals for fetal renal arteries however was not truly longitudinal as although the design was longitudinal, the data were not analysed as longitudinal data.[17] The mean of the renal Doppler measurements was calculated for each gestational age group disregarding the repeated measures and non-independence of the data.[17] Minimal change in renal haemodynamics during pregnancy is

likely due to the fetal kidneys having limited true function *in-utero* as the placenta performs most of the prenatal renal excretory functions and the proportion of cardiac output to the fetal kidneys is low at only 3 to 5%.[1, 20]

Our study had adequate intraobserver reliability for RI and PI, however, the interobserver reliability was poor for both indices. Few studies have assessed intra and interobserver reliability of the RI and PI of the fetal renal artery. One recent study assessed the reliability of the fetal renal artery PI and, similar to our study, found adequate intraobserver reliability (ICC = 0.528) but poor interobserver reliability (ICC = 0.114).[17] Obstetric care is becoming increasingly dependent on fetal Dopplers; however, most studies do not investigate or report on intra and interobserver reliability.[21] Currents studies on maternal and fetal blood flow that have reported on reliability of these Dopplers have revealed poor-to-moderately-poor results.[22, 23] It can be argued that there is known physiological variations in spectral Doppler traces of fetal blood flow due to fetal movements, breathing and heart rate changes that should be factored into reliability of these Doppler indices.[24] We need to acknowledge that the variability of fetal Doppler traces will always be more than a fixed two-dimensional measurement of a fetal structure and that innovative techniques will likely not initially have a high enough reliability for clinical decision making. They may require further development and refinement. These are important concerns that should not be underrated. Care should be employed in having too much confidence in a diagnostic test with unclear or questionable reliability.

These normative ranges of renal artery RI and PI, which include multiple different centiles, could be useful for future studies to investigate the redistribution of blood flow away from the kidneys that is thought to occur in most growth restricted fetuses.[4, 25] If changes in the renal artery blood flow profile could be quantified, these alterations in renal haemodynamics might become a useful diagnostic tool for fetal growth restriction. Renal blood flow indices may also have the potential to predict the severity of fetal growth restriction or be a novel marker for postnatal renal function,

particularly in instances of renal abnormalities. At this stage, however, the findings for RI and PI of fetal renal artery Dopplers are variable and need to be validated. They should be utilised with care as the usefulness of fetal renal artery blood flow measurements in clinical practice is still unclear. It should also be noted that RI and PI are not measures of perfusion but rather a reflection of vascularity and flow intensity. Further larger studies utilising newer enhanced Doppler techniques with standardised methods may identify and refine their future value.

Recently, novel 3D volume Doppler flow techniques have been investigated to obtain vascular indices of flow index (FI), vascular index (VI) and vascularisation flow index (VFI) of the fetal renal arteries.[26, 27] They are showing promise to assess the renal haemodynamic characteristics and their relationship to variations in flow associated with fetal growth restriction. Currently, they suffer from poor reproducibility due to technical matters such as multiple non-standardised machine settings to select prior to acquisition of data, depth of insonation, patient habitus and fetal movements which result in measurements with high variability.[26, 28, 29]

A relationship between amniotic fluid levels and changes in fetal renal perfusion seems plausible as a simple indicator for fetal renal function. However, fetal urine production is determined by both renal perfusion and tubular reabsorption, and regulation of amniotic fluid volumes is a complex interaction of many different fetal systems not just the urinary tract.[30, 31] Methods of assessing amniotic fluids levels are only estimates and there is currently no particularly accurate non-invasive method.[30, 32] Our study found, as others have, no good correlation between amniotic fluid levels and renal artery blood flow.[17, 33]

A limitation of the study was the small sample size at gestational ages prior to 20-weeks and after 36-weeks. The charts would be most reliable in the GA range from 20 to 36 weeks.

5.6 Conclusion

Pulse wave Doppler of the fetal renal arteries allows detailed analysis of the haemodynamic characteristics of the blood supply to the developing kidneys. Our standard charts provide the normal ranges of the RI and PI of the fetal renal arteries during pregnancy so that potential physiological or pathological alterations in renal blood flow can be investigated in high-risk pregnancy. Considering the problems with reliability, these charts do need to be used with caution. No correlation was found between amniotic fluid levels and fetal renal artery RI or PI. Further studies of fetal renal artery blood flow are needed to evaluate improved techniques and assess the value of fetal renal artery Dopplers in clinical practice.

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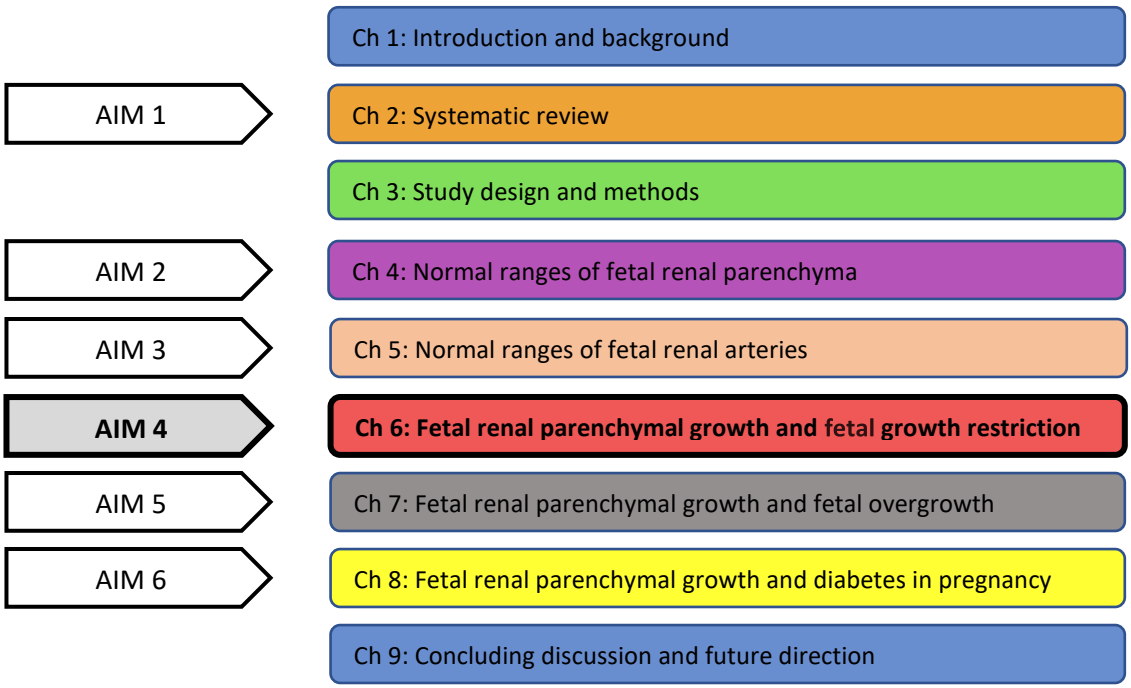
Chapter. 6 Can Measurement of the Fetal Renal Parenchymal Thickness with Ultrasound be Used as an Indirect Measure of Nephron Number?

Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number? J Dev Orig. Health Dis. 2020:1-9. https://doi.org/10.1017/S204017442000015X.



This chapter is a copy of the journal paper, referenced above and attached in Appendix D, except for minor textural modifications. The formatting of section sub-headings and references, and numbering of figures and tables have been modified from the original publication to match the thesis format. Supplementary figures and tables have also been included as part of the main text. This was an invited publication for the special themed edition "Advance Imaging in DoHaD" for The Journal of Developmental Origins of Health and Disease.

The purpose of this chapter was to provide in-vivo evidence of the effect of fetal growth restriction on the developing renal parenchyma and evaluate the use of measuring the fetal renal parenchyma as an indirect estimate of nephron number.

6.1 Abstract

Chronic kidney disease continues to be under recognised and is associated with a significant global health burden and costs. An adverse intrauterine environment may result in a depleted nephron number and an increased risk of chronic kidney disease. Antenatal ultrasound was used to measure the fetal renal parenchymal thickness, as a novel method to estimate nephron number. Fetal renal artery blood flow was also assessed. This prospective, longitudinal study evaluated the fetal kidneys of 102 appropriately grown and 30 fetal growth restricted fetuses between 20 weeks and 37 weeks gestational age to provide vital knowledge on the influences fetal growth restriction has on the developing kidneys. The fetal renal parenchymal thickness and renal artery blood flow was measured at least every four weeks using ultrasound. The renal parenchymal thickness was found to be significantly thinner in growth restricted fetuses compared to appropriately grown fetuses (likelihood ratio (LR) = 21.06, p = <0.0001) and the difference increases with gestational age. In fetuses with the same head circumference, a growth restricted fetus was more likely to have a thinner parenchyma than an appropriately grown fetus (LR=8.9, p=0.0028), supporting the principle that growth restricted fetuses preferentially shunt blood towards the brain. No significant difference was seen in the renal arteries between appropriately grown and growth restricted fetuses. Measurement of the renal parenchymal thickness appears to be a more sensitive measure than current methods. It has the potential to identify infants with a possible reduced nephron endowment allowing for monitoring and interventions to be focused on individuals at a higher risk of developing future hypertension and chronic kidney disease.

6.2 Introduction

Globally, it is estimated that between 5 to 10 million people die annually due to kidney disease.[1] Chronic kidney disease is a significant and often neglected chronic disease which continues to be under recognised, despite being identified as a huge economic burden.[1] Its link with other major diseases such as cardiovascular disease, hypertension, and diabetes is often underestimated.[1, 2] Effective screening, prevention and early treatment can slow or reduce the incidence of chronic kidney disease.[3] Understanding the influences for the healthy development of the kidneys and subsequent kidney function is a priority.

It is well established that developmental programming of the fetal kidney can affect kidney evolution *in-utero* and in early life, which can in turn impact kidney growth patterns and function.[4,5] The association between an adverse intrauterine environment and the development of chronic kidney disease and hypertension later in life is compelling.[6-8] Low birth weight (defined as birth weight < 2500g) is associated with a 70% increased risk of developing chronic kidney disease.[9]

Low birth weight, or small for gestational age (SGA) (defined as birth weight < 10th centile), is often used as a proxy for fetal growth restriction, previously known as intrauterine growth restriction (IUGR). The two terms, however, are different, as not all SGA infants are growth restricted and not all growth restricted infants are SGA. True fetal growth restriction (FGR) is a major cause of morbidity and mortality and is believed to predispose to a range of diseases later in life.[10-12] Serial antenatal ultrasound growth measurements and uteroplacental and fetal Dopplers are employed to diagnose FGR.[10, 13]

A reduced nephron endowment is associated with an increased susceptibility to hypertension and renal disease.[4, 6, 14] Nephrogenesis *in-utero* is the main determinant of life-long nephron number and so it is vital to consider the impact of fetal life programming, such as fetal growth restriction, on

the risks of developing kidney disease.[8, 15] The challenge remains to find a method to quantify nephron numbers *in-utero* and develop useful early prognostic factors for future renal function.[7,16,17]

Measurement of the fetal renal parenchymal thickness with antenatal ultrasound is a novel, non-invasive method to assess changes in kidney growth. The parenchymal tissue of the kidney comprises the renal cortex and medulla, which contain the functional units of the kidney – the nephrons and glomeruli. The renal parenchyma measurement is a single, easily performed measurement focusing on the nephron rich area. Additionally, quantifying fetal renal artery blood flow may be valuable to investigate alterations in perfusion, as it is well established that during fetal hypoxia, such as seen in fetal growth restriction, blood flow is preferentially shunted away from the kidneys to more essential organs such as the heart, brain and adrenals.[18] There is very little information on the usefulness of assessing the fetal renal parenchyma as a prognostic tool for renal function.

The aim of this study was to determine the effect of fetal growth restriction on the development of the fetal kidneys by evaluating the renal parenchymal thickness during consecutive ultrasound examinations between 20- and 36-weeks gestational age. The primary outcome measure was the difference in renal parenchymal thickness between appropriately grown and growth restricted fetuses and the secondary outcome measure was the blood flow to the fetal kidneys between these two groups. We hypothesised that fetal growth restriction impairs renal parenchymal thickness growth.

6.3 Method

This prospective, longitudinal, observational study was conducted between May 2017 and February 2019 in the Maternal-Fetal-Medicine unit and Ultrasound Department at the Townsville Hospital, Australia.

6.3.1 Study population

The Townsville Hospital and Health Service provides tertiary, perinatal services and receives public and private referrals for obstetric care from all over North Queensland, with a catchment population of around 700,000 and 10,000 births per year.[19] Pregnant patients aged 18 years or older, who presented to the Townsville Hospital for a second trimester obstetric ultrasound scan between May 2017 and October 2018 were invited to participate, or they were informed about the study by their treating obstetrician, midwife or sonographer.

Women were included if they had a singleton pregnancy up to 30 weeks gestation with an accurately dated pregnancy based on last normal menstrual period (LNMP) and 1st trimester ultrasound, that correlated with each other within seven days, or on 1st trimester ultrasound if LNMP was uncertain. Women were excluded if they had a multiple pregnancy, uncertain dates or any major congenital fetal abnormality or chromosomal abnormality. Detailed written information was given to the patient and written consent was obtained.

6.3.2 Study process

Participants completed a questionnaire, which included demographic, medical and obstetric data. The first ultrasound was most commonly performed between 16 weeks to 26 weeks gestational age (GA); however, nine women had their first ultrasound between 28- and 30-weeks GA and one at 30 weeks. To obtain robust longitudinal data, women were asked to attend ultrasound scans every four weeks from their first ultrasound until delivery. Some women, particularly those with high risk pregnancies, had additional clinically indicated ultrasounds. If an ultrasound was performed at two or more weeks from the previous ultrasound recorded for the study, renal measurements were performed again for the study.

6.3.3 Ultrasound examination

Three Australian Accredited Medical Sonographers, with at least two years post ultrasound qualification experience, performed all examinations. A documented protocol outlined the required renal measurements and how they were to be performed for the study. Training of the sonographers was conducted prior to commencement of the study. An audit and follow-up were conducted with all participating sonographers three months after commencement of the study to confirm adherence to the study protocol. A Voluson E8 (GE Healthcare Ultrasound, Milwaukee, WI, USA) or an Epiq 7 (Philips Ultrasound, Bothell, WA, USA) were used for the ultrasound examinations and the highest frequency transducer possible, matching the mother's body habitus (1–5MHz), was selected to obtain the highest image resolution for each participant.

Where possible the fetal kidneys were measured with the fetal spine positioned anteriorly, or as close as possible to this position. The image was magnified so that the kidney occupied most of the image and one focus was placed at the level of the kidney. The renal parenchymal thickness was measured in the midsagittal plane of the kidney. It was measured from the inner aspect of the renal capsule to the sinus-pyramidal apex interface in two directions - from the posterior aspect of the kidney to the sinus-pyramidal apex (posterior parenchyma) and from the anterior border of the kidney to the sinus-pyramidal apex (anterior parenchyma) (Fig 6.1). Each measurement was performed twice and the mean of the two measurements were recorded. Both kidneys were measured.

Bilateral fetal renal artery Dopplers were performed in a coronal view of the kidneys. Colour flow was employed to identify the renal artery arising from the aorta and entering the kidney. A low wall filter of between 30 to 60 Hertz was used and a sample gate of size 2mm to 3mm was placed in the mid-trunk of the main renal artery. A pulse wave signal was obtained using an angle as close to 0 degrees as possible and when there was no fetal movement or breathing (Fig 6.2). The average

of at least three consistent consecutive waveforms was used to calculate the resistivity index (RI) and pulsatility index (PI).

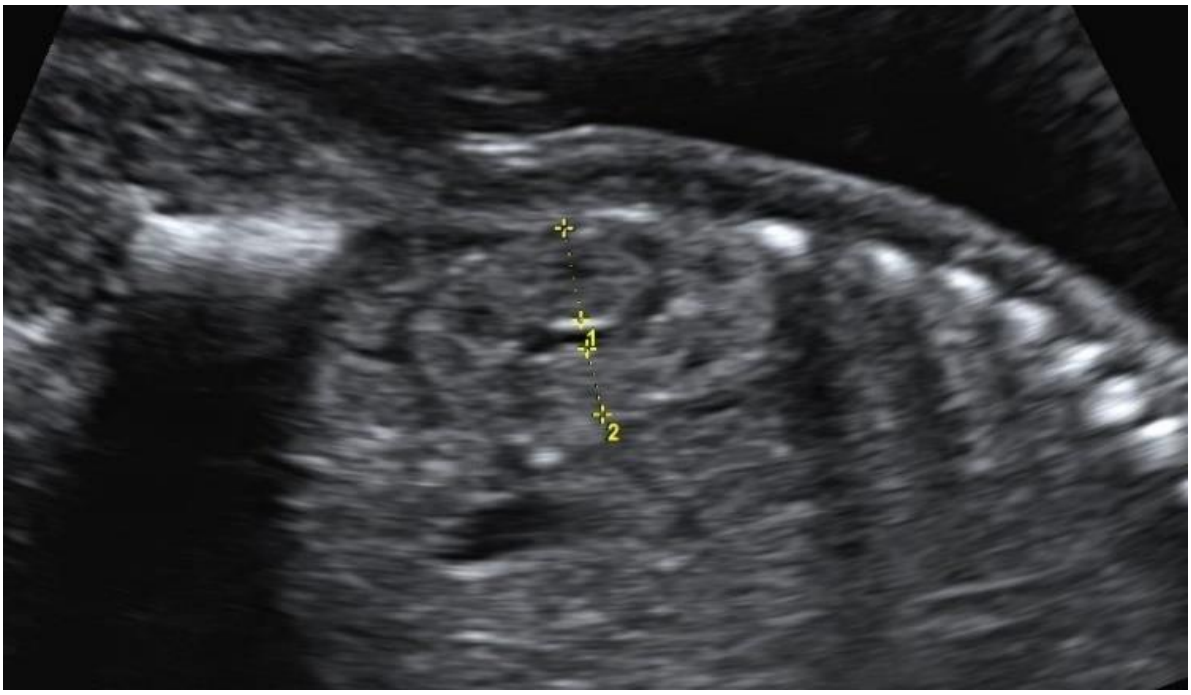


Figure 6.1 Measurement of the renal parenchymal thickness posteriorly (1) and anteriorly (2) from the inner aspect of the renal capsule to the sinus-pyramidal apex interface at 20 weeks gestational age. Source: Townsville University hospital.

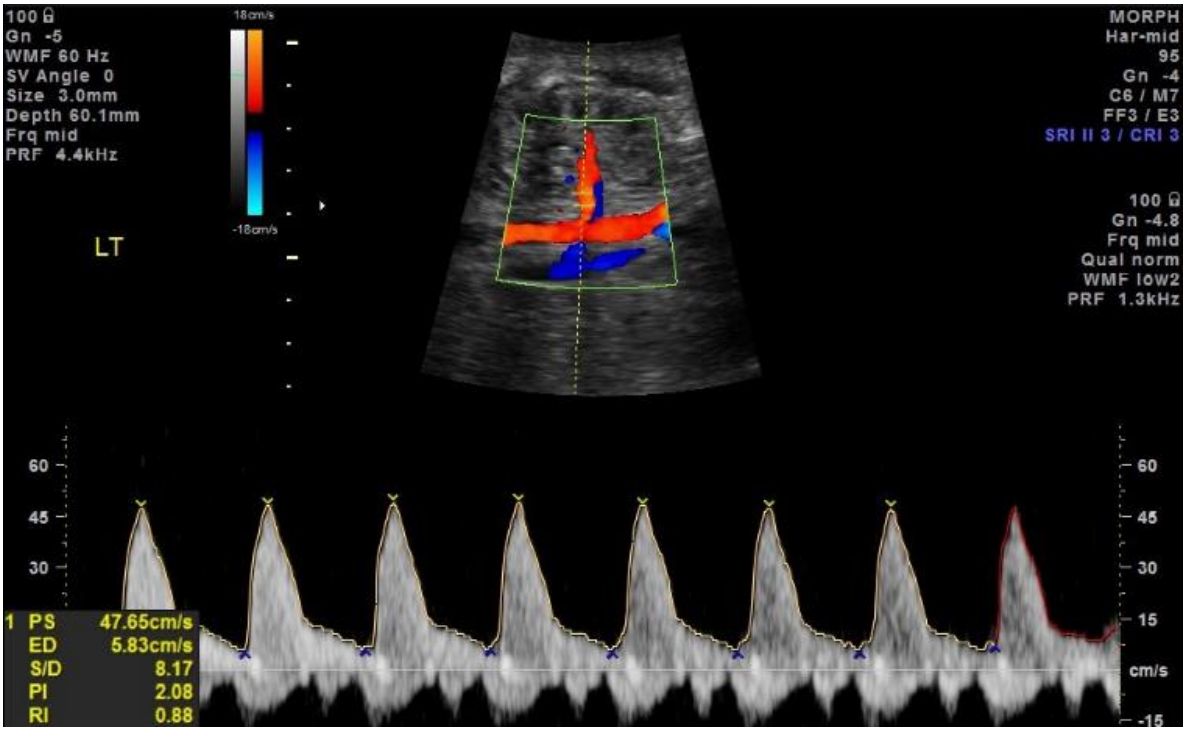


Figure 6.2 Colour and pulse wave Doppler from the mid-trunk of the left main renal artery at 33 weeks gestational age. Source: Townsville University Hospital.

6.3.4 Sample size

The sample size was calculated based on a statistical power of 80% and a significance level of 0.05 (two-tailed). Data from our previously published study demonstrated that the mean renal parenchymal thickness was 9.4mm (± 1.1mm) for normal birth weight neonates and 8.3mm (± 1.0) mm for low birth weight neonates at term.[20] Therefore, it was estimated that a sample size of 30 would be needed (15 growth restricted fetuses and 15 appropriate-for-gestational-age). Allowing for the possibility of loss to follow-up, at least 20 participants would be recruited for each group resulting in a total of 40 participants, each having ultrasound scans at least every four weeks.

6.3.5 Analysis

After birth, the infants were assigned to one of two groups – appropriate-for-gestational age (AGA) or fetal growth restriction (FGR). These groups were defined *a priori*.[13] Birth weight was plotted on Hadlock et al. fetal weight charts[21], as it has been demonstrated that neonatal charts do

not represent a random sample of the population at a given GA.[10] Infants born preterm are over-represented with cases of FGR and therefore fetal growth should be assessed against measurements of on-going pregnancies at that gestational age as opposed to a birth weight of infants born at a given gestational age.[10, 22] Those infants with a birth weight above the 90th centile were considered large for gestational age (LGA) and were excluded from this analysis. The criteria for classification of FGR is shown in Table 6.1 and was based on a consensus definition of FGR obtained by Delphi survey of 45 international experts in the field.[13] Infants who were neither LGA nor FGR were considered AGA.

Table 6.1
Classification of Fetal Growth Restriction (FGR)

Early FGR: Gestational Age < 32 weeks	**Late FGR: Gestational Age ≥ 32 weeks**
• AC or EFW < 3rd centile or UA - AEDF Or • AC or EFW < 10th centile combined with • Uterine artery - PI > 95th centile and/or • UA-PI > 95th centile	• AC or EFW < 3rd centile Or at least two of the following • AC or EFW < 10th centile • AC or EFW crossing centiles > 2 quartiles • CPR < 5th centile or UA-PI > 95th centile

Note: AC, abdominal circumference; AEDF, absent end diastolic flow; CPR, cerebroplacental ratio; EFW, estimated fetal weight; FGR, fetal growth restriction; PI, pulsatility index; UA, umbilical artery (based on Gordijn et al).[13]

Analysis of maternal and birth characteristics was performed using IBM SPSS version 25, Armonk, NY, USA. Normality of the demographic data was tested using a Kolmogorov-Smirnov test and visually inspecting the histograms. Normally distributed variables were reported as a mean and standard deviation (SD) and non-normally distributed variables as a median and interquartile range. All other analyses were conducted using R Statistical Language in R Studio (version 1.2.1335, Vienna, Austria).[23,24] Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. No outliers were removed. The *nlme* package (version 3.1-139)[25] was used to fit a random slopes linear mixed effects model to describe the effects of explanatory

variables on renal parenchymal thickness. The graphics were created with ggplot2.[26] Two models were fitted:

The first model focused on the relationship between the renal parenchymal thickness and gestational age. For this analysis, the response variable was renal parenchymal thickness and fixed effects in the model were gestational age (GA), growth (either AGA or FGR), kidney side (right or left), and the interaction between GA and growth. The relationship between parenchymal thickness and GA showed significant curvature, so a quadratic term was also included in the model. Other fixed effects were also tested (anterior or posterior parenchyma and gender), however they did not improve the fit. Random effects were participants, with random intercepts as well as random slopes for the effect of GA. Alternative models of different complexity were compared using likelihood ratio (LR) tests and Akaike's Information Criteria (AIC).

The second model assessed the effects of growth (AGA vs FGA) on the relationship between the thickness of the fetal renal parenchyma and the head circumference. We assumed a power function of the form:

$$y = ax^b$$

was appropriate to describe this relationship, where y = parenchymal thickness and x = head circumference. Since y and x are both linear measurements, the value of b should equal 1 if both grow at the same rate. In order to fit the model, the renal parenchyma and head circumference measurements were log transformed to convert the power function to a linear equation of the form:

$$\log(y) = \log(a) + b\ (\log(x))$$

The fixed effects in the model were head circumference (log10), growth (either AGA or FGR) and kidney side (right or left). In this case the interaction term did not improve the model fit and is omitted from the final model, as are other fixed effects tested (anterior or posterior parenchyma

and gender). Random effects were participants with random intercepts as well as random slopes for head circumference (log10).

Analysis of the fetal renal arteries (for both resistivity and pulsatility index) used fixed effects of GA (as a quadratic fit), growth (AGA or FGR), with interaction, and kidney side (right or left). Other fixed effects tested which did not improve the model included - anterior or posterior parenchyma and gender. Random effects were participants with random intercepts as well as random slopes for the effect of GA. As in previous models, likelihood ratio tests and Akaike's Information Criteria (AIC) were used to compare alternative models.

6.4 Results

One hundred and fifty-five pregnant women were recruited for the study, with 23 excluded (Fig 6.3). Among the remaining 132 pregnancies, 102 were AGA and 30 were FGR. The characteristics of the mother and baby are summarised in Table 6.2. FGR was associated with a significantly lower birth weight, an earlier GA at birth and a lower rate of diabetes.

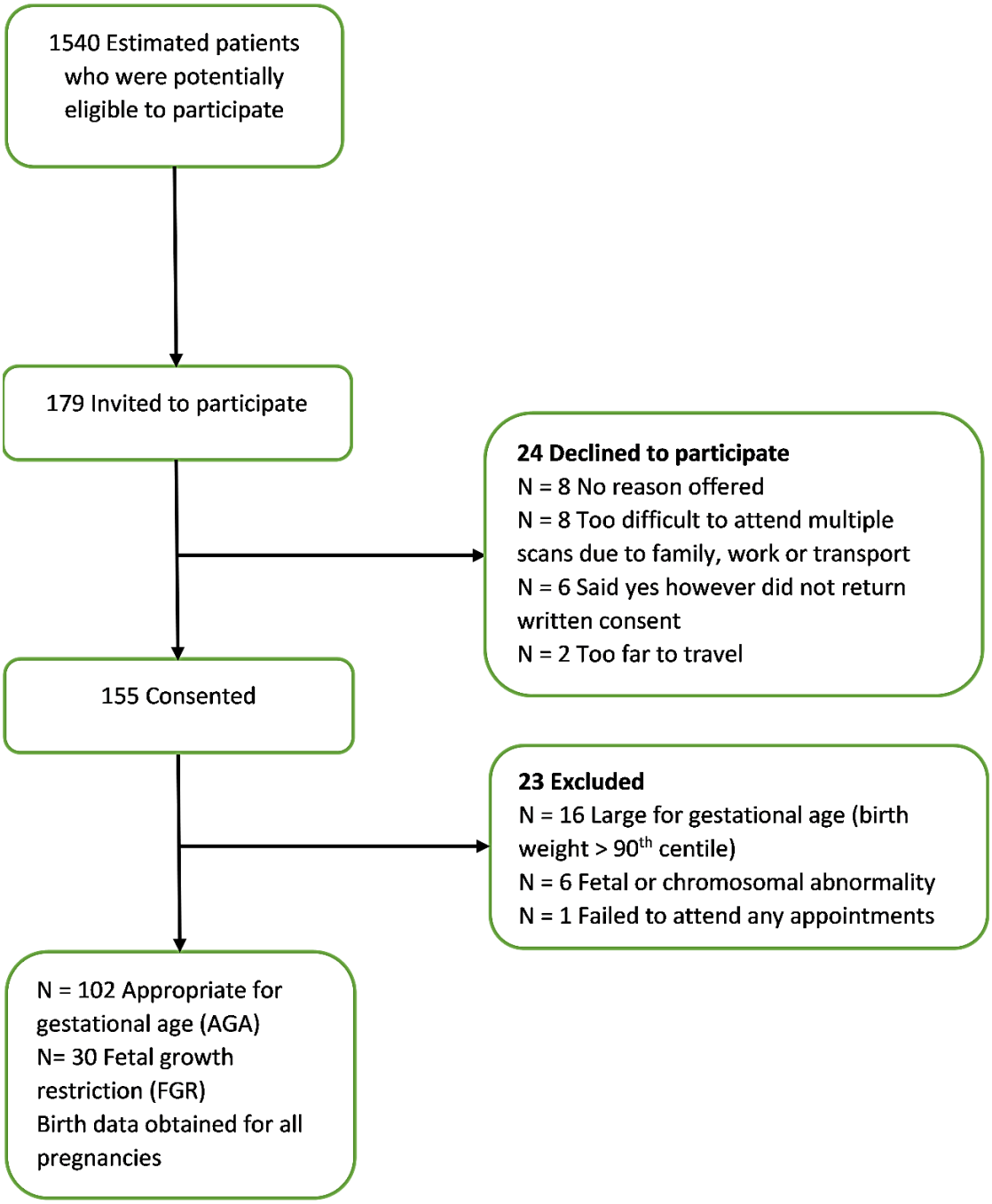


Figure 6.3 Flowchart of participant inclusion and exclusion process.

Table 6.2
Characteristics of 102 appropriate-for-gestational age (AGA) and 30 fetal growth restricted (FGR) pregnancies and their infants

Participant Characteristics	AGA (N=102)	FGR (N=30)	*p* value
MATERNAL			
Maternal age (years) (means ± SD)	29.6 ± 5.2	32.0 ± 6.4	0.099[a]
Maternal height (cm) (means ± SD)	1.65 ± 0.06	1.62 ± 0.07	0.411[a]
Maternal weight (kg) (M, IQR)	72.0 (60.0–86.5)	70.8 (55.0 - 87.7)	0.615[b]
Maternal BMI (kg/cm2) (M, IQR)	25.8 (22.7 – 31.6)	25.6 (23.2 – 33.9)	0.996[b]
Maternal race origin, *n* (%)	N=82[#]	N=25[#]	0.354[c]
• Aboriginal/Torres Strait Islander	7 (6.9%)	5 (16.7%)	
• Asian	3 (2.9%)	0 (0%)	
• Caucasian	69 (67.6%)	19 (63.3%)	
• Indian	1 (1.0%)	0 (0%)	
• Other	2 (2.0%)	1 (3.3%)	
Parity, *n* (%)			0.584[d]
• Nulliparous	50 (49.0%)	13 (43.3%)	
• Parous	52 (51.0%)	17 (56.7%)	
Maternal Medical Disorders, *n* (%)			
Pregestational Diabetes	3 (3.0%)	1 (3.3%)	1.000[e]
Gestational diabetes	35(34.3%)	4 (13.3%)	0.039[e]*
Thyroid disease	14 (13.7%)	2 (6.7%)	0.524[e]
Hypertension (needing treatment)	6 (5.9%)	4 (13.3%)	0.234[e]
Other maternal medical disorders	15 (14.7%)	7 (23.3%)	0.274[e]
NEONATAL			
GA at birth (weeks) (M, IQR)	38.7 (38.0 – 39.3)	37.4 (35.2- 38.2)	<0.0001[b]*
Birth weight (grams) (M, IQR)	3390 (2978 - 3603)	2345 (1811 - 2820)	<0.0001[b]*
Male	52 (51%)	15 (50%)	0.925[d]

Note: AGA, appropriate for gestational age; FGR, fetal growth restriction; GA, gestational age; IQR, interquartile range; M, median; SD, standard deviation.
[#]20 (19.6%) AGA and 5 (16.7%) FGR participants declined to answer maternal race.
* = p<0.05. [a] Independent t-test; [b] Mann-Whitney U; [c] Likelihood Ratio; [d] Pearson Chi-Squared; [e] Fisher's exact test.

Due to the small numbers of examinations below 20 weeks and over 38 weeks GA, data was only included from between 20 weeks 0 days and 37 weeks 6 days GA. Measurements were obtained from both fetal kidneys and renal arteries with a total of 638 separate ultrasound examinations performed between 20 to 37 weeks GA. The median number of scans per pregnancy was five (range was one to eight). The full set of planned examinations were not completed in some cases as the participant delivered prior to the end of the study.

6.4.1 Renal parenchymal thickness

In total, 2556 renal parenchymal thickness measurements were made - four measurements on each fetus at each GA, corresponding to one each by side (right or left) and anterior and posterior. During modelling, no significant effect was found according to gender (p=0.177) or whether the anterior and posterior parenchyma was measured (p=0.163) and therefore these were not included in the model. There was a significant difference in the renal parenchymal thickness between the right and left kidneys with the left parenchyma measuring significantly thinner (p = 0.001) and therefore kidney side was included in the model.

The findings have demonstrated that the renal parenchymal thickness is significantly thinner in growth restricted fetuses when compared to appropriately grown fetuses and the effect is strong (LR = 21.06, p = <0.0001). P-values are obtained by likelihood ratio (LR) tests of the full model with the growth of the fetus (whether they are appropriately grown or not) in the model against a model without the fetal growth included. With increasing gestational age, the difference between the thickness of the parenchyma of appropriately grown and growth restricted fetuses increases. The overall regression line (assuming independence) is illustrated in Fig 6.4. Table 6.3 displays the fixed effects estimates and Table 6.4 displays the random effects. The equations for renal parenchymal thickness (RPT) are:

- Right AGA RPT = 4.37 + 0.448GA – 0.00885(GA^2);
- Right FGR RPT = 4.37 + (0.448 – 0.0383)GA – 0.00885(GA^2);
- Left AGA RPT = (4.37 – 0.108) + 0.448GA – 0.00885(GA^2); and
- Left FGR RPT = (4.37 – 0.108) + (0.448 – 0.0383)GA – 0.00885(GA^2).

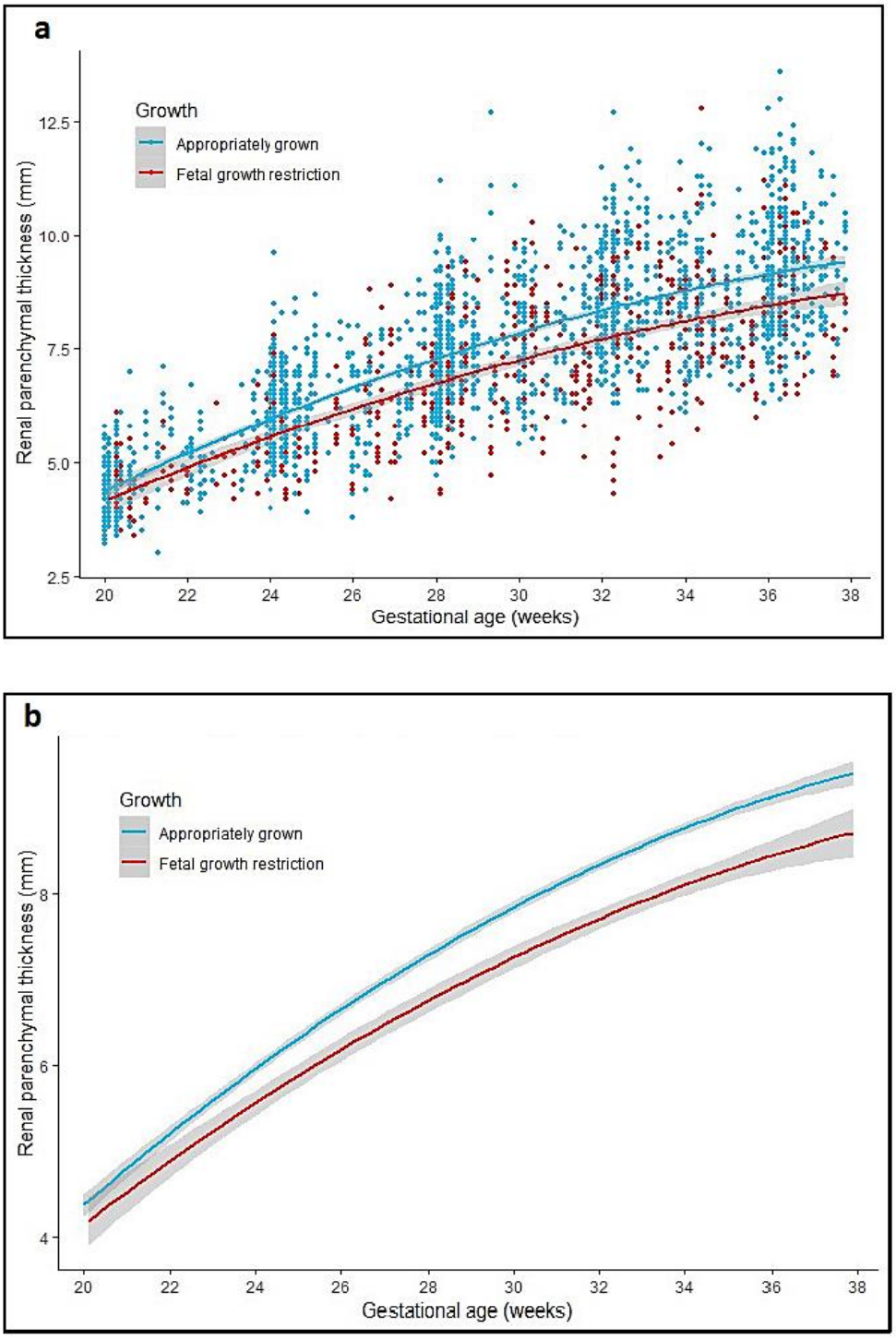


Figure 6.4 Renal parenchymal thickness by gestational age for appropriately grown and fetal growth restricted fetuses (a) overall regression lines with all data points (b) overall regression lines. Shades denote 95% confidence interval.

Table 6.3
Fixed effects estimates for renal parenchymal thickness by gestational age modelling

	Estimate	Confidence Interval	SE	p-value
Intercept	4.372	4.222 – 4.520	0.0754	<0.0001
Gestational age	0.448	0.419 – 4.476	0.0146	<0.0001
Growth (AGA to FGR)	-0.364	-0.646 - - 0.082	0.1412	0.0110
Gestational age (quadratic)	-0.009	-0.010 - - 0.007	0.0007	<0.0001
Side (right to left)	-0.108	-0.173 - -0.043	0.0331	0.0011
Gestational age: Growth interaction	-0.038	-0.070 - -0.006	0.0161	0.0181

Note: 95% confidence intervals. AGA, appropriate-for-gestational-age; FGR, Fetal growth restriction; SE, standard error.

Table 6.4
Random effects estimates for participants

Participant Level	Estimate	Confidence Interval
SD Intercept	0.484	0.380 – 0.617
SD Gestational Age	0.062	0.051 – 0.075
Cor (intercept, Gestational Age)	-0.132	-0.412 – 0.171

Note: 95% confidence intervals. SD, standard deviation.

6.4.2 Renal parenchymal thickness compared to head circumference

Growth of the renal parenchymal thickness was compared to head circumference (HC) (Fig 6.5) and this showed a significant difference between AGA and FGR fetuses (LR=8.9, p=0.0028) with the renal parenchymal thickness growing at a slower rate compared to HC in FGR than in AGA fetuses. There was, however, no difference in the slope of the growth. Fixed and random effect estimates are provided in Tables 6.5 and 6.6.

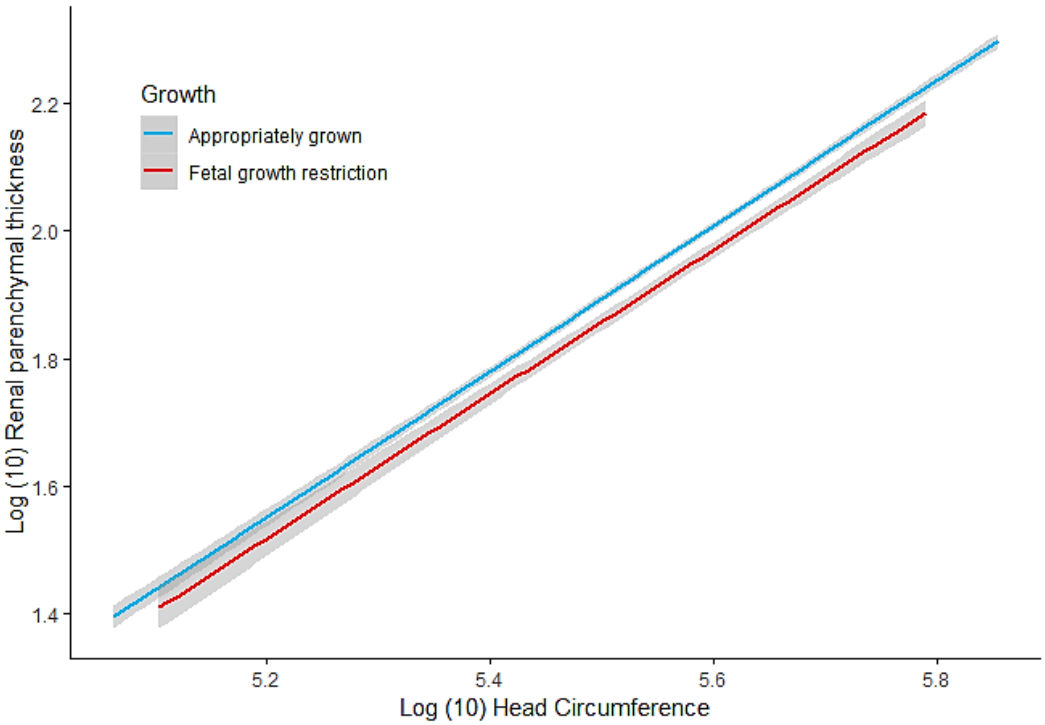


Figure 6.5 Relationship between log(10) transformed renal parenchymal thickness and head circumference for appropriately grown and fetal growth restricted fetuses. Shades denote 95% confidence interval.

Table 6.5
Fixed effects estimates for Renal parenchymal thickness(log10) to Head Circumference(log10)

	Estimate	Confidence Interval	SE	p-value
Intercept	-4.515	-4.760 - -4.269	0.1252	<0.0001
Head Circumference (log10)	0.195	1.122 – 1.211	0.0225	<0.0001
Growth (AGA to FGR)	-0.0.57	-0.095 - -0.019	0.0191	0.0032
Side (right to left)	-0.015	-0.023 - -0.005	0.0046	0.0014

Note: 95% confidence intervals. AGA, appropriate-for-gestational-age; FGR, Fetal growth restriction; SE, standard error.

Table 6.6
Random effects estimates for participants for Renal parenchymal thickness(log10) to Head Circumference(log10)

Participant Level	Estimate	Confidence Interval
SD Intercept	1.080	0.888 – 1.314
SD Head Circumference (log10)	0.195	0.161 – 0.237
Cor (intercept, head circumference(log10))	-0.997	-0.998 - -0.995

Note: 95% confidence intervals. SD, standard deviation.

6.4.3 Renal artery Dopplers

In total 1235 renal artery Dopplers were carried out. Doppler of the renal artery was not able to be obtained for one kidney in 25 scans and for both kidneys in 12 scans due to fetal position and/or persistent movement. No significant difference was seen between AGA and FGR fetuses in the resistivity index (RI) ($p = 0.182$) or pulsatility index (PI) ($p = 0.554$) of the renal arteries. Table 6.7 to 6.10 show the fixed and random effects estimates respectively.

Table 6.7
Fixed effects estimates for Renal artery resistivity index (RI)

	Estimate	Confidence Interval	SE	p-value
Intercept	0.874	0.865 – 0.884	0.0047	<0.0001
Gestational age	0.006	0.004 – 0.007	0.0009	<0.0001
Growth (AGA to FGR)	0.004	-0.014 – 0.021	0.0090	0.6856
Side (right to left)	0.005	0.001 – 0.009	0.0022	0.0224
Gestational age (quadratic)	-0.000	<-0.001 - <-0.001	<0.0001	<0.0001
Gestational age: Growth	-0.001	-0.003 – <0.001	0.0008	0.2031

Note: 95% confidence intervals. AGA, appropriate-for-gestational-age; FGR, Fetal growth restriction; SE, standard error.

Table 6.8
Random effects estimates for Renal artery resistivity index (RI)

Participant Level	Estimate	Confidence Interval
SD Intercept	0.029	0.033 – 0.046
SD Gestational age	0.003	0.003 – 0.004
Cor (intercept, gestational age)	-0.804	-0.891 - -0.662

Note: 95% confidence intervals. SD, standard deviation.

Table 6.9
Fixed effects estimates for Renal artery pulsatility index (PI)

	Estimate	Confidence Interval	SE	p-value
Intercept	2.245	2.177 – 2.311	0.0341	<0.0001
Gestational age	0.025	0.011 – 0.038	0.0068	0.0003
Growth (AGA to FGR)	0.056	-0.068 – 0.179	0.0626	0.3762
Side (right to left)	0.023	-0.010 – 0.056	0.0168	0.1632
Gestational age (quadratic)	-0.002	-0.002 - -0.001	0.0003	<0.0001
Gestational age: Growth	-0.007	-0.019 – 0.005	0.0061	0.2787

Note: 95% confidence intervals. AGA, appropriate-for-gestational-age; FGR, Fetal growth restriction; SE, standard error.

Table 6.10
Random effects estimates for Renal artery pulsatility index (PI)

Participant Level	Estimate	Confidence Interval
SD Intercept	0.180	0.132 – 0.246
SD Gestational age	0.019	0.015 – 0.025
Cor (intercept, gestational age)	-0.697	-0.839 - -0.466

Note: 95% confidence intervals. SD, standard deviation.

6.5 Discussion

6.5.1 Renal parenchymal thickness and fetal growth restriction

Our study demonstrates that the renal parenchymal thickness is significantly thinner in growth restricted fetuses when compared to appropriately grown fetuses. A point of difference with this study is that fetal size and Doppler criteria were used to classify true fetal growth restriction.[13] Almost all previous fetal and kidney studies use small for gestational age (SGA) as a surrogate for FGR.[6, 27-29] Recent advances in medical imaging technology and publication of an international consensus on FGR classification[13] enables clinicians and researchers to improve the diagnose of FGR and understand FGR is a failure to achieve optimal growth and not just smallness.

SGA is based only on a weight cut off after birth, such as a birth weight less than 2500g, and therefore includes genetically small fetuses, but healthy, and excludes infants within the normal weight range but who are truly growth restricted. FGR is defined as a pathologically small fetus who does not meet its optimal growth and will usually be associated with abnormal uteroplacental or fetal blood flow.[10, 13] It is largely independent of absolute growth and is principally based on growth trajectory.[30] If fetal growth drops from the 80th centile to the 20th centile over time the fetus is considered growth restricted even though the fetal weight is within the normal range.

As this was a longitudinal study, we can truly assess the growth of the parenchyma in real-time. In the literature, only limited data is available on actual fetal kidney growth, as although some studies report kidney growth the studies are cross-sectional in design and therefore unsuitable to assess growth.[16] A strength of our study was having longitudinal data analysed by mixed effects modelling. Mixed effects modelling is much more flexible and powerful than traditional analyses that perform overall averaging.[31] Every data point is considered using fixed and random effects in a single model to account for all sources of variation. Mixed effects models can deal with missing data and naturally handles unevenly spaced repeated measures which commonly occurs in human studies.

Our study demonstrated a significant difference in thickness and growth trajectory of the renal parenchyma between AGA and FGR fetuses. With increasing gestational age, the difference between thickness of the parenchyma in the two groups increased. Placental insufficiency is the most common cause of FGR.[10, 11] It is therefore plausible that this deceleration in growth of the parenchyma of FGR fetuses may be at least partly due to increasing placental insufficiency and redistribution of fetal blood supply away from the fetal kidneys. This is particularly important for kidney development as nephrogenesis continues up until 36 weeks GA, with 60% of nephrons formed in the third trimester.[32] Ultrasound studies also indicate maximum kidney growth occurs in the third trimester.[33 ,34] This coincides with the timing of incidence of the majority of FGR.[35]

Our analysis has shown that the right fetal renal parenchyma was thicker than the left by 0.11mm. This is not thought to be clinically important. In a recent systematic review completed by our group on the evaluation of fetal kidney growth using ultrasound we discovered almost all studies found no significant difference between right and left fetal kidney size.[16] One large study (n=1215) did find that the right kidney was significantly wider and deeper than the left kidney, however, not longer.[33] This is consistent with our study demonstrating a thicker parenchyma in right kidneys. Our ability to detect this difference may be due to the higher sensitivity provided by the mixed effects modelling in our study.

Fetal and neonatal kidney volumes have been used as a surrogate measure of nephron number and kidney function.[36-39] There are some limitations, however, with using kidney volume as an estimate of nephron number. Obtaining a kidney volume involves acquiring three orthogonal measurements and then applying an ellipsoid formula. There is error associated with each measurement and the formula. A study we conducted in neonates demonstrated that kidney volume measurements had a significantly higher variance than renal parenchymal thickness measurements.[20] Ultrasound kidney volumes calculated using the ellipsoid formula have also been found to underestimate actual kidney volume compared to *in vivo and ex vivo* models by more than

20%.[40, 41]The advantage of measuring the renal parenchymal thickness is that instead of measuring the entire kidney, a single measurement is performed of the functional, nephron containing region and the collecting system is not included. For example, in cases of hydronephrosis measurements of kidney volume could significantly overestimate nephron number due to the enlargement of the collecting system when in fact the renal parenchyma could be thinner than normal, and the kidney may have impaired function.

Kadioglu (2010) appears to be the first author to report normative ultrasound values for renal parenchymal thickness for children to assess for alterations in normal growth[42]. Our studies since on the renal parenchymal thickness of neonates and other studies in children highlight the potential of the parenchymal thickness measurement as a possible marker for renal function and to monitor renal parenchymal changes[20, 43-45]. One study has reported some normal ranges for fetal renal parenchymal thickness[46], however, to our knowledge no study has investigated the growth of the renal parenchyma with gestational age in growth restricted fetuses. This new parenchymal thickness measurement is a more specific, indirect evaluation of nephron endowment.

Measurement of fetal renal parenchymal thickness could be used to monitor the effects of FGR on fetal kidney growth and the effects of any possible interventions for FGR treatment. FGR can arise from fetal, placental and/or maternal disorders and often may be due to a combination of more than one cause.[11, 35] When placental abnormalities or maternal disease is the cause, nutrients and oxygen flow to the fetus may be impaired. The fetus compensates for this by preferentially shunting blood away from organs such as the kidneys, towards the more essential organs of the brain (known as "brain sparing"), heart and adrenals.[18]

Considering that there may be brain sparing in the FGR fetuses, the growth of the renal parenchymal thickness was compared to the growth of the head circumference between the AGA and FGR groups and a significant difference was seen in our study. In fetuses with the same head circumference, a growth restricted fetus was more likely to have a thinner parenchyma than an

appropriately grown fetus. This suggests that in small growth restricted fetuses the renal parenchyma is thinner than could be expected purely based on fetal size compared to an appropriately grown fetus. A possible mechanism for this differential renal parenchyma growth is preferential shunting of fetal blood away from the kidneys to the brain due to fetal hypoxia which impacts on appropriate nephrogenesis. The fact that the slopes are the same for both groups may imply that the "brain-sparing" effect happens earlier than 20 weeks gestation and that the kidneys never catch up once they have been compromised.

6.5.2 Fetal renal arteries

The renal arteries were analysed for any changes in blood flow to the kidneys. No significant difference in the resistivity or pulsatility index of the fetal renal arteries between AGA and FGR fetuses was seen. This is consistent with the findings from other studies.[27, 47] This observation may be due to several reasons. 1. Fetal blood flow to the kidneys is very low with only 5% of cardiac output going to the kidneys compared to 9% after birth.[48] Therefore, any change in fetal blood flow may be too subtle for us to detect using ultrasound. It is also possible that our study was not powered to specifically detect a difference in the renal blood flow. 2. A much larger study of FGR fetuses with identifiable abnormal uteroplacental or fetal blood flow is needed to detect a difference.

6.5.3 Limitations of the study

There were some limitations to our study. The lack of blinding of the sonographers could have potentially introduced measurement bias. It was difficult to blind the sonographer to all clinical and biometric information as most of the studies included a diagnostic scan. Sonographers are generally specifically trained not to look at the measurements at the time that they are being performed. Additionally, the infants were not assigned to AGA and FGR groups until after birth and it was based on birth weight and not the estimated fetal birth weight calculated from the measurements done by the sonographer. Having multiple sonographers performing the

examinations rather than only one reduces some of the bias. Another limitation was the number of the FGR group compared to the AGA group. The fetuses in the FGR group were more likely to be delivered earlier before all planned ultrasound examinations could be performed.

6.5.4 Future direction

Although it is widely accepted that fetal growth restriction has an effect on nephron number and future kidney function, there is a lack of *in vivo* proof of the mechanisms occurring *in utero*.[6] This study provides evidence of an effect on the development of the renal parenchyma which likely represents a reduced nephron number, in circumstances of true fetal growth restriction.

Life-long monitoring of growth restricted, low birth weight and preterm infants along with those exposed to pre-eclampsia or gestational diabetes is advocated.[49] Such an implementation would involve a significant number of the population and be a significant health cost burden. Measurement of the renal parenchymal thickness, in contrast, has the potential to more appropriately and accurately identify infants with a reduced nephron endowment so that monitoring and interventions can be focused on those individuals at a higher risk of developing neonatal acute kidney injury and future hypertension and chronic kidney disease.

6.6 Conclusion

Kidney disease is associated with a significant global burden and health costs and this study improves our understanding and assists in identifying adverse effects on the kidney during gestation. Utilising ultrasound to measure the fetal renal parenchymal thickness provides a simple, non-invasive estimate of nephron number. Our data suggests that fetal growth restriction has a negative influence on nephron numbers as it is associated with a significantly thinner parenchyma and slower growth trajectory. It should be remembered that having a reduced nephron number alone does not mean hypertension or chronic kidney disease is inevitable, but that the kidney may be less able to endure future kidney injury in later life. Using the approach outlined in our study,

there is the potential to prevent or reduce the adverse outcomes of kidney disease for future generations.

6.7 References

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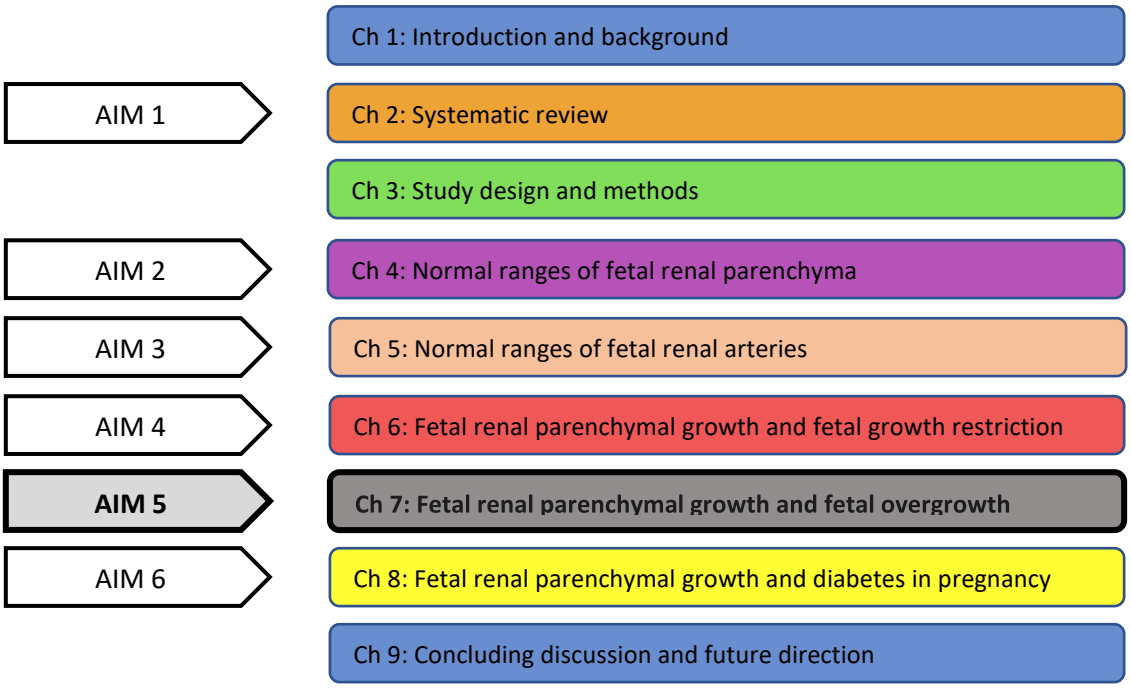
Chapter. 7 Is Bigger Better? The Large for Gestational Age Fetus and Kidney Development

Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Is bigger better? The large for gestational age fetus and kidney development. **In Preparation**.



There is growing evidence of the long-term health effects of infants born large for gestational age and recently emerging links between a high birth weight and chronic kidney disease. To our knowledge no study had yet investigated the possible effects of fetal overgrowth on fetal kidney development. The aim of this chapter was to address the lack of research in this area. This paper is currently being prepared for submission to a journal.

7.1 Abstract

Objective:

A non-invasive ultrasound measurement of the fetal renal parenchymal thickness was employed to investigate possible adverse effects of fetal overgrowth on fetal kidney development and renal perfusion. The aim of this study was to measure the fetal renal parenchymal thickness and evaluate whether fetal overgrowth affects the growth of the fetal renal parenchyma. The fetal renal artery blood flow was also assessed.

Methods

This prospective, observational study used serial ultrasound measurements to assess the growth of the fetal renal parenchyma. Mixed effects modelling was used to compare 16 large-for- gestational age (LGA) fetuses with 102 appropriate-for-gestational (AGA) age fetuses.

Results

The fetal renal parenchyma measurement of LGA fetuses was significantly thicker than the AGA fetuses (LR Chisq=6.1, df=1, p=0.013), however, this thickness was proportional to the increased size of the LGA fetuses. No significant difference was seen between the fetal renal artery resistivity index and pulsatility index of LGA and AGA fetuses.

Conclusion

This is the first study, to our knowledge, to investigate any possible effects of fetal overgrowth on the developing kidneys. The increased thickness in the renal parenchyma of LGA fetuses was proportional to the increased size of these fetuses and therefore no significant adverse effect on the growth of the fetal renal parenchyma were demonstrated. Most LGA infants were born to mothers with diabetes and therefore it would be of value to evaluate the influence of diabetes on the developing fetal renal parenchyma.

7.2 Introduction

Fetal overgrowth is associated with increased risks to the mother and infant, such as emergency caesarean section, instrumental delivery, shoulder dystocia and trauma to the birth canal.[1, 2] The large for gestational age (LGA) fetus is defined as having a weight greater than the 90th centile for a given gestational age (GA).[1, 3] The term LGA is preferred to macrosomia as macrosomia is based on a birth weight cut off and does not consider GA. Fetal growth is influenced by nutritional, hormonal, genetic and environmental factors, many of which are modifiable.[3]

The long-term risks for infants born LGA includes diabetes mellitus, obesity and metabolic syndrome.[4, 5] These are also strong risk factors for chronic kidney disease (CKD) and hypertension, with diabetes being the leading cause of CKD.[6, 7] Current research supports renal programming in-utero, with a high birth weight now becoming apparent as a developmental risk for future kidney disease and hypertension.[8-10]

Nephrons are the functional units of the kidneys and their number and function are determined *in-utero.*[11, 12] Abnormal fetal growth can influence kidney growth and may yield a lower compliment of nephrons, thus exposing the kidneys to impaired renal function and an increased risk of future kidney disease.[13, 14] It is well established that fetal growth restriction is associated with fewer nephron numbers,[15-17] however, there are currently no known studies assessing the impact of fetal overgrowth on the growing kidneys.[18] Additionally, evidence of any alterations in renal perfusion *in-utero* and its role in any possible reduction in future renal function is gaining interest.[19, 20] To our knowledge, there are no previous studies investigating the possible association between LGA and fetal renal blood flow.

It is important to understand what, if any, adverse effects of fetal overgrowth (including fetal renal perfusion) have on kidney development, throughout pregnancy. An ultrasound measurement of the fetal renal parenchyma is a non-invasive method to evaluate renal growth and can provide a

surrogate estimate of nephron number.[21] The main aim of this study was to measure the fetal renal parenchymal thickness of LGA fetuses and compare this to appropriate for gestational age (AGA) fetuses. These measurements allow evaluation of the possible effects of fetal overgrowth on the growth of the fetal kidneys. Evaluation of the fetal renal artery blood flow was also assessed. We hypothesised that the thickness of the fetal renal parenchyma of LGA fetuses would differ from AGA fetuses.

7.3 Methodology

The overall research methodology and the methodology for the ultrasound measurements performed for this study have been outlined in Chapter 3. Methods specific to this analysis are described below.

7.3.1 Study design

After birth, infants were assigned to one of two groups – LGA or AGA. These groups were defined *a priori*. LGA was defined as infants with a birth weight above the 90th centile.[1, 3] Infants who were classified as having fetal growth restriction (FGR), as described in Chapter 6, were excluded from this analysis. Infants who were neither LGA nor FGR were considered AGA. Birth weight was plotted on Hadlock et al.[22] fetal weight charts, rather than neonatal charts, as it has been demonstrated that neonatal charts do not represent a random sample of the population at a given GA.[23] Infants born preterm are over-represented with cases of FGR and therefore fetal growth should be assessed against measurements of on-going pregnancies at that GA as opposed to a birth weight of infants born at a given GA.[23, 24]

7.3.2 Statistical analyses

All statistical analyses were performed using R Statistical Language in R Studio (version 1.2.1335)[25,26] and the ggplot2 package was used to create the graphics.[27] Normality of the maternal and neonatal demographic data was assessed by visually inspecting the histograms, normal Q-Q

plots and box plots. Normally distributed variables were reported as a mean and standard deviation (SD) and non-normally distributed variables as a median and interquartile range.

The nlme package (version 3.1-139)[28] was used to fit a random slopes linear mixed effects model to describe the effects of explanatory variables on fetal renal parenchymal thickness and renal artery blood flow. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. No outliers were removed.

Fixed effects of gender and anterior vs posterior (in the case of parenchymal thickness) were tested in all models but did not improve the fit. Determining whether or not to include different effects in the final model was done by comparing alternative models using likelihood ratio (LR) test and Akaike's Information Criteria (AIC). The GA variable was modified to 16 weeks = 0, to ensure the intercept term in the summary output would represent the parenchymal thickness or renal artery value for the AGA group at 16 weeks. Three different models were fitted:

7.3.2.1 Model 1. Renal parenchymal thickness growth

The relationship between the renal parenchymal thickness and GA of LGA and AGA fetuses was compared. The response variable was renal parenchymal thickness and fixed effects in the model were GA, growth (either LGA or AGA), kidney side (right or left), and the interaction between GA and growth. The relationship between parenchymal thickness and GA showed significant curvature, so GA was included as a quadratic orthogonal polynomial term. Random effects were a random intercept for each participant and the random slopes element used the orthogonal polynomial term for the effect of GA. Appendix C2 provides the details of the models fitted with their outputs.

7.3.2.2 Model 2. Renal parenchymal thickness growth compared to abdominal circumference (AC) growth

The relationship between the thickness of the fetal renal parenchyma and the abdominal circumference (AC) was analysed by assuming a power function to describe the relationship of the form:

$$y = axb$$

where y = parenchymal thickness and x = AC. Y and x are both linear measurements and therefore, the value of b should equal 1 if both grow at the same rate. To enable the model to be fitted, the renal parenchymal thickness and AC measurements were log transformed to translate the power function to the following linear equation:

$$\log(y) = \log(a) + b\ (\log(x)).$$

For these models, the response variable was the renal parenchyma ($\log_{10}$) and the fixed effects were AC ($\log_{10}$), growth (either LGA or AGA) and kidney side (right or left). An interaction between AC ($\log_{10}$) and growth was included so that the slopes of the regression lines for the LGA or AGA groups could be compared. A random intercept was included for each participant and a random slope for the effect of AC ($\log_{10}$).

7.3.2.3 Model 3. Renal artery blood flow

These models evaluated the resistivity index (RI) and pulsatility index (PI) of the fetal renal arteries with the RI and PI being the response variable in each of their respective models. The fixed effects were GA, growth (LGA or AGA) and kidney side (right or left), and an interaction between GA and growth. GA was again included as a quadratic orthogonal polynomial to allow for curvature in the relationship between GA and RI or PI. Each participant had a random intercept and the random slopes component used the orthogonal polynomial for the effect of GA.

7.4 Results

A flowchart of the recruitment data is shown in Figure 7.1. A total of 155 pregnant women were recruited. Thirty-seven were excluded, mostly due to fetal growth restriction, resulting in 16 LGA infants and 102 AGA infants. There were 633 ultrasound examinations performed between 16- to 39 weeks GA. The mean (SD) number of scans per pregnancy was 5.4 ± 1.4. The first ultrasound was usually completed between 16- and 26-weeks, however, six women had their first ultrasound at 28 weeks.

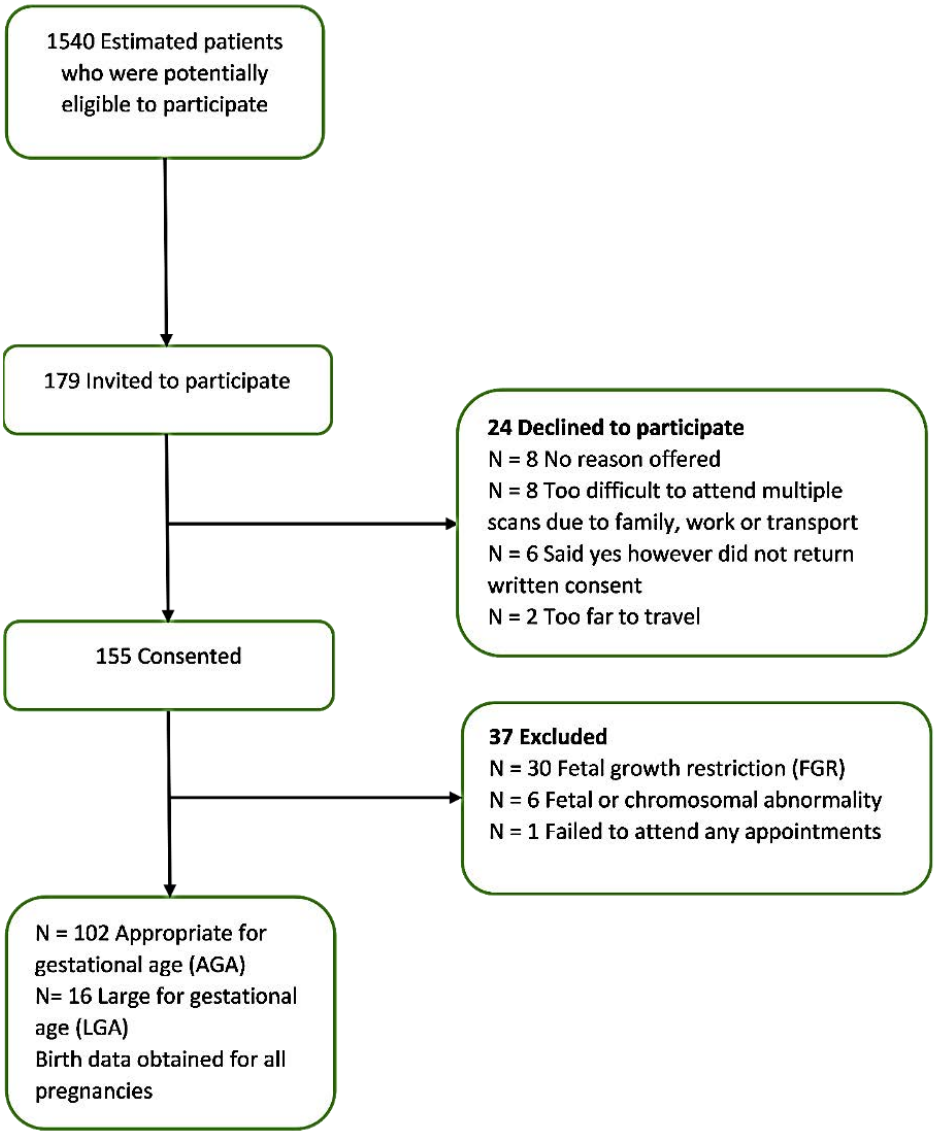


Figure 7.1 Flowchart of participant inclusion and exclusion process.

The characteristics of the mothers and infants are summarised in Table 7.1. Mothers of LGA infants had a significantly higher pre-pregnancy weight and BMI (median weight 93kg; BMI 32.3) when compared to mothers of AGA infants (median weight 72kg; BMI 25.8; p = <0.05). In 75% of the LGA infants the mother had pregestational or gestational diabetes mellitus during pregnancy. Despite their lower gestational age at birth, the LGA infants had a significantly higher birth weight and birth weight centile than the AGA infants. A comparison of maternal BMI between the LGA or AGA groups are shown in Figure 7.2. There were no stillbirths or neonatal deaths.

Table 7.1
Characteristics of appropriate-for-gestational age (AGA) and large-for-gestational age (LGA) pregnancies and their infants

Participant Characteristics	AGA (N=102)	LGA (N=16)	*p* value
MATERNAL			
Maternal age (years)	29.6 ± 5.2	30.0 ± 5.0	0.758[a]
Maternal height (cm)	165 ± 6	165 ± 6	0.789[a]
Maternal weight (kg) (M, IQR)	72.0 (60.0 – 86.5)	93.0 (75.3 – 106.8)	0.015[b]*
Maternal BMI (kg/cm2) (M, IQR)	25.8 (22.7 – 31.6)	32.3 (38.0 – 39.8)	0.010[b]*
Maternal race origin	N = 82#	N = 14#	
• Aboriginal/Torres Strait Islander	7 (8.5)	2 (14.3)	0.798[c]
• Asian	3 (3.7)	1 (7.1)	
• Caucasian	69 (84.1)	11 (78.6)	
• Indian	1 (1.2)	0	
• Other	2 (2.4)	0	
Multiparous	52 (51.0)	13 (81.2)	0.024[d]*
Diabetes			0.0001[c]*
• No diabetes	64 (62.7)	4 (25.0)	
• Pregestational Diabetes	3 (3.0)	6 (37.5)	
• Gestational diabetes	35 (34.3)	6 (37.5)	
NEONATAL			
GA at birth (weeks) (M, IQR)	38.7 (38.0 – 39.3)	38.3 (36.3 – 38.8)	0.035[b]*
Preterm (<37 weeks GA)	8 (7.8)	5 (31.3)	0.037[c]*
Birth weight (grams) (M, IQR)	3390 (2978 – 3603)	3905 (3684 – 4123)	<0.0001[b]*
Birth centile	44.5 (24.0 – 67.5)	94.5 (91.5 – 97.5)	<0.0001[b]*
Male	52 (51.0)	11 (68.8)	0.185[d]

Note: [#]20 (19.6%) AGA and 2(12.5%) LGA participants declined to answer maternal race. Means ± SD. M, median; IQR, interquartile range or n (%). GA, gestational age. * = p<0.05
[a] Independent t-test; [b] Mann-Whitney U; [c] Likelihood Ratio; [d] Pearson Chi-Squared.

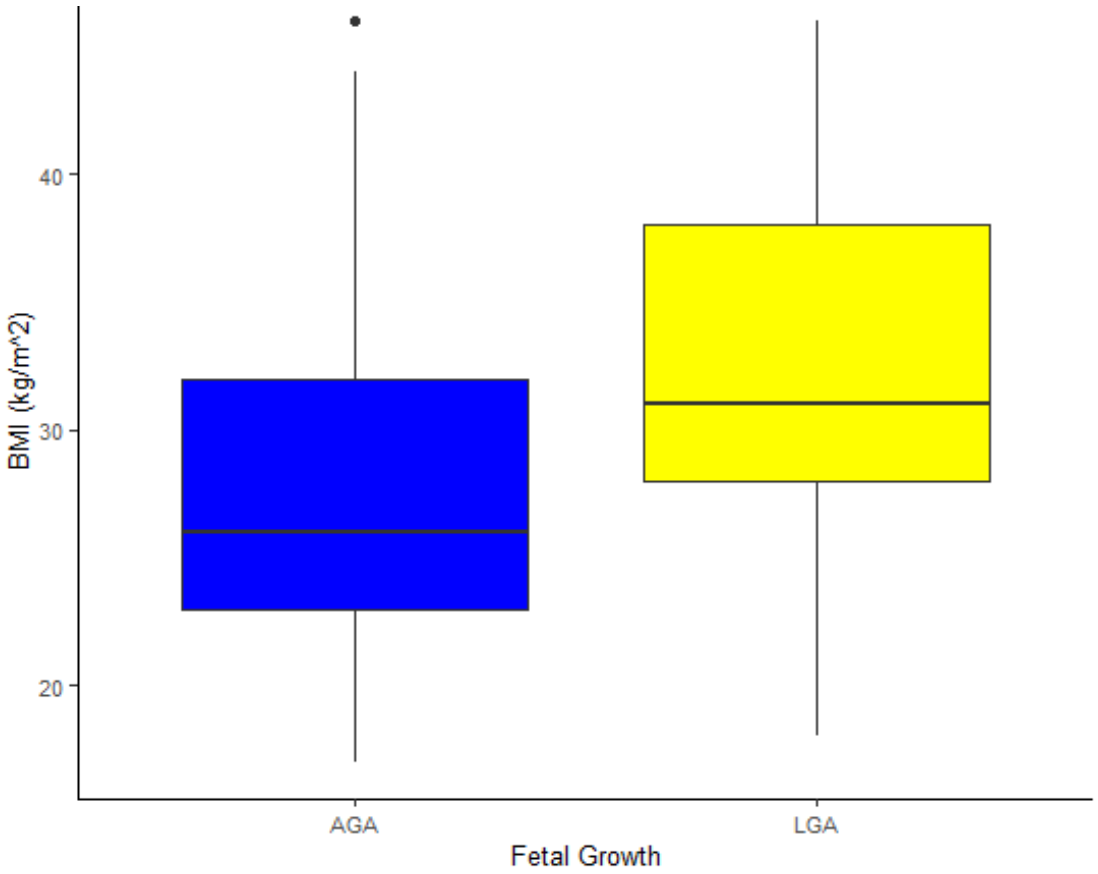


Figure 7.2 Comparison of appropriate for gestational age (AGA; N=102) and large for gestational age (LGA; N=16) fetuses according to the mother's BMI (p = 0.010, Mann-Whitney).

7.4.1 Renal parenchymal thickness growth

A total of 2536 renal parenchymal thickness measurements were obtained. At each ultrasound examination four measurements were performed, corresponding to the anterior and posterior thickness of both the right and left kidneys. Statistical modelling found no significant difference in the renal parenchymal thickness for fetal gender or whether the anterior or posterior parenchyma was measured and therefore these were not included in the final model. Kidney side (right or left) was included as a fixed effect in all models, as the renal parenchymal measurement of the right kidney was significantly thicker than the left (p=<0.05). The complete output of the models is provided in Appendix C2.

The fetal renal parenchyma measurement of LGA fetuses was significantly thicker than the AGA fetuses (LR Chisq=6.1, df=1, p=0.013) (Fig 7.3). No significant interaction was seen between GA and whether the fetus was LGA or AGA, indicating that the parenchymal growth rate of both groups over time was not significantly different (Appendix C2 part 2).

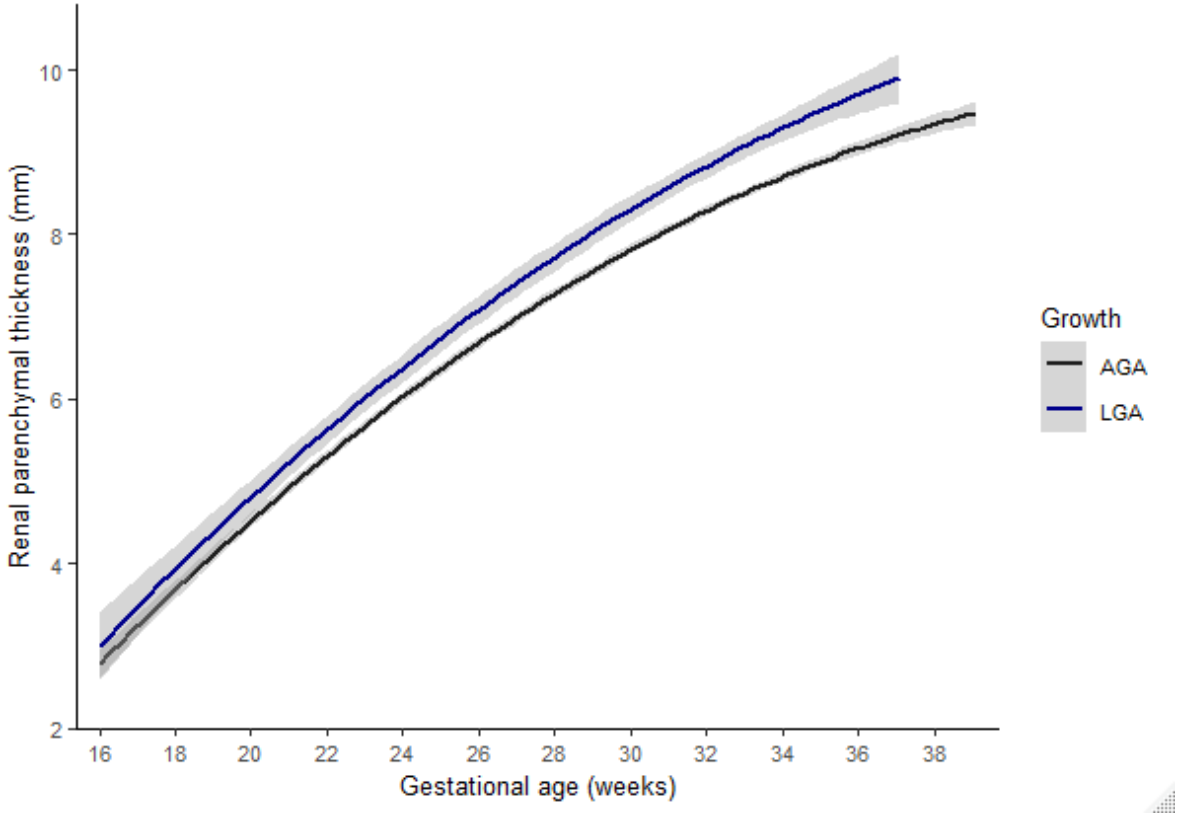


Figure 7.3 Overall regression lines of fetal renal parenchymal thickness for AGA and LGA groups (p= 0.013). Shaded areas denote 95% confidence interval.

7.4.2 Renal parenchymal thickness growth compared to AC growth

Renal parenchyma growth was compared to growth of the AC throughout the pregnancy for the LGA or AGA fetuses. There was no significant difference demonstrated between the LGA or AGA groups (LR Chisq=1.1, df=1, p=0.290). Renal parenchymal thickness grew proportional with AC (Fig 7.4), as demonstrated by the slope of both lines approximating 1 (Appendix C2 part 3).

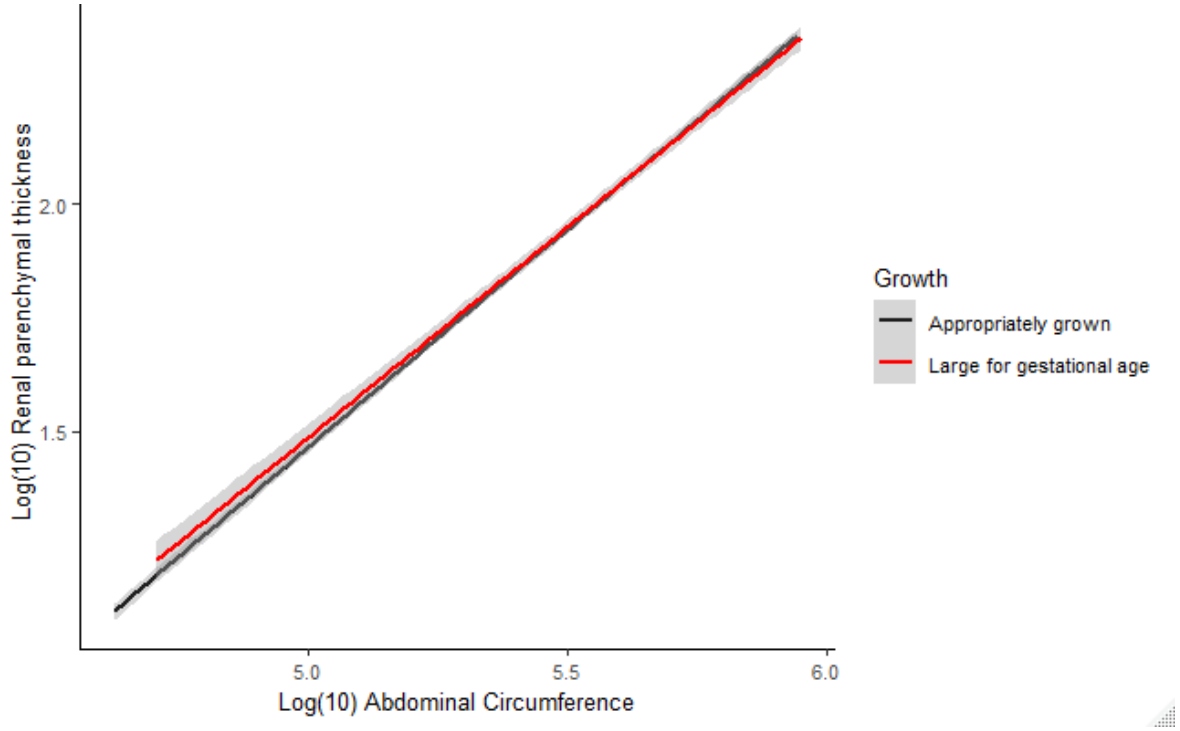


Figure 7.4 Overall regression lines of the relationship between fetal renal parenchymal thickness (log10) and abdominal circumference (log10) for AGA and LGA groups. Shades denote 95% confidence interval.

7.4.3 Renal artery Dopplers

Fetal renal artery Dopplers were also measured during the ultrasound examination. In total 1203 renal artery Dopplers were carried out. Due to fetal position and/or persistent fetal movements, the renal artery Doppler could not be performed for both kidneys in 28 scans and for one kidney in 35 scans. No significant difference was seen between LGA or AGA fetuses in the RI (LR Chisq=0.698, df=1, p=0.403) or PI (LR Chisq=0.005, df=1, p=0.956) (Fig 7.5), (Appendix C2 part 4).

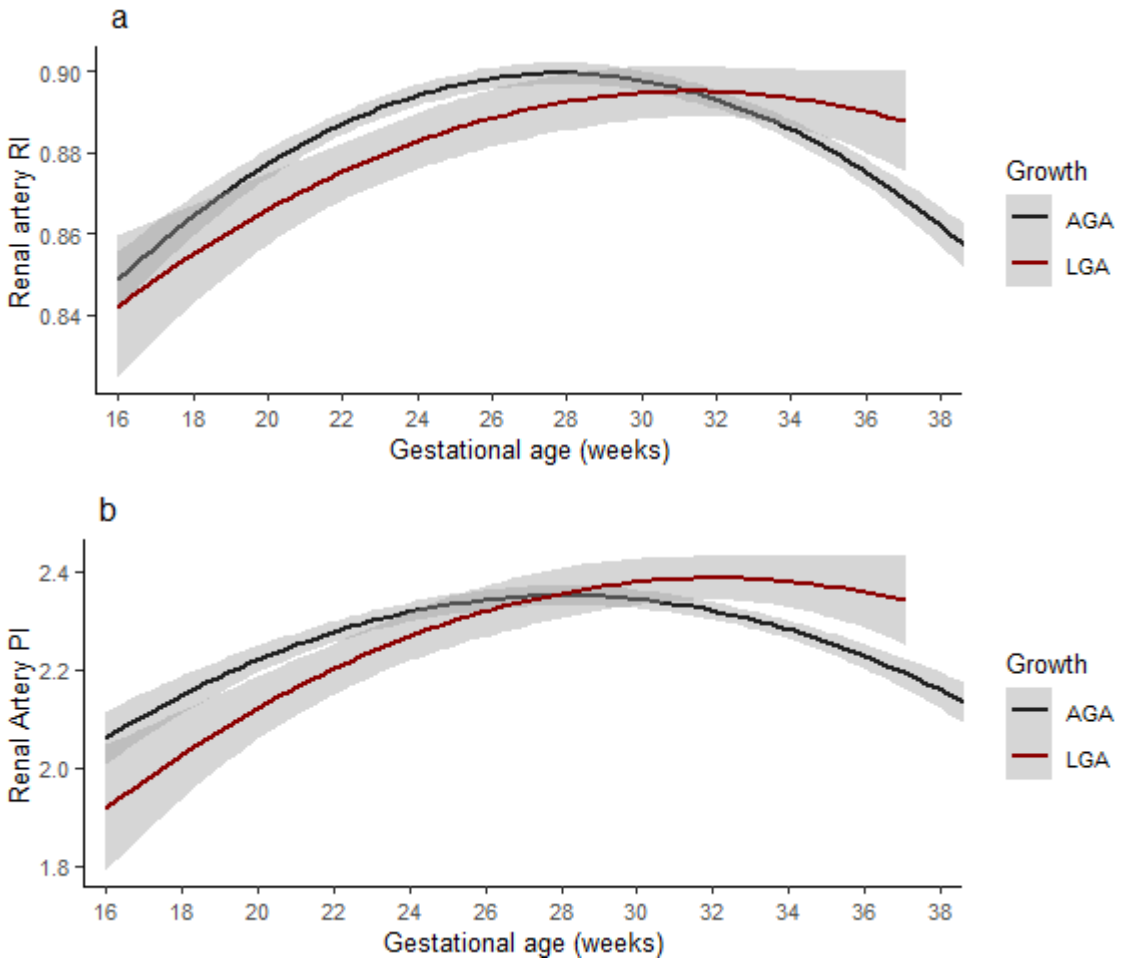


Figure 7.5 Overall regression lines of AGA and LGA groups for fetal renal artery (a) resistivity index (RI) and (b) pulsatility index (PI). Shades denote 95% confidence interval.

7.5 Discussion

The aim of this study was to investigate the effect of fetal overgrowth on the developing fetal kidneys through the use of a novel ultrasound measurement of renal parenchymal thickness and renal artery Doppler in AGA and LGA pregnancies. LGA is a significant problem, associated with many fetal and maternal complications, and should be taken as seriously as other obstetric conditions.[2] The role of fetal overgrowth in fetal renal programming is also under-researched and not well understood.[9,29] Some links with increased risks of kidney disease and hypertension have been demonstrated, however, to our knowledge, no study has previously analysed the growth of

the kidneys in LGA fetuses.[18] It is important to try and understand the effect of fetal overgrowth on the developing kidneys.

7.5.1 Fetal renal parenchymal thickness growth

The results from this study demonstrated that the renal parenchyma of fetuses born LGA was significantly thicker than AGA fetuses. The birth weight and birth centiles are significantly higher in the LGA group compared to the AGA group and, therefore the kidneys of the LGA fetuses appeared to be proportionately larger, in-line with the larger infants. To test this, the renal parenchymal growth was compared to abdominal circumference growth for LGA and AGA groups. This demonstrated no difference in the growth profile between the two groups indicating that the larger fetus had a proportionately thicker renal parenchyma.

7.5.2 Influences of diabetes in pregnancy on fetal renal parenchymal thickness growth

LGA infants are a heterogeneous population which include individuals from diabetic pregnancies, obese mothers and normal genetically large infants and a combination of these. Often in studies the aetiology of fetal overgrowth is not reported.[8] The results from this study did indicate an association between LGA and maternal pre-pregnancy weight and BMI. The mothers of LGA infants were significantly heavier and had a higher BMI. The mothers of LGA infants were also more likely to have pregnancies complicated by diabetes, with 75% having some type of diabetes. Diabetes in pregnancy is a leading cause for having a LGA infant.[1, 30]

There is a difference in the body composition and distribution of muscle and fat of infants who are born to a mother with diabetes.[30] LGA infants of diabetic mothers have a higher fat to muscle ratio and tend to have asymmetric growth of the abdominal circumference and thorax, whereas LGA infants of mothers without diabetes tend to be symmetrically large and have a similar fat to muscle ratio as appropriately grown infants.[1, 30]

7.5.3 Fetal renal artery Doppler analysis

This study investigated the fetal renal artery Doppler measurements for LGA and AGA pregnancies. The fetal renal artery blood flow did not demonstrate any significant difference in the RI or PI between LGA or AGA fetuses. Blood flow in the fetal renal arteries of LGA fetuses, to our knowledge, has not been previously reported and no significant changes in fetal blood flow have been associated with fetal overgrowth. There is significant overlap of confidence intervals for both RI and PI between the LGA or AGA groups which is likely due to the relatively poor interobserver reliability of these measurements that we have previously reported (Chapter 5). Additionally, the fetal kidneys receive only 3 - 5% of overall cardiac output and therefore any changes in this blood flow would likely be very subtle.[31, 32] A much larger study of LGA fetuses would be required to detect any significant difference from AGA pregnancies.

There is evidence of a link between maternal obesity, diabetes, LGA infants and future kidney disease. However, the precise effect of each and the combined implications on fetal programming remains unclear.[8, 29] Our study did not find any adverse effect of fetal overgrowth on the growth of the parenchyma during the pregnancy which is quite different to the adverse effect on the fetal renal parenchyma growth seen in growth restricted fetuses.[15] LGA is associated with childhood obesity and metabolic syndromes, which are strong risk factors for hypertension and CKD later in life.[5, 7, 9, 33] This is a small study and therefore at this stage we cannot say there is no effect on the developing kidneys until further larger studies are conducted. Also, in these infants, it may be in early childhood that the bulk of programming of the kidneys occurs.[34, 35]

7.5.4 Limitations

There was only a small number of LGA infants in our cohort which limited our analyses. Larger studies are needed to achieve more conclusive findings.

7.6 Conclusions

No adverse effect on the growth of the renal parenchyma was demonstrated in fetuses who were LGA. The majority of LGA infants were born to mothers with diabetes in pregnancy. It would therefore be of value to further investigate the influence of diabetes on the developing fetal renal parenchyma as it may be that hyperglycaemia results in a detrimental effect on nephron endowment.

7.7 References

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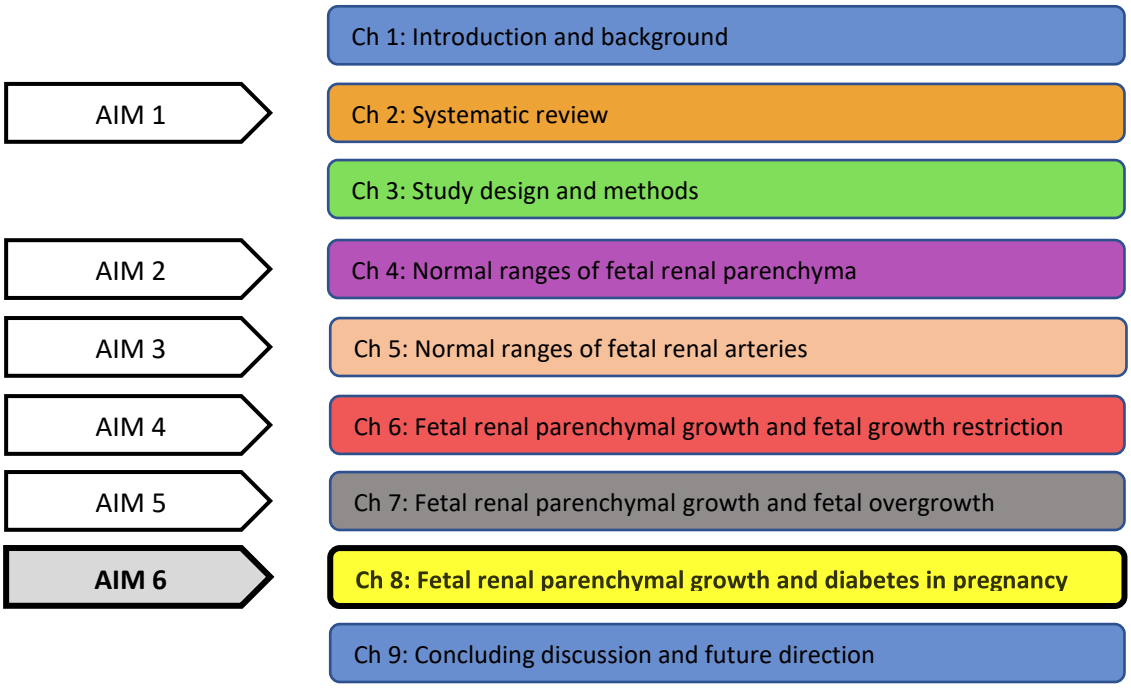
Chapter. 8 The Effect of Diabetes During Pregnancy on Fetal Renal Parenchymal Growth

Brennan S, Kandasamy Y, Rudd DM, Schneider ME, Jones RE, Watson DL. J. The effect of diabetes during pregnancy on fetal renal parenchymal growth. J Nephrol. **In press**.



This chapter is a copy of the journal paper, referenced above, except for minor textural modifications. The formatting of section sub-headings and references, and numbering of figures and tables have been modified from the original publication to match the thesis format. This paper has been accepted for publication and is currently in press.

Diabetes in pregnancy is thought to adversely affect the developing fetal kidneys however it is unclear how hyperglycaemia impacts the development of the kidneys. Our study uses a novel ultrasound measurement to provide unique data on how diabetes in pregnancy influences fetal kidney growth.

8.1 Abstract

Aims

Diabetes in pregnancy is thought to adversely affect the developing kidneys. The rate of gestational diabetes is increasing globally with major consequences for future renal function. Very little is known about the impact of hyperglycaemia on the fetal renal parenchyma which contains the developing nephrons. The aim of this study was to measure the fetal renal parenchymal thickness and evaluate whether diabetes during pregnancy affects the growth of the fetal kidneys.

Methods

This prospective, observational study used serial ultrasound measurements to evaluate the fetal renal parenchymal growth of 55 pregnancies with diabetes compared to 72 control pregnancies. Mixed effects modelling was used to analyse the data.

Results

The renal parenchyma of fetuses from mothers with gestational diabetes was significantly thicker than those from the control group (LR Chisq=4.8, df=1, p=0.029), however, the difference was proportional to the larger size of these fetuses. Fetuses of pregestational diabetics demonstrated no significant difference in renal parenchymal thickness compared to the control group even though they were also larger fetuses. Parenchymal growth slowed with increasing abdominal circumference in the pregestational diabetic group, suggesting an adverse effect on nephrogenesis, however this did not reach statistical significance.

Conclusions

Our study provides unique data on how diabetes during pregnancy influences fetal kidney growth. Appropriate management of diabetic pregnancies may mitigate some of the adverse effects on the fetal kidneys. Increasing degrees of hyperglycaemia, as seen sometimes in pregestational diabetes, may affect nephrogenesis; however larger studies are needed.

8.2 Introduction

Hyperglycaemia in pregnancy is an increasingly common condition compounded by an increase in obesity and maternal age.[1, 2] Pregestational diabetes refers to type 1 and type 2 diabetes mellitus that exists prior to pregnancy, while gestational diabetes mellitus refers to diabetes identified during pregnancy.[2, 3] The prevalence of type 2 diabetes in pregnancy and gestational diabetes mellitus is increasing.[1, 4] The global incidence of gestational diabetes has been reported to be around 20%; however, variations in screening protocols, criteria for diagnosis and risk factors means that the incidence varies between different pregnant populations.[4] Glucose is an essential nutrient for the fetus. To facilitate glucose transfer to the fetus, maternal insulin sensitivity decreases during pregnancy by 50-60%.[1] Elevated maternal glucose levels, however, are known to be teratogenic and are associated with an increased risk of adverse perinatal and maternal outcomes such as abnormal fetal growth, preterm birth, birth trauma and operative delivery.[2, 5]

Hyperglycaemia in pregnancy is also associated with adverse effects on the developing kidneys. One study demonstrated a three-fold increase in renal dysgenesis and agenesis.[6] Studies using animal models have confirmed this association, with offspring of hyperglycaemic mothers demonstrating reduced nephron numbers, increased blood pressure, microalbuminuria and a reduced glomerular filtration rate.[7, 8]. Human studies have demonstrated that adults born to mothers with diabetes in pregnancy have an increased risk of hypertension, chronic kidney disease and diabetes mellitus.[9, 10]. Current evidence suggests an adverse programming effect on the fetus due to exposure to hyperglycaemia during pregnancy, however human studies are still limited.[8, 9] It is unclear how diabetes in pregnancy impacts the developing kidneys *in-utero*. Given the increasing incidence of diabetes during pregnancy, it is critical that further investigations are carried out to understand the effects of hyperglycaemia during pregnancy on kidney development and long-term function.[6, 11]

We have previously demonstrated that an ultrasound measurement of the renal parenchymal thickness in fetuses and neonates presents a novel, non-invasive method to assess kidney growth and can provide a surrogate estimate of nephron numbers.[12-14] The aim of this study was to use an ultrasound measurement of the fetal renal parenchymal thickness to evaluate whether diabetes during pregnancy affects the growth of the fetal kidneys. We hypothesised that the renal parenchymal thickness in fetuses from women with diabetes during pregnancy would differ from fetuses in the control group.

8.3 Research Design and Methods

8.3.1 Study design and recruitment

This prospective, longitudinal, observational study was conducted between May 2017 and February 2019 at the Maternal-Fetal-Medicine Unit and Ultrasound Department of the Townsville University Hospital, Australia. The Townsville University Hospital is a large regional hospital that provides tertiary perinatal services to the large geographical area of North Queensland.[15] Recruitment was between May 2017 to October 2018. All neonates were born by March 2019 and data collection complete by December 2019.

This study was part of a larger study assessing multiple factors which affect fetal kidney growth. Pregnant patients aged 18 years or older, who presented to the Townsville University Hospital were informed about the study and invited to participate by their treating obstetrician, midwife or sonographer at their second trimester obstetric ultrasound scan up until 28 weeks gestation. Inclusion criteria were an accurately dated singleton pregnancy based on last normal menstrual period (LNMP) and 1st trimester ultrasound, that correlated with each other within seven days, or if LNMP was uncertain, by 1st trimester ultrasound alone. Patients were excluded if they had a multiple pregnancy, uncertain dates or any major congenital fetal abnormality or chromosomal abnormality.

The subgroup of pregnant women with hyperglycaemia were diagnosed either with pregestational diabetes (type 1 or 2) or with gestational diabetes. Gestational diabetes was identified with a glucose tolerance test before 24 weeks, if they were assessed as high risk for gestational diabetes, or between 24 to 28 weeks as per routine management for all pregnant women. The diagnosis of gestational diabetes was based on the Australasian Diabetes in Pregnancy Society (ADIPS) guidelines,[16] which are consistent with the International Association of Diabetes and Pregnancy Study Groups Consensus Panel,[3] specifying if their plasma glucose levels met one or more of the following criteria:

- Fasting of ≥5.1mmol/L;
- 1-hour ≥10.0mmol/L following an oral 75g glucose load; and
- 2-hour ≥8.5mmol/L following an oral 75g glucose load.

Pregnant women with diagnosed diabetes were managed in a multidisciplinary diabetes clinic which included an endocrinologist or obstetric physician, maternal fetal medicine sub-specialist, diabetes educator, dietitian and midwife where they received specialist support for monitoring of glucose, diet and exercise. Patients generally attended the clinic every 1 to 4 weeks and were instructed to test their blood glucose levels four times a day: fasting and 2 hours after each meal. The aim was to maintain fasting glucose levels < 5.1mmol/L and < 7.0mmol/L 2 hours after meals for gestational as well as pregestational diabetes. If glycemic controls could not be met with appropriate diet, medications were added and adjusted to try and achieve these goals.[16]

The control group was a cohort of low-risk pregnancies without underlying maternal diseases which may affect fetal growth (diabetes mellitus, heart disease, hypertension requiring treatment, kidney disease, pre-eclampsia), and who did not give birth prematurely before 32 weeks.

8.3.2 Sample size

In order to determine sample size, a power analysis was carried out for a statistical power of 80% and a significance level of 0.05 (two-tailed). Data from our previous study found the mean (SD) renal parenchymal thickness for neonates of normal birth weight was 9.4mm (± 1.1) and for low birth weight neonates it was 8.3mm (± 1.0) (p=0.01).[12] Therefore, a sample size of 15 would be needed for each group. To allow for loss to follow-up, we aimed to recruit a minimum of 20 participants for each group (40 in total). Ultrasound examinations were scheduled for each participant at least every four weeks.

8.3.3 Study process

Participants completed a questionnaire which included demographic, medical and obstetric data. The first ultrasound was most commonly performed between 16- and 26-weeks gestational age (GA); however, eight women had their first ultrasound at 28 weeks GA. Women were asked to attend ultrasound scans every four weeks from their first ultrasound until delivery. Some women, with high risk pregnancies, had additional clinically indicated ultrasounds. When their ultrasound examination was more than two weeks from the previous ultrasound recorded for the study, renal measurements were again performed for the study. After birth, perinatal data was collected from the mother and baby's electronic medical record around onset of labour, model of delivery, gestational age at birth, birth weight, gender, condition of neonate and maternal medical history and medications.

8.3.4 Ultrasound examination

All examinations were performed by three Australian Accredited Medical Sonographers with more than two years post ultrasound qualification experience. Prior to commencement of the study, sonographers were trained to use a standardised protocol for renal measurements by authors DLW and SB. A follow-up audit, to ensure measurement consistency between sonographers, was conducted three months after commencement of the study. The sonographers were not blinded

to all clinical and biometric information as most studies included a diagnostic scan. A Voluson E8 (GE Healthcare Ultrasound, Milwaukee, WI, USA) or an Epiq 7 (Philips Ultrasound, Bothell, WA, USA) were used for the ultrasound examinations. The highest frequency transducer possible (1-5MHz), which matched the mother's body habitus was selected.

All measurements were performed on both kidneys. The images of the fetal kidneys were magnified so the kidney occupied the majority of the image. The renal parenchymal thickness was obtained from a midsagittal plane of the kidney by measuring from the inner aspect of the renal capsule to the sinus-pyramidal apex in both the anterior and posterior aspects (Fig 8.1a). The maximum kidney length was also measured in this midsagittal plane. In a transverse section of the fetal kidney, at the level of the renal pelvis, the maximum anteroposterior diameter (H) and transverse diameter (W) were measured (Fig 8.1b). Every measurement was performed twice, with the mean recorded. The kidney volume was calculated using the ellipsoid formula: KV = 0.523 × L × W × AP (π/6 x L x W x AP).[17]

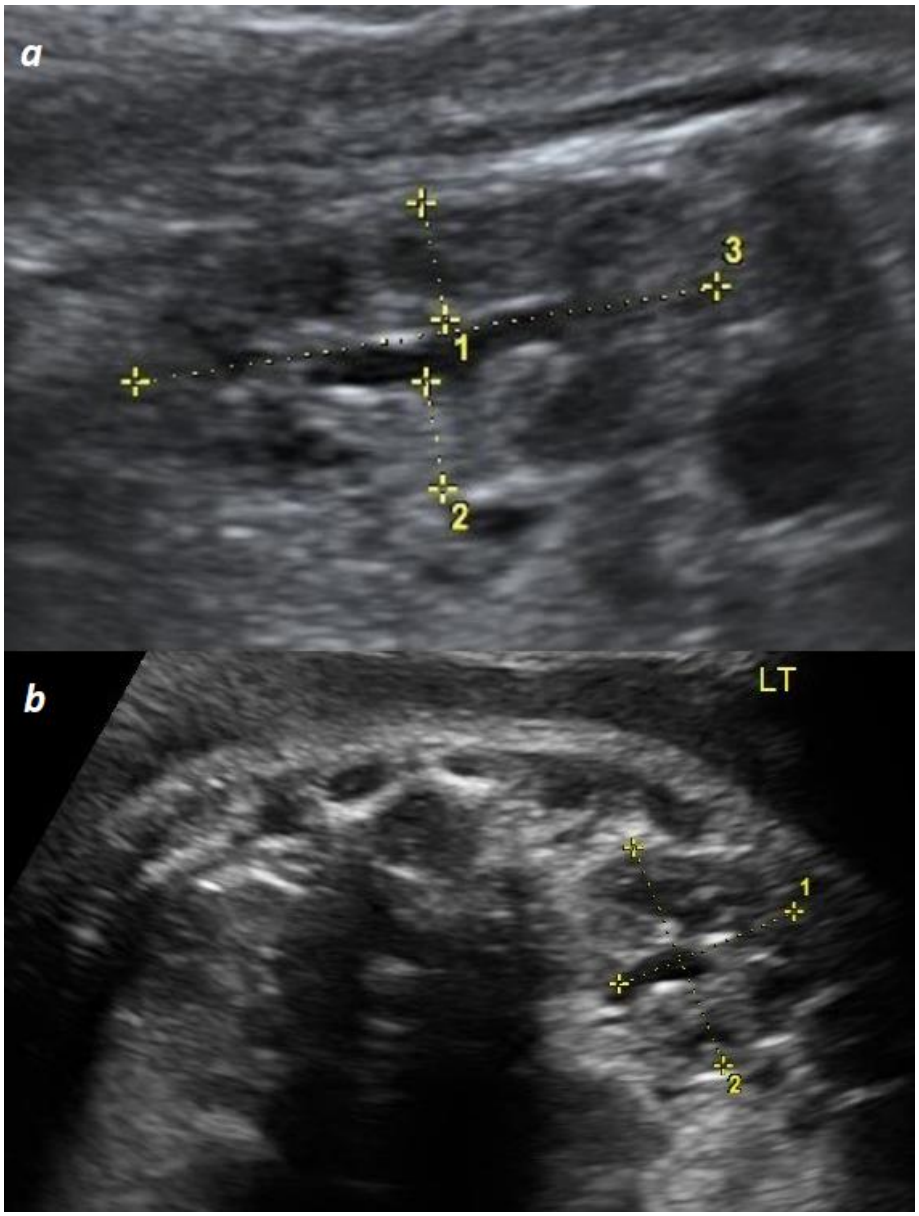


Figure 8.1 30 weeks gestational age (a) Measurement of the anterior (1) and posterior (2) fetal renal parenchymal thickness from the inner aspect of the renal capsule to the sinus-pyramidal apex interface and of the kidney length (3). (b) Measurement of kidney transverse (1) and antero-posterior (2) dimensions.

8.3.5 Statistical analyses

Hadlock et al.[18] fetal weight charts were used to plot the birth weight. Fetal weight charts are preferred to neonatal weight charts as neonatal charts do not represent a random sample of the population at a given GA, particularly those neonates born prematurely. It has been recommended that fetal growth be assessed against measurements of on-going pregnancies at that GA as opposed

to a birth weight of neonates born at a given GA.[19, 20] R Statistical Language in R Studio (version 1.2.1335)[21, 22] was used for all analyses and graphics.

Visual inspection of the histograms, normal Q-Q plots and box plots showed the maternal and birth data were approximately normally distributed. Each measurement is described as means +/- SD or N (%) and statistical significance was set as $p<0.05$.

8.3.5.1 Modelling analysis

As repeated measurements were made on each fetus, the effects of maternal diabetes on the relationship between parenchymal thickness and GA were analysed using random slopes linear mixed effects models. These allow for individual differences between fetuses in kidney size and growth rate. The nlme package (version 3.1-139).[23] was used to fit the models and the ggplot2 package[24] was used to create the graphics. All measurements on each participant, for each visit, were included in the analysis with no outliers removed. Normality and homoscedasticity were assessed using visual inspection of residual plots.

Fixed effects of gender and anterior vs posterior parenchymal thickness were tested in all the models but did not improve the fit. Whether or not to include them in the final model was determined by comparing alternative models of different complexity using likelihood ratio (LR) tests and Akaike's Information Criteria (AIC). Three groups of models were fitted.

8.3.5.2 Renal parenchymal thickness growth

The relationship between the fetal renal parenchymal thickness and GA in fetuses of mothers with diabetes and the control group was analysed. Renal parenchymal thickness was the response variable and the fixed effects were GA, diabetes status (no diabetes or diabetes), kidney side (right or left) and an interaction between GA and diabetes status. To allow for curvature in the relationship between parenchymal thickness and GA, GA was included as a quadratic orthogonal polynomial. The diabetes status variable was dependent on which data set was used: it was either

no diabetes versus gestational diabetes, or no diabetes versus pregestational diabetes. The variable for GA was adjusted to 16 weeks = 0, so that the intercept term in the summary output would represent the parenchymal thickness value of the control group at 16 weeks, the lowest gestational age included in the study.

Participant ID was treated as a random effect and the random slopes component used the orthogonal polynomial for the effect of GA. The fitted models showed greater residual variation as renal parenchymal thickness increased, so this was allowed for in the model. Details of the models fitted, with their outputs, are provided in Appendix C3.

8.3.5.3 Abdominal circumference growth

The relationship between the fetal abdominal circumference (AC) and GA was analysed with the AC as the response variable and the fixed effects were GA (again using a quadratic polynomial to allow for curvature), diabetes status, kidney side and an interaction between GA and diabetes status. As for models in the first group, participant ID and the GA polynomial were incorporated as random effects and the increase in residual variance with increasing AC was allowed for in the model.

8.3.5.4 Fetal renal parenchymal thickness growth compared to AC growth

The effects of hyperglycaemia during pregnancy on the relationship between the thickness of the fetal renal parenchyma and the AC were assessed. We assumed a power function of the form:

$$y = ax^b$$

was suitable to define this relationship, where y = parenchymal thickness and x = AC. Since y and x are both linear measurements, if they both grow at the same rate then the value of b should be 1. To fit the model, the renal parenchyma and the AC were log transformed to convert the power function to the following linear equation:

$$\log(y) = \log(a) + b(\log(x))$$

The response variable for these models was the $\log_{10}$-transformed parenchymal thickness and the fixed effects in each model were diabetes status, $\log_{10}$-transformed AC and an interaction between the two to examine whether the regression lines for each type of diabetes status had different slopes. A random intercept for each participant and a random slope for the effect of AC ($\log_{10}$) was used.

8.4 Results

Overall, 155 pregnant women were recruited. Fifty-five of the women had diabetes in pregnancy: 46 gestational diabetes and 9 pregestational diabetes (type 1 $n = 3$ and type 2 $n = 6$). Although the number of women presenting with pregestational diabetes was small, it consisted of serial data which provided important, novel measurements and so was retained as a separate group in the analysis. Seventy-two women with low risk pregnancies were used as the control group. Twenty-one women were excluded due to maternal disease classifying the pregnancy as high risk, six were excluded due to fetal abnormality and one was excluded as she failed to attend any ultrasound examinations (Fig 8.2).

The characteristics of the mothers and babies are summarised in Table 8.1. Pregnancies that were complicated by either gestational diabetes or pregestational diabetes were associated with a significantly higher pre-pregnancy weight and BMI. Neonates of mothers with diabetes were born at a lower gestational age, however, at a higher birth weight centile. There were no stillbirths or neonatal deaths.

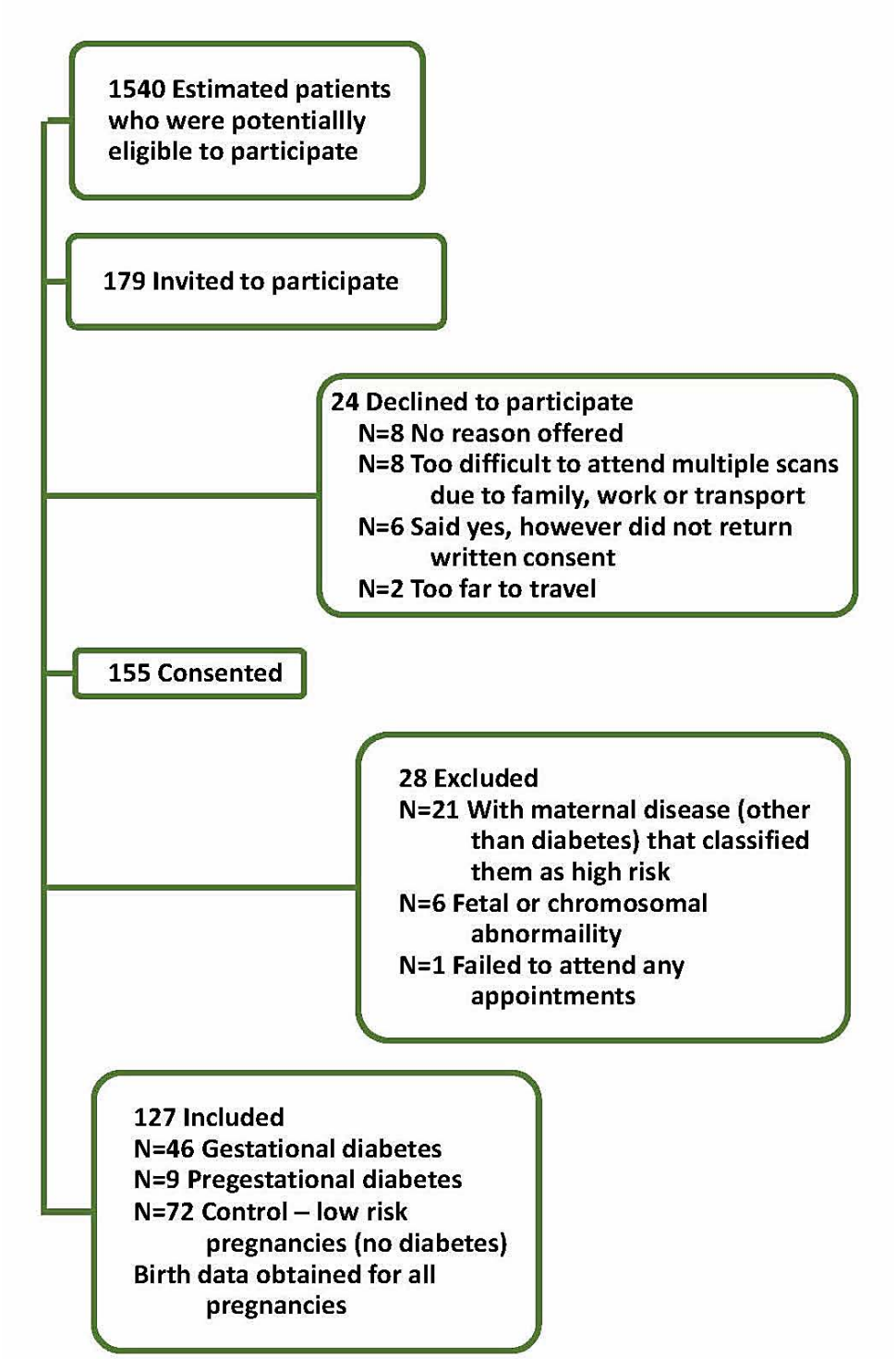


Figure 8.2 Flowchart of participant inclusion and exclusion process.

A total of 690 ultrasound examinations were performed between 16- to 39-weeks GA with a mean (SD) number of scans per pregnancy of 5.4 ± 1.4. A single participant had only one scan. This participant had gestational diabetes and delivered preterm at 27 weeks GA. Some participants delivered early and therefore the full set of planned ultrasounds could not be completed.

Table 8.1
Characteristics of diabetic pregnancies and control pregnancies and their infants

Participant Characteristics	Control (N=72)	Gestational diabetes (N=46)	Pregestational diabetes (N = 9)	*p* value
MATERNAL				
Maternal age (years)	29.1 ± 5.3	30.5 ± 4.7	31.2 ± 5.1	$F_{2,124}$ = 0.216[a]
Maternal height (cm)	164 ± 6.2	1.66 ± 6.6	165 ± 8.2	$F_{2,124}$ = 0.233[a]
Maternal pre-pregnancy weight (kg)	68.5 ± 15.9	87.0 ± 20.5	98.9 ± 26.7	$F_{2,124}$ = <0.000*[ab]
Maternal BMI (kg/cm2) (M, IQR)	25.5 ± 5.4	31.5 ± 6.7	36.1 ± 7.8	$F_{2,124}$ = <0.000*[ab]
Multiparous	33 (45.8%)	32 (69.9%)	7 (77.8%)	0.020*[c]
MATERNAL – Diabetic groups only				
HbA1c (% (mmol/mol))		5.12 (32) ± 0.33 (N=33[f])	6.91 (52) ± 1.28	$F_{2,124}$ = <0.000*[d]
Fasting GTT (for gestational diabetes only)		5.02 ± 0.69		
Treatment				0.284[c]
• Diet only		10 (22%)	0 (0%)	
• Oral hyperglycemic agent (OHA)		9 (20%)	1 (11%)	
• Insulin		14 (30%)	5 (56%)	
• Insulin & OHA		13 (28%)	4 (33%)	

Participant Characteristics	Control (N=72)	Gestational diabetes (N=46)	Pregestational diabetes (N = 9)	*p* value
NEONATAL				
GA at birth (weeks)	38.7 ± 1.5	37.9 ± 2.3	37.3 ± 1.2	$F_{2,124}$ = 0.026[ae]
Preterm	8 (11.1%)	5 (10.9%)	4 (44.4%)	0.040*[c]
Birth weight (grams)	3189 ± 516	3288 ± 678	3560 ± 659	$F_{2,124}$ = 0.184[a]
Weight centile	37.6 ± 25.6	56.3 ± 29.7	73.6 ± 37.1	$F_{2,124}$ = <0.000*[ab]
Male	41 (56.9%)	20 (43.5%)	7 (77.8%)	0.126[c]

Note: Means ± SD or N (%). GTT, glucose tolerance test; GA, gestational age. * = p<0.05; a one - way ANOVA; b Tukey individual pairwise comparison shows significant difference is between the control group and both groups of diabetes; c Fisher's Exact Test; d Independent t-test; e Tukey individual pairwise comparisons did not achieve statistical significance. f 13 (28.3%) of gestational diabetics did not have a HbA1c.

8.4.1 Renal parenchymal thickness growth

There were 2556 renal parenchymal thickness measurements for the comparison between the gestational diabetes and the control groups and 1780 for the comparison of the pregestational diabetes to the control groups. At each examination four measurements of each fetus were completed, corresponding to one each by right or left kidney and anterior or posterior portion of the kidney. During modelling, no difference between the anterior or posterior parenchymal thickness or gender was found and therefore these were not included in the final model. Whether the measurement was from the left or the right kidney was included as a fixed effect in this, and all subsequent models, as the renal parenchymal measurement of the left kidney was found to be significantly thinner than the right ($p=<0.05$).

The fetal renal parenchymal thickness for pregnancies with gestational diabetes was significantly thicker than the control group (LR Chisq=4.8, df=1, p=0.029) (Fig 8.3a). There was no significant interaction between diabetes and the GA, indicating that the parenchymal growth rate of the gestational diabetes group was not significantly different from the control group (Appendix C3 part 2). The fetal renal parenchymal thickness of pregnancies with pregestational diabetes showed no significant differences from the control group, although the pregestational diabetes group was small (Fig 8.3b). The full output is provided in Appendix C3 part 2.

The aim of the study did not include evaluating the impact of HbA1c levels and diabetic management of the different types of diabetes on renal growth, however, we did perform these analyses. HbA1c levels (LR Chisq=0.001, df=1, p=0.972) and diabetic management (diet only, OHA or insulin) (LR Chisq=2.79, df=3, p=0.424) were tested in the model, however there was no significant effect of either variable on renal parenchymal thickness. Although the mean HbA1c levels of mothers with pregestational diabetes was significantly higher than mothers with gestational diabetes ($p=<0.0001$), the number of cases is small so HbA1c impact in the model may

be underestimated. Analysis of the effect of these variables would require a larger study that is designed and powered for this purpose.

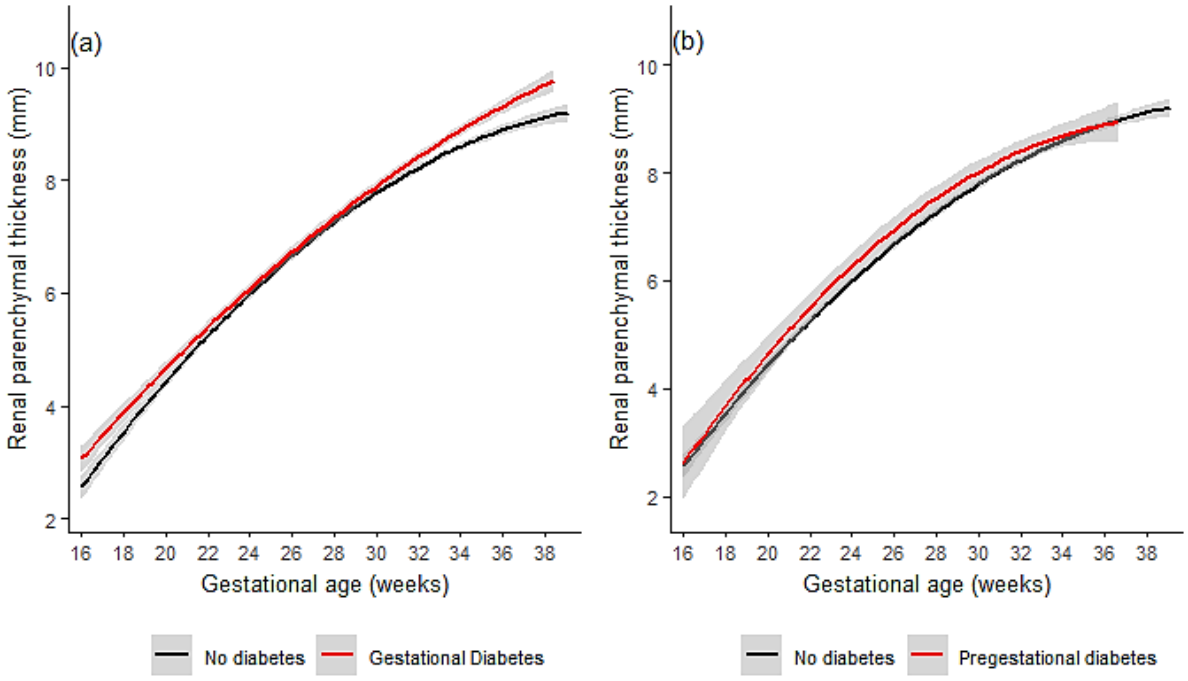


Figure 8.3 Overall regression line for fetal renal parenchymal thickness by gestational age for (a) gestational diabetes and control groups and (b) pregestational diabetes and control. Shades denote 95% confidence interval.

8.4.2 Abdominal circumference growth

The interaction between diabetes type and GA was significant, with the results demonstrating that the growth rate of the fetal AC was higher for pregnancies with either gestational diabetes (LR Chisq=23.9, df=2, p=<0.0001) or pregestational diabetes (LR Chisq=12.8, df=2, p= 0.0015) (Fig 8.4). The full output is provided in Appendix C3 part 3.

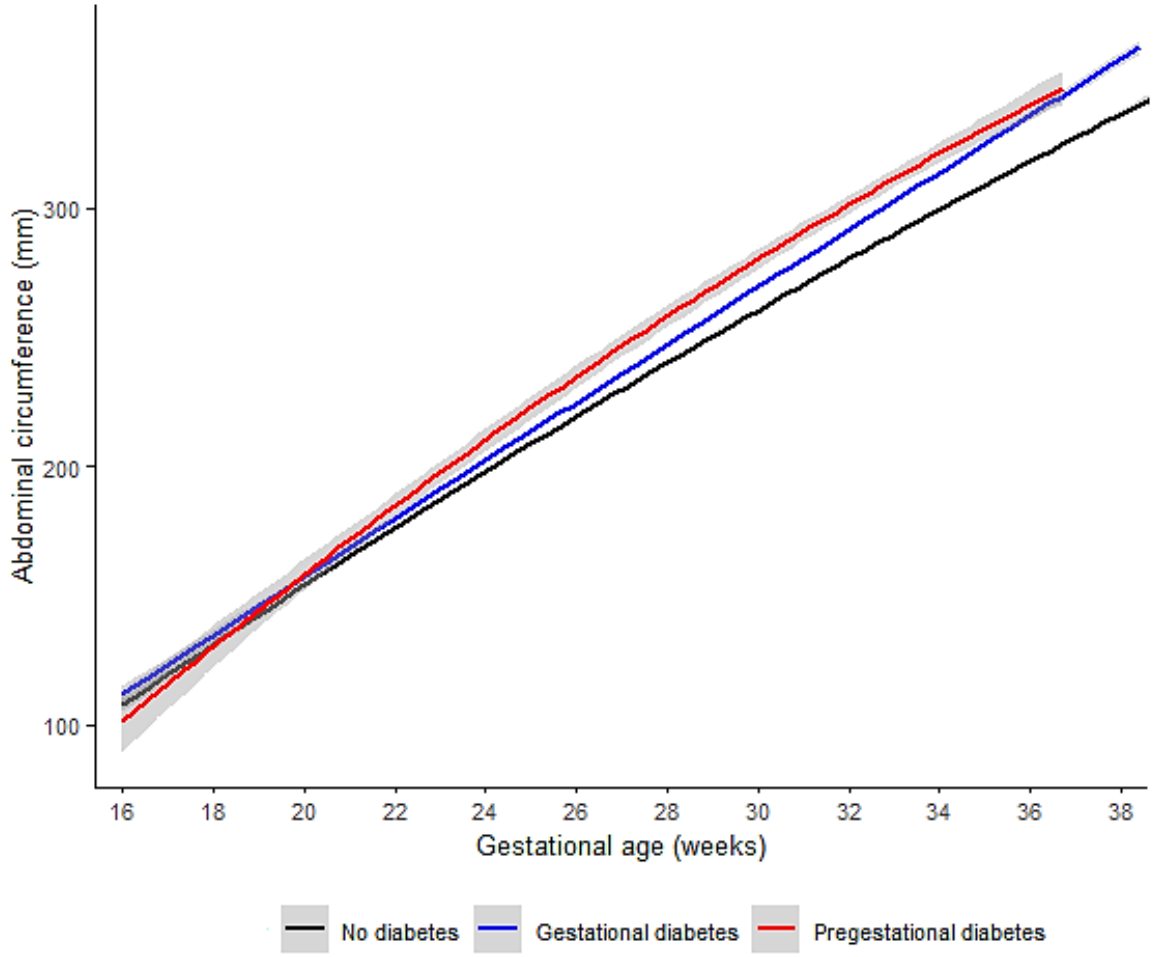


Figure 8.4 Overall regression line for abdominal circumference by gestational age for control (black line), gestational diabetes (blue line) and pregestational diabetes (red line) groups. Shades denote 95% confidence interval.

8.4.3 Fetal renal parenchymal thickness growth relative to AC growth

The $\log_{10}$-transformed relationship between fetal renal parenchymal thickness and AC did not differ significantly between the groups of pregnancies with gestational diabetes and the control group (LR Chisq=1.37, df=1, p=0.242), and pregestational diabetes and the control group (LR Chisq=2.67, df=1, p=0.103) (Fig 8.5). There was some flattening of the slope of the pregestational group, however this did not reach significance. Similar findings were seen when the parenchymal growth relative to the AC growth was compared between the gestational and pregestational groups (LR Chisq=1.59, df=1, p=0.201) (Appendix C3 part 4).

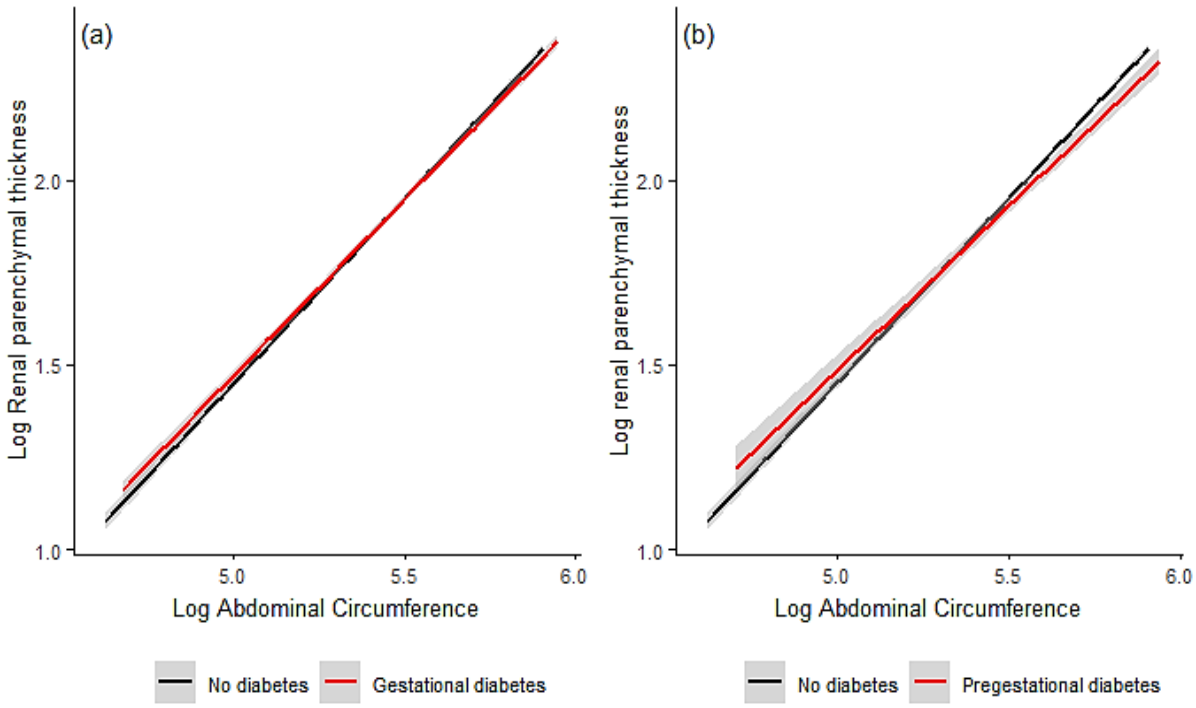


Figure 8.5 Relationship between log10 transformed fetal renal parenchymal thickness and abdominal circumference for (a) gestational diabetes and control groups and (b) pregestational diabetes and control groups. Shades denote 95% confidence interval.

8.5 Discussion

Diabetes in pregnancy is a high-risk condition for mothers and babies and the incidence is increasing globally.[1, 2, 25] This is the first study, to our knowledge, that compared the growth of the fetal renal parenchyma in diabetic pregnancies to a control group of low-risk pregnancies. The longitudinal nature of our data and the use of mixed effects modelling is a strength of our study. Our findings demonstrated that the fetal renal parenchyma of pregnancies with gestational diabetes was significantly thicker than in the control group. However, there was no significant difference in fetal renal parenchymal thickness between the pregestational diabetes group when compared to the control group.

Mothers with pregestational and gestational diabetes both had a significantly higher pre-pregnancy weight and BMI than the control group. Comparing the birth weight centiles of the neonates in lieu of GA allowed for the differences in GA at birth. Neonates born to mothers with gestational

and pregestational diabetes were significantly heavier when compared to the control group. The growth of the fetal AC in these groups was also significantly larger than the control group, supporting the findings of previous studies that mothers with diabetes have larger fetuses.[26] Glucose readily crosses the placenta, however insulin does not. Fetal overgrowth frequently occurs in diabetic pregnancies and this is referred to as the Pedersen hypothesis.[27]

The Pedersen hypothesis provides an explanation for fetal overgrowth and is supported by other studies such as the HAPO trial.[5, 27] The hypothesis states that maternal hyperglycaemia results in fetal hyperglycaemia and hypertrophy of fetal islet tissue leading to insulin hypersecretion, increased growth hormones and insulin-like growth factors (IGF), which promote fetal growth with increased deposition of fat and protein production.[27] Diabetes in pregnancy is a leading cause for a large-for-gestational age (> 90th centile for GA) neonate with a disproportionate growth of the AC due to an increase in excessive fat and muscle in this region.[1, 28]

If newborns of mothers with hyperglycaemia are larger and have organomegaly, due to fetal overgrowth, then it may be hypothesised that the kidneys should be proportionally bigger than kidneys from normo-glycemic mothers. This is consistent with our findings of a thicker fetal renal parenchyma in the gestational diabetes group when compared to the control group, however, when renal parenchymal growth was corrected for AC growth in these fetuses the difference disappeared. Thus indicating, that at the same AC both groups have a similar parenchymal thickness: the thicker renal parenchyma of fetuses of mothers with gestational diabetes was proportionally grown to the overall larger size of these fetuses.

Few studies have investigated the impact of hyperglycaemia in pregnancy on the fetal kidneys. One longitudinal study demonstrated a significant increase in fetal kidney volume of fetuses of mothers with gestational diabetes.[29] They did not report on any maternal or neonatal demographics. Another cross-sectional study measured the fetal kidney volume at one point in time between 32-34-weeks gestation and found no difference in the kidney volumes of fetuses of mothers with

gestational diabetes and those that did not.[30] As opposed to our study, this study did not find any differences in maternal weight, BMI or parity between gestational diabetes group and their control group. No neonatal data, such as birth weight, was reported.[30]

Studies of adults born to mothers with diabetes during pregnancy indicate that they are more likely to develop chronic kidney disease, hypertension, obesity and diabetes.[9, 11] Animal studies of rodents have also demonstrated that the kidneys from offspring of mothers with diabetes have smaller kidney volumes, fewer nephrons and a greater proportion of damaged nephrons which is thought to be due to the toxic effects of hyperglycaemia.[7, 31] It is important to consider that animal studies generally model severe hyperglycaemia in pregnancy which also frequently results in growth restriction.[7, 8, 31] This does not reflect what usually occurs in humans.

Participants in this study with gestational diabetes did not have severe hyperglycaemia at the time of diagnosis, which is supported by the fasting GTT and HbA1c. Over the last few decades there has been significant advances in the management of diabetes during pregnancy resulting in earlier diagnosis, better surveillance and improved management which may result in better outcomes in adulthood for these children.[2] The findings from this study support the role for good glycemic control for healthy kidney development. Appropriately managed gestational diabetes results in normal fetal kidney growth, although larger than those of low risk pregnancies, with no discernible adverse effect to their growth pattern. This does not mean there is no impact, but rather that the impact is not significant enough to be detected by our current methods and it may emerge later in adulthood, which remains to be explored further.

In the pregestational diabetes group, despite the overall larger birth weight of these babies compared to the control group, the fetal renal parenchyma was not significantly thicker than those in the control group. Relative to the size of the fetus, the renal parenchyma was not as thick as expected, and as was seen in the gestational diabetes group. When comparing the fetal renal parenchyma to AC growth of the pregestational diabetes group and the control group, there was

a flattening in the slope of the pregestational diabetes groups, however, this did not reach statistical significance. The findings suggest renal parenchymal growth slowed with increasing AC in the pregestational diabetes group compared to the control group and with a larger sample size this may reach statistical significance. When the fetal renal parenchyma to AC growth was compared between the gestational diabetes and pregestational diabetes groups, again the growth rate of the fetal renal parenchyma to AC of the pregestational group appears slightly slower than the gestational diabetes group, however, was not statistically significant.

Maternal hyperglycaemia is more severe in pregestational compared to gestational diabetes and pregestational diabetes is associated with increased fetal abnormalities and adverse outcomes.[2,5,25] This suggests that the difference in the growth rate of the fetal renal parenchyma for the pregestational diabetes group may be due to the fetus being exposed to hyperglycaemia from conception to throughout their gestation and to higher glucose levels. Maintaining adequate glycemic control in pregestational diabetes is known to be more difficult during pregnancy.[32] More severe hyperglycaemia in pregnancy may result in a reduced nephron endowment, which is reflected by a thinner than expected parenchyma, and may lead to an increased risk of kidney disease and hypertension in future life. Although the number of women with pregestational diabetes was small, the findings were interesting and warrant further investigation. If we were able to obtain a large cohort of untreated or poorly controlled women with diabetes in pregnancy, we might see the findings demonstrated in animal studies.

8.5.1 Limitations

There was a small number of women with pregestational diabetes, however, novel, longitudinal data was obtained which allowed our group to explore possible trends. There is also potential measurement bias as the sonographers were not blinded during the study. Sonographers are trained not to look at their measurements at the time they are performing them and three sonographers, rather than one, performed the examinations to reduce this bias.

8.5.2 Future research

Larger studies evaluating the different subtypes of diabetes in pregnancy, timing of diagnosis, glycaemic control over the duration of the pregnancy and the effects of different treatments are needed to further improve our understanding of the impacts on fetal kidney development, both adverse and beneficial. Further studies utilising ultrasound to evaluate the renal parenchymal thickness of children and adults born to mothers with diabetes in pregnancy would be useful to gain additional information on long-term effects.

8.6 Conclusion

In-utero kidney development influences future kidney function and it is thought that diabetes in pregnancy may have an adverse effect on kidney development. Our study of gestational diabetes in pregnancy did not find any significant effect on the fetal renal parenchyma relative to the size of the fetus. However, in women with pregestational diabetes our finding of a relatively thinner renal parenchyma than expected suggests these fetuses may have fewer nephrons. Our study on the growth of the fetal renal parenchyma using ultrasound provides unique data to gain insight into the effect of maternal diabetes in kidney development. It is plausible that appropriate management and treatment of diabetes in pregnancy may mitigate some of the adverse impacts on the fetal kidneys.

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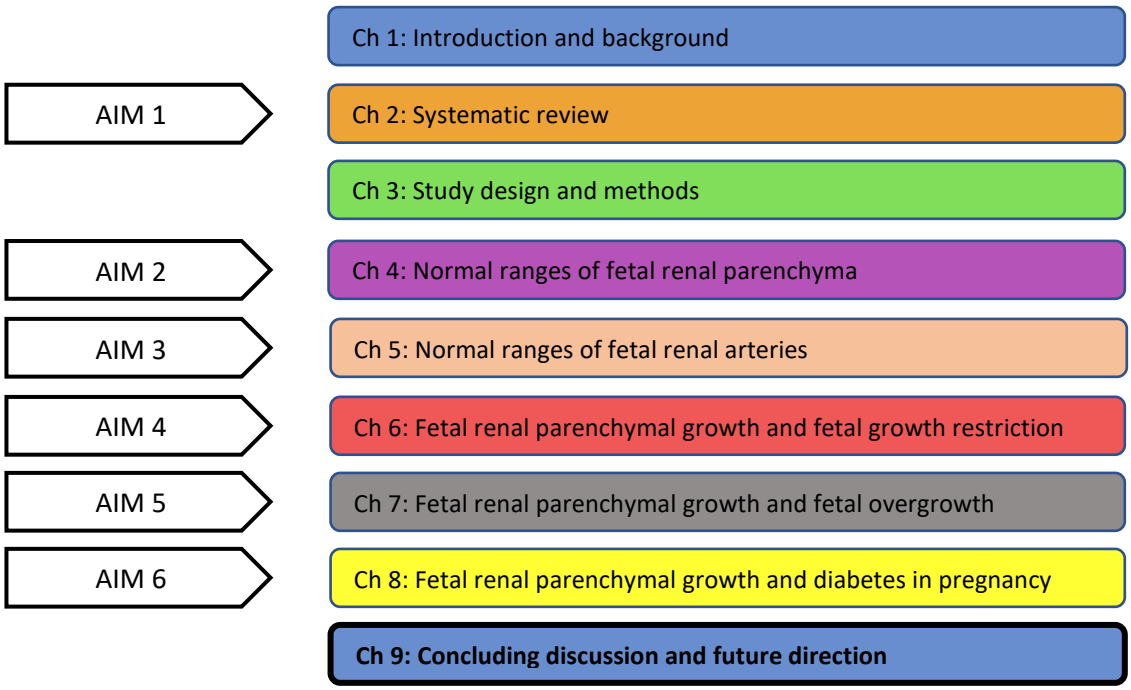
Chapter. 9 Concluding Discussion and Future Direction



Chronic kidney disease (CKD) and hypertension are mostly preventable diseases.[1, 2] The optimal way to truly tackle these problems is to start at the very beginning – where life begins. This thesis is concerned with the utility of antenatal ultrasound to examine methods to support the discovery of solutions to reduce the future risk of CKD and hypertension. The thesis presents a logical progression of studies undertaken to investigate novel measurements using antenatal ultrasound, of the fetal renal parenchyma and the fetal renal arteries. The reliability of these measurements was also assessed. These measurements were then further explored to investigate the effects of abnormal fetal growth and diabetes in pregnancy on the growth of the fetal renal parenchyma. Growth of the renal parenchymal thickness over the pregnancy should indirectly reflect nephron number. This chapter will summarise the overall findings of this thesis, the applications to clinical practice and suggestions for future research.

9.1 Statistical Methods

9.1.1 Study design

This study was a prospective, longitudinal, cohort, observational study. As the aim was to assess the growth of the fetal kidneys, a longitudinal design was selected for several reasons. Longitudinal studies tend to be more efficient and have greater power than cross-sectional studies.[3] Charts created from cross-sectional data can only be used for size not growth, even though, this incorrect use of cross-sectional data it is frequently encountered.[4-6] There is active debate in the literature about the construction of fetal centile charts, as in the last decade there has been a push to improve the quality and methodological consistency of these charts.[4, 7, 8] Large longitudinal cohort studies of healthy participants are the gold standard for the creation of standard charts.[9] Fetuses grow at different velocities, and therefore the goal of performing multiple, serial measurements on the fetus during gestation is to quantify these variations in growth.

The study was designed so that the participants would attend ultrasound examinations at approximately the same and equally spaced gestational ages. Yet, due to multiple different issues, such as family illness and transport, some examinations were missed or delayed, or at times additional examinations were required for clinical reasons. Women commenced the study at various gestational ages depending on when they presented to the hospital. This added heterogeneity and complexity to the data and meant that traditional statistical methods of comparisons would not be appropriate and would not utilise the full potential of the longitudinal data.

9.1.2 Statistical analysis

To maximise the potential of the longitudinal data, modern, advanced statistical methods were required for accurate assessment of the repeated measures and multiple variabilities within the data, and to produce high quality standardised charts for use in clinical practice. Mixed effects

models are emerging as the method of choice for the analysis of longitudinal data.[10, 11] Mixed effects modelling is a sophisticated, powerful statistical method that analyses every data point and better accounts for the heterogeneity of the timing of the scans and missing data.[10, 12] These models can simultaneously account for fixed and random effects that potentially contribute to the understanding of the data.[10] To develop the charts from this study, methods of the recent large INTERGROWTH 21st Project for fetal growth, which produced international growth standards for fetuses, were employed.[13] This resulted in charts with centiles that provide a good fit to the raw data and change smoothly with gestational age (GA) as recommended by the literature.[4, 6, 8]

9.2 Renal Parenchyma Thickness: A Non-Invasive In-Vivo Method to Estimate Nephron Endowment

It is a challenge to obtain a non-invasive in-vivo estimation of nephron number during fetal development. It is known that nephrons grow by branching outwards in concentric layers.[14, 15] Therefore, if there is an adverse interruption to their growth, fewer layers of nephrons develop and hence we expect that this would result in a thinner renal parenchyma. The uniqueness of the measurement of the renal parenchyma is its specificity and its simplicity. Specific, in that it is directly measuring the functional kidney tissue (layers of nephrons) and does not include the collecting system of the kidney. Simplistic, in that it is a single linear measurement which should have less variation and be more reliable than multiple renal measurements or renal volume sets which have traditionally been used to investigate renal development. Ultrasound is a non-invasive, non-ionising, relatively cheap imaging modality which is widely available, including in rural and remote areas, around the world.[16] If found to be validated by other clinicians and populations, measurement of the renal parenchymal thickness could be easily learnt by sonographers and sonologists and applied to clinical practice.

9.3 Standardised Charts for Normal Ranges of Renal Parenchymal Thickness Throughout Pregnancy

Standard charts reflecting normal ranges of fetal renal parenchymal thickness, kidney length and kidney volume from 16 to 38 weeks were developed in the first study in order to address the need for a more sensitive method to evaluate fetal kidney growth and nephron number. These charts of normal ranges of renal parenchymal thickness, kidney length and kidney volume could be used in clinical practice. The charts require further validation in other populations and cohorts. This study revealed that the fetal renal parenchymal thickness increased with increasing GA up until 34 to 36 weeks when the thickness of the parenchyma starts to plateau. This appropriately correlates with our understanding of nephrogenesis and its completion by 36 weeks' gestation.[17]

Renal parenchymal measurements can now be plotted on the graph that we have developed.[18] Deviation from the normal range presents a new criterion for the diagnosis of renal parenchymal pathologies and/or reduced nephron endowment. Providing multiple centiles allows the charts to be used as a potential screening tool (using the 10^{th} and 90^{th} centiles as lower and upper limits) or as a diagnostic tool (using the 3^{rd} and 97^{th} centiles as lower and upper limits). The fetal renal parenchymal thickness of participant 111, plotted in Figure 9.1, demonstrates slowing of growth of the renal parenchyma so that by 37 weeks GA the parenchyma was well below the 10th centile. This infant had fetal growth restriction (FGR) and was delivered at 37 weeks and 5 days with a birth weight of 2,225 grams. Charts, developed from this study, for fetal renal parenchymal thickness and those for fetal kidney length and volume, have been submitted for consideration for addition to Viewpoint 6 (GE Healthcare, Milwaukee, WI, USA), an advanced fetal imaging reporting system. Therefore, these fetal kidney charts could be utilised and verified by clinicians all over the world.

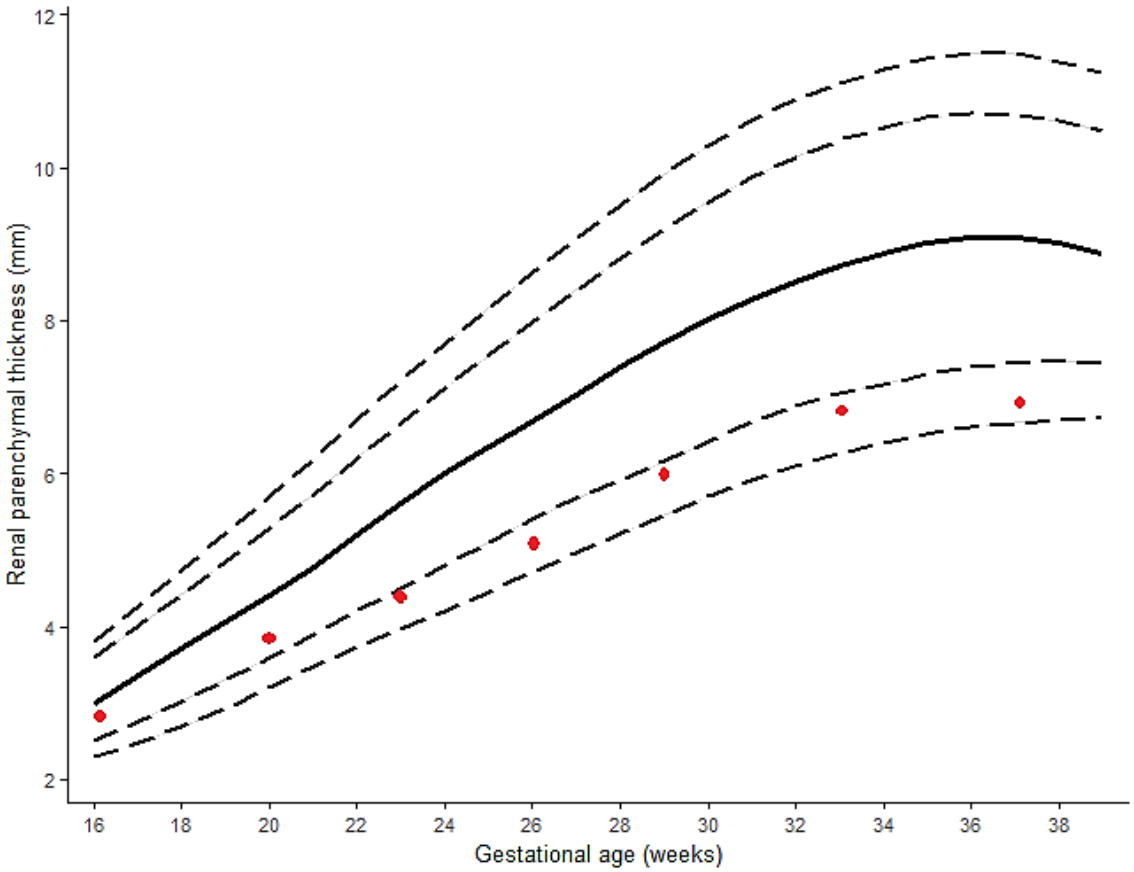


Figure 9.1 Standard chart of fetal renal Parenchymal Thickness with 3rd, 10th, 50th, 90th & 97th Centiles and the renal parenchymal thickness (red dots) for participant 111 demonstrating slowing of growth of the renal parenchyma so that at 37 weeks GA the thickness of the renal parenchyma was well below the 10th centile.

9.4 Reliability of the Measurements

Fetal measurements are important to enable the correct prenatal diagnosis; however, clinicians need to have confidence in the validity of the measurement on which they base their clinical decisions. Therefore, it is important when developing a measuring technique to also assess its reliability. For this reason, a separate study was conducted to assess the reliability of the fetal renal measurements performed in the study. In this quality assurance study, all three sonographers were blinded to their own and each other's measurements by concealing the measurement on the monitor. Following analysis, it was found that measurement of the renal parenchyma had excellent intraobserver and interobserver reliability. Excellent intraobserver and interobserver reliability was also demonstrated for the renal length, transverse and antero-posterior dimensions.[18]

In contrast to the fetal renal parenchymal measurements, measurements of the resistivity index (RI) and pulsatility index (PI) of the fetal renal arteries were only adequate for intraobserver reliability and poor for interobserver reliability. This finding is similar to other studies in the literature.[19-21] A recent review of the reliability of ultrasound measurements in obstetrics and gynaecology studies found most studies had significant flaws in study design, interpretation and/or reporting which tended to result in overrating of the reproducibility.[22] Fetal Doppler studies, in particular, often do not report reliability at all. This non-reporting of measurement reliability may be due to a concern that the measurement will not be considered for clinical use if there is poor reliability.[20, 22] Points that remain to be considered, include, whether known physiological variations due to fetal movements, breathing and heart rate changes, be factored in when assessing the reliability of fetal Doppler.[23-25] Additionally, new techniques being investigated may need refinement to improve their reliability and therefore should not be discounted based on early poor reliability studies. It is essential to appreciate the reliability of the measurements that are being utilised in clinical practice. Once measurement of the fetal renal parenchyma was shown to have excellent reliability, we then wanted to assess if this measurement could be applied to detect adverse effects of FGR on the development of the fetal kidneys.

9.5 Effects of Fetal Growth Restriction on Fetal Kidney Growth

Having defined the normal ranges of fetal renal parenchymal thickness, the aim of the subsequent study was to utilise this novel measurement to determine the effect of FGR on the development of the renal parenchyma. It is widely accepted that FGR can affect nephron number and future kidney function, however, there is a lack of in-vivo, in-utero proof of this concept which was based on animal and ex-vivo studies.[26-28] This study provided evidence of the negative effects of FGR on the renal parenchymal growth and therefore the development of the functional component of the fetal kidney. It demonstrated that measurement of the renal parenchyma was able to detect changes in parenchymal growth of the fetal kidney.

The findings from this study supported the theory that FGR has a direct adverse effect on nephrogenesis. Growth restricted fetuses had a significantly thinner parenchyma and a slower growth trajectory of the parenchyma than appropriately grown fetuses. Placental insufficiency is the most common cause of FGR, with the majority of FGR occurring in the 3rd trimester of pregnancy.[29, 30] The 3rd trimester (28 weeks to term) is also when 60% of nephrons are formed. [17] Therefore, it is likely that this deceleration in the growth of the renal parenchyma in FGR fetuses may stem from increasing placental insufficiency and slowing of fetal growth in the third trimester, at the time of maximum kidney growth.

A key strength of our study was that a single birth weight centile cut-off (such as below the 10th centile) was not used as a proxy for FGR. Only fetuses that met an internationally recognised criteria for growth restriction were defined and included as FGR.[31] These criteria for FGR incorporate a decline in overall growth as well as demonstrated changes in feto-maternal circulation on pulse Doppler. A fetus may be within the normal weight range, however, may still be growth restricted and the development of their kidneys may be compromised. This study attempted to analyse fetuses who were truly growth restricted and not just those who were small for gestational age.

It could be argued that smaller babies have proportionally smaller kidneys and therefore there are no negative effects from FGR on kidney growth. By comparing the growth of the fetal head to the growth of the fetal renal parenchyma between FGR and appropriate-for-gestational age (AGA) fetuses, our study has shown that in growth restricted fetuses the renal parenchyma was significantly thinner than expected based on fetal head size alone. To our knowledge this comparison has not been done before and provides good evidence that FGR does have a detrimental effect on the renal development, as fetuses with the same head circumference had a significantly thinner renal parenchyma if they were truly growth restricted. A thinner fetal renal

parenchyma implies fewer nephrons and consequently a fetus who is at a higher risk of future decreased kidney function and disease.

A reduced nephron number does not directly correlate with inevitable renal damage and future CKD, however the remaining nephrons will have an increased demand, making the kidneys more vulnerable to future kidney injury.[32, 33] Some experts are advocating for life-long monitoring of kidney disease of all growth restricted, low birth weight and preterm infants along with those exposed to pre-eclampsia or diabetes.[28] This would require the implementation of enormous resources and represent a significant health cost burden. The renal parenchymal measurement could be employed to assist in more appropriately identifying infants at the highest risk of developing CKD and to provide a more complete picture of the infant's renal function. Functional renal biomarkers could be evaluated after birth and combined with the information around possible nephron endowment from the renal parenchymal thickness to gain a more complete picture of renal function. More focused monitoring through a combination of postnatal renal ultrasound imaging and biomarkers could be applied. Early interventions such as healthy lifestyle programs providing information around nutrition and physical activity can also be concentrated on this group to try and reduce the occurrence of CKD and hypertension in later life.

9.6 Effects of Fetal Overgrowth on Fetal Kidney Growth

There is a paucity of studies generally on fetal well-being in the setting of fetal overgrowth not associated with diabetes. Most studies focus on delivery and birth trauma to the mother and infant.[34, 35] No study had yet investigated the effects of fetal overgrowth on fetal kidney development.[36] Renal parenchymal thickness was measured in both large-for-gestational age (LGA) and AGA fetuses to detect any possible effects of fetal overgrowth on the developing kidneys. The fetal renal parenchyma was found to be significantly thicker in LGA fetuses when compared to the AGA fetuses. The LGA infants were, however, significantly heavier at birth and had a higher birth weight centile. To account for this, the growth of the fetal renal parenchyma

was compared to the growth of the AC between the LGA and AGA groups. No difference was found in their growth trajectory, thus, indicating the growth in thickness of the renal parenchyma was in proportion to the larger size of the fetuses.

Fetal overgrowth could not be demonstrated to have an adverse effect on the growth of the fetal renal parenchyma. This finding is not surprising as it has been demonstrated that there is a wide variation in nephron number in a normal population from autopsy studies.[37, 38] The limitation of *in-vivo* studies is that it is not possible to determine the number of nephrons, this can only be estimated. Additionally, the quality of the nephrons and glomeruli cannot be assessed. Autopsy studies have demonstrated significant effects on glomeruli due to low birth weight and preterm birth,[39, 40] however, these studies have not investigated nephron number and quality in fetal overgrowth. Animal and human autopsy studies are warranted to investigate fetal overgrowth and kidney development and to guide future clinical studies. Our study recruited a relatively small sample of LGA fetuses (16) and therefore, larger longitudinal studies following the infants into adulthood would be required to confirm any potential effects on renal parenchymal growth. LGA has been linked with childhood obesity and metabolic syndromes, which are strong risk factors for future CKD and hypertension.[41-43] It is possible that programming of the kidneys might occur in early childhood rather than fetal life.[44, 45]

Most of the literature on postnatal growth and risks of CKD and hypertension focus on infants affected by FGR, while there is little evidence on postnatal growth of infants born LGA.[44, 46] A recent review based on human and animal studies showed that early and excessive weight gain of the child was associated with an increased risk of CKD and hypertension in adulthood and that many studies highlighted the potential role of early maternal nutrition in the development of future CKD.[44] There were, however, some conflicting findings with 5 of 24 studies finding that increased growth in infancy and childhood appeared to be protective for developing CKD and hypertension.[44] The etiology of fetal overgrowth and childhood obesity, and the links to future

kidney health is complex and involves many factors that likely interact and remain to be fully elucidated.[41, 42, 46] One important factor is the role of diabetes in pregnancy. Most of the LGA infants in this study were born to mothers with diabetes in pregnancy. Therefore, the aim of the subsequent study was to investigate growth of the fetal renal parenchyma in pregnancies complicated by diabetes.

9.7 Effects of Diabetes in Pregnancy

Diabetes in pregnancy is a leading cause of LGA.[34, 47] This study investigated the effect of hyperglycaemia during pregnancy on nephrogenesis by comparing the renal parenchyma of fetuses of mothers with diabetes to a control group of low risk pregnancies. There were two subgroups of pregnant women with diabetes: a larger group of women with gestational diabetes (46) and a smaller group of women with pregestational diabetes (type 1 or 2) (9).

The fetal renal parenchyma was significantly thicker in fetuses of mothers with gestational diabetes compared to the control group of low-risk pregnancies, however, there was no significant difference in the growth trajectory of the renal parenchyma between the two groups.[48] The infants of mothers with gestational diabetes were seen to be significantly bigger and therefore their parenchyma was proportionally thicker. This was surprising as animal studies and human studies of adults born to mothers with diabetes suggest a resultant reduced renal reserve and function.[49-52] It was therefore thought that we might have found the renal parenchymal to be relatively thinner due to adverse effects on nephrogenesis. The finding of no significant effect on the renal parenchymal in fetuses of mothers with gestational diabetes does not mean there is no effect, but rather no effect *in-utero* was detected using current methods. Another possible explanation for this result is that recent advances in management and treatment of diabetes in pregnancy over the last decade may be diminishing any adverse impacts hyperglycaemia may be having on the developing fetal kidneys. We are yet to carry out the long term follow up into adulthood of these infants.

Therefore, it is yet to be elucidated whether these improvements in management and treatment will result in a reduction in CKD and hypertension in the future.

The findings were different in pregnancies complicated by pregestational diabetes. Infants of mothers with pregestational diabetes were also significantly larger than the control group, however, the fetal renal parenchyma was not found to be significantly thicker.[48] It was expected that the renal parenchyma would be thicker in these fetuses considering their significantly larger size compared to the control group. A thinner than expected renal parenchyma may be due to hyperglycaemia adversely effecting nephrogenesis.

Hyperglycaemia is known to be teratogenic to the kidneys during embryogenesis and is thought to effect nephrogenesis.[53-55] Maternal hyperglycaemia is more severe in pregestational compared to gestational diabetes. The fetus is exposed to higher levels of maternal glucose from conception and throughout gestation and this is associated with increased fetal abnormalities and adverse outcomes.[56, 57] One study showed an almost 30% increase in fetal abnormalities for each percentage rise in HbA1C on presentation[58] and in yet another study, mothers with diabetes were found to have a three-fold increase in fetal renal dysgenesis or agenesis.[54] Our study demonstrated a trend towards a thinner than expected renal parenchyma in the pregestational diabetes group. Hyperglycaemia is present throughout the entire pregnancy in this group, whereas the gestational diabetes group, have less prolonged, milder hyperglycaemia and the hyperglycaemia predominantly presents during the third trimester. This hyperglycaemia throughout pregnancy in the pregestational diabetes group may be adversely affecting nephron endowment, however, the sample size of pregestational diabetic pregnancies was not large enough to make any concrete conclusions. The renal parenchymal measurement could be used in a larger study and correlated with maternal glucose levels to investigate if higher levels of hyperglycaemia present throughout the pregnancy affect the renal parenchymal growth.

This study offered unique information to gain a better understanding of the effects that diabetes in pregnancy may have on the developing fetal kidneys, however there are many unanswered questions and multiple valuable opportunities for further research.

9.8 Fetal Renal Arterial Measurements

This section of the thesis aimed at gaining more information on the role of blood flow to the fetal kidneys in fetuses with normal and abnormal fetal growth. In situations of chronic fetal hypoxia and/or nutrient deficiency, the fetus will preferentially shunt blood flow away from organs, such as the kidneys to more essential organs such as the brain, heart and adrenal glands.[59, 60] This reduced perfusion to the fetal kidneys, due to preferential shunting, is thought to inhibit normal nephrogenesis.[61, 62] We firstly established normal ranges of blood flow to the developing kidneys during pregnancy and then investigated to see if any alterations in the blood flow could be detected in situations of abnormal fetal growth.

Charts of the normal ranges of the fetal renal artery RI and PI from 16 to 38 weeks gestation were developed. The RI and PI of the fetal renal arteries showed little variation during pregnancy. These charts may be used to detect physiological or pathological changes in the fetal renal blood flow. When the RI and PI of the fetal renal arteries were compared between FGR and AGA, as well as LGA and AGA fetuses there was no significant difference. It was thought that some reduction in the renal blood flow may have been detected in our FGR group to support the theory of preferential shunting away from the kidneys, however, no evidence of this was found.

The findings indicate a lack of sensitivity in the measurements of RI and PI in the fetal renal artery. Blood flow to the fetal kidneys is relatively low, with only 3-5% of cardiac output directed to the kidneys compared to 15% after birth.[63, 64] Changes to fetal renal blood flow may be too subtle to be detected using current methods. Perhaps analysing the fetal renal blood flow over a longer time, such as several minutes, may improve the sensitivity of the measurements and elucidate differences

in the blood flow. The reliability of the RI and PI measurements from our study was poor, further indicating the low sensitivity of the measurement. However, blood flow in the fetal renal arteries was not the primary objective of this thesis and the study was not specifically powered to detect changes in blood flow. A larger study, particularly with a larger group of severe FGR fetuses and utilising emerging blood flow techniques, such as 3D volume flow, may demonstrate detectable changes in blood flow to these fetal kidneys compared to healthy fetuses. The true value of fetal renal artery Dopplers in clinical practice is still unanswered.

9.9 Fetal Kidney Function and Amniotic Fluid

Assessing fetal kidney function, non-invasively is challenging and complicated. From mid-gestation, around 90% of amniotic fluid is provided through urination from the fetal kidneys, making it plausible to hypothesise that amniotic fluid levels reflect fetal kidney function.[65] Regulation of amniotic fluid is, however, a complex collaboration of many different fetal systems, not just the urinary tract, and the production of fetal urine is controlled by renal perfusion and tubular reabsorption.[66,67] Furthermore, the kidneys have little function *in-utero* as the placenta performs most of the excretory functions that are performed by the postnatal kidneys.[63] Therefore, perhaps we should be focusing on anatomical measurements rather than functional parameters *in-utero* to predict future kidney function.

In this thesis, amniotic fluid was tested as a fixed effect, in the mixed effects models comparing fetal renal parenchymal growth between FGR and AGA fetuses and LGA and AGA fetuses. There was, however, no significant effect. There was also no correlation between amniotic fluid levels and the RI and PI of the fetal renal arteries. Being unable to demonstrate any significant difference in amniotic fluid levels in abnormal fetal growth compared to normal fetal growth was disappointing but not surprising. It has been shown that amniotic fluid levels give some prognostic signs of fetal renal function, however, unfortunately these are not sensitive or specific enough to provide accurate quantification of fetal kidney function.[67] Evaluation of amniotic fluid levels does

offer some information about the function of the fetal kidneys. For example, in cases of severe bilateral urinary tract abnormalities oligohydramnios may occur, or in chronic FGR there may be in a decline of amniotic fluid levels over time.[63, 67] Currently we can only rely on invasive sampling of the amniotic or fetal blood to give any real quantification of fetal kidney function and the value of these procedures is controversial.[68] Furthermore, the risks associated with these invasive tests should be considered. A systematic review in 2007 of 23 articles found that none of the analytes obtained from fetal urine could accurately predict poor postnatal kidney function.[68] The challenge continues to find a non-invasive measure of fetal renal function.

9.10 Limitations of the Study

This was an observational study and therefore fundamentally subject to some selection bias. The participants were recruited through a mixed risk hospital service and therefore do not represent the whole community. Subsequentially, caution should be employed in extrapolating these results to different populations.

Another limitation of the study was the inability to get the women to attend at the same gestational age and at set intervals. This is often a problem with clinical studies and can result in missing data and drop out of participants. Fortunately, only one participant failed to attend any ultrasound examinations. Most women were keen to attend and therefore if they were unable to come for an examination they attended at another time. This did add more heterogeneity to the data than was planned at the onset.

Longitudinal studies are more efficient and powerful than cross-sectional studies and therefore require fewer participants, however, the sample size of some of the groups analysed were small. It was difficult to obtain a large group of infants born LGA and those with pregestational diabetes within the time allocated for recruitment. Study recruitment was initially set for a year - May 2017 – April 2018. This was extended for a further six months until October 2018 to obtain a larger

cohort. Due to time constraints from grants and access to ultrasound equipment it was not possible to extend the recruitment further.

A further limitation was the lack of blinding of the sonographers, which could have introduced possible measurement bias. Most ultrasound examinations included a diagnostic scan and therefore it was difficult to blind the sonographer to all clinical and biometric information. Having multiple sonographers undertaking the ultrasound examinations reduces some of the bias. Sonographers are also generally trained not to look at the measurements at the time that they are being performed. Additionally, the infants were not assigned to groups until after birth and the groups were based on birth weight and not the fetal birth weight calculated from the measurements by the sonographers.

Antenatal ultrasound measurements of fetal structures require small-scale measurements. Due to maternal body habitus and/or fetal position during the scan, some measurements were not able to be obtained. Almost all linear kidney measurements were completed at every ultrasound examination, however, some renal Dopplers were not. The fetal renal arteries were the most technically difficult measurement to obtain as fetal movement and breathing also contributed to the ability to obtain a good quality Doppler signal.

9.11 Conclusion

The main aim of this thesis was to use antenatal ultrasound to assess the fetal renal parenchymal growth in a pregnant population and determine if abnormal growth or diabetes in pregnancy impacts fetal renal parenchymal thickness. Using mixed effects modelling, it was demonstrated that measurement of the fetal renal parenchyma could detect changes in kidney growth and provide an indirect estimate of nephron number. Measurement of the fetal renal parenchyma offers an enhanced, easy to perform, non-invasive single measurement to assess fetal kidney growth.

The standard charts developed in these studies will provide normal ranges of fetal renal parenchymal thickness, kidney length and kidney volume for use in clinical practice to identify alterations in fetal kidney growth. These charts will provide a useful resource for high-risk pregnancies. The thickness of the fetal renal parenchyma, when combined with other markers such as echogenicity of the parenchyma and the presence or absence of cysts will assist in the diagnosis of renal parenchymal pathologies. The standard charts describing the normal range of fetal renal artery RI and PI will also be a possible helpful resource in clinical practice. Presently, however, these charts and the findings from this research around fetal renal arteries, might be most valuable to guide and inform future research in this area and as an additional tool when investigating fetal renal development.

The most significant finding from these studies was that measurement of the fetal renal parenchymal thickness could be used to detect changes in the growth of the renal parenchyma in situations of abnormal fetal conditions. Our work showed that FGR appears to adversely affect the growth of the fetal renal parenchyma. This implies that renal parenchymal thickness is providing an indirect estimate of nephron number – with a thinner renal parenchyma suggesting fewer layers of nephrons.

Fetal renal parenchymal thickness presents a new marker that may indicate a reduced nephron number, which could be used in clinical practice to improve the identification of infants at a higher risk of future kidney disease and hypertension. Then suitable screening and support could be implemented early, such as performing postnatal renal ultrasounds and renal function tests, optimising childhood nutrition and facilitating involvement in programs to reduce the risk of obesity. We should also remember that maternal characteristics and health play a significant role in fetal growth and fetal development. Consequently, optimising maternal health before and during pregnancy, along with improving child health has the greatest potential economic and health community benefits to prevent adult CKD and hypertension.

This was the first study to investigate if fetal overgrowth affected fetal kidney growth and one of only a few studies into the effects of diabetes on fetal kidney growth. Although no significant changes to renal parenchymal thickness relative to the size of the fetus were definitively demonstrated in these circumstances, there were some interesting findings which should be evaluated in future research. The study of the effects of diabetes in pregnancy on the renal parenchymal thickness suggests the possibility that more severe levels of hyperglycaemia, present throughout pregnancy, may affect the growth of the parenchyma. It also provided some evidence that good management of glucose levels may counteract possible adverse effects on the renal parenchymal growth. Our work in this area also highlighted the many gaps in our understanding of the effects of fetal overgrowth and diabetes in pregnancy on the developing kidneys.

We have demonstrated that the measurement of the fetal renal parenchyma shows promise as the most accurate, indirect method to estimate nephron numbers and that it can detect changes in fetal kidney growth. It could now be utilised in future studies to further increase our understanding of fetal renal development.

9.12 Future Research

Future investigations could emulate this study design with mixed effects modelling analysis of different populations and larger cohorts of FGR and LGA pregnancies to refine and validate measurement of the fetal renal parenchymal thickness across different populations of pregnant women. To really be able to elucidate the many complex factors that could impact the growth of the fetal kidneys in pregnancies complicated by diabetes, a large, dedicated study focusing on the effects of different types of diabetes, GA at diagnosis, adequacy of BSL control and types and timing of treatment would be valuable. This should be followed by a long-term study following the growth of the renal parenchymal thickness of infants, born to diabetic mothers, into adulthood. This should also include correlation with renal function tests. Only then can we truly start to demonstrate the long-term outcomes of the intrauterine environment on later life.

Although this study did not demonstrate any significant changes in the fetal renal blood flow in FGR fetuses this does still warrant refinement and improvement. Ultrasound imaging and, in particular, blood flow analysis, is rapidly improving with implementation of new techniques. Therefore, a study examining the renal arteries of growth restricted fetuses that is powered to detect a difference in blood flow and which investigates various different emerging Doppler techniques, such as volume flow measurements and 3D flow imaging, could be conducted and may be able to detect the subtle changes in renal perfusion that is thought to occur with FGR.

Now we have renal parenchymal thickness as a marker of nephron endowment, it could be used in multiple different study settings to assess for any adverse changes to the renal parenchymal growth. For example, in the future if we discover interventions for the treatment of FGR, measuring the fetal renal parenchyma could be used to monitor the effects of the intervention on fetal kidney growth. Similar studies could be performed for treatments of diabetes in pregnancy.

A natural progression of this work is to analyse how the renal parenchymal thickness, as a surrogate marker of nephron endowment, could be extended into the postnatal period and beyond. We have already completed pilot studies comparing the renal parenchyma of low birth and preterm neonates to normal birth weight term neonates and shown the usefulness of this measurement in the neonatal period. The preterm neonate in the neonatal intensive care unit usually suffers from a range of complex conditions and receives multiple different interventions. We know that nephrogenesis can be adversely impacted by preterm birth and growth restriction, however there are many other factors such as medications and types of ventilation which may also influence nephrogenesis. The renal parenchyma measurement could be used to assess effects of the various treatments on renal growth to assist in achieving optimal renal growth in this high-risk group.

Extending on from this, is investigating the role that the renal parenchymal measurement could have in monitoring children and adults in cases of declining renal function, CKD or post kidney surgery. The renal parenchymal measurement is easily performed and uses ultrasound imaging

which is widely available. This makes the future applications of the renal parenchymal measurement exciting and varied.

9.13 References

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Appendices

Appendix A – Ethics

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Appendix B -Research Documents

Appendix B1: Patient/Participant Information and Consent Form V2

PATIENT/PARTICIPANT INFORMATION SHEET and CONSENT FORM

PROTOCOL NAME: The renal parenchyma – Evaluation of a novel ultrasound measurement

FeRP Study – Fetal Renal Parenchyma

INVESTIGATORS:

Mrs Sonja Brennan
Ultrasound Department
Townsville Hospital
James Cook University, Qld

A/Prof Yoga Kandasamy
Neonatal Department
The Townsville Hospital
James Cook University, Qld

Dr David Watson
Maternal-Fetal-Medicine
Townsville Hospital
James Cook University, Qld

Dr Donna Rudd
College of Public Health, Medical
and Veterinary Sciences
James Cook University, Qld

A/ Prof Michal Schneider
Department of Medical Imaging
and Radiation Sciences
Monash University, Vic

1 Introduction

You are invited to take part in the FeRP research study. Before you decide whether to participate or not, it is important for you to understand why this research is being done and what it will involve. This information sheet tells you about the study. Please take the time to read through this and discuss it with others if you wish. Ask questions about anything you don't understand or would like to know more about.

This study is entirely voluntary (your choice). If you decide you want to take part in this study, you will be asked to sign the consent form. You will be given a copy of this information sheet and consent form to keep.

Thank you for your interest and for taking the time to consider being involved.

2 What is the purpose of this study?

Abnormal fetal growth, such as growth restriction, overgrowth and preterm birth, have been found to affect the growth of the kidneys. Abnormal growth of the kidneys in the fetus is linked to high blood pressure and kidney disease later in life. Currently there is no easy, accurate method to assess the growth of the fetal kidneys.

The purpose of this study is to investigate whether a new ultrasound measurement will improve our understanding of the growth of the kidneys and how abnormal fetal growth affects the kidney (renal) development. This study will also assess the blood flow to the kidneys and if abnormal fetal growth affects this blood flow.

This new measurement looks at the parenchyma of the kidney. The parenchyma is where the functional units of the kidney are. This study will measure how thick the parenchyma is and its blood flow in normally grown fetuses, growth restricted (small) fetuses and overgrown (large) fetuses and compare the measurements between these three groups. It is possible that the results of this study will help us find factors we can modify to improve fetal kidney growth.

This study is being undertaken at the Townsville Hospital Ultrasound Department working in collaboration with James Cook University (JCU) and Monash University, Melbourne. At least 60 participants will be taking part in this study.

The results of this study will be used by the principal researcher, Mrs Brennan, to obtain a Doctor of Philosophy (JCU).

3 Who can participate?

You can participate in this study if you are 18 years of age or older and are carrying one baby with a known due date.

4 What does participation involve?

The care for you and your pregnancy will be provided as normal by your doctors and midwives. If you agree to take part in this study, we will ask you to sign a consent form.

You will need to have an ultrasound scan every four weeks from when you have your first scan after 16 weeks, until you deliver your baby. For example, if your first scan after 16 weeks was at 20 weeks, we would ask you to come for other scans for the research at 24, 28, 32, 36 and 40 weeks, whereas if your first scan was at 22 weeks it would be every four weeks from then (26, 30, 34, 38).

Pregnant women, where the baby is not growing properly (too small or too large), may be referred by their doctor to have follow-up ultrasound scans every one to four weeks to watch the growth of the baby. If this is the case, you will already be coming for scans at least every four weeks and will not need extra scans. The ultrasound scan that your doctor referred you for will be the first priority. After this is done some extra kidney measurements on your baby will be done. This should only add about five minutes to the overall scan time.

Pregnant women with a well grown baby, may only need one or two ultrasound scans normally between 16 to 40 weeks. If your doctor does not require a scan for clinical reasons, we will organise a scan for you every four weeks until you deliver your baby. This ultrasound scan will be similar to a routine growth scan. Measurements of baby, blood flow to your baby and the fluid around baby will be done and then the extra kidney measurements will be done. This scan should take around 20 to 30 minutes to be done.

Before your first ultrasound for the study you will be asked to fill in a questionnaire telling us about your current pregnancy, your past pregnancies and your general health. We will also ask you for contact details in case we need to contact you after your baby is born.

A member of the research team will collect information about your pregnancy and your baby from your medical notes. We may contact you after your baby is born, particularly if you did not deliver at Townsville Hospital. We may ask how baby is going and ask information around their birth, for example, your baby's birth weight, sex and health.

5 Does the participant have to take part in this study?

Participation in this study is voluntary and is up to you if you wish to take part. If you decide to participate or not, it will not affect the care you will receive at any time and

will not affect your relationship with the staff caring for you. If you do decide to take part, you can still withdraw from the study at any time and you don't have to give a reason.

6 What are the possible benefits of taking part?
There are no benefits to participating.

7 What are the possible risks and disadvantages of taking part?
Ultrasound is a part of routine obstetric care and there are no known risks to the mother or their baby. There are no disadvantages in taking part in this study.

8 What will happen to the participant's ultrasound results?
All ultrasound images will be stored on Queensland Health Enterprise Picture Archiving and Communication System (ePACS) which is normal practice for all medical images and is part of the patient's medical record. The scan will be reported by a radiologist or maternal-fetal-medicine specialist and this report is stored with images on ePACS as part of the patient's record.

10 Can the participant have other treatments during this study?
Yes. You can receive whatever treatment you and your medical team think is suitable for your pregnancy.

11 What if I join the study but then change my mind and want to withdraw?
You can withdraw from the study at any time and you do not have to give a reason. Please let a member of the research team know and they will get you to complete a "Withdrawal of Consent" form.

If you decide to no longer take part in the study no further information will be collected about you or your baby. You should be aware that data already collected will be kept and will form part of the study results.

A decision to withdraw from the study will not affect the care you receive now or in the future and will not affect your relationship with the staff caring for you.

12 What happens when the study ends?
The results from this study will be analysed. They will then be presented at conferences and professional forums and written up and submitted to medical journals to publish. They will also be written up in the format of a PhD thesis.

In any publication, presentation or report, information will be presented as summary data so no participant or their baby can be identified. Summary results of the data will also be provided to you by a letter from the researchers after publication, if you wish.

13 What will happen to information about the participant?
All information collected about participants during the study will be kept strictly confidential. No material that could potentially identify any participant or their baby will be used in any report of this study. Your personal details will be held securely within the Townsville Hospital and only members of the research team will have access to them.

14 Will I be paid for taking part in this study?
There is no payment or incentive for participating in this study. For participants who are asked to come for additional scans for the research, who would not have needed to come otherwise, the cost of the hospital parking fee will be repaid.

15 Who is organising and funding the study?

The study is being organised and funded by the Medical Imaging Ultrasound Department of the Townsville Hospital**.** The study will be the subject of funding applications in the future. Whether funding applications are successful or not, no money will be paid directly to any member of the research team.

16 Who has reviewed the study?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this study have been reviewed and approved by the HREC of the Townsville Hospital and Health Service Human Ethics Committee (EC00183).

17 Further information and who to contact

After you have read this information, a member of the research team will discuss the study with you again and answer any questions you may have. You will be given the chance to discuss participating in this study with whomever you wish, such as your partner, other family, friends or your doctors and midwives providing your care. If you would like to know more at any stage, please do not hesitate to contact the research contact person below.

Research Contact Person:
Name: Mrs Sonja Brennan
Position: Consultant Senior Sonographer
Phone:
Email:

This project has been reviewed and approved by the Townsville Hospital and Health Service Human Research Ethics Committee. For concerns relating to the conduct of this project contact:

HREC Chairperson
Phone: 07 4433 1440
Email: TSV-Ethics-Committee@health.qld.gov.au

Thank you for taking the time to read this and consider being involved in the study.

You will be provided with a copy of this form to keep.

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Appendix B2: Participant Questionnaire

PARTICIPANT QUESTIONNAIRE

FeRP Study - Fetal Renal Parenchyma

SECTION 1: Mother's Details

Date:	UR:
Name:	DOB:
How many weeks pregnant are you? -	Due Date:

Mother's height: _____cm Mother's weight (pre-pregnancy): _____kg

Contact phone number: _____

Address: _____

Email: _____

Partner's name: _____

Partner 's contact phone number: _____

Other contact name and phone: _____

Mother's Medical History

Please check yes or no	Y	N	Please check yes or no	Y	N
Diabetes			Epilepsy, seizures		
Thyroid disease			Blood clots		
High blood pressure			Chrohn's or coeliac disease		
Heart disease			Rheumatoid arthritis, lupus		
Kidney disease			Autoimmune disease		
Liver disease, hepatitis			HIV or AIDS		
Lung problem, chronic asthma			Any malignancy/cancer		
Anaemia or blood disorder			Other medical condition		

Additional details: _____
_____
_____

SECTION 2: Obstetric Details

Previous pregnancies:

	Date	Outcome*	Gestation age (e.g. 37 weeks)	Alive Y / N	Birth weight (kg)	Sex M / F	Baby name	Complications
1								
2								
3								
4								
5								
6								

*Outcomes include vaginal delivery, caesarean, miscarriage, ectopic pregnancy

Current pregnancy:

Did you have fertility treatment with this pregnancy? ☐ No ☐ Yes

Have you had any previous ultrasounds on this pregnancy? ☐ No ☐ Yes

If yes, please list when and where: _____

Have you smoked during this pregnancy?

☐ No ☐ Yes If yes, how many per day _____

Have you consumed alcohol during this pregnancy?

☐ No ☐ Yes If yes, how many drinks per week_____

Do you have gestational diabetes? ☐ No ☐ Yes

If yes, is it controlled with ☐ diet, ☐ insulin, and/or ☐ oral tablets

Mother of Baby – Ancestry

☐ Aboriginal / Torres Strait Islander
☐ African
☐ Asian
☐ Caucasian
☐ Indian
☐ Middle Eastern
☐ Other_____

Father of Baby – Ancestry

☐ Aboriginal / Torres Strait Islander
☐ African
☐ Asian
☐ Caucasian
☐ Indian
☐ Middle Eastern
☐ Other_____

Appendix C: Miscellaneous

Appendix C1: Search Strategy

Medline Ovid (R) 1946 to 7th Jan 2017:

1 Ultrasonography (MeSH term exploded) — "computer echotomography" OR "diagnostic ultrasound*" OR echography OR echotomography OR "medical sonography" OR "ultrasonic diagnoses" OR "ultrasonic diagnosis" OR "ultrasonic imaging" OR "ultrasonic tomography" OR "ultrasound imaging*" OR sonography
2 Kidney* (MeSH term exploded)
3 Renal (keyword)
4 2 OR 3
5 Fetus (MeSH term exploded) – "fetal structure*" OR "fetal tissue*" OR fetuses
6 Prenatal (MeSH term exploded) – "antenatal diagnoses" OR antenatal diagnosis OR "antenatal screening*" OR "intrauterine diagnoses" OR "intrauterine diagnosis" OR "prenatal diagnoses" OR "prenatal diagnosis" OR "prenatal screening*"
7 Foetus OR Foetal (keyword)
8 5 OR 6 OR 7
9 1 AND 4 AND 8
10 Limit 9 to yr = "1996 – 2016"
11 Limit 10 to English language

Appendix C2 – Chapter 7: Analysis For: Is bigger better? Large for Gestational Age and Kidney Growth

Analysis for: Is bigger better? Large for gestational age and kidney growth

Part 1: Set up of libraries and data

Load needed libraries

```
library(dplyr)
library(nlme)
library(car)
library(ggplot2)
```

Load data sets

```
LGA = read.csv("FINALLGAvsAGA.csv", header=T, sep=",")
```

Factor variables

```
LGA[,c("PtCode","AntPos", "SideRtLt", "Growth", "GendF1", "DM", "DMtype")]=
  lapply(LGA[,c("PtCode","AntPos", "SideRtLt", "Growth", "GendF1", "DM", "DMtype")],factor)
```

Re-name factors

```
LGA$AntPos=factor(LGA$AntPos, labels=c("Ant", "Post"))
LGA$SideRtLt=factor(LGA$SideRtLt, labels=c("Rt", "Lt"))
LGA$Growth=factor(LGA$Growth, labels=c("AGA", "LGA"))
LGA$GendF1=factor(LGA$GendF1, labels=c("F", "M"))
LGA$DM=factor(LGA$DM, labels=c("N", "Y"))
LGA$DMtype=factor(LGA$DMtype, labels = c("none", "T1", "T2", "GDM"))
```

Remove all NAs from GAcont

```
LGA.complete = LGA[!is.na(LGA$GAcont),]
```

Change intercept so 16 starts at 0

```
LGA.complete$GAcont=LGA.complete$GAcont-16
```

Part 2: Renal parenchymal thickness (RPT) growth

Model for RPT vs gestational age (GA) to compare appropriately for gestational age (AGA) to large for gestational age (LGA)

Best model - Rerun with REML

```
LGA11 = lme(RPT~ poly(GAcont,2)+Growth +SideRtLt, random= ~poly(GAcont,2)|P
tCode,
            method = "REML",
            na.action="na.omit",
            weights = varPower(form=~fitted(.)),
            data=LGA.complete, control = lmeControl(opt = "optim"))
```

Anova(LGA11)

```
## Analysis of Deviance Table (Type II tests)
##
## Response: RPT
##                     Chisq Df Pr(>Chisq)
## poly(GAcont, 2) 2630.0193  2  < 2.2e-16 ***
## Growth             6.1236  1    0.01334 *
## SideRtLt          17.8700  1  2.365e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

summary(LGA11)

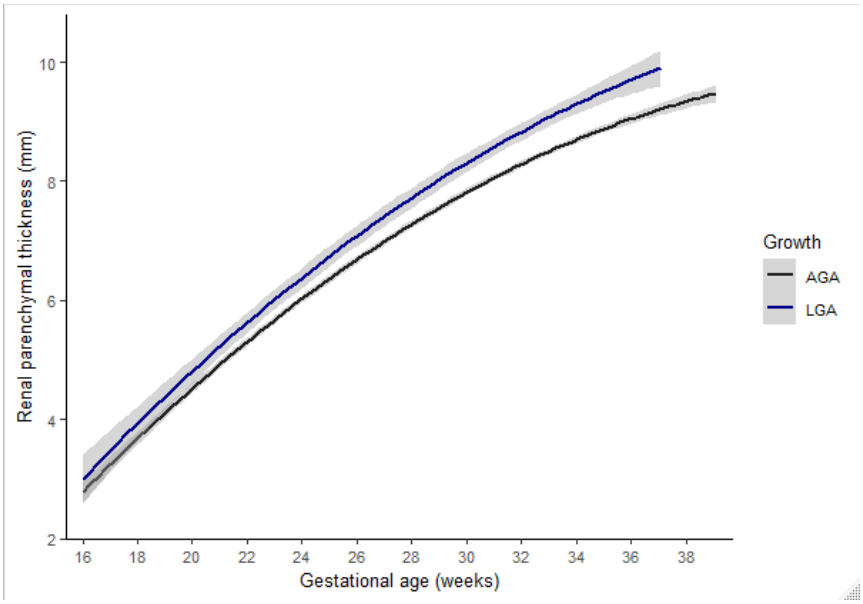
```
## Linear mixed-effects model fit by REML
##  Data: LGA.complete
##        AIC      BIC    logLik
##   6457.323 6533.196 -3215.662
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                  StdDev     Corr
## (Intercept)       0.6337342 (Intr) p(GA,2
## poly(GAcont, 2)1 17.7227171 0.741
## poly(GAcont, 2)2 10.6686602 0.033  0.232
## Residual          0.1560605
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##     power
## 0.8273603
## Fixed effects: RPT ~ poly(GAcont, 2) + Growth + SideRtLt
##                      Value Std.Error   DF   t-value p-value
## (Intercept)        7.26745 0.0656708 2415 110.66486  0.0000
## poly(GAcont, 2)1  91.70038 1.9062724 2415  48.10455  0.0000
## poly(GAcont, 2)2 -12.20847 1.3739818 2415  -8.88547  0.0000
## GrowthLGA          0.33871 0.1368743  116   2.47460  0.0148
## SideRtLtLt        -0.12042 0.0284872 2415  -4.22730  0.0000
##  Correlation:
##                  (Intr) p(GA,2)1 p(GA,2)2 GrwLGA
## poly(GAcont, 2)1  0.604
## poly(GAcont, 2)2  0.074  0.179
## GrowthLGA        -0.281 -0.002   -0.012
## SideRtLtLt       -0.220  0.002   -0.001    0.000
##
```

```
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.98110238 -0.62300863 -0.04924571  0.60903970  3.79301690
##
## Number of Observations: 2536
## Number of Groups: 118
```

intervals(LGA11)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                           lower        est.       upper
## (Intercept)         7.13867397   7.2674509  7.39622789
## poly(GAcont, 2)1   87.96228536  91.7003841 95.43848277
## poly(GAcont, 2)2 -14.90277289 -12.2084677 -9.51416260
## GrowthLGA           0.06761216   0.3387089  0.60980572
## SideRtLtLt         -0.17628567  -0.1204238 -0.06456195
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                             lower        est.      uppe
r
## sd((Intercept))                          0.54912509  0.63373422  0.731379
9
## sd(poly(GAcont, 2)1)                    14.81076354 17.72271712 21.207191
7
## sd(poly(GAcont, 2)2)                     7.88602219 10.66866016 14.433171
4
## cor((Intercept),poly(GAcont, 2)1)        0.59359263  0.74100829  0.840310
3
## cor((Intercept),poly(GAcont, 2)2)       -0.09230832  0.03288405  0.157053
0
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2) -0.04379457  0.23156819  0.474230
6
##
##  Variance function:
##           lower      est.     upper
## power 0.7168314 0.8273603 0.9378892
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1256245 0.1560605 0.1938704
```

Graph RPT vs GA for AGA and LGA



Part 3: RPT growth compared to AC growth for AGA and LGA

Power curves - log transform data so both intercept at 0

```
log.LGA = mutate(LGA.complete,
                 logRPT=log(RPT),
                 logHC=log(HC),
                 logEFW=log(EFW),
                 logAC =log(AC),
                 logFL=log(FL))
```

Model for RPT growth compared to AC growth for AGA and LGA Best model- re-run with REML

```
LGAgrowth4 = lme(logRPT ~ logAC*Growth + SideRtLt, random=~logAC|PtCode,
                 method = "REML",na.action="na.omit", data=log.LGA,
                 control = lmeControl(msMaxIter = 200))
```

```
Anova(LGAgrowth4)
## Analysis of Deviance Table (Type II tests)
##
## Response: logRPT
##                 Chisq Df Pr(>Chisq)
## logAC        2667.6462  1  < 2.2e-16 ***
## Growth          0.0050  1     0.9437
## SideRtLt       18.5835  1  1.626e-05 ***
## logAC:Growth    1.1204  1     0.2898
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

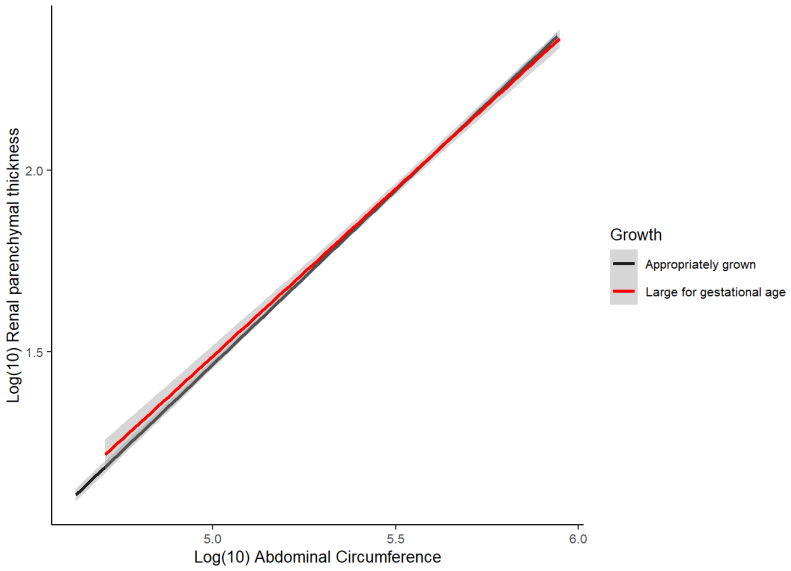
```
summary(LGAgrowth4)
## Linear mixed-effects model fit by REML
##  Data: log.LGA
##         AIC       BIC   logLik
```

```
##   -3180.477 -3127.963 1599.238
##
## Random effects:
##  Formula: ~logAC | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##             StdDev    Corr
## (Intercept) 0.8811569 (Intr)
## logAC       0.1624066 -0.996
## Residual    0.1178400
##
## Fixed effects: logRPT ~ logAC * Growth + SideRtLt
##                     Value  Std.Error   DF   t-value p-value
## (Intercept)     -3.217452 0.10623361 2411 -30.28657  0.0000
## logAC            0.942259 0.01945883 2411  48.42322  0.0000
## GrowthLGA        0.307815 0.29016680  116   1.06082  0.2910
## SideRtLtLt      -0.020191 0.00468372 2411  -4.31085  0.0000
## logAC:GrowthLGA -0.056041 0.05294316 2411  -1.05851  0.2899
##  Correlation:
##                 (Intr) logAC  GrwLGA SdRtLL
## logAC           -0.997
## GrowthLGA       -0.366  0.365
## SideRtLtLt      -0.022  0.000  0.000
## logAC:GrowthLGA  0.366 -0.368 -0.997  0.000
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -4.029098804 -0.645188691 -0.005399481  0.644233639  3.469127118
##
## Number of Observations: 2532
## Number of Groups: 118
```

intervals(LGAgrowth4)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                         lower        est.       upper
## (Intercept)     -3.42577045 -3.21745183 -3.00913320
## logAC            0.90410140  0.94225916  0.98041692
## GrowthLGA       -0.26689686  0.30781505  0.88252696
## SideRtLtLt      -0.02937537 -0.02019083 -0.01100630
## logAC:GrowthLGA -0.15985947 -0.05604066  0.04777815
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                lower       est.      upper
## sd((Intercept))         0.7107334  0.8811569  1.0924454
## sd(logAC)               0.1315787  0.1624066  0.2004572
## cor((Intercept),logAC) -0.9978904 -0.9964768 -0.9941188
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1144578 0.1178400 0.1213222
```

Graph for RPT(log10) vs AC(log10) for LGA and AGA



Part 4: Renal arteries RI and PI for AGA and LGA

Model for for RI vs GA for AGA and LGA

```
LGAri8=lme(RI~ poly(GAcont,2)*Growth +SideRtLt, random= ~poly(GAcont,2)|PtC
ode,
           method = "REML",
           na.action="na.omit",
           data= LGA.complete, weights = varPower(form=~fitted(.)),
           control = lmeControl(opt = "optim"))

Anova(LGAri8)
## Analysis of Deviance Table (Type II tests)
##
## Response: RI
##                          Chisq Df Pr(>Chisq)
## poly(GAcont, 2)        52.1052  2  4.847e-12 ***
## Growth                  0.6980  1  0.4034493
## SideRtLt               11.7396  1  0.0006119 ***
## poly(GAcont, 2):Growth  5.5377  2  0.0627326 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(LGAri8)
## Linear mixed-effects model fit by REML
##  Data: LGA.complete
##        AIC       BIC  logLik
##   -8750.42 -8663.678 4390.21
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                  StdDev     Corr
## (Intercept)      0.01744079 (Intr) p(GA,2
## poly(GAcont, 2)1 0.91954104 -0.061
## poly(GAcont, 2)2 0.82465751  0.197 -0.358
## Residual         0.02618637
##
```

```
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##     power
## -2.320179
## Fixed effects: RI ~ poly(GAcont, 2) * Growth + SideRtLt
##                                Value  Std.Error   DF  t-value p-value
## (Intercept)                 0.8844856 0.00212007 2283 417.1971  0.0000
## poly(GAcont, 2)1            0.0339716 0.10829763 2283   0.3137  0.7538
## poly(GAcont, 2)2           -0.6740581 0.10012551 2283  -6.7321  0.0000
## GrowthLGA                  -0.0041984 0.00556876  116  -0.7539  0.4524
## SideRtLtLt                  0.0048392 0.00141237 2283   3.4263  0.0006
## poly(GAcont, 2)1:GrowthLGA  0.5938162 0.30120248 2283   1.9715  0.0488
## poly(GAcont, 2)2:GrowthLGA  0.1381872 0.28087357 2283   0.4920  0.6228
##  Correlation:
##                            (Intr) pl(GA,2)1 pl(GA,2)2 GrwLGA SdRtLL p(GA
,2)1:
## poly(GAcont, 2)1           -0.122
## poly(GAcont, 2)2            0.257 -0.372
## GrowthLGA                  -0.337  0.046    -0.098
## SideRtLtLt                 -0.336  0.004     0.003    -0.001
## poly(GAcont, 2)1:GrowthLGA  0.046 -0.360     0.134    -0.152 -0.007
## poly(GAcont, 2)2:GrowthLGA -0.093  0.133    -0.356     0.303  0.002 -0.3
59
##
## Standardized Within-Group Residuals:
##           Min            Q1           Med            Q3           Max
## -2.9284721597 -0.6184383751 -0.0006439879  0.5772429722  3.3180871580
##
## Number of Observations: 2406
## Number of Groups: 118
```

intervals(LGAri8)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                                 lower         est.         upper
## (Intercept)                 0.880328118  0.884485577  0.888643035
## poly(GAcont, 2)1           -0.178400441  0.033971600  0.246343640
## poly(GAcont, 2)2           -0.870404566 -0.674058077 -0.477711588
## GrowthLGA                  -0.015228021 -0.004198390  0.006831241
## SideRtLtLt                  0.002069543  0.004839202  0.007608861
## poly(GAcont, 2)1:GrowthLGA  0.003157034  0.593816192  1.184475350
## poly(GAcont, 2)2:GrowthLGA -0.412606899  0.138187196  0.688981292
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                              lower        est.       upp
er
## sd((Intercept))                          0.01461665  0.01744079  0.020810
58
## sd(poly(GAcont, 2)1)                     0.77876821  0.91954104  1.085760
46
## sd(poly(GAcont, 2)2)                     0.66542578  0.82465751  1.021992
27
## cor((Intercept),poly(GAcont, 2)1)       -0.29404119 -0.06053193  0.179796
29
```

```
## cor((Intercept),poly(GAcont, 2)2)      -0.04700223  0.19724996  0.419245
28
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2) -0.57158388 -0.35799004 -0.098960
07
##
##  Variance function:
##           lower      est.     upper
## power -3.467513 -2.320179 -1.172845
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##      lower       est.      upper
## 0.02275063 0.02618637 0.03014096
```

Model for for PI vs GA for AGA and LGA Best model for PI - re-run using REML

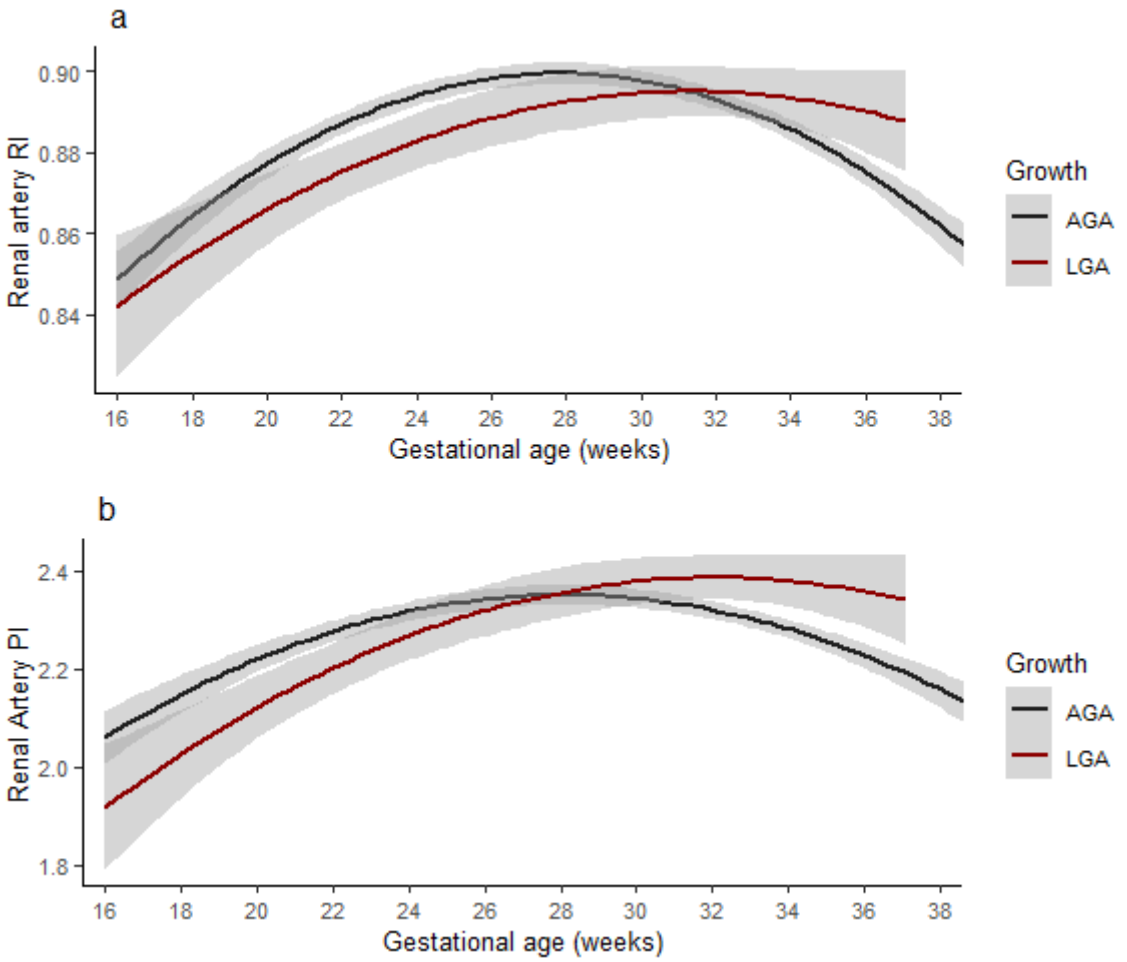
```
LGApi1=lme(PI~ poly(GAcont,2)*Growth +SideRtLt, random= ~poly(GAcont,2)|PtC
ode,
           method = "REML",
           na.action="na.omit",
           data= LGA.complete, weights = varPower(form=~fitted(.)),
           control = lmeControl(opt = "optim"))

Anova(LGApi1)
## Analysis of Deviance Table (Type II tests)
##
## Response: PI
##                          Chisq Df Pr(>Chisq)
## poly(GAcont, 2)        17.5720  2  0.0001529 ***
## Growth                  0.0046  1  0.9457488
## SideRtLt                1.7249  1  0.1890689
## poly(GAcont, 2):Growth  3.5370  2  0.1705875
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(LGApi1)
## Linear mixed-effects model fit by REML
##  Data: LGA.complete
##        AIC      BIC    logLik
##   903.0603 989.8024 -436.5301
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                  StdDev    Corr
## (Intercept)      0.1505377 (Intr) p(GA,2
## poly(GAcont, 2)1 6.6174758  0.197
## poly(GAcont, 2)2 6.7811936  0.250 -0.154
## Residual         0.1147190
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##     power
## 0.9811413
## Fixed effects: PI ~ poly(GAcont, 2) * Growth + SideRtLt
```

```
##                                Value Std.Error   DF   t-value p-value
## (Intercept)                 2.272742 0.0174594 2283 130.17335  0.0000
## poly(GAcont, 2)1           -0.116593 0.7830765 2283  -0.14889  0.8817
## poly(GAcont, 2)2           -3.145205 0.7938186 2283  -3.96212  0.0001
## GrowthLGA                   0.012254 0.0461858  116   0.26532  0.7912
## SideRtLtLt                  0.013685 0.0104199 2283   1.31334  0.1892
## poly(GAcont, 2)1:GrowthLGA  3.892908 2.1714894 2283   1.79274  0.0731
## poly(GAcont, 2)2:GrowthLGA  0.477373 2.2255649 2283   0.21450  0.8302
##  Correlation:
##                            (Intr) pl(GA,2)1 pl(GA,2)2 GrwLGA SdRtLL p(GA
,2)1:
## poly(GAcont, 2)1            0.081
## poly(GAcont, 2)2            0.273 -0.213
## GrowthLGA                  -0.345 -0.031    -0.104
## SideRtLtLt                 -0.296  0.005     0.003    -0.001
## poly(GAcont, 2)1:GrowthLGA -0.028 -0.361     0.077     0.077 -0.007
## poly(GAcont, 2)2:GrowthLGA -0.098  0.076    -0.357     0.315  0.001 -0.1
92
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.57339146 -0.61952610 -0.01784109  0.62608968  4.88878722
##
## Number of Observations: 2406
## Number of Groups: 118
```

intervals(LGApi1)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                                 lower        est.       upper
## (Intercept)                 2.238504413  2.27274227  2.30698012
## poly(GAcont, 2)1           -1.652208764 -0.11659292  1.41902292
## poly(GAcont, 2)2           -4.701885721 -3.14520452 -1.58852331
## GrowthLGA                  -0.079222896  0.01225387  0.10373063
## SideRtLtLt                 -0.006748613  0.01368487  0.03411836
## poly(GAcont, 2)1:GrowthLGA -0.365390927  3.89290772  8.15120636
## poly(GAcont, 2)2:GrowthLGA -3.886967508  0.47737339  4.84171428
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                                lower       est.     upper
## sd((Intercept))                           0.127250024  0.1505377 0.1780871
## sd(poly(GAcont, 2)1)                      5.410884667  6.6174758 8.0931287
## sd(poly(GAcont, 2)2)                      5.538414956  6.7811936 8.3028425
## cor((Intercept),poly(GAcont, 2)1)        -0.102719046  0.1971167 0.4641159
## cor((Intercept),poly(GAcont, 2)2)        -0.008724587  0.2496544 0.4767835
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2) -0.408234892 -0.1543860 0.1216252
##
##  Variance function:
##           lower      est.    upper
## power 0.6012455 0.9811413 1.361037
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##      lower       est.      upper
## 0.08394216 0.11471897 0.15677988
```

Graph RI vs GA for AGA vs LGA and PI vs GA for AGA vs LGA



Appendix C3 – Chapter 8: Analysis for: The Effect of Diabetes During Pregnancy on Fetal Renal Parenchymal Growth

Analysis for: The effect of diabetes during pregnancy on fetal renal parenchymal growth

Journal of Nephrology

Sonja Brennan, Yogavijayan Kandasamy, Donna M Rudd, Michal E Schneider, Rhondda E Jones, David L Watson

Part 1: Set up of libraries and data

```
library(dplyr)
library(nlme)
library(car)
library(ggplot2)
```

Load data sets

```
DM=read.csv("DM2.csv")
```

Subset out only gestational diabetes (GDM) vs Control

```
GDM = subset(DM, (DMtype=="0"|DMtype=="3"),
             select =c(PtCode,GAcont, RPT, AntPos, SideRtLt,
                       GendF1, DM,DMtype, BMI,
                       HC, AC, FL, EFW, Length, Trans, AP, RVol))
```

Subset out Pregestational vs control

```
DM_pregest=subset(DM, (DMtype=="0"|DMtype=="1"|DMtype=="2"),
                  select =c(PtCode,GAcont, RPT, AntPos, SideRtLt,
                            GendF1,DM,DMtype, BMI,
                            HC, AC, FL, EFW, Length, Trans, AP,RVol))
```

Remove NAs from gestational age variable (GAcont)

```
GDM.complete = GDM[!is.na(GDM$GAcont),]
PreGD.complete = DM_pregest[!is.na(DM_pregest$GAcont),]
```

Change intercept so 16 starts at 0

```
GDM.complete$GAcont=GDM.complete$GAcont-16
PreGD.complete$GAcont=PreGD.complete$GAcont-16
```

Part 2: Renal parenchymal thickness (RPT) growth

Model for RPT vs gestational age (GA) to compare GDM to control

Best model is GDM5 - re-run with REML

```
GDM5 = lme(RPT~ poly(GAcont,2)*DM +SideRtLt, random= ~poly(GAcont,2)|PtCode
,
           method = "REML",
           na.action="na.omit",
           weights = varPower(form=~fitted(.)),
           data=GDM.complete)


summary(GDM5)
```

```
## Linear mixed-effects model fit by REML
##  Data: GDM.complete
##        AIC      BIC    logLik
##   6471.402 6559.054 -3220.701
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                   StdDev     Corr
## (Intercept)       0.6812192 (Intr) p(GA,2
## poly(GAcont, 2)1 16.5691877  0.765
## poly(GAcont, 2)2  9.3826482 -0.062  0.325
## Residual          0.1625385
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##     power
## 0.8069518
## Fixed effects: RPT ~ poly(GAcont, 2) * DM + SideRtLt
##                           Value Std.Error   DF  t-value p-value
## (Intercept)             7.08178  0.084664 2433 83.64605  0.0000
## poly(GAcont, 2)1       90.65337  2.276828 2433 39.81564  0.0000
## poly(GAcont, 2)2      -13.61145  1.585395 2433 -8.58553  0.0000
## DMY                     0.21158  0.134046  116  1.57839  0.1172
## SideRtLtRt              0.09999  0.028430 2433  3.51715  0.0004
## poly(GAcont, 2)1:DMY    1.66424  3.678743 2433  0.45239  0.6510
## poly(GAcont, 2)2:DMY    3.64535  2.532931 2433  1.43918  0.1502
##  Correlation:
##                       (Intr) pl(GA,2)1 pl(GA,2)2 DMY    SdRtLR p(GA,2)1:
## poly(GAcont, 2)1       0.662
## poly(GAcont, 2)2      -0.007  0.253
## DMY                   -0.614 -0.418     0.004
## SideRtLtRt            -0.166 -0.001     0.001     0.000
## poly(GAcont, 2)1:DMY -0.409 -0.619    -0.157     0.675  0.000
## poly(GAcont, 2)2:DMY  0.004 -0.159    -0.626    -0.002  0.000  0.283
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.92995204 -0.62816629 -0.02979786  0.60368922  3.77055641
##
## Number of Observations: 2556
## Number of Groups: 118
```

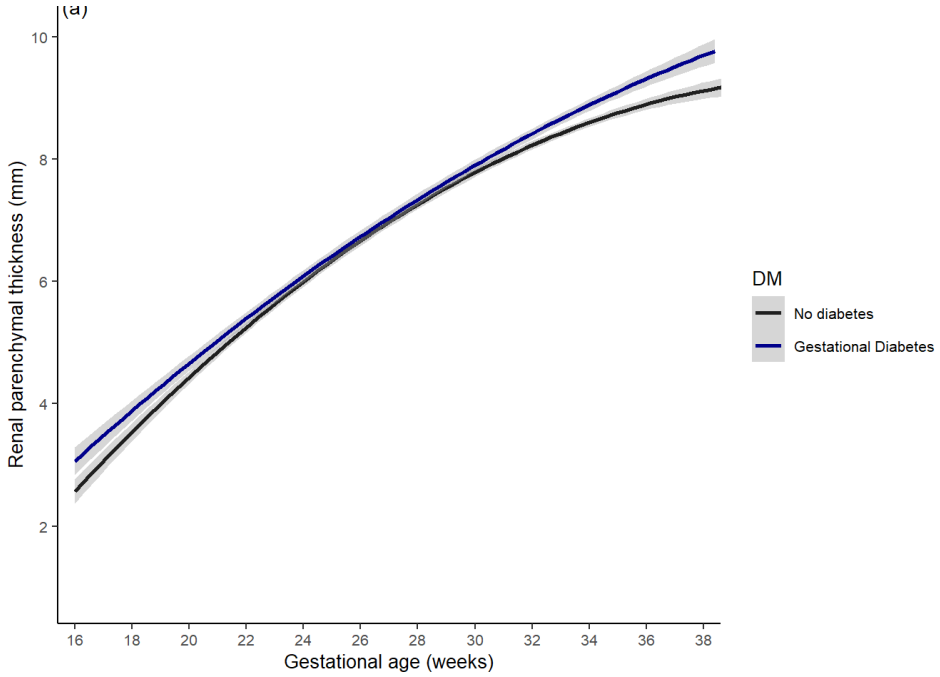
Anova(GDM5)

```
## Analysis of Deviance Table (Type II tests)
##
## Response: RPT
##                       Chisq Df Pr(>Chisq)
## poly(GAcont, 2)    3214.5919  2  < 2.2e-16 ***
## DM                    4.7581  1  0.0291613 *
## SideRtLt             12.3703  1  0.0004362 ***
## poly(GAcont, 2):DM    2.0734  2  0.3546233
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

intervals(GDM5)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                              lower         est.       upper
## (Intercept)            6.91575875   7.08177902   7.2477993
## poly(GAcont, 2)1      86.18864533  90.65336689  95.1180885
## poly(GAcont, 2)2     -16.72031887 -13.61145480 -10.5025907
## DMY                   -0.05391847   0.21157653   0.4770715
## SideRtLtRt             0.04424309   0.09999278   0.1557425
## poly(GAcont, 2)1:DMY  -5.54955261   1.66424052   8.8780336
## poly(GAcont, 2)2:DMY  -1.32157292   3.64535093   8.6122748
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                                lower        est.      upp
er
## sd((Intercept))                           0.590506901  0.68121923  0.78586
66
## sd(poly(GAcont, 2)1)                    13.743157960 16.56918774 19.97633
90
## sd(poly(GAcont, 2)2)                     6.968197009  9.38264818 12.63369
66
## cor((Intercept),poly(GAcont, 2)1)        0.624526414  0.76487260  0.85736
55
## cor((Intercept),poly(GAcont, 2)2)       -0.305239552 -0.06174329  0.18933
13
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2)  0.006599854  0.32502800  0.58360
85
##
##  Variance function:
##           lower      est.     upper
## power 0.6964282 0.8069518 0.9174754
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1309424 0.1625385 0.2017587
```

Graph of RPT vs GA for GDM and Control



Model for RPT vs GA to compare pregestational diabetes to control

Pregest5 is best model

```
Pregest5 = lme(RPT~ poly(GAcont,2)*DM +SideRtLt, random= ~poly(GAcont,2)|Pt
Code,
           method = "ML",
           na.action="na.omit",
           weights = varPower(form=~fitted(.)),
           data=PreGD.complete, control = lmeControl(opt = "optim"))

summary(Pregest5)
## Linear mixed-effects model fit by maximum likelihood
##  Data: PreGD.complete
##        AIC      BIC    logLik
##   4572.846 4655.112 -2271.423
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                   StdDev     Corr
## (Intercept)       0.6528489 (Intr) p(GA,2
## poly(GAcont, 2)1 13.8865035 0.792
## poly(GAcont, 2)2  9.3562617 0.054  0.062
## Residual          0.1323798
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##     power
## 0.9186026
```

```
## Fixed effects: RPT ~ poly(GAcont, 2) * DM + SideRtLt
##                          Value Std.Error   DF  t-value p-value
## (Intercept)            7.16355  0.082494 1694 86.83697  0.0000
## poly(GAcont, 2)1      74.09936  1.945246 1694 38.09254  0.0000
## poly(GAcont, 2)2     -11.36670  1.527425 1694 -7.44174  0.0000
## DMY                    0.15105  0.241291   79  0.62600  0.5331
## SideRtLtRt             0.04942  0.033856 1694  1.45976  0.1445
## poly(GAcont, 2)1:DMY  -3.67667  5.743090 1694 -0.64019  0.5221
## poly(GAcont, 2)2:DMY  -6.56468  4.748648 1694 -1.38243  0.1670
##  Correlation:
##                      (Intr) pl(GA,2)1 pl(GA,2)2 DMY    SdRtLR p(GA,2)1:
## poly(GAcont, 2)1      0.664
## poly(GAcont, 2)2      0.084  0.053
## DMY                  -0.328 -0.227    -0.029
## SideRtLtRt           -0.204 -0.001     0.001     0.000
## poly(GAcont, 2)1:DMY -0.225 -0.339    -0.018     0.690  0.000
## poly(GAcont, 2)2:DMY -0.027 -0.017    -0.322     0.084  0.000  0.093
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.97657950 -0.60539159 -0.04743634  0.60957532  3.45017476
##
## Number of Observations: 1780
## Number of Groups: 81
```

Anova(Pregest5)

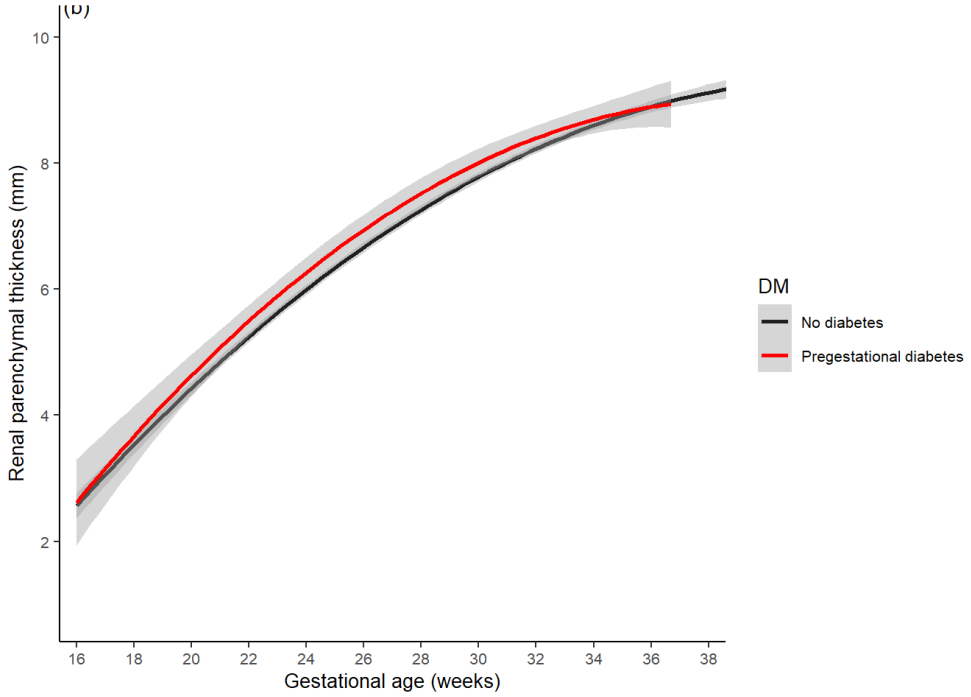
```
## Analysis of Deviance Table (Type II tests)
##
## Response: RPT
##                       Chisq Df Pr(>Chisq)
## poly(GAcont, 2)    1743.1681  2     <2e-16 ***
## DM                    2.2975  1     0.1296
## SideRtLt              2.1393  1     0.1436
## poly(GAcont, 2):DM    2.1839  2     0.3356
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

intervals(Pregest5)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                             lower         est.      upper
## (Intercept)            7.00207071   7.16355368  7.3250366
## poly(GAcont, 2)1      70.29152683  74.09935556 77.9071843
## poly(GAcont, 2)2     -14.35664100 -11.36669862 -8.3767562
## DMY                   -0.32828476   0.15104714  0.6303790
## SideRtLtRt            -0.01685129   0.04942112  0.1156935
## poly(GAcont, 2)1:DMY -14.91880103  -3.67667474  7.5654516
## poly(GAcont, 2)2:DMY -15.86017844  -6.56467642  2.7308256
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                             lower        est.      upper
## sd((Intercept))                         0.5503656  0.65284892  0.7744156
## sd(poly(GAcont, 2)1)                   11.0924388 13.88650351 17.3843627
## sd(poly(GAcont, 2)2)                    6.1301555  9.35626170 14.2801651
## cor((Intercept),poly(GAcont, 2)1)       0.6232270  0.79234828  0.8906695
## cor((Intercept),poly(GAcont, 2)2)      -0.2519524  0.05375325  0.3497042
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2) -0.2987676  0.06164774  0.4066725
```

```
##
##  Variance function:
##           lower      est.    upper
## power 0.7826365 0.9186026 1.054569
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1014177 0.1323798 0.1727946
```

Graph of RPT vs GA for Pregestational diabetes and Control groups



Part 3: Abdominal circumference (AC) growth

Model for AC vs GA to compare GDM to control

Best model GDMac5 - change to REML

```
GDMac5 = lme(AC~ poly(GAcont,2)*DM, random= ~poly(GAcont,2)|PtCode,
             method = "REML",
             na.action="na.omit",
             weights = varPower(form=~fitted(.)),
             data=GDM.complete, control=lmeControl(opt = "optim"))

summary(GDMac5)
## Linear mixed-effects model fit by REML
##  Data: GDM.complete
##        AIC      BIC    logLik
##   15483.06 15564.88 -7727.531
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
```

```
##                   StdDev      Corr
## (Intercept)       12.0236330 (Intr) p(GA,2
## poly(GAcont, 2)1 297.1461245  0.735
## poly(GAcont, 2)2 270.8745754  0.100 -0.104
## Residual           0.2655375
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##    power
## 0.490408
## Fixed effects: AC ~ poly(GAcont, 2) * DM
##                         Value Std.Error   DF   t-value p-value
## (Intercept)           245.706   1.42850 2434 172.00296  0.0000
## poly(GAcont, 2)1     3260.539  36.71709 2434  88.80165  0.0000
## poly(GAcont, 2)2     -166.129  33.42844 2434  -4.96970  0.0000
## DMY                     9.195   2.29021  116   4.01512  0.0001
## poly(GAcont, 2)1:DMY  238.257  58.82853 2434   4.05003  0.0001
## poly(GAcont, 2)2:DMY  117.386  53.86519 2434   2.17925  0.0294
##  Correlation:
##                      (Intr) pl(GA,2)1 pl(GA,2)2 DMY    p(GA,2)1:
## poly(GAcont, 2)1      0.684
## poly(GAcont, 2)2      0.118 -0.137
## DMY                  -0.624 -0.427    -0.074
## poly(GAcont, 2)1:DMY -0.427 -0.624     0.085     0.686
## poly(GAcont, 2)2:DMY -0.073  0.085    -0.621     0.123 -0.132
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.32175728 -0.56120251  0.01299609  0.51433392  3.20138174
##
## Number of Observations: 2556
## Number of Groups: 118
```

Anova(GDMac5)

```
## Analysis of Deviance Table (Type II tests)
##
## Response: AC
##                       Chisq Df Pr(>Chisq)
## poly(GAcont, 2)    13784.137  2  < 2.2e-16 ***
## DM                     0.877  1      0.349
## poly(GAcont, 2):DM    23.889  2  6.495e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

intervals(GDMac5)

```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                            lower        est.     upper
## (Intercept)           242.905279  245.706484  248.5077
## poly(GAcont, 2)1     3188.538568 3260.538553 3332.5385
## poly(GAcont, 2)2     -231.680475 -166.129336 -100.5782
## DMY                     4.659417    9.195459   13.7315
## poly(GAcont, 2)1:DMY  122.897879  238.257045  353.6162
## poly(GAcont, 2)2:DMY   11.759209  117.385557  223.0119
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
```

```
##   Level: PtCode
##                                                 lower        est.        u
pper
## sd((Intercept))                            10.56390163  12.0236330  13.6850
7169
## sd(poly(GAcont, 2)1)                      256.10223685 297.1461245 344.7678
5683
## sd(poly(GAcont, 2)2)                      232.86153898 270.8745754 315.0929
7714
## cor((Intercept),poly(GAcont, 2)1)          0.61063628   0.7349709   0.8239
4200
## cor((Intercept),poly(GAcont, 2)2)         -0.05730062   0.1000135   0.2524
8144
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2)  -0.27543722  -0.1041160   0.0736
1516
##
##  Variance function:
##          lower     est.    upper
## power 0.342002 0.490408 0.638814
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1168555 0.2655375 0.6033959
```

Model for AC vs GA to compare pregestational diabetes to control

Best model Pregac5 - change to REML

```
Pregac5 = lme(AC~ poly(GAcont,2)*DM, random= ~poly(GAcont,2)|PtCode,
              method = "REML",
              na.action="na.omit",
              weights = varPower(form=~fitted(.)),
              data=PreGD.complete, control = lmeControl(msMaxIter = 200))

summary(Pregac5)
```

```
## Linear mixed-effects model fit by REML
##  Data: PreGD.complete
##        AIC      BIC    logLik
##   10582.36 10659.07 -5277.182
##
## Random effects:
##  Formula: ~poly(GAcont, 2) | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##                   StdDev       Corr
## (Intercept)      1.252347e+01 (Intr) p(GA,2
## poly(GAcont, 2)1 3.006259e+02  0.746
## poly(GAcont, 2)2 2.443911e+02  0.106 -0.137
## Residual         6.987270e-04
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~fitted(.)
##  Parameter estimates:
##   power
## 1.56092
## Fixed effects: AC ~ poly(GAcont, 2) * DM
##                           Value Std.Error   DF   t-value p-value
## (Intercept)           247.8149   1.48815 1691 166.52535  0.0000
## poly(GAcont, 2)1     2671.7814  36.64231 1691  72.91520  0.0000
## poly(GAcont, 2)2     -135.2711  30.04207 1691  -4.50272  0.0000
```

```
## DMY                    19.8031   4.47122   79   4.42902  0.0000
## poly(GAcont, 2)1:DMY  367.9845 109.37263 1691   3.36450  0.0008
## poly(GAcont, 2)2:DMY   66.3260  91.72530 1691   0.72309  0.4697
##  Correlation:
##                       (Intr) pl(GA,2)1 pl(GA,2)2 DMY    p(GA,2)1:
## poly(GAcont, 2)1       0.710
## poly(GAcont, 2)2       0.125 -0.157
## DMY                   -0.333 -0.236    -0.042
## poly(GAcont, 2)1:DMY -0.238 -0.335     0.053     0.712
## poly(GAcont, 2)2:DMY -0.041  0.051    -0.328     0.134 -0.148
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.93730036 -0.54228041  0.00563303  0.58062035  3.39023834
##
## Number of Observations: 1776
## Number of Groups: 81
```

Anova(Pregac5)

```
## Analysis of Deviance Table (Type II tests)
##
## Response: AC
##                       Chisq Df Pr(>Chisq)
## poly(GAcont, 2)    6236.2808  2  < 2.2e-16 ***
## DM                    6.9224  1   0.008512 **
## poly(GAcont, 2):DM   12.8479  2   0.001622 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

intervals(Pregac5)

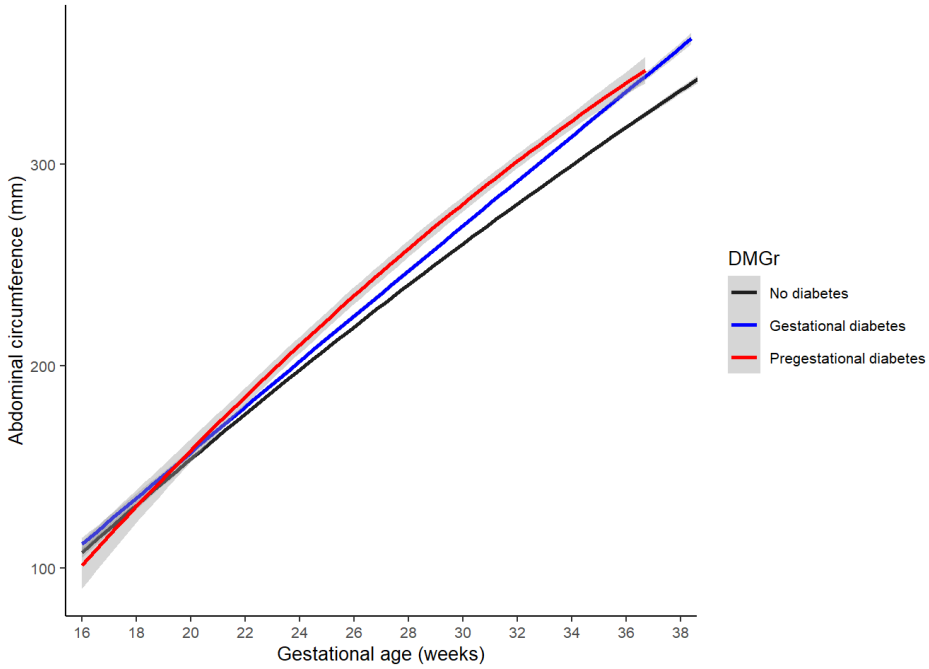
```
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                           lower       est.      upper
## (Intercept)           244.89610  247.81491  250.73373
## poly(GAcont, 2)1     2599.91234 2671.78139 2743.65044
## poly(GAcont, 2)2     -194.19467 -135.27112  -76.34758
## DMY                    10.90337   19.80311   28.70285
## poly(GAcont, 2)1:DMY  153.46455  367.98451  582.50447
## poly(GAcont, 2)2:DMY -113.58104   66.32603  246.23309
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                                lower        est.       upp
er
## sd((Intercept))                           10.6512565  12.5234744  14.72478
03
## sd(poly(GAcont, 2)1)                     249.4033068 300.6258717 362.36855
03
## sd(poly(GAcont, 2)2)                     204.0064095 244.3910908 292.77023
91
## cor((Intercept),poly(GAcont, 2)1)          0.6021273   0.7456422   0.84248
02
## cor((Intercept),poly(GAcont, 2)2)         -0.1490315   0.1060003   0.34780
96
## cor(poly(GAcont, 2)1,poly(GAcont, 2)2)  -0.4050833  -0.1365864   0.15359
91
##
##  Variance function:
##          lower    est.    upper
```

```
## power 1.370619 1.56092 1.751221
## attr(,"label")
## [1] "Variance function:"
##
##  Within-group standard error:
##        lower         est.        upper
## 0.0002456354 0.0006987270 0.0019875774
```

Graph gestational, pregestational and control on one graph Load data set and setup

```
DM3=read.csv("DM3_Reviewers.csv")

##Factor viables
library(dplyr)
DM3[,c("PtCode","AntPos", "SideRtLt", "GendF1","DM", "DMtype", "DMGr", "GTT
Scale","Treat")]=
  lapply(DM3[,c("PtCode","AntPos", "SideRtLt", "GendF1", "DM", "DMtype", "D
MGr", "GTTScale","Treat")],factor)

##Change factor names
DM3$AntPos=factor(DM3$AntPos, labels=c("Ant", "Post"))
DM3$SideRtLt=factor(DM3$SideRtLt, labels=c("Rt", "Lt"))
DM3$GendF1=factor(DM3$GendF1, labels = c("F", "M"))
DM3$DM=factor(DM3$DM, labels = c("N", "Y"))
DM3$DM_type=factor(DM3$DMtype, labels=c("None", "T1", "T2", "GDM"))
DM3$DMGr=factor(DM3$DMGr, labels=c("control", "GDM", "Pregest"))
DM3$Treat=factor(DM3$Treat, labels=c("none", "Diet", "OHA", "Insulin"))

##Remove all NAs from GAcont
DM3.complete = DM3[!is.na(DM3$GAcont),]

##Change intercept so 16 starts at 0
DM3.complete$GAcont=DM3.complete$GAcont-16
```

Graph of AC vs GA for gestational and pregestation diabetes and Control groups



Part 4: RPT growth compared to AC growth for GDM and pregestational diabetes

Model for RPT growth compared to AC growth for GDM and control

```
##Power curves - log transform data so both intercept at 0

log.DM = mutate(DM,
                logRPT=log(RPT),
                logHC=log(HC),
                logEFW=log(EFW),
                logAC =log(AC),
                logFL=log(FL))
```

Subset out GDM vs control from log.DM

```
log_GDM=subset(log.DM, (DMtype=="0"|DMtype=="3"),
               select =c(PtCode,GAcont, RPT, AntPos, SideRtLt,
                         GendF1, DM,DMtype, DMGr, BMI,HC, AC, FL, EFW,
                         Length, Trans, AP,RVol, logRPT, logHC, logEFW,
                         logAC, logFL))
```

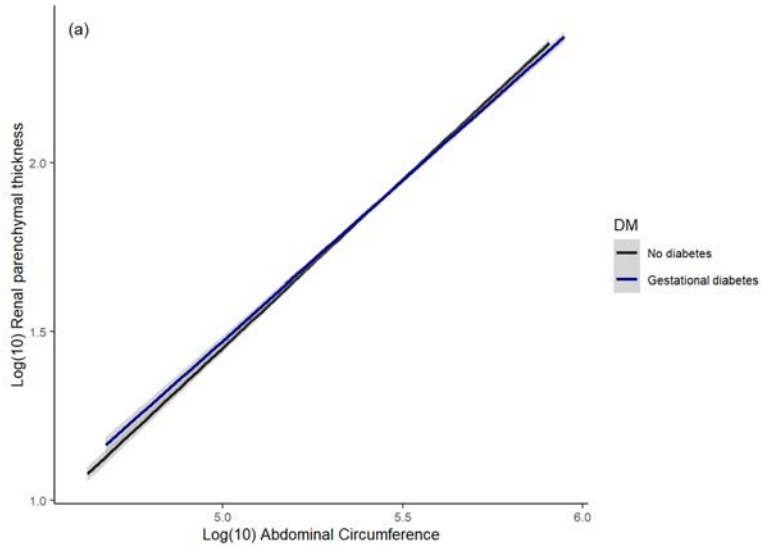
Power curve RPT to AC for GDM vs control

GDMgrowth3 best model - change to REML

```
GDMgrowth3 = lme(logRPT ~ logAC*DM + SideRtLt, random=~logAC|PtCode,
             method = "REML",na.action="na.omit", data=log_GDM,
             control = lmeControl(msMaxIter = 200))


summary(GDMgrowth3)
## Linear mixed-effects model fit by REML
##  Data: log_GDM
##         AIC       BIC   logLik
##   -3261.793 -3209.195 1639.897
##
## Random effects:
##  Formula: ~logAC | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##             StdDev    Corr
## (Intercept) 0.8622425 (Intr)
## logAC       0.1593078 -0.996
## Residual    0.1167751
##
## Fixed effects: logRPT ~ logAC * DM + SideRtLt
##                 Value  Std.Error   DF   t-value p-value
## (Intercept) -3.444753 0.12602929 2435 -27.33295  0.0000
## logAC        0.980422 0.02311627 2435  42.41265  0.0000
## DMY          0.231434 0.20008793  116   1.15666  0.2498
## SideRtLtRt   0.017114 0.00461955 2435   3.70467  0.0002
## logAC:DMY   -0.042936 0.03671102 2435  -1.16957  0.2423
##  Correlation:
##             (Intr) logAC  DMY    SdRtLR
## logAC       -0.997
## DMY         -0.630  0.628
## SideRtLtRt -0.018  0.000  0.000
## logAC:DMY   0.628 -0.630 -0.997  0.000
##
## Standardized Within-Group Residuals:
```

```
##          Min           Q1          Med           Q3          Max
## -3.98164034 -0.65163417  0.01276083  0.63774356  3.48621096
##
## Number of Observations: 2556
## Number of Groups: 118
Anova(GDMgrowth3)
## Analysis of Deviance Table (Type II tests)
##
## Response: logRPT
##              Chisq Df Pr(>Chisq)
## logAC    2878.0506  1  < 2.2e-16 ***
## DM          0.0161  1  0.8990371
## SideRtLt   13.7246  1  0.0002117 ***
## logAC:DM    1.3679  1  0.2421746
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
intervals(GDMgrowth3)
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                     lower        est.       upper
## (Intercept) -3.691888511 -3.44475280 -3.19761709
## logAC        0.935092672  0.98042226  1.02575185
## DMY         -0.164865690  0.23143367  0.62773303
## SideRtLtRt   0.008055243  0.01711390  0.02617256
## logAC:DMY   -0.114924115 -0.04293606  0.02905200
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                               lower       est.      upper
## sd((Intercept))         0.6965259  0.8622425  1.0673862
## sd(logAC)               0.1292732  0.1593078  0.1963206
## cor((Intercept),logAC) -0.9977837 -0.9963207 -0.9938949
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1134394 0.1167751 0.1202089
```

Graph of RPT vs AC for GDM and Control groups



Model for RPT growth compared to AC growth for pregestational and control

Subset out pregest vs control

```
log_pregest=subset(log.DM, (DMtype=="0"|DMtype=="1"|DMtype=="2"),
                   select =c(PtCode,GAcont, RPT, AntPos, SideRtLt,
                             GendF1, DM,DMtype, DMGr, BMI,
                             HC, AC, FL, EFW, Length, Trans, AP,RVol,
                             logRPT, logHC, logEFW, logAC, logFL))
```

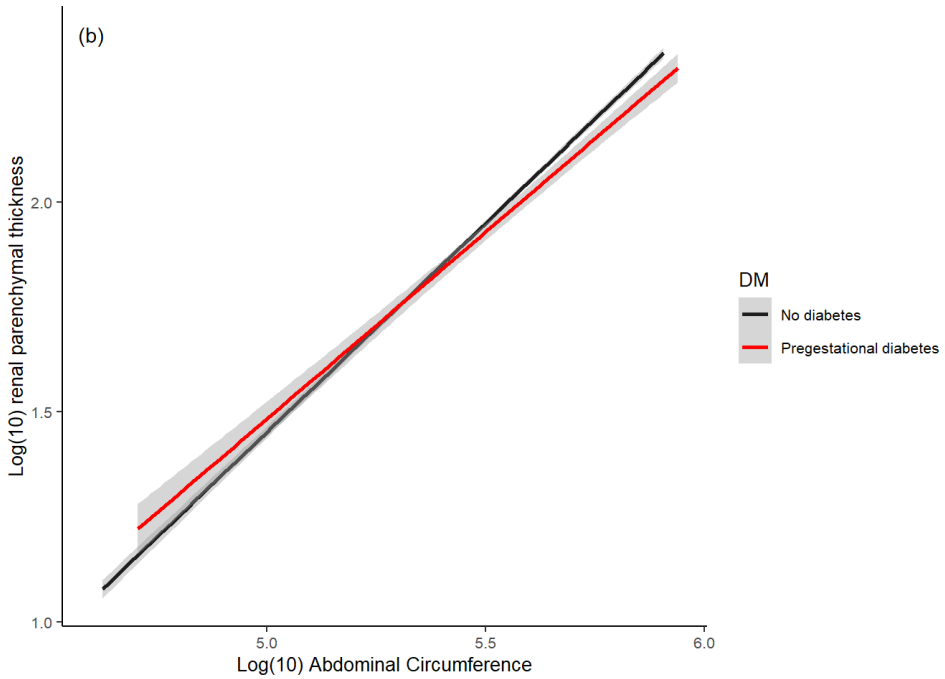
Best model 3 - change to REML

```
pregestgrowth3 = lme(logRPT ~ logAC *DM +SideRtLt, random=~logAC|PtCode,
                     method = "REML",na.action="na.omit",
                     data=log_pregest,
                     control = lmeControl(opt = ("optim")))

summary(pregestgrowth3)
## Linear mixed-effects model fit by REML
##  Data: log_pregest
##         AIC      BIC   logLik
##   -2220.764 -2171.45 1119.382
##
## Random effects:
##  Formula: ~logAC | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##             StdDev    Corr
## (Intercept) 0.9798013 (Intr)
## logAC       0.1815529 -0.997
## Residual    0.1179587
##
## Fixed effects: logRPT ~ logAC * DM + SideRtLt
##                 Value Std.Error   DF   t-value p-value
## (Intercept) -3.434973 0.1382659 1692 -24.84324  0.0000
## logAC        0.979359 0.0254353 1692  38.50398  0.0000
## DMY          0.646008 0.4068848   79   1.58769  0.1164
## SideRtLtRt   0.010171 0.0055981 1692   1.81684  0.0694
## logAC:DMY   -0.121865 0.0746864 1692  -1.63169  0.1029
##  Correlation:
##             (Intr) logAC  DMY    SdRtLR
## logAC       -0.998
## DMY         -0.340  0.339
## SideRtLtRt -0.020  0.000  0.000
## logAC:DMY   0.340 -0.341 -0.998  0.000
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -3.910485269 -0.657655213 -0.007770504  0.635791145  3.149876796
##
## Number of Observations: 1776
## Number of Groups: 81

Anova(pregestgrowth3)
## Analysis of Deviance Table (Type II tests)
##
## Response: logRPT
##              Chisq Df Pr(>Chisq)
## logAC    1629.0077  1    < 2e-16 ***
## DM          0.3648  1    0.54585
```

```
## SideRtLt    3.3009  1    0.06924 .
## logAC:DM    2.6624  1    0.10274
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
intervals(pregestgrowth3)
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                     lower        est.       upper
## (Intercept) -3.7061636482 -3.43497340 -3.16378316
## logAC        0.9294706538  0.97935852  1.02924638
## DMY         -0.1638757797  0.64600815  1.45589208
## SideRtLtRt  -0.0008090544  0.01017082  0.02115070
## logAC:DMY   -0.2683527862 -0.12186527  0.02462225
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                               lower       est.      upper
## sd((Intercept))         0.7626885  0.9798013  1.2587191
## sd(logAC)               0.1420963  0.1815529  0.2319656
## cor((Intercept),logAC) -0.9986405 -0.9974975 -0.9953956
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1139383 0.1179587 0.1221210
```

Graph of RPT vs AC for pregestational diabetes and Control groups



Model for RPT growth compared to AC growth for GDM and pregestational

Subset out only diabetes patients (no control)

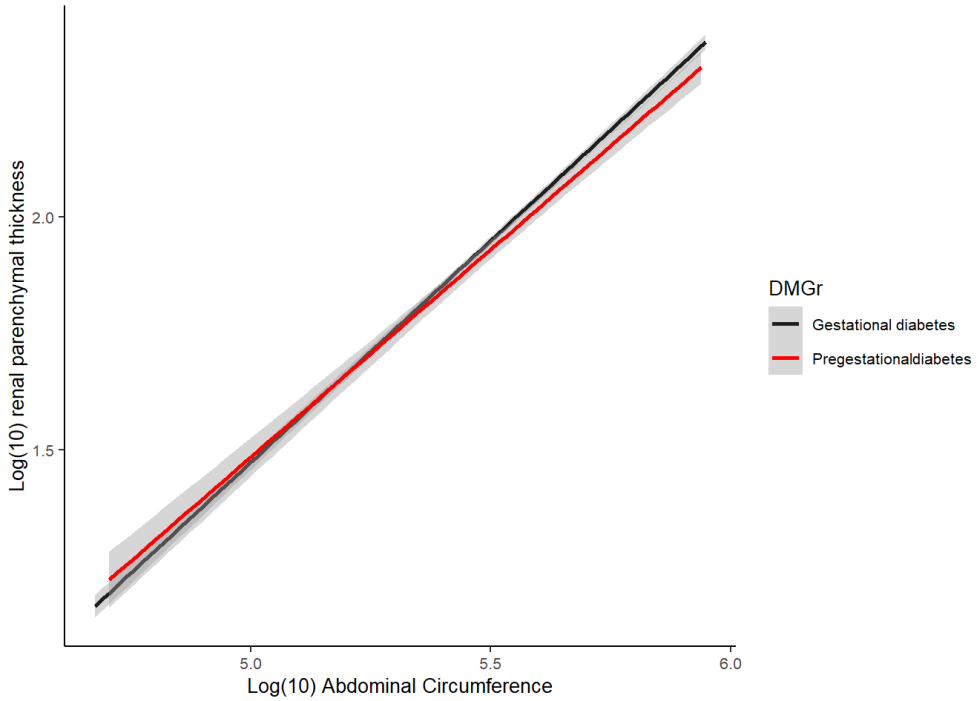
```
DMOnly=subset(log.DM, (DMGr=="Pregest"|DMGr=="GDM"),
              select =c(PtCode,GAcont, RPT, AntPos, SideRtLt,
                        GendF1, DM, DMtype, DMGr, BMI,HC, AC, FL, EFW,
                        Length, Trans, AP,RVol,
                        logRPT, logHC, logEFW,logAC, logFL))
```

Best model 3 - change to REML

```
GDMvsPDGgrowth3 = lme(logRPT ~ logAC *DMGr + SideRtLt, random=~logAC|PtCode
,
                      method = "REML",na.action="na.omit", data=DMOnly,
                      control = lmeControl(opt = ("optim")))

summary(GDMvsPDGgrowth3)
## Linear mixed-effects model fit by REML
##  Data: DMOnly
##         AIC       BIC   logLik
##   -1494.217 -1448.596 756.1084
##
## Random effects:
##  Formula: ~logAC | PtCode
##  Structure: General positive-definite, Log-Cholesky parametrization
##             StdDev    Corr
## (Intercept) 0.6914525 (Intr)
## logAC       0.1270452 -0.994
## Residual    0.1166929
##
## Fixed effects: logRPT ~ logAC * DMGr + SideRtLt
##                       Value Std.Error   DF   t-value p-value
## (Intercept)       -3.233875 0.1333902 1122 -24.24372  0.0000
## logAC              0.940781 0.0243450 1122  38.64365  0.0000
## DMGrPregest        0.393306 0.3287192   53   1.19648  0.2368
## SideRtLtRt         0.021284 0.0067941 1122   3.13267  0.0018
## logAC:DMGrPregest -0.075308 0.0597792 1122  -1.25977  0.2080
##  Correlation:
##                   (Intr) logAC  DMGrPr SdRtLR
## logAC             -0.996
## DMGrPregest       -0.406  0.404
## SideRtLtRt        -0.025  0.000  0.000
## logAC:DMGrPregest  0.405 -0.407 -0.996  0.000
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.34878388 -0.66269527 -0.02239424  0.67312143  3.46624600
##
## Number of Observations: 1180
## Number of Groups: 55

anova(GDMvsPDGgrowth3)
##             numDF denDF   F-value p-value
## (Intercept)     1  1122 29501.614  <.0001
## logAC           1  1122  1742.650  <.0001
## DMGr            1    53     0.400  0.5297
## SideRtLt        1  1122     9.814  0.0018
## logAC:DMGr      1  1122     1.587  0.2080
```

```
intervals(GDMvsPDGgrowth3)
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                          lower        est.       upper
## (Intercept)       -3.495597641 -3.23387526 -2.97215288
## logAC              0.893014244  0.94078117  0.98854810
## DMGrPregest       -0.266021708  0.39330556  1.05263282
## SideRtLtRt         0.007953113  0.02128373  0.03461435
## logAC:DMGrPregest -0.192599328 -0.07530774  0.04198385
## attr(,"label")
## [1] "Fixed effects:"
##
##  Random Effects:
##   Level: PtCode
##                                 lower       est.      upper
## sd((Intercept))          0.47303760  0.6914525  1.0107156
## sd(logAC)                0.08749299  0.1270452  0.1844773
## cor((Intercept),logAC) -0.99739637 -0.9938017 -0.9852807
##
##  Within-group standard error:
##     lower      est.     upper
## 0.1118062 0.1166929 0.1217931
```

Graph of RPT vs AC for GDM and Pregestational groups



Appendix D: Published Papers

Appendix D1:
Brennan S, Watson D, Rudd D, Schneider M, Kandasamy Y. Evaluation of fetal kidney growth using ultrasound: A systematic review. Eur J Radiol. 2017; 96:55-64

European Journal of Radiology 96 (2017) 55–64

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Review article

Evaluation of fetal kidney growth using ultrasound: A systematic review

Sonja Brennan[a,b,*], David Watson[c], Donna Rudd[b], Michal Schneider[d], Yogavijayan Kandasamy[b,e,f]

[a] Ultrasound Department, The Townsville Hospital, IMB 47 P.O. Box 670, Douglas, Townsville, Queensland, 4810, Australia
[b] College of Public Health, Medical and Veterinary Sciences, James Cook University, 1 James Cook Drive, Townsville, Queensland 4811, Australia
[c] Department of Obstetrics and Gynaecology, The Townsville Hospital, IMB 85 P.O. Box 670, Townsville, Queensland 4810, Australia
[d] Department of Medical Imaging and Radiation Sciences, School of Primary And Allied Health Care, 10 Chancellors Way, Monash University, Clayton, Victoria 3800, Australia
[e] Department of Neonatology, The Townsville Hospital, IMB 51 P.O. Box 670, Townsville, Queensland 4810, Australia
[f] Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, The University of Newcastle,University Drive, Callaghan, NSW 2308, Australia

ARTICLE INFO

Keywords:
Fetal kidney
Renal
Prenatal diagnosis
Ultrasound
Obstetric ultrasonography

ABSTRACT

Purpose: To determine the role of ultrasound imaging in evaluating fetal kidney growth.
Methods: MEDLINE, CINAHL and EMBASE databases were electronically searched for studies between 1996 and January 2017 and limited to English language. Studies were included if they reported on an ultrasound technique to assess fetal kidney growth and they were not a case report or case series. There was independent selection of studies by two reviewers in consensus with one other reviewer. Data were extracted by one reviewer in consensus with two other reviewers.
Results: A total of 1785 articles were identified. The full text of 39 of these were assessed for eligibility for inclusion. Twenty-eight studies were then included in the review. Standard two dimensional (2D) fetal renal measurements are easy to perform, however, this review identified that most studies had some methodological limitations. The disadvantage with 2D and three dimensional (3D) fetal renal volumes are that they include the entire kidney and good reproducibility of 3D volumes has not yet been demonstrated. Currently there is limited research on fetal kidney growth in the setting of abnormal fetal growth. Research focussing directly on fetal kidney parenchyma and blood flow is scarce.
Conclusions: Some nomograms of 2D and 3D fetal kidney size and volume have been developed. Kidney length is the most popular single fetal kidney measurement; however, it does not seem to be a good indicator of growth. In IUGR fetuses, kidney length remained similar to appropriately grown fetuses whereas AP and TS dimensions were significantly decreased. New ultrasound techniques focusing on the parenchyma of the kidney and perfusion to the kidney should be explored as they may provide more meaningful information on kidney development in the fetus and future kidney function.

1. Introduction

It is well established that an adverse intrauterine environment can affect fetal kidney development resulting in possible hypertension and chronic kidney disease later in life [1,2]. Intrauterine growth restriction (IUGR) can result in significant reductions in nephron number [3] which may ultimately result in decreased renal function [4]. Although most studies concentrate on IUGR and low birth weight infants, overgrowth or large for gestational age (LGA) are also emerging as factors that can disrupt normal fetal kidney development and increase risks for hypertension and chronic kidney disease [5]. The normal development of the fetal kidneys can be crucial to an individual's long-term health outcomes.

The human kidney develops through three successive embryonic stages. Transient development and regression of the primary (pronephros) and secondary (mesonephros) fetal kidneys occurs between day 23 and day 112 [6]. These primitive fetal kidneys have no impact on fetal renal function. The definitive, tertiary fetal kidney is the metanephros and this is the permanent functional kidney. It begins developing on day 30 leading to the formation of nephrons − the functional units within the kidney [6,7]. Fetal kidneys are unlike most other organs in that the maximum cell proliferation occurs in the third trimester. Nephrogenesis continues up until 34–36 weeks gestation with approximately 60% of nephrons formed in the third trimester [8].

Assessment of the fetal kidneys is an essential part of an obstetric ultrasound. Accurate information regarding kidney size is crucial to

* Corresponding author at: Ultrasound Department, The Townsville Hospital, IMB 47 P.O. Box 670, Douglas, Townsville, Queensland 4810, Australia.
E-mail addresses: sonja.brennan@my.jcu.edu.au, sonja.brennan@health.qld.gov.au (S. Brennan).

http://dx.doi.org/10.1016/j.ejrad.2017.09.017
Received 30 May 2017; Received in revised form 6 August 2017; Accepted 22 September 2017

identifying kidney abnormalities and detecting changes in fetal kidney growth. Ultrasound imaging is safe, cost effective and widely available to evaluate fetal kidney size, echotexture and perfusion. A variety of two and three-dimensional ultrasound techniques have emerged and advanced to evaluate kidney development. The aim of this review was to systematically review the literature to determine what role ultrasound plays in evaluating fetal kidney growth. Current ultrasound imaging techniques and accuracy will be reviewed.

2. Method

A systematic review of observational studies was conducted using a protocol designed *a priori* and following the PRISMA guidelines for systematic reviews. Author SB developed and conducted the search strategy using medical subject headings (MeSH) and keywords and this was reviewed by author YK (Appendix A). MEDLINE (ovid); CINAHL and EMBASE electronic databases were electronically searched in August 2016 and again in January 2017; for publications from the year 1996 onwards. The literature search was limited to the English language. The reference lists of relevant articles were hand-searched for additional relevant studies.

Human observational ultrasound studies that were not a case report or case series were included. Only studies reporting on an ultrasound technique assessing fetal kidney growth were included. Studies that only assessed fetal pelvic renal dilatation were excluded.

Only studies published from the last 20 years (from 1996) were included as it was felt that the significant advances in ultrasound techniques and improvements in diagnosis and definition in prenatal imaging made these older studies less relevant. Also excluded were unpublished studies, non-peer-reviewed, conference abstracts, letters to the editor and opinion articles. If data from a single study population was reported more than once, the publication containing the most complete information was included.

Study selection was performed in two sequential steps, firstly assessing articles by title and abstract and secondly by full text of the article. Two reviewers (SB and YK) independently screened the titles and abstracts of all identified citations and potentially eligible studies were selected. The full text of these potentially eligible studies was screened by the same two reviewers. Any discrepancies between the reviewers were resolved by consultation with a third reviewer (DW).

A data extraction sheet was developed. Only pre-specified outcomes of interest in the review were collected. Review author SB extracted the data from the studies and the second review author YK checked the extracted data. Any disagreements were resolved by discussion with a third reviewer (DW). Fig. 1 outlines study selection process. A narrative synthesis, including tables, was done on the extracted data to explain and summarise the characteristics and findings of the included studies.

3. Results

A total of 1785 articles were identified and after review of the title and abstract, the full text of 39 of these were assessed for eligibility for inclusion (Fig. 1). Four papers from the Generation R study reported the same renal data [9–12] and therefore these data were only considered once using the paper by Verburg et al. [9] as it contained the most relevant and complete data assessing fetal kidney measurements. Finally, 28 studies were reviewed.

Relevant characteristics of these included studies are presented in Tables 1–3. Most studies were prospective in design with only 2 of the 28 studies retrospective [13,14]. A cross-sectional design was utilised by 21 studies while 7 studies had a longitudinal design. Selected studies were divided into three groups depending on the ultrasound technique used to assess the kidneys. Some studies reported on more than one ultrasound technique (Tables 1–3).

Generally, the study time and duration, how participants were recruited and missing participants and data was poorly reported.

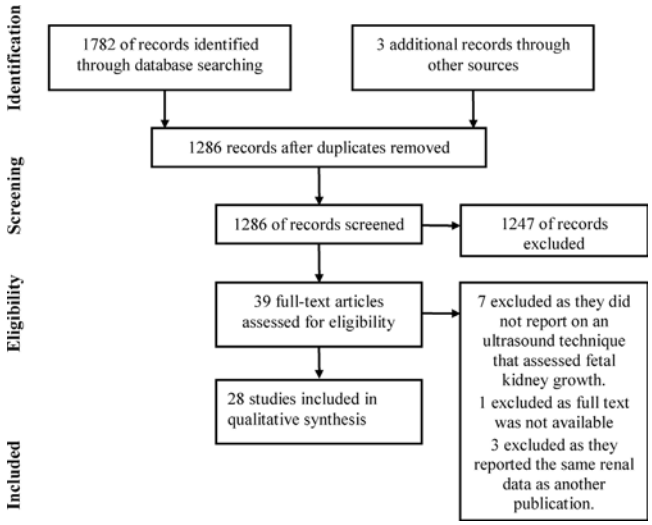


Fig. 1. Study selection process.

Calculation of estimated gestational age (GA) was most commonly achieved using the last normal menstrual period (LNMP) correlated with a first or early second trimester ultrasound (18 studies) [13–30]. Six studies used ultrasound dating only [9,31–35], one used only an accurate LNMP [36] and three did not report how GA was determined [37–39].

Overall ultrasound features of the studies and measurement methods were well described. Most studies focussed on the mid-second trimester to third trimester [9,13,14,16,18–31,33–35,37–39], as imaging the fetal kidneys well under 20 weeks can be difficult [33,40]. The three studies that reported data below 14 weeks GA used transvaginal scanning [15,17,36]. The GA range assessed was very variable between studies. Two studies showed only a snap shot in time with a GA range of 15 days (around 34 weeks) [34] and 4 weeks (28–32 weeks) [9]. One study measured the fetal kidneys at 23 weeks and again at 32 weeks [18]. Studies covering the longest GA ranges were Chitty and Altman [20] 16–42 weeks, van Vuuren et al. [32] 16–42 weeks and Hsieh et al. [25] 15–40 weeks. The GA range was unclear in one study [23].

3.1. Differences between right and left kidneys and gender

Overall the evidence strongly supported no significant difference between right and left fetal kidney size (17 of the 18 studies) for all ultrasound measurements regardless of the technique used [13,15,16,17,19,23–26,28–33,35,38]. Six of the seven studies that examined gender differences found no significant difference between fetal kidney measurements [17–19,23,29,30]. Only one study demonstrated a difference in size between right and left kidneys and males and females [9]. This was a large study, however, it had a small four-week gestational window (28.4–32.6 weeks) when each fetus was measured once. The study revealed right kidneys had a larger transverse and antero-posterior dimension when compared to left kidneys, resulting in larger calculated renal volumes. No difference, however, was found between kidney lengths. All kidney measurements were smaller in females than males [9].

3.2. Standard two dimensional (2D) measurements

Nineteen studies reported on a standard two-dimensional (2D) ultrasound measurement [9,13,15–23,29,31,32,34,36–39]. Standard two-dimensional (2D) measurements of the fetal kidneys was the earliest and simplest method utilised to assess kidney size at different gestational ages [41,42]. Most reviewed studies involved a low risk, uncomplicated pregnancy to obtain normal fetal kidney nomograms

Table 1
Main characteristics of studies included for standard 2D whole kidney measurements.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Konje et al 1996 [37]	UK	P/Long	Singleton preg AGA & SGA	AGA = 50 SGA = 37	NR	20–38	Every 2 weeks after recruitment	TA, 2D, No operators NR KL, TS, AP & circumference	SGA different growth to AGA. AGA acceleration of growth 26–34 weeks (critical period) not seen in SGA. No difference in KL.
Rosati et al 1996 [15]	Italy	P/CS	Normal AGA singleton preg high risk population	489	LNMP & US	11 − 16	Once	TV, 2D, 2 operators KL, TS, AP, circumference & KC/AC	Normal fetal kidney size in early pregnancy. There was linear growth of all kidney measurements throughout early pregnancy.
Konje et al 1997 [31]	UK	P/CS	Singleton preg AGA & SGA	AGA = 129 SGA = 90	10–12 week US	22 − 38	Once	TA, 2D, 1 operator KL, TS, AP & circumference	Between AGA & SGA − no difference in KL, significant difference in TS & AP from 26 weeks. SGA resulted in long thin kidneys = "sausage shaped" kidneys.
Gloor et al 1997 [38]	USA	P/CS	Normal AGA singleton preg	100	NR	18–39	Once	TA, 2D, 4 operators KL, TS, AP, 2D volume, EFW	Provides normal fetal kidney size relative to both GA & EFW. Constant ratio between KL to weight. Ratio of KL to weight declines.
Ansari et al 1997 [16]	Bangladesh	P/CS	Normal AGA singleton preg	793	LNMP & BPD & FL	16 − 40	Once	TA, 2D, No operators NR. KL, BPD & FL	Good correlation between KL, BPD & FL. GA can be assessed by BPD, FL & KL
Guariglia et al 1998 [36]	Italy	P/CS	Normal AGA singleton preg	807	LNMP	11 − 16	Once	TV, 2D, 2 operators KL, BPD, BPD/KL	BPD/KL plotted against GA for early pregnancy & appears constant through 11–16wks.
Zalel et al 2002 [17]	Israel	P/CS	Normal AGA singleton preg	269	LNMP & CRL	13 − 22	Once	TV, TA, 2D, 1 operator KL	Normal range of KL in early pregnancy (13 −22weeks).
Lampl et al 2002 [18]	Belgium	P/CS	Normal AGA singleton preg	25	LNMP & CRL 8–10 weeks	23 & 32	Twice (23 weeks & 32 weeks)	TA, 2D, 1 operator KL, TS, AP & 2D volume	Thinner neonates at birth have smaller kidneys relative to body size. Mean KV/EFW ratio did not significantly change between 23 and 32 weeks. KL not related to birthweight & ponderal index, however, TS & AP related positively to both.
Konje et al 2002 [19]	UK	P/Long	Normal AGA singleton preg	73	LNMP & CRL	24 − 38	Every 2 weeks from 24 weeks	TA, 2D, 2 operators KL, BPD, HC, AC & FL	KL predicted GA better than AC & FL.
Chitty et al 2003 [20]	UK	P/CS	Normal AGA singleton preg	661	LNMP & US 18–20weeks	14 − 42	Once	TA, 2D, No operators NR KL, TS, AP, 2D volume	Normal ranges of KL, TS & AP for 14–42 weeks.
Silver et al 2003 [21]	USA	P/CS	Singleton preg IUGR & SGA	AGA = 43 IUGR = 34	LNMP & CRL or 2nd US	27 − 41	Once	TA, 2D, 2 operators KL, TS, AP, circumference & 2D volume Renal artery Doppler	Kidney volume in IUGR fetuses was 31% less than in AGA fetuses & 15% less when adjusted for fetal weight. No difference in renal artery blood flow AGA vs SGA.
Lampl et al 2005 [22]	Belgium	P/Long	Singleton preg smoking exposure & none	Smokers = 10 Non-smokers = 24	LNMP & CRL	19 − NR	Every week from 19 weeks	TA, 2D, 1 operator KL, TS, AP, 2D volume	Recorded birth outcomes for 6 smokers & 21 non-smokers this was insufficient for meaningful results. Smoke exposed fetal kidneys were thicker in 2nd trimester and then became thinner in 3rd trimester than non-exposed kidneys.
Verburg et al (Gen R) 2007 [9]	Netherlands	P/CS	Singleton preg, population based cohort	1215	CRL or BPD if > 12 weeks	28.4 − 32.6	Once	TA, 2D,?3operators KL, TS, AP, 2D volume, EFW, UA, MCA & UA/MCA ratio	Normal ranges of KL, TS, AP & 2D volume for 28 to 34wks. Maternal weight & height positively associated with kidney volume. Kidney volume is positively associated with all growth characteristics & amniotic fluid. Umbilical artery RI's & CPR negatively associated with kidney volume.
Van Vuuren et al 2012 [32]	Netherlands	P/Long	Normal AGA singleton preg	96	CRL	16 − 42	Every 4 weeks, 2 groups staggered by 2 weeks	TA, 2D, 1 operator KL, TS, AP, 2D volume	Normal ranges of KL, TS, AP & 2D volume for 16–42 weeks.
Neves et al 2013 [33]	Brazil	P/Long	Singleton preg hyperglycaemic & normoglycaemic	Hyperglycaemic = 92 normoglycaemic = 339	US 11–14 weeks	22 − 38	Every 2 weeks, not all women attended all	TA, 2D, 1 operator KL, TS, AP, 2D volume	Significant difference between groups. Median kidney volume of hyperglycaemic group > 75th percentile for normoglycaemic group. Maternal hyperglycaemia is associated with fetal kidney growth modification.

(*continued on next page*)

Table 1 (*continued*)

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Seilanian Toosi et al 2013 [23]	Iran	P/CS	Normal AGA singleton preg	89	LNMP & US 8–10weeks	NR?3rd trim	Once	TA, 2D, 2 operators KL, BPD, HC, AC, FL	KL, AC, FL & HC had a linear & strong correlation with GA. Best GA predictor combined HC, BPD, FL & KL (± 14.2 days).
Roderick et al 2016 [34]	UK	P/CS	Normal AGA singleton preg South Asian & White British	South Asian = 715 White British = 872	US 8–14 weeks	32 + 3 days to 34 + 4 days	Once	TA, 2D, 4 sonographers. KL, TS, AP, 2D volume, kidney circumference in TS	After adjusting for GA, all renal dimensions for South Asians were significantly smaller than for White British. Proportion reduction greatest for TS and AP. Kidney volume reduced with adjusted BW, kidney volume still higher in White British. Findings may partly explain the increased risk of adult chronic kidney disease in South Asians.

AC, abdominal circumference; AGA, appropriate-for-gestational age; AP, anterio-posterior; BPD, biparietal diameter; Btw, between; BW, birth weight; CPR, cerebroplacental ratio; CS, cross-sectional; CRL, crown rump length; EFW, estimated fetal weight; FL, femur length; GA, gestational age; HC, head circumference; IUGR, intrauterine growth restriction; KC, kidney circumference; KL, kidney length; LNMP, last normal menstrual period; Long, longitudinal; NR, not recorded; P, prospective; Preg, pregnancy; R, retrospective; RI, resistive index; SGA, small for gestational age; TA, transabdominal; TS, transverse; TV, transvaginal; US, ultrasound.

[16–20,23,32,36,38].

A longitudinal design was used by four studies that reported on 2D measurements [19,22,32,37], with the best quality longitudinal study being Van Vuuren et al. [32]. This study of 96 participants measured all three dimensions of the kidney and clearly defined how GA was determined. Good longitudinal data was obtained every two weeks. Participants were divided into two groups. Each group came every four weeks, however, one group started at 16 weeks and the other at 18 weeks. Reference charts were constructed for kidney length, antero-posterior diameter, transverse diameter and volume [32]. In the other longitudinal studies, one did not report how GA was determined [37], another only measured kidney length [19] and the third study had a small sample size of ten normal pregnancies [22].

Overall, evidence from the selected studies using standard 2D ultrasound measurements suggest fetal kidney growth correlates positively with GA. The velocity of kidney growth is highest between 26 and 34 weeks in appropriately grown fetuses. This was termed the "critical period" for fetal kidney growth [37] and was supported by other studies [9,34]. Kidney size was linked positively with fetal weight and size [9,18,38].

Few studies used standard 2D methods to investigate fetal kidney growth in abnormal fetal growth. Compared to appropriately grown fetuses, kidneys of IUGR fetuses demonstrated significant reductions in transverse and antero-posterior dimensions [31]. This is particularly marked during the critical kidney growth period (26–34 weeks). No significant difference was demonstrated between the renal lengths of IUGR fetuses compared to appropriately grown fetuses.

3.3. Renal volumes – calculated from two dimensional (2D) measurements

Kidney volume calculations should technically be a better estimate of overall kidney size and shape than single linear measurements [43,44]. Traditionally fetal renal volumes were calculated using 2D ultrasound measures. Three orthogonal kidney diameters are applied to the volume formula of an ellipsoid shape to obtain a volume estimate (length x transverse x antero-posterior x 0.523) [45]. The ellipsoid formula was used to calculate renal volumes in nine studies [9,18,20–22,32–34,38]. Findings from the studies of appropriately grown fetuses in normal pregnancies demonstrated fetal kidney volume increases exponentially until birth [32] or with some slowing of growth velocity after 36 weeks [20].

Studies assessing fetal renal 2D volumes during abnormal fetal growth were scarce. One such study was a large study, however was cross-sectional in design and examined a very narrow GA window of 28.4 weeks to 32.6 weeks (median age 30.4 weeks) [9]. Their findings suggested that IUGR, placental insufficiency and fetal redistribution of blood flow result in decreased fetal kidney volumes, around the critical kidney growth period of 26–34 weeks. Smaller kidney volumes were also associated with reduced amniotic fluid suggesting an association with fetal kidney function [9]. Another cross-sectional study of IUGR fetuses reported considerable reductions in the kidney volumes of IUGR fetuses [21]. When corrected for fetal weight, there was a 15% reduction of kidney volume for IUGR fetuses compared to appropriately grown fetuses [21].

No studies were found evaluating fetal kidney growth in large for gestational age (LGA) fetuses. Fetal kidney growth in hyperglycaemic pregnancies was investigated by one study [33]. This longitudinal study compared 2D kidney volumes of normoglycaemic pregnancies with hyperglycaemic pregnancies. Often fetuses of hyperglycaemic mothers are LGA due to organomegaly and increased fat deposition from the increased glycogen to the fetus [33], however, the relationship between fetal size and kidney size was not reported. This study demonstrated maternal hyperglycaemia was associated with alterations in fetal kidney volume. The median fetal kidney volumes of hyperglycaemic pregnancies were significantly larger than the 75th percentile for normoglycaemic pregnancies [33]. This finding warrants further

Table 2
Main characteristics of studies included for 3D techniques.

Study & Date	Country	Study Design	Population	Fetuses (n)	GA Estimate Method	GA Range	Time-points	US Renal Measures	Summary of Reported Findings
Yu et al 2000 [24]	Taiwan	P/CS	Normal AGA singleton pregnancy	152	LNMP & CRL	20 –40wks	Once	TA, 3D, No operators NR. 3D kidney multiplanar volume	Good correlation between GA and kidney volume. Normal ranges of 3D kidney volumes.
Hsieh et al 2000 [25]	Taiwan	P/CS	Normal AGA singleton pregnancy	112	LNMP & CRL	15 –40wks	Once	TA, 2D & 3D, 1 operator. 2D calculated volume & 3D kidney multiplanar volume	Normal ranges of 3D kidney volumes. Due to limitations of 3D volumes this study found the constant for volume to be calculated using 2D measures for right & left kidneys.
Kuno et al 2006 [26]	Japan	P/Long	Normal AGA singleton pregnancy	13	LNMP & CRL or early 2nd US	20wks to birth	Every 2–3 wks from 20 wks to birth	TA, 3D, No operators of acquisition NR. 1 operator analysed 3D data. 3D kidney multiplanar volume	Normal ranges of 3D kidney volumes. Significant difference found between their study and two others [24,25]. They were doubtful about reproducibility of 3D intrauterine volumes.
Chang et al 2008 [27]	Taiwan	P/CS	Singleton pregnancy AGA & IUGR	AGA = 221 IUGR = 28	LNMP & CRL or early 2nd US	20 –40wks	Once	TA, 3D, 1 operator but unclear if they did acquisition as well. 3D kidney multiplanar volume	Using 3D kidney volumes, the best screening criteria for detection of a growth restricted fetus is the 10th percentile − sensitivity = 96.4%, specificity = 95.9%, PPV = 75%, NPV = 99.5%, accuracy = 96%.
Tedesco et al 2009 [28]	Brazil	P/Long	Normal AGA singleton pregnancy	57	LNMP & CRL or early 2nd US	24 –34wks	Every 2 wks − some started 24 wks others 25 wks until 34 wks	TA, 3D, 1 operator 3D kidney VOCAL volume	Strong correlation between GA and kidney volume. Normal ranges of 3D kidney volumes using VOCAL.. Kidney volume highly correlated with other fetal biometry and EFW
Yoshizaki et al 2013 [35]	Brazil	P/CS	Normal AGA singleton pregnancy	213	CRL	20 –40wks	Once	TA, 3D, 1 operator 3D kidney VOCAL volume	Normal ranges of 3D kidney volumes using VOCAL.

AGA, appropriate-for-gestational age; CS, cross-sectional; CRL, crown rump length; GA, gestational age; IUGR, intrauterine growth restriction; LNMP, last normal menstrual period; Long, longitudinal; NR, not recorded; P, prospective; TA, transabdominal; TS, transverse; VOCAL, virtual organ computer-aided analysis; Wks, weeks.

Table 3
Main characteristics of studies included for other novel ultrasound measurements.

Study & Date	Country	Study Design	Population	Fetuses (n)		GA Range (weeks)	Time-points	US Renal Measures	Summary of Reported Findings
Suranyi et al 2003 [29]	Hungry	P/CS	AGA & IUGR Singleton pregnancy	IUGR with hyperechoic kidney = 28 IUGR without hyperechoic kidneys = 62 AGA No NR	LNMP & CRL	Unclear – states 3 stages 1st, 2nd & 3rd trimesters	Once	TA, 2D, No operators NR. Assess for renal medullary hyperechogenicity. KL & BPD/KL ratio	IUGR fetuses divided into those with hyperechoic kidneys and those without. Normal nomogram BPD/KL correlated with GA. Significant difference btw KL of normal fetuses and IUGR fetuses with hyperechoic kidneys, but no difference btw KL of normal fetuses and IUGR fetuses without hyperechoic kidneys.
Kennedy et al 2003 [14]	USA	R/CS	Normal AGA singleton pregnancy	123	LNMP & 2nd trimester ultrasound	16–38	Once	TA, 2D, No readers NR. Reviewed US images to find max KL image then measured area of kidney.	Normal ranges of total fetal renal parenchymal area. Combined fetal nomogram with their previously devised birth to adolescent nomogram of total parenchymal area.
Shin et al 2007 [13]	Korea	R/CS	Normal AGA singleton pregnancy	216	LNMP or CRL	16– 41	Once	TA, 2D, 1 reader. Reviewed US images to find max KL image & TS image then measured length, TS & area of kidney in long & TS.	Normal ranges of total fetal renal parenchymal area in long and TS and normal ranges of KL. KL of Korean fetuses are similar to other races.
Hadar et al 2012 [30]	Israel	P/CS	Normal AGA singleton pregnancy	128	LNMP & CRL	20–40	Once	TA, 2D, 2 operators. Anterior & posterior parenchymal thickness measured in long & TS planes.	Normal ranges of ant & post parenchymal thickness in long & TS. Gradual linear growth of parenchyma throughout pregnancy. Findings indicate parenchymal thickness measured on long section are more reliable & reproducible than those made on transverse section.
Devriendt et al 2013 [39]	Belgium/ France	P/CS	Normal AGA singleton pregnancy	156	"Known GA" NR how GA determined	21– 37	Once	TA, 2D, 2 readers did offline measurements, operators that obtained images NR. Length, cortical thickness (CT) & medullary thickness (MT) both in long, assessed cortical echogenicity.	KL increased with GA. Cortical echogenicity (CE) evolved with GA. After 32 weeks GA, the CE should not be higher than the liver or spleen. Normal ranges of CT and MT according to GA. CT/MT ratio calculated and decreased with increasing GA.

AGA, appropriate-for-gestational age; BPD, biparietal diameter; Btw, between; CRL, crown rump length; CS, cross-sectional; CT, cortical thickness; GA, gestational age; IUGR, intrauterine growth restriction; KL, kidney length; LNMP, last normal menstrual period; Long, longitudinal; MT. medullary thickness; NR, not recorded; P, prospective; R, retrospective; TA, transabdominal; TS, transverse.

examination.

3.4. Renal volumes – three dimensional (3D)

Three dimensional (3D) techniques were used to calculate fetal kidney volumes in six studies. One of two methods were used to obtain 3D volumes: the multiple parallel plane method (multiplanar) or the rotational Virtual Organ Computer-aided AnaLysis (VOCAL) method. The older multiplanar technique is utilised in the four earlier studies, between 2000 and 2008 [24–27] and involves manually tracing multiple adjacent sequential planes of the organ. VOCAL is the newer, more automated volumetric tool employed by the two later 3D studies (2009 and 2013) [28–35].

Most of the included 3D studies focused on constructing reference curves for 3D fetal kidney volumes correlating to GA. Only one of these studies was longitudinal in design and able to truly assess growth, unfortunately this study had the narrowest gestational range of between 24–34 weeks [26]. All studies reporting on 3D kidney volumes demonstrated substantial differences of reported "normal" values. For example, two similarly designed studies published the same year, from the same country, using the same equipment reported at 35 weeks a kidney volume of 7.9 mL as the 50th centile in one study [24] and below the 5th centile for the other [25]. Even when reviewing two studies using the newer VOCAL method, one study reported the 50th centile of the right kidney at 34 weeks as 21.8 cm^3 [35] and the other reported 11.7 cm^3 as their 50th centile at 34 weeks [28]. These considerable variations are highly unlikely to be due to different population characteristics. In clinical practice, it would be impossible to know which reference curves to use and these results should therefore be used with caution.

3D ultrasound assessment of abnormal fetal kidney growth was evaluated in only one study. This study compared 3D fetal renal volumes between IUGR fetuses and appropriately grown fetuses [27]. Volumes in IUGR fetuses were significantly smaller when compared to appropriately grown fetuses [27]. This evidence is not strong as this one cross-sectional study measured volumes only once in 28 IUGR fetuses and the considerable variation in normal 3D fetal kidney volumes is likely to also be an issue in measurements of abnormally grown fetuses.

3.5. Other ultrasound techniques

Five studies investigated other ultrasound techniques to assess fetal renal growth or size, with a focus on the kidney parenchyma [13,14,29,30,39]. The kidney parenchyma has more recently become an emphasis of investigation as it contains the nephrons. In a hydronephrotic kidney, the length, transverse and antero-posterior dimensions and kidney volume may be normal or above normal. However, the parenchyma may be thinner than normal and the kidney may have impaired function [46].

The study by Devriendt et al. [39] measured cortical thickness (from the outer renal capsule to the external limit of the pyramids) and medullary thickness (the pyramid from the papilla to its base) of the parenchyma separately and demonstrated that these measures increased with GA. There was poor reproducibility with an inter-observer variability of 16.5% for cortical thickness and 28.6% for medullary thickness [39]. This was likely due to the various and changing shape of the renal pyramids, making accurate and reproducible placement of the callipers difficult.

In contrast, Hadar et al. [30] conducted a study measuring the entire kidney parenchyma from the renal capsule to the sinus-pyramidal interface and indicated there was a gradual linear growth of the parenchyma with increasing GA [30]. Both the anterior and posterior parenchyma thicknesses were measured in transverse and longitudinal planes. Their findings demonstrated that measuring parenchymal thickness on longitudinal sections was more reliable and reproducible than transverse measurements and had significantly better intra and inter-observer variability than Devriendt et al. [39] at 0.6% and 8.8% for anterior parenchyma and 3.5% and 2.4% for posterior parenchyma respectively [30]. Supporting the consistency of this study [30] is that measurements were completed while performing the ultrasound rather than measured offline, by a reader, from archived images, as was done in the study by Devriendt et al. [39].

The echogenicity of the parenchyma was subjectively evaluated and compared to kidney size by one study [29]. This study reported a significantly higher biparietal diameter/kidney length ratio in those fetuses with hyperechogenicity of the kidney parenchyma and proposed this parenchymal hyperechogenicity is an indicator of depression of fetal renal perfusion. These results should be interpreted with caution as their standard deviation for fetal kidney length was large at 5.4 mm and the study had other technical issues, including a non-validated technique to assess fetal hypoxia [29].

Kidney parenchymal area was measured in two other studies [13,14]. Unfortunately, they were retrospective in design and the entire area of the kidney in transverse and longitudinal was measured. These two studies in fact did not measure renal parenchyma and actually measured kidney area. Both used their data to develop nomograms of kidney area.

4. Discussion

After reviewing 28 studies using ultrasound to assess fetal kidney development, this systematic review revealed several ultrasound techniques to evaluate fetal kidney growth. These techniques had a wide variety of sensitivity and reproducibility. Identification of abnormal kidney morphology or growth is aided by availability of normal fetal kidney biometry charts. Unfortunately, this review identified most studies had some methodological limitations.

There is limited data on actual kidney growth. Some studies reported kidney growth as an outcome, however, they were cross-sectional in design [13,14,24,25,30,38]. It is common for size and growth to be confused and used interchangeably. A limitation of cross-sectional studies is they are not appropriate to evaluate growth and can only be used to produce kidney size reference curves [47]. Only one cross-sectional study recognised this limitation [20]. Longitudinal studies can overcome this limitation as the same fetal kidneys are measured at multiple time periods during the pregnancy. A large, longitudinal study of kidney size and volume would provide reference curves for kidney size and growth [47].

In IUGR fetuses, AP and TS dimensions were significantly decreased, but kidney length remained similar to appropriately grown fetuses. The kidney shape changes to long and skinny or "sausage-shaped" as described by Konje et al. [31] and suggests the thinning of the kidney could be due to fewer layers of nephrons. Lampl et al. [22] investigated effects of maternal smoking during pregnancy on fetal kidney growth, proposing maternal smoking affected growth patterns, resulting in long thin "sausage-shaped" kidneys late in the third trimester. Unfortunately, the number of participants with recorded birth outcomes was small (6 smokers and 21 non-smokers) and thus the results deemed insufficient to suggest any meaningful outcomes [22].

Kidney length is the most popular single fetal kidney 2D measurement used in current clinical practice and in the selected studies. It was not, however, seen to be a good indicator of growth. This may make kidney length more useful for estimating GA, where dates are uncertain [16,19,23] and highlights its lack of sensitivity in assessing alterations in fetal kidney growth.

Kidney volume calculations estimate overall kidney size and shape. 2D volumes are simpler and quicker than 3D volume calculations and can be done with any basic ultrasound equipment. 3D volume calculations require higher-level ultrasound equipment with additional 3D-specific transducers and proprietary software. The disadvantage of calculating fetal kidney volumes from 2D measurements is it erroneously assumes that the kidney is an ellipsoid shape. Compared to the

gold standard of fluid displacement, an *in vitro* study demonstrated using the ellipsoid formula underestimated actual renal volumes by 24% [48].

3D ultrasound volume calculations are not dependant on an assumed geometric shape and therefore are thought to more precisely estimate volumes of irregular shaped organs [49]. Large variations between reported results for these 3D studies is likely due to methodological inconsistencies. It is apparent from the selected studies that there is substantial variation in data collection, analysis and presentation of 3D volumes. This is mostly due to ultrasound machines having proprietary file formats that prevent viewing and analysis of data sets outside the specific machine brand. Universal standardisation of 3D file formats and software, regardless of equipment used, is overdue.

All studies describe the technique utilised to acquire 3D data sets and subsequent 3D volume calculations, however, the studies lack explicit image landmarks for volume acquisition and calliper placement for volume measurement. This is necessary for any ultrasound measurement to provide accurate and reproducible results. This is even more crucial when calculating 3D volumes as several planes need to be consistently demarcated and any error in calliper placement is multiplied over the volume [50].

By far the biggest issue compromising the evidence from all the reviewed 3D volume studies is the lack of acceptable reproducibility data. Two studies did not report any intra or inter-observer variability [24,25]. The other four reported either only intra-observer variability, when there was one operator [26,27], or both intra and inter-observability, if there was more than one operator [28,35]. Surprisingly all four studies only assessed reproducibility on the analysis and measurement of the already obtained 3D data set. Errors introduced during acquisition of the 3D data set can be a considerable source of error. Factors such as the depth of the kidney, the number and orientation of the slices and movement of the patient, the fetus or the probe all affect spatial accuracy and can significantly influence the 3D kidney volume obtained [26]. It is important to assess the variability of the post-processed images; however, it is illogical for all studies to ignore the variability associated with 3D data acquisition.

Additionally, it was not clear in most studies how many operators had obtained the 3D data sets and no information on their qualifications and skill level. It was also unclear if the same person acquiring the data was analysing the data. These omissions may considerably influence the quality of the results. In most clinical settings ultrasound scans are performed by multiple operators and variability is unavoidable. This needs to be accounted for, or at least, it needs to be reported along with what quality assurance steps were taken to try and maintain some consistency and standardisation of measurements.

3D ultrasound equipment and software is expensive and not as readily available as 2D ultrasound equipment. 3D imaging has a higher workload than 2D measurements: longer acquisition times, followed by time for post processing the volume of interest. The quality of the acquired image significantly affects the 3D outcome. All studies report that performing the 3D acquisition requires a “quiet” fetus which can take substantial time to achieve [24]. In summary, the reviewed 3D ultrasound studies had no acceptable evidence of true intra or inter-observer variability and therefore good reliability and reproducibility of 3D volumes has not yet been demonstrated.

Hyperechogenicity of the fetal kidneys may be associated with disruption of kidney growth and changes in kidney function. Suranyi et al. [29] investigated the echogenicity of the parenchyma and fetal kidney size to establish a correlation with fetal hypoxia. Their findings, however, are questionable for several reasons. Hyperechogenicity of the kidney medullae was subjectively established by comparing the echogenicity of the kidney to the liver or spleen. The authors stated that kidney hyperechogenicity was a sensitive sign of fetal hypoxia and they appear to use this to determine severity of fetal hypoxia [29]. Renal parenchymal echogenicity and corticomedullary differentiation are reported in the literature, however, are yet to be validated [39]. In 2003, as it is today, fetal medullary hyperechogenicity is not a validated or clinically used method to establish the presence or absence of fetal hypoxia.

During fetal hypoxia, blood flow is redistributed away from the kidneys to more essential organs [51]. Suranyi et al. [29] propose that this reduced kidney perfusion possibly delays fetal kidney development resulting in hypoplasia of the kidney and a hyperechogenic appearance. The role of the kidney parenchyma and blood flow in assessing fetal kidney growth and future renal function has not yet been well evaluated. Preliminary data from term neonates [52] and children [53] indicates parenchymal thickness may be a more reliable investigative method to define normal and abnormal kidney development. More research is needed to validate these propositions.

Magnetic resonance imaging (MRI) is being increasingly utilised to image the fetal kidneys and provides excellent delineation of anatomy [54]. Fetal kidney measurements and volumes have been described with MRI [55,56]. There are still some safety concerns around MRI for the fetus, such as biological effects and acoustic noise [54]. MRI is more expensive than ultrasound, is limited by fetal movement, has several contraindications and has similar problems to ultrasound with regards to maternal obesity. Ultrasound imaging provides real-time images, is non-invasive and relatively inexpensive and is still the modality of choice for routine evaluation of fetal kidney growth [54,57].

4.1. Limitations

The search was restricted to English language and studies published from the last 20 years (from 1996) as it was felt that the significant advances in ultrasound imaging made these older studies less relevant. The major limitation of this systematic review is most of the included studies were of a cross-sectional design rather than longitudinal. Widely variable gestational age ranges were analysed. Most studies involved low-risk, uncomplicated pregnancies as most studies were establishing normal ranges.

5. Conclusions

Following a review of 28 studies investigating fetal kidney growth using ultrasound, we can conclude that the collective results provide some normal ranges of 2D and 3D fetal kidney size and volume. However, there are few large, good quality, longitudinal studies. There is also a paucity of research into the effects of abnormal fetal growth, particularly overgrowth, on fetal kidney development. Kidney length is the most popular single fetal kidney measurement; however, it does not seem to be a good indicator of growth. In IUGR fetuses, kidney length remained similar to appropriately grown fetuses whereas antero-posterior and transverse dimensions were significantly decreased. Currently there is no easily reproducible, sensitive method for measuring changes in fetal kidney growth. New ultrasound techniques concentrating on the parenchyma of the kidney and the perfusion to the kidney may provide improved information on fetal kidney development.

Conflicts of interest

All authors have no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgement

none

Appendix A. Medline Ovid (R) 1946 to 7th Jan 2017

1 Ultrasonography (MeSH term exploded) — "computer echotomography" OR "diagnostic ultrasound*" OR echography OR echotomography OR "medical sonography" OR "ultrasonic diagnoses" OR "ultrasonic diagnosis" OR "ultrasonic imaging" OR "ultrasonic tomography" OR "ultrasound imaging*" OR sonography
2 Kidney* (MeSH term exploded)
3 Renal (keyword)
4 2 OR 3
5 Fetus (MeSH term exploded) – "fetal structure*" OR "fetal tissue*" OR fetuses
6 Prenatal (MeSH term exploded) – "antenatal diagnoses" OR antenatal diagnosis OR "antenatal screening*" OR "intrauterine diagnoses" OR "intrauterine diagnosis" OR "prenatal diagnoses" OR "prenatal diagnosis" OR "prenatal screening*"
7 Foetus OR Foetal (keyword)
8 5 OR 6 OR 7
9 1 AND 4 AND 8
10 Limit 9 to yr = "1996–2016"
11 Limit 10 to English language

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Appendix D2:
Brennan S, Schneider M, Watson D, Kandasamy Y, Rudd D. The renal parenchyma—evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study. BMJ Open. 2017; 7(12).

BMJ Open

The renal parenchyma—evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study

Sonja Brennan,[1,2] Michal Schneider,[3] David Watson,[2,4] Yogavijayan Kandasamy,[2,5,6] Donna Rudd[2]

To cite: Brennan S, Schneider M, Watson D, *et al*. The renal parenchyma—evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study. *BMJ Open* 2017;**7**:e019369. doi:10.1136/bmjopen-2017-019369

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-019369).

Received 5 September 2017
Revised 4 November 2017
Accepted 24 November 2017

For numbered affiliations see end of article.

Correspondence to
Sonja Brennan;
sonja.brennan@my.jcu.edu.au

ABSTRACT

Introduction Disorders of fetal growth, such as intrauterine growth restriction (IUGR) and large for gestational age (LGA), have been found to have a profound effect on the development of the fetal kidney. Abnormal kidney development is associated with hypertension and chronic kidney disease later in life. This study will use a novel ultrasound measurement to assess the renal parenchymal growth and kidney arterial blood flow in the fetus to evaluate the development of the fetal kidneys and provide an indirect estimate of nephron number. Measurements in normally grown, IUGR and LGA fetuses will be compared to determine if changes in renal parenchymal growth can be detected in utero.

Methods and analysis This longitudinal, prospective, observational study will be conducted over 12 months in the Ultrasound Department of the Townsville Hospital, Australia. The study will compare fetal renal parenchymal thickness (RPT) and renal artery Doppler flow between IUGR fetuses and appropriately grown fetuses, and LGA fetuses and appropriately grown fetuses between 16 and 40 weeks. The fetal RPT to renal volume ratio will also be compared, and correlations between RPT, renal parenchymal echogenicity, fetal Doppler indices and amniotic fluid levels will be analysed.

Ethics and dissemination This study was approved by the Townsville Health District Human Research Ethics Committee. The study results will form part of a thesis and will be published in peer-reviewed journals and disseminated at international conferences.

Strengths and limitations of this study

- This will be the first study to use a novel ultrasound measurement of the fetal renal parenchyma and measurements of renal blood flow to assess fetal kidney growth.
- Fetal kidney growth will be assessed not only in normally grown but also in growth-restricted and large-for-gestational-age fetuses.
- This is a prospective, longitudinal, rather than cross-sectional, ultrasound study and should enhance our understanding of how fetal kidneys grow.
- This study is the first of a series of studies investigating kidney growth, and although renal function and renal parenchymal thickness of the infants are not included in this study, follow-up of the infants will be included in future studies.
- Due to fetal position and/or maternal habitus, not all kidney measurements may be obtainable at every scan.

INTRODUCTION

Chronic kidney disease is an increasing contributor to the global burden of disease, with hypertension now the leading risk factor.[1] Recognition of the risk factors and implementation of preventive strategies is crucial to reducing hypertension and kidney disease. Abnormalities in fetal growth, such as intrauterine growth restriction (IUGR), have a profound effect on the kidney development.[2 3] The association between an adverse intrauterine environment and chronic kidney disease later in life is now compelling.[4–6]

During early fetal life, there is transient development and regression of the primary (pronephros) and secondary (mesonephros) fetal kidneys between day 23 and day 112 of embryonic life.[7] The permanent functional tertiary fetal kidney is the metanephros which begins developing on day 30.[7] Nephrogenesis involves the formation of the functional units of the kidney called nephrons and continues up to 36 weeks' gestational age.[8 9] It is essential that appropriate nephrogenesis is achieved in utero as the number and quality of nephrons directly influences lifetime kidney function.[10]

IUGR can result in a significant reduction in nephron number; however, large for gestational age (LGA), particularly related to maternal hyperglycaemia, is also associated with abnormal fetal kidney development and an increased risk of hypertension and chronic kidney disease.[11] A better understanding of the relationship between abnormal fetal growth and nephrogenesis is needed.

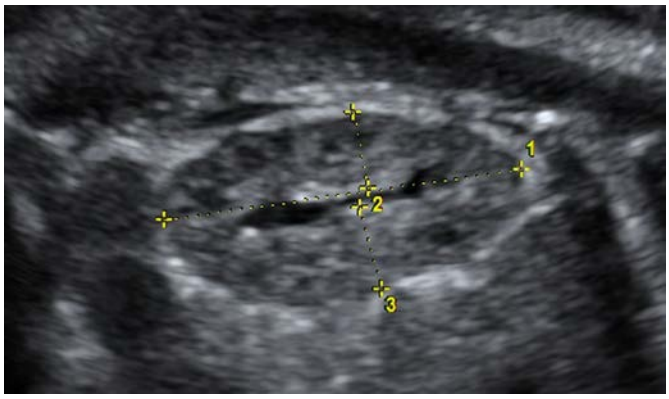

Figure 1 Measurement of kidney length (1) and the anterior (2) and posterior (3) fetal renal parenchymal thickness from the inner aspect of the renal capsule to the sinus–pyramidal apex interface.

Presently, the only accurate method of calculating human nephron number is during an autopsy.[12] A non-invasive measure of nephron endowment is needed.

Ultrasound is the primary imaging modality for evaluating fetal kidneys. We conducted a systematic review on the evaluation of fetal kidney growth using ultrasound which revealed that there are few good-quality, longitudinal studies.[13] The most commonly reported ultrasound measurement was renal length; however, renal length alone was not found to be very sensitive to evaluate disruptions in fetal kidney growth in the presence of fetal growth restriction.[13] Few studies analysed the effects of IUGR on fetal kidney growth, and no studies have, to date, analysed if LGA has an effect on fetal kidney growth. Results from two-dimensional (2D) and three-dimensional (3D) renal volume (RV) calculations were disappointing. Volumes calculated from 2D measurements underestimate RVs by as much as 24%.[14] Substantial variations were reported for 'normal' 3D kidney volumes, and good reliability and reproducibility has not yet been demonstrated. Currently, there is no easily repeatable, sensitive method of measuring changes in fetal kidney growth.[13]

Measuring the renal parenchyma with ultrasound is a novel method to assess fetal kidney development and predict future renal function. Measuring just the renal parenchyma will measure only the important functional part of the kidney which contains the nephrons (figure 1). One small cross-sectional study measured fetal renal parenchyma in normally grown fetuses[15]; however, no studies have evaluated the fetal renal parenchyma in abnormally grown fetuses. Preliminary data from term neonates[16] and children[17] indicate that the parenchymal thickness may be a more reliable investigative method to define normal and abnormal kidney development. Methods such as measuring the parenchymal thickness, RV to parenchymal thickness ratio, renal artery Dopplers and echogenicity of the renal parenchyma are potential non-invasive methods to evaluate nephron endowment and future renal function.

The aim of this study is to use ultrasound to assess the fetal renal parenchymal growth in a pregnant population demonstrating either normal or abnormal growth and determine if abnormal fetal growth influences fetal renal parenchymal thickness (RPT). Non-invasive ultrasound techniques are used. The results could help identify factors that adversely affect kidney development so that they could be modified by public health interventions and education programmes. This may promote improved fetal nephron number and quality at birth and reduce susceptibility to chronic disease in later life.

METHODS AND ANALYSIS

Objectives

Primary objectives

- Determine normal RPT of fetuses from 16 to 40 weeks' gestation from a group of normally grown fetuses.
- Determine the effects of IUGR and LGA on RPT in a group of abnormally grown fetuses.

Secondary objectives

- Assess the relationship between RPT and renal artery, umbilical artery and middle cerebral artery Doppler indices, and amniotic fluid levels.
- Assess the relationship between renal parenchymal echogenicity, renal artery Doppler flow and IUGR or LGA.

Study design and setting

This is a prospective, longitudinal, observational study being conducted over 12 months, commencing in May 2017, in the Ultrasound Department of the Townsville Hospital, Australia.

Participants

Patients who are referred for a diagnostic second trimester ultrasound scan will be recruited for this study. Pregnant women of 18 years or older, with an accurately dated singleton pregnancy of 16 weeks' gestation or more, will be included. Pregnant women with uncertain dates, multiple pregnancy or any known major congenital fetal abnormality or chromosomal fetal abnormality will be excluded.

Recruitment and consent of participants

Pregnant patients 18 years of age or older, who present to the Medical Imaging Department at the Townsville Hospital for an obstetric ultrasound, will be invited to participate. In addition, mixed risk patients may also be informed about the study by their treating obstetrician, midwife or sonographer. Detailed written information will be given to the patient and written consent obtained.

Study process

Figure 2 outlines the flow schedule of the participants. The first ultrasound scan will be performed from 16 weeks and then follow-up ultrasounds will be performed at least every 4 weeks from the first ultrasound scan. Some women, particularly with high-risk pregnancies, will

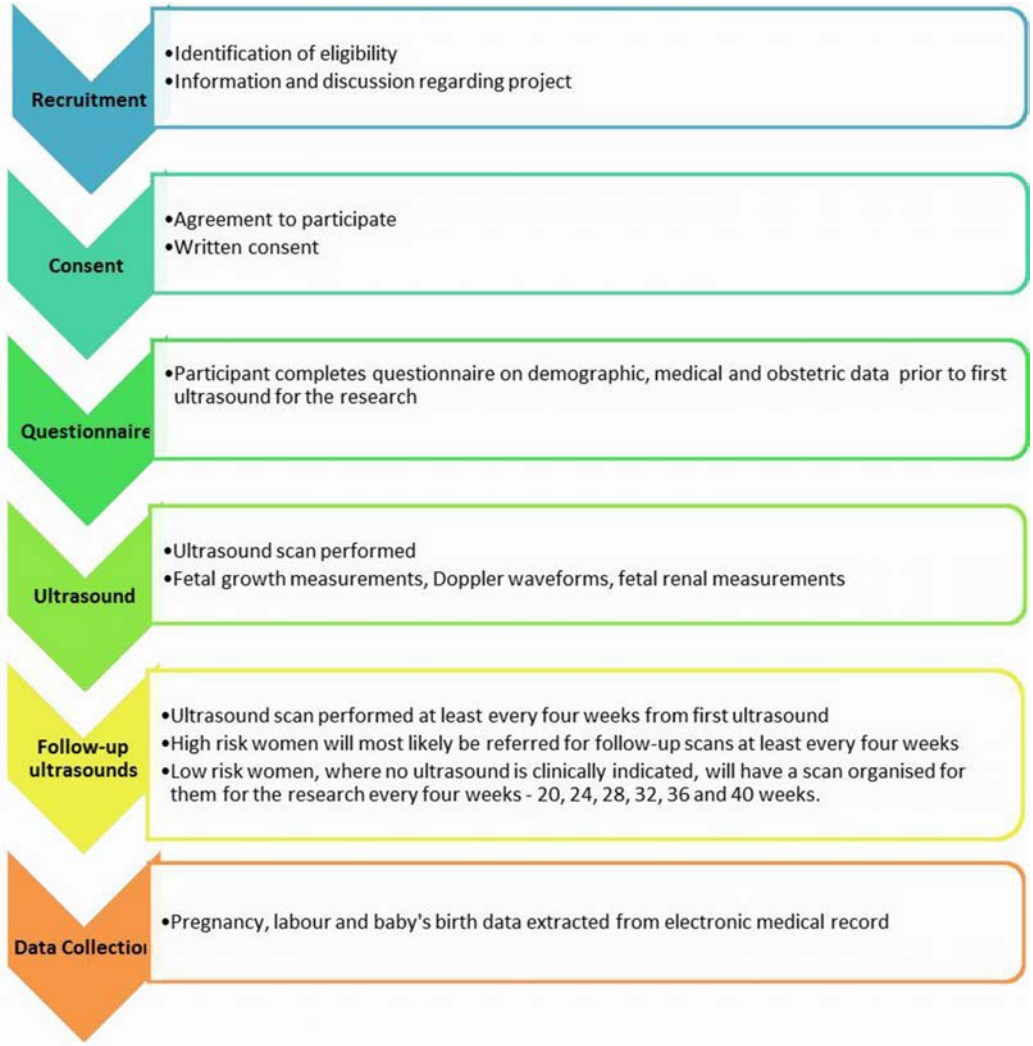

Figure 2 Flow chart of study participants.

require more than one clinically indicated ultrasound. For example, women with a growth-restricted fetus may need to be monitored by ultrasound monthly or more frequently. However, the control group of healthy women, with appropriately grown fetuses, may only require one to two clinically indicated scans between 16 and 40 weeks' gestation. To obtain good longitudinal data, particularly for the control group, the women will be asked to attend for additional research scans every 4 weeks until delivery.

Ultrasound examinations

Australian Accredited Medical Sonographers with at least 2 years' postultrasound qualification experience will perform all ultrasound examinations. A high-level ultrasound machine with pulse wave and colour Doppler flow will be used, and the highest frequency transducer possible, matching the mother's body habitus, will be selected. When a woman attends for a clinically indicated scan, this will be the priority, and then the additional fetal renal measurements required for the research study will be performed thereafter.

Where possible, the fetal kidneys should be measured with the fetal spine up (anterior) or as close as possible to this position. The image is magnified so that the kidney occupies most of the image and both kidneys can be identified. A midsagittal scan of both kidneys along their longest length will be recorded and the longest length (L) of both kidneys measured. The parenchymal thickness will be measured in two directions: from the posterior aspect of the kidney to the pelvis (posterior parenchyma) and from the anterior border of the kidney to the pelvis (anterior parenchyma) (figure 1). A transverse section of the fetal abdomen at the level of each renal pelvis will be imaged. The maximum anteroposterior diameter (H) and transverse diameter (W) will be measured for both kidneys.

Bilateral fetal renal artery Dopplers will be performed in the coronal view of the kidneys. Colour flow should be used to identify the renal artery entering the kidney. A low wall filter of between 30 and 60 Hz will be used, and a sample gate of size 2–3 mm will be placed in the mid trunk of the renal artery. Using an angle as close to 0° as possible, a pulse wave signal will be obtained. The average of three consecutive waveforms will be used to calculate the resistivity index (RI) and pulsatility index (PI).

Table 1 After baby's birth, perinatal data to be collected from the mother and baby's electronic medical record

	Birth data to be collected	
Onset of labour	Gestational age at birth	Antenatal steroids
Mode of delivery	Birth weight	Other antenatal medications
Placental histopathology	Gender	Maternal medical history:
	Apgar scores at 1 and 5 min	► Diabetes
	Umbilical artery cord potential hydrogen (pH)	► Renal disease
	Base excess	► Hypertension
	Lactate	Demographic, medical and obstetric history from participant questionnaire

The following routinely performed obstetric measurements will also be recorded for the study:

- Single deepest pool amniotic fluid measurement;
- Umbilical artery Doppler;
- Middle cerebral artery Doppler (where clinically indicated or 30 weeks' gestation and over);
- Ductus venous, where clinically indicated;
- Biometries—head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL).

Birth data

Outcome measures

Perinatal data will be collected from the mother and baby's electronic medical record (table 1).

Primary outcome measure

- RPT: anterior and posterior thickness in longitudinal plane.

Secondary outcome measures

- RV: calculated using the formula RV=length×width×height×0.523;
- Fetal growth biometries: HC, BPD, AC and FL;
- Amniotic fluid: single deepest pool;
- Umbilical artery Doppler flow: RI and PI calculated from the average of at least three consecutive waveforms;
- Middle cerebral artery Doppler flow: RI and PI calculated from the average of at least three consecutive waveforms;
- Renal parenchymal echogenicity: subjectively assessed by the sonographer as either normal echogenicity, more hyperechoic than normal or more hypoechoic than normal;
- Renal artery Doppler flow: RI and PI calculated from the average of at least three consecutive waveforms.

Sample size

Optimal sample size has been calculated based on a statistical power of 80% and a significance level of 0.05 (two-tailed). Data from a previously published study[16] have demonstrated that the RPT was 9.4 mm (±1.1 mm) for normal birth weight neonates and 8.3 mm (±1.0 mm) for low birth weight neonates at term. Therefore, it is estimated that a sample size of 45 will be needed (15 intrauterine growth-restricted fetuses, 15 LGA fetuses and 15 appropriate for gestational age (AGA)). Allowing for the possibility of loss to follow-up, 20 participants will be recruited for each group resulting in a total of 60 participants, each having an ultrasound scan every 4 weeks.

Data analysis

After the ultrasound examination is complete, and the baby is born, all data will be collated and divided into three groups: AGA, IUGR and LGA. Comparisons of RPT and renal artery Dopplers between IUGR and AGA fetuses, and LGA and AGA fetuses between 16 and 40 weeks will be analysed. RV will be compared with RPT in each fetal group to obtain an RPT to RV ratio. Correlation between RPT, renal parenchymal echogenicity, fetal Doppler indices and amniotic fluid levels will be carried out, and intraobserver and interobserver variability will be assessed.

Statistical analysis will be performed using IBM SPSS Statistics, V.24.0. The normality of the variables will be determined by the Kolmogorov-Smirnov test. Renal measurements will be expressed as means±SDs for continuous, normally distributed data and as a median (IQR) for continuous, non-normally distributed data. Paired/unpaired t-tests will be used to compare means of normally distributed data and Mann-Whitney or Kruskal-Wallis tests for non-normally distributed data. A value of $P<0.05$ will be considered statistically significant. Univariate and multivariate analysis will be carried out to determine the association between RPT and other variables. Intraobserver variability will be determined by calculating the differences between the two measurements made by the same sonographer. Interobserver variability will be assessed by calculating the differences between two measurements carried out on the same patient by different sonographers.

Data management

Data collection commenced in May 2017 and is planned to finish in December 2018 once the birth data of all participants are obtained. Participant data will be deidentified and assigned a number code to ensure confidentiality for each woman and baby.

Electronic data will be stored and saved on a password-protected computer. Hard (paper) copies of the consent form, questionnaire and data sheets from the ultrasound examination will be stored in a locked filing cabinet in the principal researcher's office. This office is a secure room within the ultrasound department of the Townsville Hospital. Only the principal researcher

and members of the research team will have access to the data.

Author affiliations
[1]Ultrasound Department, The Townsville Hospital, Townsville, Queensland, Australia
[2]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia
[3]Medical Imaging & Radiation Sciences, Monash University, Melbourne, Victoria, Australia
[4]Department of Obstetrics and Gynaecology, The Townsville Hospital, Townsville, Queensland, Australia
[5]Department of Neonatology, The Townsville Hospital, Townsville, Queensland, Australia
[6]Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, The University of Newcastle, Newcastle, Australia

Contributors SB conceived the study and drafted the study design under the supervision of YK, DW, DR and MS. All authors contributed to the conception, design and development of the study protocol. MS provided her expertise for the study design, ethics and critical revision of the manuscript. DW provided his expertise for the ultrasound protocol, recruitment of participants and revision of the protocol. YK provided his expertise for ethics, statistics and drafting of the study protocol. DR provided her expertise for the study design, data management and analysis. All authors approved the final version and agree to be accountable for the contents and integrity of this manuscript.

Funding This study is partly funded by a Townsville Hospital and Health Service Research Grant.

Competing interests None declared.

Ethics approval This study was approved by the Townsville Health District Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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The renal parenchyma––evaluation of a novel ultrasound measurement to assess fetal renal development: protocol for an observational longitudinal study

Sonja Brennan, Michal Schneider, David Watson, Yogavijayan Kandasamy and Donna Rudd

*BMJ Open*2017 7:
doi: 10.1136/bmjopen-2017-019369

Appendix D3:
Brennan S, Kandasamy Y, Rudd D, Schneider M, Watson D. Fetal kidney charts of a novel measurement of the renal parenchymal thickness to evaluate fetal kidney growth and potential function. Prenat. Diagn.2020;40(7):860-9.

Received: 1 February 2020 | Revised: 31 March 2020 | Accepted: 2 April 2020

DOI: 10.1002/pd.5701

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Fetal kidney charts of a novel measurement of the renal parenchymal thickness to evaluate fetal kidney growth and potential function

Sonja Brennan[1,2] | Yogavijayan Kandasamy[3,4,5] | Donna Rudd[2] | Michal Schneider[6] | David Watson[3,7]

[1]Ultrasound Department, Townsville University Hospital, Douglas, Townsville, Australia

[2]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia

[3]College of Medicine and Dentistry, James Cook University, Townsville, Australia

[4]Department of Neonatology, Townsville University Hospital, Townsville, Australia

[5]Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, The University of Newcastle, Newcastle, Australia

[6]Department of Medical Imaging & Radiation Sciences, Monash University, Melbourne, Australia

[7]Maternal Fetal Medicine Unit and Department of Obstetrics and Gynaecology, Townsville University Hospital, Townsville, Australia

Correspondence
Sonja Brennan, Ultrasound Department, Townsville University Hospital, IMB 47 P.O. Box 670, Douglas, Townsville, Queensland 4810, Australia.
Email: sonja.brennan@my.jcu.edu.au, sonja.brennan@health.qld.gov.au.

Funding information
Australasian Society for Ultrasound in Medicine (ASUM), Grant/Award Number: ASUM research grant 2018; Townsville Hospital Study, Education and Research Trust Account (SERTA), Grant/Award Number: SERTA grant:27_2016

Abstract

Objective: The objective of this study was to develop new standard growth charts for fetal renal parenchymal thickness, length, and volume to define normal ranges for use in clinical practice and to assess the reliability of these measurements.

Methods: This was a prospective, longitudinal study of 72 low-risk singleton pregnancies undergoing serial ultrasound examinations at least every four weeks. Multiple renal measurements were performed on both kidneys at each scan. The renal parenchymal thickness was measured in the mid-sagittal plane. Standard charts were developed and the intra and interobserver reliability for the renal measurements was analysed.

Results: Standard charts were developed for fetal renal parenchymal thickness, length, and volume.

Conclusion: We present novel charts, which demonstrate the growth of the fetal renal parenchyma during pregnancy. They will be useful in clinical practice to identify any alterations from these normal ranges, which may be an important criterion for assisting prenatal diagnosis of renal pathologies and future studies in the prediction of kidney function.

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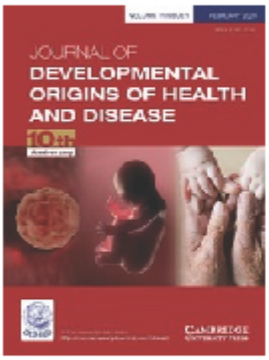
Appendix D4:
Brennan S, Watson D, Schneider M, Rudd D, Kandasamy Y. Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number? J Dev Orig. Health Dis. 2020:1-9.



Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number?

Journal of Developmental Origins of Health and Disease

SONJA BRENNAN, DAVID WATSON, MICHAL SCHNEIDER, DONNA RUDD, YOGAVIJAYAN KANDASAMY

DOI: 10.1017/S204017442000015X

Published online: 15 April 2020, pp. 1-9

Abstract

Chronic kidney disease continues to be under recognised and is associated with a significant global health burden and costs. An adverse intrauterine environment may result in a depleted nephron number and an increased risk of chronic kidney disease. Antenatal ultrasound was used to measure the foetal renal parenchymal thickness (RPT), as a novel method to estimate nephron number. Foetal renal artery blood flow was also assessed. This prospective, longitudinal study evaluated the foetal kidneys of 102 appropriately grown and 30 foetal growth-restricted foetuses between 20 and 37 weeks gestational age (GA) to provide vital knowledge on the influences foetal growth restriction has on the developing kidneys. The foetal RPT and renal artery blood flow were measured at least every 4 weeks using ultrasound. The RPT was found to be significantly thinner in growth-restricted foetuses compared to appropriately grown foetuses [likelihood ratio (LR) = 21.06, $P \leq 0.0001$] and the difference increases with GA. In foetuses with the same head circumference, a growth-restricted foetus was more likely to have a thinner parenchyma than an appropriately grown foetus (LR = 8.9, $P = 0.0028$), supporting the principle that growth-restricted foetuses preferentially shunt blood towards the brain. No significant difference was seen in the renal arteries between appropriately grown and growth-restricted foetuses. Measurement of the RPT appears to be a more sensitive measure than current methods. It has the potential to identify infants with a possible reduced nephron endowment allowing for monitoring and interventions to be focused on individuals at a higher risk of developing future hypertension and chronic kidney disease.

Journal of Developmental Origins of Health and Disease

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Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number?

Sonja Brennan[1,2], David Watson[3], Michal Schneider[4], Donna Rudd[2] and Yogavijayan Kandasamy[2,5,6]

[1]Ultrasound Department, The Townsville Hospital, Douglas, Townsville, Australia; [2]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia; [3]Maternal Fetal Medicine Unit, Department of Obstetrics and Gynaecology, The Townsville Hospital, Townsville, Australia; [4]Department of Medical Imaging & Radiation Sciences, Monash University, Melbourne, Australia; [5]Department of Neonatology, The Townsville Hospital, Townsville, Australia and [6]Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, The University of Newcastle, Newcastle, Australia

Original Article

Cite this article: Brennan S, Watson D, Schneider M, Rudd D, and Kandasamy Y. Can measurement of the foetal renal parenchymal thickness with ultrasound be used as an indirect measure of nephron number? *Journal of Developmental Origins of Health and Disease* doi: 10.1017/S204017442000015X

Received: 30 October 2019
Revised: 30 November 2019
Accepted: 19 January 2020

Keywords:
Renal parenchyma; foetal renal artery; foetal growth restriction; nephron number; ultrasound

Address for correspondence: Sonja Brennan, Ultrasound Department, The Townsville Hospital, IMB 47, P.O. Box 670, Douglas, Townsville, QLD 4810, Australia.
Email: sonja.brennan@my.jcu.edu.au; sonja.brennan@health.qld.gov.au

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