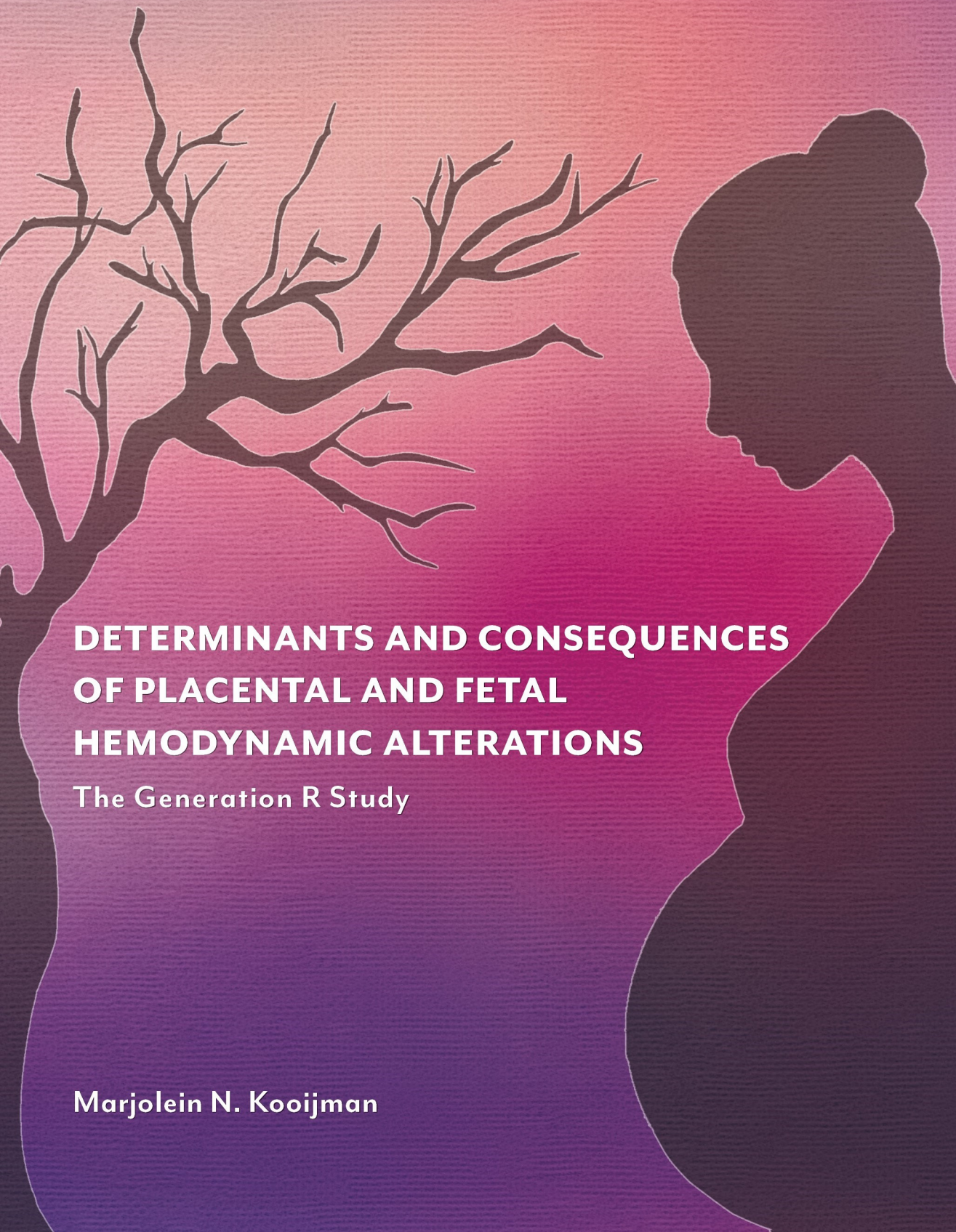

DETERMINANTS AND CONSEQUENCES OF PLACENTAL AND FETAL HEMODYNAMIC ALTERATIONS

The Generation R Study

Marjolein N. Kooijman

Determinants and consequences of placental and fetal hemodynamic alterations

Propositions

1. Higher maternal cholesterol and triglyceride concentrations are, within their normal ranges, associated with increased fetal middle cerebral artery resistance (This thesis)
2. Fetal blood flow redistribution is associated with lower fetal growth, adverse birth outcomes and may have persistent consequences for childhood growth (This thesis)
3. Third trimester fetal aorta ascendens diameter and fetal left cardiac output are positively associated with childhood aortic root diameter and childhood left ventricular mass (This thesis)
4. Fetal blood flow redistribution and fetal pulmonary blood flow is not consistently associated with childhood respiratory health (This thesis)
5. Fetal blood flow redistribution is associated with smaller childhood kidney volume (This thesis)
6. Longitudinal cohort studies can provide important evidence about preventable causes of disease, but the success relies on the commitment of their participants, both at recruitment and during follow up (Nohr et al., Acta Obstet Gynecol Scand., 2018)
7. Simple exercise programs in schools and sports clubs are probably the most cost-effective investments a society can make in its psychological and physical health (Noakes & Spedding, Nature, 2012)
8. Excellent data-stewardship is often overlooked, but a prerequisite and basic principle for long-term use of data of cohort studies
9. To increase effectiveness of lifestyle interventions in pregnancy they should be focused on the whole family rather than on mothers only
10. No single life fits within the median of a statistical model (Troonrede, 2019)
11. Wat er ook speelt in een land, laat het vooral de kinderen zijn (Loesje)

Marjolein Kooijman, 19 oktober 2020

Determinants and Consequences of Placental and Fetal Hemodynamic Alterations

The Generation R Study

Marjolein N. Kooijman

The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families.

Publication of this thesis was kindly supported by the Generation R Study Group. Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

ISBN: 978-94-6416-086-4

Cover design & Thesis Lay-out: Iliana Boshoven-Gkini | AgileColor.com
Printing: Ridderprint | www.ridderprint.nl

Determinants and Consequences of Placental and Fetal Hemodynamic Alterations

The Generation R Study

Determinanten en consequenties van placentale en foetale hemodynamische veranderingen

Het Generation R onderzoek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
19 oktober 2020

door
Marjolein Nel Kooijman
geboren te Cromstrijen

Erasmus University Rotterdam

Promotiecommissie

Promotoren	Prof. dr. V.W.V. Jaddoe Prof. dr. E.A.P. Steegers
Overige leden	Prof. dr. I.K.M. Reiss Prof. dr. A. Franx Prof. dr. C.J.M. de Groot
Copromotor	Dr. R. Gaillard

Paranimfen	Heleen G. Kooijman Florianne O.L. Vehmeijer

Contents

Manuscripts based on this thesis

Chapter 1.2
Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IJzendoorn MH, de Jongste JC, Klaver CC, van der Lugt A, Mackenbach JP, Moll HA, Peeters RP, Raat H, Rings EH, Rivadeneira F, van der Schroeff MP, Steegers EAP, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius E, Felix JF, Jaddoe VWV. *The Generation R Study: design and cohort update 2017*. Eur J Epidemiol. 2016;31(12):1243-1264

Chapter 2
Kooijman MN, Jaddoe VWV, Steegers EAP, Gaillard R. *Associations of maternal metabolic profile with placental, and fetal cerebral and cardiac hemodynamics* Submitted

Chapter 3.1
Kooijman MN, Gaillard R, Reiss IKM, Hofman A, Steegers EAP, Jaddoe VWV. *Influence of fetal blood flow redistribution on fetal and childhood growth and fat distribution: the Generation R Study*. BJOG. 2016;123(13):2104-2112

Chapter 3.2
Kooijman MN, de Jonge LL, Steegers EAP, van Osch-Gevers L, Verburg BO, Hofman A, Helbing WA, Jaddoe VWV. *Third trimester fetal hemodynamics and cardiovascular outcomes in childhood: the Generation R study*. J Hypertens. 2014;32(6):1275-82

Chapter 3.3
Kooijman MN, van Meel ER, Steegers EAP, Reiss IKM, de Jongste JC, Jaddoe VWV, Duijts L. *Fetal umbilical, cerebral and pulmonary blood flow patterns in relation to lung function and asthma in childhood. The Generation R Study*. Pediatr Allergy Immunol. 2019;30(4):443-450

Chapter 3.4
Kooijman MN*, Bakker H*, van der Heijden AJ, Hofman A, Franco OH, Steegers EAP, Taal HR, Jaddoe VWV. *Childhood kidney outcomes in relation to fetal blood flow and kidney size*. J Am Soc Nephrol. 2014;25(11):2616-24

Chapter 4.1
Kooijman MN, Gaillard R. *Maternal and offspring health consequences of placental and fetal hemodynamic alterations throughout pregnancy: a narrative review*. Submitted (partly adapted)

* These authors contributed equally

Chapter 1

Introduction and design

Chapter 1

Chapter 1.1

General Introduction

Placental and fetal hemodynamic patterns

The placenta is the interface between the maternal and fetal blood circulation and is responsible for the maternal to fetal transfer of nutrients essential for fetal growth and development.[1] The efficiency of placental exchange is a complex interplay between placental growth and development, rates of placental blood flow and metabolic demands of placental tissue. This interplay is arranged by maternal, placental and fetal hormones, and under favorable conditions ensures an adequate supply to the fetus.[2] Early in pregnancy, placental tissue develops by trophoblast shell plugs invasion of the utero-placental vessels.[3] After 10 weeks of gestation, these plugs are repressed and blood flows into the intervillous space to exchange oxygen and nutrient with the developing fetus.[4]

Suboptimal early placental development leads to placental and fetal hemodynamic alterations.[5] Fetal hemodynamic alterations are important mechanisms by which the fetus protects the most important organs such as the brain and heart from an adverse fetal environment.[6] The first signs of these fetal hemodynamic alterations in response to an adverse fetal environment can be detected by a decrease in umbilical vein blood flow. A compensatory increase in ductus venous diameter increases the blood flow to the heart.[7, 8] This is followed by fetal blood flow redistribution. The umbilical artery resistance increases and the cerebral artery resistance decreases (**Table 1.1.1**).[9]

Parameters of fetal blood flow redistribution are not implemented in routine clinical practice. More common parameters used in clinical practice are the use of the ductus venosus pulsatility index (PI) and the absence or reversed diastolic flow in the umbilical artery.[10, 11] However, evaluation of the fetal cerebral circulation and fetal blood flow redistribution is often part of clinical follow-up in growth restricted fetuses.[12] Combined measurements of different placenta and fetal hemodynamic parameters may be of prognostic value in predicting fetal outcome.[6] For example, growth restricted fetuses with fetal blood flow redistribution had an 11-fold increased risk of an adverse perinatal outcome compared with growth restricted fetuses without fetal blood flow redistribution.[13]

TABLE 1.1.1 | Placental and fetal hemodynamic alterations in complicated pregnancies.

1. Umbilical vein blood flow	↓		
2. Ductus venous diameter	↑		
3. Umbilical artery resistance	↑	and Cerebral artery resistance	↓

Placental and fetal hemodynamics and later life outcomes

Population based studies have demonstrated associations of preterm birth or small for gestational age at birth, as reflection of an adverse fetal and neonatal environment, with diseases later in life.[14, 15] Morrison et al showed that adults born with an extremely low birth weight had higher increased body fat, lower lean mass, and a higher systolic and diastolic blood pressure compared with normal birth weight participants.[16] Low birth weight is also associated with a higher incidence of chronic kidney disease or impairment of renal function in adulthood.[17] However, studies also showed that a high birth weight is a risk factor for later obesity and diabetes.[18-21] Clearly, birth weight is not the causal factor per se leading to diseases in later life. Only limited studies focused on the associations of placental and fetal hemodynamics with birth outcomes and childhood developmental outcomes. An increase in the fetal umbilical artery/cerebral artery PI ratio was associated with a low birth weight and Apgar scores, higher risks of admission to the intensive care unit and perinatal death.[22, 23] Furthermore, an increased umbilical artery vascular resistance was associated with a higher childhood BMI, fat mass, systolic blood pressure and a lower left ventricular mass.[24] An animal study showed that increased utero-placental insufficiency was associated with a larger left ventricular mass and fewer glomeruli.[25] Altogether, these studies suggest that changes in placental and fetal hemodynamics affect organ development and may have long term consequences. Identifying more detailed measures of placental and fetal hemodynamic alterations might give further insight in long-term consequences of an unfavorable fetal environment. Therefore, studies presented in this thesis were designed to identify alterations in placental and fetal hemodynamics which are associated with childhood growth, cardiovascular development, lung function and kidney outcomes (**Figure 1.1.1**).

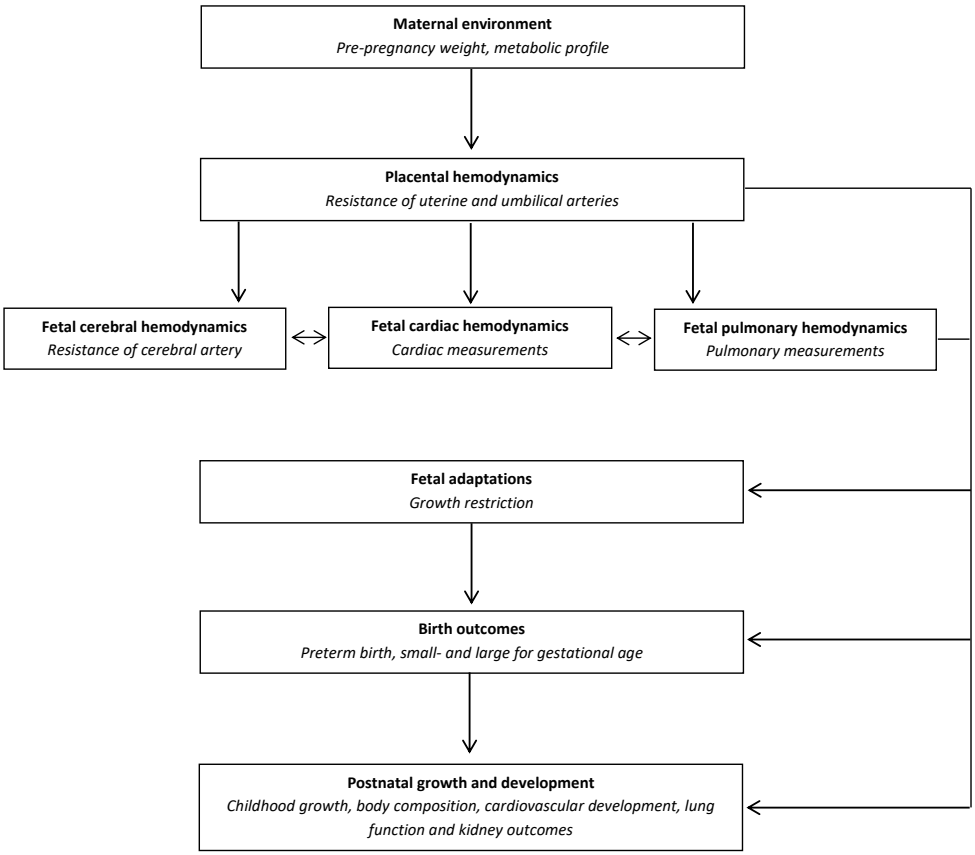

FIGURE 1.1.1 | Overview of the pathways of maternal determinants and childhood consequences of placental and fetal hemodynamic alterations.

Placental and fetal hemodynamic measurements

For the studies presented in this thesis, third trimester fetal Doppler ultrasound examinations were performed (**Table 1.1.2** and **textbox 1.1.1**). Placental vascular resistance was evaluated with flow-velocity waveforms from the uterine and umbilical arteries. A higher PI and uterine artery resistance index (RI) is an indication of an increased vascular resistance and a decrease in blood flow.[26, 27] The PI of the fetal cerebral arteries provide insight into fetal cerebral blood flow patterns. Reductions in middle cerebral artery PI are valid indicators of the brain-sparing effect and fetal blood flow redistribution.[28, 29] Fetal blood flow redistribution in favor to the brain at expense of the trunk is indicated by an increased ratio between the umbilical (U) artery PI and the cerebral (C) artery PI.[30] Cardiac outflow measures from the aorta were recorded; the inner diameter, peak systolic velocity (PSV) and the time velocity integral (TVI). A smaller diameter

in fetuses with abnormal placental or fetal hemodynamics was seen. A low PSV and TVI can be a sign of reduced cardiac function, raised afterload or decreased vascular compliance.[31, 32] Left cardiac output was calculated by multiplying the vessel area by the TVI by fetal heart rate. A reduced cardiac output is a sign of deterioration of cardiac function.[33] Cardiac flow-velocity waveforms at the level of the mitral valves were recorded. The early (E) diastolic phase is caused by accumulation of blood in the atria during previous systole and a measure of early passive ventricular filling. Active (A) filling during atrial contraction follows. The E/A ratio, which is an index for ventricular diastolic function and expresses both cardiac compliance and preload conditions, was calculated. Early in pregnancy, in healthy fetuses, the A-wave is higher compared to the E-wave. As gestation progresses, the E/A ratio increases, approaching postnatal values. Both, reductions and increases in E/A wave has been shown in complicated pregnancies.[34] From the pulmonary artery the TVI was recorded. A higher pulmonary artery TVI indicated higher pulmonary vascular resistance, this might also be a sign of underdevelopment of the fetal airways, such as fewer but larger alveoli and impaired growth of the airways and lungs.[35, 36] **Figure 1.1.2** shows the placental and fetal circulation.

TEXTBOX 1.1.1 | Formula's to quantify placenta and fetal hemodynamics.

$$\text{Resistance Index (RI)} = \frac{\text{Peak systolic velocity}}{\text{Peak systolic velocity+Lowest diastolic velocity}}$$

$$\text{Pulsatiliy index (PI)} = \frac{\text{Peak systolic velocity-lowest diastolic velocity}}{\text{Mean peak systolic velocity}}$$

$$\text{Umbilical (U) /(middle) Cerebral (C) artery PI ratio} = \frac{\text{PI umbilical artery}}{\text{PI middle cerebral artery}}$$

$$\text{Time-velocity integral (TVI)} = \int \text{of each point under the curve with time}$$

$$\text{Left cardiac output} = \text{Vessel area} * \text{TVI} * \text{Fetal heart rate}$$

$$\text{Early (E) passive ventricular filling/Active (A) filling ratio} = \frac{\text{E wave}}{\text{A wave}}$$

TABLE 1.1.2 | Placental and fetal hemodynamic measures.

		Hemodynamic alterations in complicated pregnancies
Placental hemodynamics		
Uterine artery resistance index (RI)	Utero-placental vascular resistance, parameter primarily of the maternal circulation	↑
Umbilical artery pulsatiliy index (PI)	Feto-placental vascular resistance, parameter primarily of the fetal circulation	↑
Fetal cerebral hemodynamics		
Middle cerebral artery PI	Fetal cerebral blood flow	↓
U/(middle)C artery PI ratio	Fetal blood flow redistribution	↑
Fetal cardiac hemodynamics		
Aorta ascendens diameter	Inner diameter of the aorta	↓
Aorta ascendens PSV	Cardiac function and vascular compliance of the aorta	↓
Aorta ascendens TVI	Cardiac function and vascular compliance of the aorta	↓
Left cardiac output		↓
Mitral valve E wave	Cardiac flow-velocity waveforms at the level of the mitral valves. Measure of early passive ventricular filling	↓↑*
Mitral valve A wave	Cardiac flow-velocity waveforms at the level of the mitral valves. Measure of active (A) filling during atrial contraction	↓↑*
Mitral valve E/A ratio	Index for ventricular diastolic function and expresses both cardiac compliance and preload conditions	↓↑*
Fetal pulmonary hemodynamics		
Pulmonary artery time-velocity integral (TVI)	Pulmonary vascular resistance	↑

RI = resistance index, PI = pulsatiliy index, U/C = umbilical/cerebral, PSV = peak systolic velocity, TVI = time-velocity integral
*See text

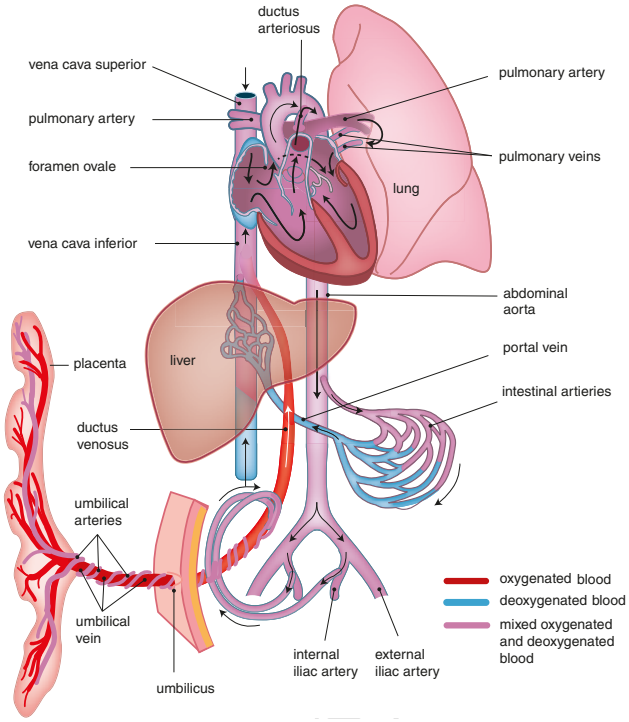

FIGURE 1.1.2 | Placental and fetal circulation[37] (*Printed with approval*).

Aim of this thesis

The aim of this thesis was to identify maternal determinants and childhood consequences of placental and fetal hemodynamic alterations.

Outline of this thesis

The objectives of this thesis are addressed in several studies. In chapter 1.2, we present the overall design of the study. Chapter 2 is focused on the potential associations of maternal metabolic profile with placenta and fetal hemodynamic alterations.

Chapter 3 presents studies on placental and fetal hemodynamics with childhood development. In chapter 3.1 we examined whether fetal blood flow redistribution is associated with childhood growth and fat distribution. The influences of fetal blood flow redistribution on childhood cardiovascular development, lung function, and kidney outcomes are presented in chapters 3.2, 3.3 and 3.4, respectively.

Finally, in chapter 4 the studies performed in this thesis are discussed, and suggestions for future research are presented.

References

1. Araujo JR, Keating E, Martel F. Impact of gestational diabetes mellitus in the maternal-to-fetal transport of nutrients. Curr Diab Rep. 2015;15(2):569.
2. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015;370(1663):20140066.
3. Carter AM, Enders AC, Pijnenborg R. The role of invasive trophoblast in implantation and placentation of primates. Philos Trans R Soc Lond B Biol Sci. 2015;370(1663):20140070.
4. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. Thromb Res. 2004;114(5-6):397-407.
5. Longtine MS, Nelson DM. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. Semin Reprod Med. 2011;29(3):187-96.
6. Degani S. Fetal cerebrovascular circulation: a review of prenatal ultrasound assessment. Gynecol Obstet Invest. 2008;66(3):184-96.
7. Bellotti M, Pennati G, De Gasperi C, Bozzo M, Battaglia FC, Ferrazzi E. Simultaneous measurements of umbilical venous, fetal hepatic, and ductus venosus blood flow in growth-restricted human fetuses. Am J Obstet Gynecol. 2004;190(5):1347-58.
8. Kiserud T, Kessler J, Ebbing C, Rasmussen S. Ductus venosus shunting in growth-restricted fetuses and the effect of umbilical circulatory compromise. Ultrasound Obstet Gynecol. 2006;28(2):143-9.
9. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. Obstet Gynecol. 1992;79(3):416-20.
10. Hernandez-Andrade E, Crispi F, Benavides-Serralde JA, Plasencia W, Diesel HF, Eixarch E, et al. Contribution of the myocardial performance index and aortic isthmus blood flow index to predicting mortality in preterm growth-restricted fetuses. Ultrasound Obstet Gynecol. 2009;34(4):430-6.
11. Figueras F, Eixarch E, Meler E, Iraola A, Figueras J, Puerto B, et al. Small-for-gestational-age fetuses with normal umbilical artery Doppler have suboptimal perinatal and neurodevelopmental outcome. Eur J Obstet Gynecol Reprod Biol. 2008;136(1):34-8.
12. Hernandez-Andrade E, Serralde JA, Cruz-Martinez R. Can anomalies of fetal brain circulation be useful in the management of growth restricted fetuses? Prenat Diagn. 2012;32(2):103-12.
13. Flood K, Unterscheider J, Daly S, Geary MP, Kennelly MM, McAuliffe FM, et al. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multicenter PORTO Study. Am J Obstet Gynecol. 2014;211(3):288 e1-5.
14. de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. Aust N Z J Obstet Gynaecol. 2006;46(1):4-14.
15. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
16. Morrison KM, Ramsingh L, Gunn E, Streiner D, Van Lieshout R, Boyle M, et al. Cardiometabolic Health in Adults Born Premature With Extremely Low Birth Weight. Pediatrics. 2016;138(4).
17. White SL, Perkovic V, Cass A, Chang CL, Poulter NR, Spector T, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Am J Kidney Dis. 2009;54(2):248-61.
18. Schellong K, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One. 2012;7(10):e47776.
19. Palatianou ME, Simos YV, Andronikou SK, Kiortsis DN. Long-term metabolic effects of high birth weight: a critical review of the literature. Horm Metab Res. 2014;46(13):911-20.
20. Skilton MR, Siitonen N, Wurtz P, Viikari JS, Juonala M, Seppala I, et al. High birth weight is associated with obesity and increased carotid wall thickness in young adults: the cardiovascular risk in young Finns study. Arterioscler Thromb Vasc Biol. 2014;34(5):1064-8.
21. Johnsson IW, Haglund B, Ahlsson F, Gustafsson J. A high birth weight is associated with increased risk of type 2 diabetes and obesity. Pediatr Obes. 2015;10(2):77-83.

22. Turner JM, Flatley C, Kumar S. A low fetal cerebroplacental ratio confers a greater risk of intrapartum fetal compromise and adverse neonatal outcomes in low risk multiparous women at term. Eur J Obstet Gynecol Reprod Biol. 2018;230:15-21.
23. Khalil AA, Morales-Rosello J, Morlando M, Hannan H, Bhide A, Papageorghiou A, et al. Is fetal cerebroplacental ratio an independent predictor of intrapartum fetal compromise and neonatal unit admission? Am J Obstet Gynecol. 2015;213(1):54 e1- e10.
24. Gaillard R, Steegers EA, Tiemeier H, Hofman A, Jaddoe VW. Placental Vascular Dysfunction, Fetal and Childhood Growth and Cardiovascular Development: The Generation R Study. Circulation. 2013;128(20):2202-10.
25. Wlodek ME, Westcott K, Siebel AL, Owens JA, Moritz KM. Growth restriction before or after birth reduces nephron number and increases blood pressure in male rats. Kidney Int. 2008;74(2):187-95.
26. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
27. Albaiges G, Missfelder-Lobos H, Parra M, Lees C, Cooper D, Nicolaides KH. Comparison of color Doppler uterine artery indices in a population at high risk for adverse outcome at 24 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21(2):170-3.
28. van den Wijngaard JA, Groenenberg IA, Wladimiroff JW, Hop WC. Cerebral Doppler ultrasound of the human fetus. Br J Obstet Gynaecol. 1989;96(7):845-9.
29. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
30. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: A pulsed Doppler study. J PERINAT MED. 1992;20(1):79-82.
31. Severi FM, Rizzo G, Bocchi C, D'Antona D, Verzuri MS, Arduini D. Intrauterine growth retardation and fetal cardiac function. Fetal Diagn Ther. 2000;15(1):8-19.
32. Gardiner H, Brodszki J, Marsal K. Ventriculovascular physiology of the growth-restricted fetus. Ultrasound Obstet Gynecol. 2001;18(1):47-53.
33. Rizzo G, Arduini D. Fetal cardiac function in intrauterine growth retardation. Am J Obstet Gynecol. 1991;165(4 Pt 1):876-82.
34. Godfrey ME, Messing B, Cohen SM, Valsky DV, Yagel S. Functional assessment of the fetal heart: a review. Ultrasound Obstet Gynecol. 2012;39(2):131-44.
35. Duijts L. Fetal and infant origins of asthma. Eur J Epidemiol. 2012;27(1):5-14.
36. Maritz GS, Cock ML, Louey S, Suzuki K, Harding R. Fetal growth restriction has long-term effects on postnatal lung structure in sheep. Pediatr Res. 2004;55(2):287-95.
37. Editors, Steegers EAP, Fauser BCJM, Hilders CGJM, Jaddoe VWV, Massuger LFAG, et al. Textbook of Obstetrics and Gynaecology - A life course approach: bohn stafleu van loghum; 2019.

Chapter 1

Chapter 1.2

The Generation R Study: design and cohort update 2017

Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IJzendoorn MH, de Jongste JC, Klaver CC, van der Lugt A, Mackenbach JP, Moll HA, Peeters RP, Raat H, Rings EH, Rivadeneira F, van der Schroeff MP, Steegers EAP, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius E, Felix JF, Jaddoe VWV

Adapted from Eur J Epidemiol. 2016;31(12):1243-1264

Abstract

The Generation R Study is a population-based prospective cohort study from fetal life until adulthood. The study is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development and health from fetal life, childhood and young adulthood. This multidisciplinary study focuses on several health outcomes including behaviour and cognition, body composition, eye development, growth, hearing, heart and vascular development, infectious disease and immunity, oral health and facial growth, respiratory health, allergy and skin disorders of children and their parents. Main exposures of interest include environmental, endocrine, genomic (genetic, epigenetic, microbiome), lifestyle related, nutritional and socio-demographic determinants. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Response at baseline was 61%, and general follow-up rates until the age of 10 years were around 80%. Data collection in children and their parents includes questionnaires, interviews, detailed physical and ultrasound examinations, behavioural observations, lung function, Magnetic Resonance Imaging (MRI) and biological sampling. Genome and epigenome wide association screens are available. Eventually, results from the Generation R Study contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Introduction

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The background and design have been described in detail previously.[1-7] Briefly, the Generation R Study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. This multidisciplinary study focuses on several health outcomes including behaviour and cognition, body composition, eye development, growth, hearing, heart and vascular development, infectious disease and immunity, oral health and facial growth, respiratory health, allergy and skin disorders of children and their parents. Main exposures of interest include environmental, endocrine, genomic (genetic, epigenetic, microbiome) lifestyle related, nutritional and socio-demographic determinants. Full lists of exposures and outcomes are presented in **Tables 1.2.1** and **1.2.2**. An important focus of the study is on the identification of new early life determinants of common non-communicable diseases in adulthood or there risk factors, on which various papers have been published recently in this journal.[8-26] A detailed and extensive data collection has been conducted over the years, starting in the early prenatal phase and currently in early adolescence (age 13 years). Data collection in parents and their children included questionnaires, interviews, detailed physical and ultrasound examinations, behavioural observations, lung function, Magnetic Resonance Imaging (MRI) and biological sampling. In this paper, we give an update of the data collection in the children and their parents until the child's age of 13 years.

TABLE 1.2.1 | Main outcomes per research area.

Maternal health	Cardiovascular health
	Endothelial (dys)function
	Pregnancy complications
	Risk factors for osteoporosis
	Risk factors for type 2 diabetes
Growth and physical development	Body composition and obesity
	Bone development
	Childhood growth patterns
	Dental development
	Dental caries
	Fetal growth patterns and organ development
	Myopia
	Physical characteristics and appearance
	Puberty stages
	Risk factors for cardiovascular disease
	Risk factors for type 2 diabetes
Behavioural and cognitive development	Attachment
	Behavioural and emotional problems
	Brain development
	Child psychopathology
	Child risk taking behaviour (alcohol, drugs, smoking)
	Child physical activity and sedentary behaviours
	Child sleeping patterns
	Compliance and moral development

Behavioural and cognitive development	Family interaction, parenting and child attachment Language delay Neuromotor development Neuropsychology – executive function Stress reactivity Use of social media Verbal and nonverbal cognitive development
Airways, asthma, allergy and skin disordes	Airways and lung structure Acne Allergy Asthma Eczema Hearing loss Lung function Physical (exercise) condition Microbiome skin Skin color
Infectious and inflammatory diseases	Celiac disease Infectious diseases and immune system
Health and healthcare	Health care utilization Social health inequalities Qualitiy of life

TABLE 1.2.2. | Main determinants.

Endocrine determinants	Maternal and fetal thyroid hormone levels Maternal thyroid autoimmunity Maternal hCG levels Childhood thyroid hormone and cortisol levels
Environmental determinants	Air pollution during pregnancy and childhood (PM10, NO_2) Bisphenol A, pesticides, phthalates Housing conditions Home environment
Genetic, epigenetic and microbiome determinants	Genetic variants (genome wide, candidate gene) DNA methylation (genome wide, candidate gene)
Lifestyle related determinants	Parental alcohol consumption Parental anthropometrics and obesity Parental smoking Parental working conditions Child anthropometrics and obesity Child music listening behaviour Child sedentary and physical activity behaviour Child smoking Dental care
Nutritional determinants	Maternal nutrition (products, patterns) Folic acid supplement use Breastfeeding Infant and childhood nutrition (timing, products, patterns) Nutritional biomarkers (folate, homocystein, vitamin B12, vitamin D)
Infection and micriobiota	Nasopharyngeal microbiota and bacterial carriage Faeces microbiota
Social-demographic determinants	Ethnicity Parental education, employment status and household income Parental marital status Parental psychopathology

Study Design

The Generation R Study is conducted in Rotterdam, the second largest city in the Netherlands. Rotterdam is situated in the Western part of the Netherlands. The study is a population-based prospective cohort study from fetal life onwards. Pregnant women with an expected delivery date between April 2002 and January 2006 living in Rotterdam were eligible for participation in the study. Extensive assessments are performed in mothers, fathers and their children. Measurements were planned in early pregnancy (gestational age <18 weeks), mid pregnancy (gestational age 18–25 weeks) and late pregnancy (gestational age > 25 weeks). The fathers were assessed once during the pregnancy of their partner. The children form a prenatally recruited birth cohort that will be followed at least until young adulthood. In the preschool period, which in the Netherlands refers to the period from birth until the age of 4 years, data collection was performed by a home-visit at the age of 3 months, and by repeated questionnaires and routine child health centers visits. Information from these routine visits was obtained and used for the study. Additional detailed measurements of fetal and postnatal growth and development were conducted in a randomly selected subgroup of Dutch children and their parents at a gestational age of 32 weeks and postnatally at the ages of 1.5, 6, 14, 24, 36 and 48 months in a dedicated research center.

Around the ages of 6 and 10 years all children and their parents were invited to visit our research center in the Erasmus MC-Sophia Children's Hospital to participate in hands-on measurements, advanced imaging modalities, behavioural observations and biological sample collection. MRI scans of all participating children were made in order to image abdominal composition, brain, lungs, cardiovascular system, fat tissue, kidney, liver, and hip development. Furthermore, the parents received 6 questionnaires during this period. Children also received their own questionnaire around the age of 10. Information from municipal health services, schools and general practicionars has also been collected.

In the current adolescence period, all children and their parents will be re-invited around the child's age of 13 and 16 years. We will again assess their growth, development and health in our research center and with questionnaires. We will perform MRI scans of the abdominal composition (fat), brain, and hip development.

Study Cohort

Eligibility and enrolment

Eligible mothers were those who were resident in the study area at their delivery date and had an expected delivery date from April 2002 until January 2006. We aimed to enrol mothers in early pregnancy but enrolment was possible until birth of their child. The enrolment procedure has been described previously in detail.[1-4] In total, 9,778 mothers were enrolled in the study. Of

these mothers, 91% (n = 8,879) was enrolled during pregnancy. Partners from mothers enrolled in pregnancy were invited to participate. In total, 71% (n = 6,347) of all fathers were included. A total of 1,232 pregnant women and their children form the subgroup of Dutch children for additional detailed studies. The overall response rate based on the number of children at birth was 61%.

The study group is an multi ethnic cohort. Ethnicity was defined according the classification of Statistics Netherlands.[27-32] Ethnic background was assessed in accordance with the country of birth of participants themselves and his or her parents. A participant was considered to have non-Dutch ethnic origin if one of her parents was born abroad. If both parents were born abroad, the country of birth of the participant's mother determined the ethnic background [33]. The largest ethnic groups were the Dutch, Surinamese, Turkish and Moroccan groups. We also constructed a dichotomous variable "Western/non-Western" ethnicity. Western ethnicity included Dutch, European, American Western (including North American), Asian Western (including Indonesian and Japanese) and Oceanian. Non-Western ethnicity included Turkish, Moroccan, Surinamese, Antillean, Cape Verdean, African, Asian (except Indonesia and Japan) and South American and Central American.[33, 34]

Response and follow-up

Figure 1.2.1 shows the enrolment and follow-up rates of the children and parents included in the Generation R Study. The 9,778 mothers enrolled in the study gave birth to 9,749 live born children. During the preschool period (0–4 years), the logistics of the postnatal follow-up studies were embedded in the municipal routine child care system and restricted to only part of the study area. In total 1,166 children lived outside this defined study area at birth and were therefore not approached for the postnatal follow-up studies during the preschool period. Of the remaining 8,583 children, 690 (8%) parents did not give consent, or their children died or were lost to follow-up, leaving 7,893 children for the preschool studies. At the age of 6 years (early school age), we invited all 9,278 children from the original cohort of 9,749 children to participate in follow-up studies. This invitation was independent of their home address and participation in the preschool period. In total, 8,305 children (90% of those who were invited (n = 9,278) and 85% of the original cohort (n = 9,749)) still participated in the study at this age, of whom 6,690 visited the research center at a median age of 6.0 years. For the follow-up phase at the age of 10 years (mid childhood period) 730 children of the 9,278 could not be invited. In total, 7,393 children (86% of those who were invited (n = 8,548) and 76% of the original cohort (n = 9,749)) participated in the study in mid childhood, of whom 5,862 visited the research center at a median age of 9.7 years. Of the 8,548 children invited in the mid childhood period, 456 had withdrawn and 124 children were lost to follow-up during this period, leaving 7,968 children for invitation around the age of 13 (early adolescence period).

Table 1.2.3 shows the general characteristics of the mothers who were enrolled in the study at baseline, and who remaind in the study until the child's age of 13 years. The median age of the women at enrolment was 30.5 (95% range, 19.3 - 39.6) years, 58% percent of those mothers were

of the Dutch nationality, 43% of the mothers were highly educated and 55% had a high household income. The mean birth weight of the children was 3,397 (SD 582) grams and they were born at a median gestational age of 40.0 (95% range, 34.9 - 42.3) weeks. Compared to the baseline characteristics, the mothers who still participated in the study at follow up were older, more frequently of Dutch nationality and higher educated.

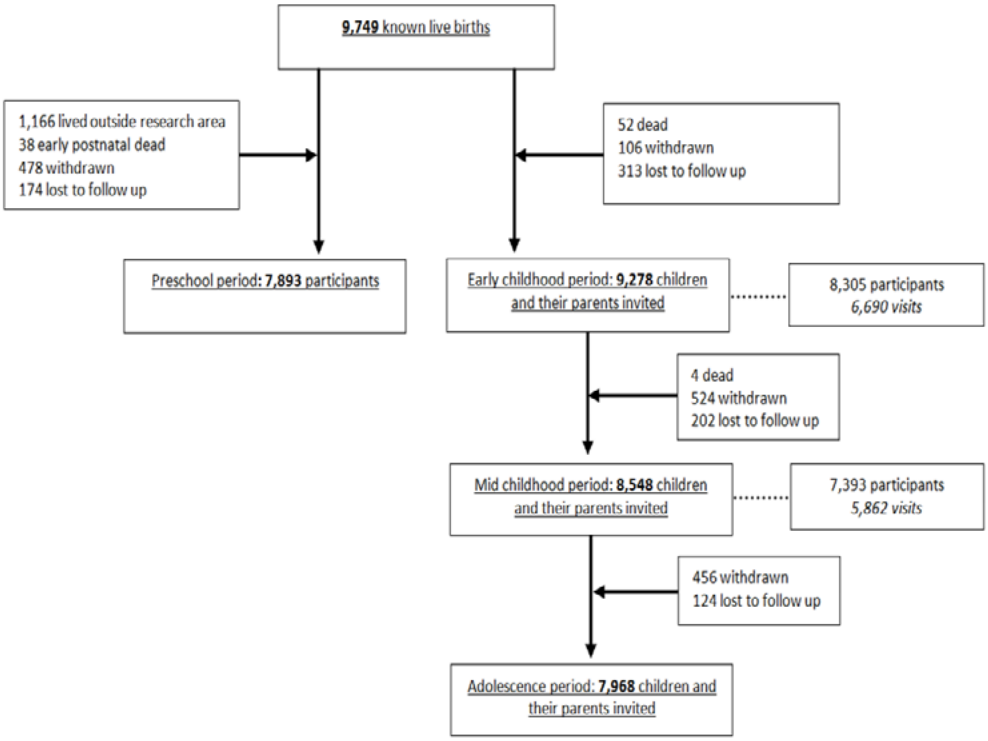

FIGURE 1.2.1 | Enrolment and follow-up rates in the Generation R Study.

TABLE 1.2.3 | General characteristics.

	Fetal period (n = 9,749)	Preschool period *0-5 years* (n = 7,893)	Early school age/ Mid childhood period *6-11 years* (n = 8,305)	Adolescence period *12-16 years* (n = 7,968)
Mothers				
Age at enrolment (years)	30.5 (19.3, 39.6)	31.0 (19.6, 39.8)	31.1 (19.9, 39.9)	31.3 (20.0, 39.9)
Ethnicity				
Dutch, other-European	58%	61%	64%	65%
Surinamese	9%	8%	8%	8%
Moroccan	6%	6%	6%	5%
Turkish	8%	8%	8%	7%
Dutch Antilles	3%	2%	2%	2%
Cape Verdian	4%	4%	4%	4%
Others	12%	11%	8%	9%
Educational level				
Low (no/primary education)	11%	10%	9%	8%
Intermediate (secondary school, vocational training)	46%	43%	42%	41%
High (Bachelor's degree, University)	43%	47%	49%	51%
Pre-pregnancy BMI	23.6 (4.4)	23.5 (4.2)	23.5 (4.1)	23.5 (4.1)
Net household income, per month				
<800 Euros	9%	8%	7%	6%
800-2200 Euros	36%	34%	32%	32%
>2200 Euros	55%	58%	61%	62%
Children				
Sex				
Male	51%	51%	51%	50%
Female	49%	49%	49%	50%
Ethnicity				
Dutch, other-European	62%	65%	67%	68%
Surinamese	8%	7%	7%	7%
Moroccan	7%	6%	6%	6%
Turkish	8%	8%	7%	6%
Dutch Antilles	4%	3%	3%	3%
Cape Verdian	3%	3%	3%	3%
Others	8%	8%	7%	7%
Birth weight (grams)	3397 (582)	3404 (572)	3412 (572)	3411 (576)
Gestational age at birth (weeks)	40.0 (34.9, 42.3)	40.0 (35.4, 42.3)	40.1 (35.4, 42.3)	40.1 (35.4, 42.3)

Values are means (standard deviation), percentages or medians (95% range)

Measurements

Data collection during pregnancy and fetal life

Physical examinations were planned at each visit in early pregnancy, mid pregnancy and late pregnancy and included height, weight and blood pressure measurements of both parents (**Table 1.2.4**). Mothers received four postal questionnaires and fathers received one postal questionnaire during pregnancy. Topics in these questionnaires were:

- Mother 1: medical and family history, previous pregnancies, quality of life, life style habits, housing conditions, ethnicity, and educational level;
- Mother 2: diet, including macronutrients and micronutrients;
- Mother 3: current pregnancy, quality of life, life style habits, and psychopathology;
- Mother 4: current pregnancy, quality of life, life style habits, working conditions, household income, and self-esteem;
- Father: medical history, family history, life style habits, educational level, and psychopathology.

Blood samples were collected in early (mother, father) and mid-pregnancy (mother) and at birth (child). A detailed overview of the design and response of the biological sample collection and available measurements is given elsewhere.[5-7]

Fetal ultrasound examinations were performed at each prenatal visit. These ultrasound examinations were used to establish gestational age and to assess fetal growth patterns.[35,36] These methods have previously been described in detail.[37-39] Longitudinal curves of all fetal growth measurements (head circumference, biparietal diameter, abdominal circumference and femur length) were created resulting in standard deviation scores for all of these specific growth measurements. Placental hemodynamics including resistance indices of the uterine and umbilical arteries have been measured in second and third trimester.[40-42] Detailed measurements of fetal brain, heart and kidney development were done in the subgroup.[40,43-48]

The obstetric records of mothers have been retrieved from hospitals and mid-wife practices to collect information about pregnancy progress and outcomes. Specialists in the relevant field coded items in these records.[49]

TABLE 1.2.4 | Assessments in mothers, fathers and their children during the fetal period.

	Early pregnancy	Mid pregnancy	Late pregnancy	Birth
Mother				
Physical examination	+	+	+	
Questionnaire	+	+	+	
Interview			S	
Fetal growth ultrasound exam	+	+	+	
Fetal organ ultrasound exam			S	
Blood sample	+	+		
Urine sample	+	+	+	
Father (or partner)				
Physical examination	+	+*	+*	
Questionnaire		+		
Psychiatric interview			S	
Blood sample	+			
Child				
Physical examination				+
Cord blood				+

+ = Assessment in whole cohort

S = Assessment only in subgroup

*In case of intake at mid- or late pregnancy

Early pregnancy: gestational age <18 weeks; mid pregnancy: gestational age 18–25 weeks; late pregnancy: gestational age >25 weeks

Data collection during the preschool period

At the age of 3 months, home visits were performed to assess neuromotor development using an adapted version of Touwen's Neurodevelopmental examination and to perform a home environment assessment.[50-53] Information about growth (length (height), weight, head circumference) was collected at each visit to the routine child health centers in the study area using standardized procedures (**Table 1.2.5**).[54]

During the preschool period, parents received 8 questionnaires, of which one was specifically for fathers. Items included in these questionnaires and their references are listed in **Table 1.2.6 and 1.2.7**. Response rates based on the number of sent questionnaires are shown in **Figure 1.2.2**. Not all children received each questionnaire due to logistical constraints and delayed implementation of some of the questionnaires after the first group of children reached the target age for those questionnaires. Thus, although response rates may be similar, the absolute number of completed questionnaires differs between different ages. Response rates presented in **Figure 1.2.2** are based on the number of sent questionnaires.

During the preschool period, children participating in the subgroup were invited six times to a dedicated research center. Measurements at these visits included physical examinations (height, weight, head circumference, skinfold thickness and waist—hip ratio, Touwen's Neurodevelopmental Examination) and ultrasound examinations (brain, cardiac and kidney structures).[44, 55-59] Dual X Energy Absorptiometry (DXA) scanning and Fractional exhaled Nitric Oxide (FeNO) measurements have been performed in a smaller subgroup.[60, 61] Blood pressure was measured at the age of 24 months.[62, 63] Observations of parent–child interaction and behaviour,

such as executive function, heart rate variability, infant-parent attachment, moral development, and compliance with mother and child have been repeatedly performed and with father and child once.[64-68] Biological materials were collected if parents gave consent.[69-71]

TABLE 1.2.5 | Assessments in mothers, fathers and children during the preschool period.

	Age (months)												
	2	**3**	**4**	**6**	**11**	**12**	**14**	**18**	**24**	**30**	**36**	**45**	**48**
Child													
Questionnaire (parent)	+	+		+		+		+	+	+	+		+
Physical examination	+	+	+	+	+		+		+		+	+	
Brain ultrasound	S												
Cardiac and renal ultrasound				S					S				
Blood pressure									S				
Airway inflammation				S					S				
Behavioural observation							S				S		S
Bacterial carriage	S			S			S		S		S		
Blood sample				S			S		S				
Mother													
Questionnaire		+		+							+		S
Interaction with child							S				S		
Father (or partner)													
Questionnaire											+		
Interaction with child													S

+ = Assessment in whole cohort

S = Assessment only in subgroup

TABLE 1.2.6 | Themes in postnatal questionnaires until early adolescence - Parental questionnaires.

Main themes	**2 months**	**6 months**	**12 months**	**18 months**	**24 months**	**30 months**	**36 months** [e]	**48 months**	**6 years** [f]	**10 years** [g]	**13 years**
Mother/father											
General health											
Quality of life [101]	+	+									
Pregnancy and complications	+								+		
Life events							+				
Medical history										+	
Lifestyle [102, 103]									+	+	+
Eating behaviour [104]											+
Social and demographic factors											
Housing and living conditions[a] [105, 106]	+	+			+			+	+	+	+
Work and working conditions		+							+		+
Educational level and household income					+		+		+	+	+
Family activities and social support [107, 108]		+								+	
Mental health and stress											
Parenting [109, 110]				+			+		+		
Depressive symptoms [111]	+									+	
Psychopathology [112-114]	+	+					+			+	
Family functioning [115, 116]									+	+	

Main themes	2 months	6 months	12 months	18 months	24 months	30 months	36 months [e]	48 months	6 years [f]	10 years [g]	13 years
Child											
Diet and physical activity											
Diet[b 117, 118]	+	+	+		+ / S				+		
Eating behaviour [119-127]					+			+	+	+	+
Television watching, use of computer and physical activity [128-131]					+		+	+	+	+	
Day-care, School		+	+				+		+	+	+
Childhood health and diseases											
Quality of life [132-135]					+		+		+	+	
Fever and infectious diseases [136]	+	+	+		+		+	+	+	+	
Asthma, Asthma related symptoms and eczema [137-140]		+	+		+		+	+	+	+	+
Acne [141]											+
Allergy		+	+							+	+
Accidents [142, 143]		+		+	+				+	+	+
Seizures[c]	+	+	+		+		+	+	+		
Abdominal pain, stool pattern [144]					+		+	+	+	+	
Doctors visit	+	+			+		+	+	+	+	
Teeth and dental care [145-148]									+		+
Physical characteristics									+		
Hearing (listen to music, use of headphone) [149]										+	+
Vision/Eyes (glasses, viewing habits ("close" and "far away"))										+	+
Behaviour and cognition											
Sleeping, crying and soothing [150-152]	+	+	+		+		+			+	
Temperament [153-156]	+								+		+
Motor development [157]		+	+	+	+			+			
Behaviour and emotional problems [158-161]		+		+			+		+	+	+
Pain perception [162-164]					+		+		+		
Language development [165]				+		+				+	+
Non-verbal cognition [166]					+	+					
Executive function [167]								+			
Prosocial behaviour [168-171]									+		+
Autistic traits [172-174]									+		+
Obsessive compulsive disorder [175]										+	+
Bullying									+		
Social media use [176, 177]											+

+ = Assessment in whole cohort. S = Assessment only in subgroup

a Housing and living conditions include information about family structure, poverty, (environmental) smoking and pets

b Diet questionnaires included in 2, 6 and 12 months questionnaire. Additional food frequency questionnaires at 12 months for all Dutch speaking children and at 24 months for the Dutch subgroup children

c Screening 10 items questionnaire on seizures. Screen positives receive additional questionnaire and are being asked for their medical records

d Infant Behaviour Questionnaire at the age of 6 months, Child Behaviour Checklist thereafter

e For parenting, psychopathology and child behaviour additional questionnaire for fathers

f Diet and part of behaviour and cognition additional at the age of 8 years

g For medical history, lifestyle, depressive symptoms, psychopathology, family activities, behaviour and emotional problems additional questionnaire for fathers

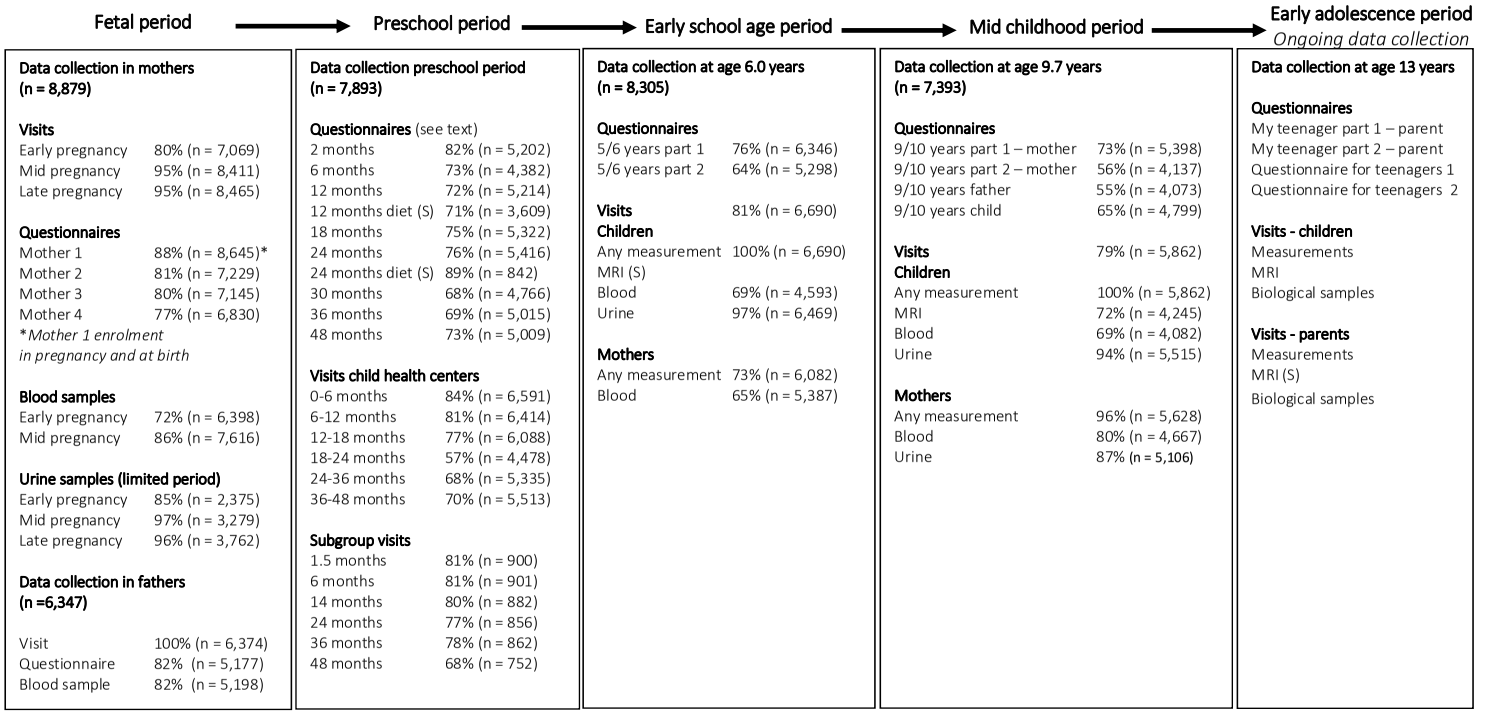

FIGURE 1.2.2 | Response to the questionnaires and visits in the Generation R Study.
S = Assessment only in subgroup

TABLE 1.2.7 | Themes in postnatal questionnaires - child questionnaire.

Main themes	10 years	13 years
Friendships [161, 178]	+	+
Bullying [179-181]		+
General health [132]		+
Abdominal pain, stool pattern [182]		+
Social status [183]		+
Development and well-being [122, 184, 185]		+
Eating behaviour [126, 127, 186-189]	+	+
Television watching and physical activity [128, 131, 190, 191]	+	+
Temperament [192, 193]		+
Behaviour [161, 175, 194, 195]	+	+
Body Image [196, 197]	+	+
Self-perception [198-200]	+	+
Sleeping behaviour [201-204]	+	+
Puberty stages [203, 205]		+
Social media [176, 177]		+
Hearing (listen to music, use of headphone)		+
Vision (viewing habits ("close" and "far away"))		+

+ = Assessment in whole cohort

Data collection during the early school age, mid childhood and adolescence period

From the age of 6 years onwards, we invite all participating children to a well-equipped and dedicated research center at the Erasmus MC-Sophia Children's Hospital every 3 to 4 years. Visits at age 6 and 10 years have been completed, at age 13 years are ongoing and age 16 years are being planned.

Currently, the total visit takes about 3 hours and all measurements are grouped in thematic 35 minutes blocks. Clinically relevant results are discussed with the children and their parents and, if needed, children or parents are referred to their general practitioner or other relevant health care provider.

At each age, we collect data using questionnaires on growth, health and physical and mental development of the children. Also, we collect information on childhood diet and behaviour (**Table 1.2.6, 1.2.7**). These questionnaires are sent to the primary caregiver. The measurements at the research center are focused on several health parameters including behaviour and cognition, body composition, bone health and muscle function, eye development, growth, hearing, heart and vascular development, infectieus diseases and immunity, oral health and facial growth, respiratory health, allergy and skin disorders (**Table 1.2.8**).[72-79] We use various advanced imaging techniques including ultrasound and Doppler (GE LOGIQ E9, Milwaukee, WI, USA) for measuring thoracic and abdominal structures, Dual X Absorptiometry for measuring body composition and bone mineral density (iDXA scanner, GE Healthcare, Madison, WI, USA) and Peripheral Quantitative Computed Tomography (PQCT, Stratec Medicin Technik, Pforzheim, Germany) for measuring bone mineral density and geometry of the tibia. We use orthopantomograms (OP 200 D, Intrumentarium Dental, Tuusula, Finland) for measuring dental development.

MRI has been used for brain imaging in a subgroup (n=801) of 6-8 year old children using a hospital-based 3.0 Tesla MRI scanner (Discovery MR750, GE Healthcare, Milwaukee, WI, USA).[80-83] From 2014 onwards, we use a dedicated 3.0 Tesla MRI (Discovery MR750, GE Healthcare, Milwaukee, WI, USA) for brain and total body imaging of all children participating in the study at the mid childhood visit (age 10 years) (see **Table 1.2.9** for the MRI outcome measures). We use a mock MRI scanner, to familiarize the children and get use to the scanning procedures. Children are scanned using standard imaging and positioning protocols, wearing light clothing without metal objects while undergoing the scanning procedure. Total scanning time amounts to approximately 60 minutes. The scanner is operated by trained research technicians and all imaging data are collected according to standardized imaging protocols. Changes or updates in hardware are avoided. Changes or updates in software configuration are minimized and regular checks with phantoms are performed to secure validity of cross-subject and cross-scan comparisons. Imaging is performed without administration of contrast agents. All imaging data are stored on a securely backed-up research picture archiving system, using programmed scripts to check for completeness of the data received. We will re-scanning the abdominal composition (fat), brain imaging and hip development during adolescence (age 13 years) of all participating children in Generation R. MRI scan of the brains will also be conducted in the parents of a subgroup of Generation R participants. This research is focused on aging effects of the brains in young adults and follow up of mothers who experienced gestational hypertensive complications.

Blood and urine samples are collected in the mothers and their children during every visit. A detailed overview of the design and response of the biological sample collection and available measures is given elsewhere.[5-7]

TABLE 1.2.8 | Assessments in mothers and children during early school age, mid childhood and early adolescence visit.

	Early school age median age 6.0 years 95% range 5.6-7.9	**Mid childhood** median age 9.7 years 95% range 9.4-10.8	**Early adolescence** 13 years ongoing datacollection
Mother			
Behaviour and cognition			
Cognition	+		
Dutch language skills			+
Interaction with child		+	
Life events		+	
Interview about health, parenting, family situation, depression			+
Maternal health			
Anthropometrics and blood pressure	+	+	+
Arterial stiffness	+		
Endothelial function			+
Body composition and bone mineral density (DXA)	+	+	
Intima-media thickness		+	
Physical appearance	+	+	
Ultrasound heart	+		
Eyes; retinal vasculature, refraction	+		

	Early school age median age 6.0 years 95% range 5.6-7.9	**Mid childhood** median age 9.7 years 95% range 9.4-10.8	**Early adolescence** 13 years ongoing datacollection
Biological samples			
Blood sample		+	+
Urine sample		+	+
Hair sample	+		
Child			
Behaviour and cognition			
Behaviour and behavioural observation	+	+	+
Cognition	+	+	+
Language development	+	+	+
Pain perception	+		
Risk taking interview			+
Cardiovascular and metabolic development			
Anthropometrics and blood pressure	+	+	+
Arterial stiffness	+		
Body composition and bone mineral density (DXA)	+	+	+
Bone mineral density and geometry of the tibia (PQCT)		+	+
Intima-media thickness		+	+
Ultrasound abdominal fat	+		+
Ultrasound heart	+	+	
Ultrasound kidney	+		
Physical appearance		+	+
Puberty stages (Tanner)			+
Eyes, ears and mouth			
Eyes; visual acuity, retinal picture, refraction, IOL master, OCT	+	+	+
Dental status and development	+	+	+
Face development		+	+
Hearing		+	+
Taste experience	+		
Lungs			
Airway inflammation	+		
Lung function	+	+	+
Exercise test (SRT)			+
Allergy test		+	
Dermatology			
Spectrophotometry			+
Biological samples			
Nasopharynx bacterial carriage	+	+	
Blood and urine sample	+	+	+
Dental plaque			
Faeces microbiota		+	
Hair sample	+	+	+
Saliva	+	+	
Skin swab (head, elbow)			+

DXA = Dual energy X-ray Absorptiometry scan, PQCT = Peripheral Quantitative Computertomografie Scan, SRT = Steep Ramp Test, IOL = Intraoculaire Measurement, OCT = Optical Coherence Tomografie

S = assessment only in subgroup

TABLE 1.2.9 | MRI measurements in children of the Generation R Study.

	Early school age median age 8.0 years 95% range 6.3-10.1	**Mid childhood** median age 9.9 years 95% range 9.5-11.9	**Early adolescence** 13 years ongoing datacollection
Children			
Brain measurements			
Structural imaging			
3D T1-weighted GRE sequence	X(S)	X	X
2D-PD-weighted TSE sequence	X(S)	X	X
Diffusion tensor imaging (DTI)	X(S)	X	X
Resting state functional MRI	X(S)	X	X
Lungs			
Inspiratory volume		X	
Expiratory volume		X	
Sizes of the trachea		X	
Sizes of the main bronchi		X	
Chronic obstructive lung problems			
Air trapping		X	
Atelectasis		X	
Cardiac measurements			
Structural cardiac measurements		X	
Diastolic volume		X	
Cardiac mass		X	
Functional cardiac measurements		X	
Systolic volume		X	
Ejection fraction		X	
Stroke volume		X	
Aortic diameter		X (S)	
Total visceral adipose tissue from top of liver to femur head			
Fat volume/mass		X	x
Subcutaneous adipose tissue from top of liver to femur head			
Fat volume/mass		X	x
Pericardial fat			
Fat volume/mass		X	x
Kidney			
Length		X	
Width		X	
Depth		X	
Volume		X	
Liver			
Fat fraction		X	
Liver volume		X	
Structure and morphology of the hipbone		X	X
Testicular volume		X	
Ovarial volume		X	

S = assessment only in subgroup

Genomics: genetic, epigenetic and microbiome biobank

DNA from parents and children has been extracted and used for genotyping using taqman analyses for individual genetic variants and using a genome-wide association scan (GWAS) using the Illumina 670 K platform in the children.[5-7] For genotyping, we used the infrastructure of the Human Genomics Facility (HuGe-F) of the Genetic Laboratory of the Department of Internal Medicine (www.glimdna.org). The GWAS dataset underwent a stringent QC process, which has been described in detail previously.[5-7, 84] Most GWAS analyses are strongly embedded in the Early Growth Genetics (EGG) (http://egg-consortium.org/) and Early Genetics and Longitudinal Epidemiology (EAGLE) Consortia, in which several birth cohort studies combine their GWAS efforts focused on multiple outcomes in fetal life, childhood and adolescence. These efforts have already led to successful identification of various common genetic variants related to birth weight, infant head circumference, childhood body mass index, bone development and obesity and atopic dermatitis.[85-91] DNA from parents is used for genotyping for candidate gene or replication studies.

DNA methylation was measured on a genome wide level in a subgroup of Dutch children, using the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). We used cord blood samples of 1,339 children, blood samples in 469 children aged 6 years and blood samples in 425 children aged 10 years. Quality control and normalization of analyzed samples was performed using standardized criteria. Many of the epigenome-wide association analyses are performed in the context of the Pregnancy And Childhood Epigenetics (PACE) Consortium (http://www.niehs.nih.gov/research/ atniehs/labs/epi/pi/genetics/pace/index.cfm), which brings together studies with epigenome-wide DNA-methylation data in pregnant women, newborns and/or children. Recent studies have identified differentially methylated sites in association with maternal smoking, maternal folate levels, maternal stress and air pollution during pregnancy.[92-95]

Gut microbiota profiles were determined by Next Generation Sequencing (on Illumina MiSeq) of the V3 and V4 variable regions of the 16S ribosomal RNA gene in DNA extracted from feacal samples. Samples were collected at mid childhood in 2414 children. Phylogenetic *de novo* profiling was performed using the QIIME [96] and USEARCH [97] software packages and resulted in an operational taxonomic unit table with 239 species, 109 genera and 8 phyla. For example, those samples can be used for studying the effects of the fecel microbiota with overweight or obesity.[98-100]

Ethics

The general design, all research aims and the specific measurements in the Generation R Study have been approved by the Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam. New measurements are only introduced into the study after approval of the Medical Ethical Committee. Participants need to give written informed consent for each phase of the study (fetal, preschool, childhood and adolescence period). From the age of 12 years onwards, children must sign their own consent form, in accordance with Dutch Law. At the start of each phase, children and their parents receive written and oral information about the study. Even with consent, when the child or the parents are not willing to participate actively, specific measurements are skipped or no measurements at all are performed.

Follow-up and retention strategies

Thus far, loss to follow-up has been lower than 10%. Major efforts are made to keep the children and parents involved in the study and to minimize loss to follow-up. Several strategies have been implemented and are currently part of the study design:

- Addresses: new addresses of participants, which are known by the municipal health service, can be retrieved by the study staff;
- Newsletters: participants receive two to four newsletters per year, in which several results of the study are presented and explained, questions of participants are answered and new research initiatives are presented;
- Facebook: every week we post a short news update about the ongoing research on our facebook page;
- Website: we have an up-to-date website where participants can find information about the ongoing research, the procedures at the dedicated research center and our contact information;
- Presents and discounts: all children who visit our research center receive small presents. Also, discount offers are regularly presented in the newsletter;
- Transport costs: all costs for transport and parking related to visits to the research center are reimbursed;
- Reminders for questionnaires: when the questionnaire has not been returned within 3 weeks, a kind reminder letter is sent to the parents. After 6 weeks, if the questionnaire still has not been returned, the parents receive a phone call. If necessary, help with completing the questionnaire is offered and the importance of filling out the questionnaire is explained once more during this phone call;
- Individual feedback: if clinically relevant, results of measurements are discussed with the parents and children at the visit. If necessary, follow-up appointments with the general practitioner are planned;

- Support for non-Dutch speaking participants: all study materials such as questionnaires, newsletters, website, and information folders are available in three languages (Dutch, English, and Turkish). Furthermore, staff from different ethnic backgrounds is available and verbally translate these materials into Arabic, French and Portuguese. As such, the study staff is able to communicate with all participants;
- Additional help: children and parents who showed low response rates for different measurements, showed difficulties in completing questionnaires or require additional explanation or support are pro-actively contacted by one dedicated member of the study staff;
- Home visits: We visit children and parents who cannot be contacted by phone, e-mail or letter. Most visits are planned in the evenings to have higher chances that both parents and children are at home.

Power, datamanagement, privacy protection

Power calculations for the Generation R Study are shown in **Tables 1.2.10** and **1.2.11**. Due to missing values and loss to follow-up, most analyses in the study are not based on data in all subjects. Therefore, these power calculations demonstrated are based on 7,000 subjects in the whole cohort and 700 subjects in the subgroup. The presented power calculations are conservative since most studies will assess the effects of continuous instead of dichotomous exposures and studies may be focused on outcomes collected in more than only 1 year.

From 2016 onwards, data collected during the measurements at the research center are entered directly into an electronic database. Data collected by questionnaires are scanned and manually entered into an electronic database by a commercial company. Random samples of all questionnaires are double checked by study staff members to monitor the quality of this manual data entry process. The percentage of mistakes does not exceed 3% per questionnaire. Open text fields are entered into the electronic database exactly as they are filled in on the questionnaires. In a secondary stage, these open text fields are cleaned and coded by a specialist in the relevant field.

All measurements are centrally checked by examination of the data including their ranges, distributions, means, standard deviations, outliers and logical errors. Data outliers and missing values are checked with the original forms. The data of one specific measurement are only distributed for analyses after data collection and preparation is completed for that measurement for the whole cohort.

Datasets needed for answering specific research questions are centrally constructed from different databases. All information in these datasets that enables identification of a particular participant, including names and dates of birth, is excluded before distribution to the researchers. The datasets for researchers include unique identification numbers for each subject that enable

feedback about individuals to the datamanager but do not enable identification of that particular subject. Currently, we are exploring possibilities for a remote access environment, in which researchers can access centrally stored research data from their own computer without storing such data locally.

TABLE 1.2.10 | Effects sizes that can minimally be detected according to the prevalence of the exposure.

Proportion exposed (%)	Whole cohort (n = 7,000)	Subgroup (n = 700)
50	0.067	0.212
25	0.077	0.276
10	0.112	0.353
5	0.154	0.486
1	0.337	1.064

The presented effect sizes are detectable proportions of the standard deviation with a type I error of 5% and a type II error of 20% (power 80%).

TABLE 1.2.11 | Relative risks that can minimally be detected according to the prevalence of the exposure.

Proportion exposed (%)	Incidence (1 year) of outcome of interest					
	Whole cohort (n = 7,000)			Subgroup (n = 700)		
	10 %	5 %	1 %	10 %	5 %	1%
50	1.23	1.33	1.83	1.83	2.28	4.94
25	1.26	1.38	1.94	1.96	2.46	5.41
10	1.39	1.56	2.42	2.48	3.26	7.92
5	1.55	1.80	3.09	3.20	4.39	11.74
1	2.36	3.04	6.83	7.75	11.61	37.55

The presented effect sizes are detectable relative risks with a type I error of 5% and a type II error of 20% (power 80%).

Collaboration

The Generation R Study is conducted by several research groups from the Erasmus MC in close collaboration with the Erasmus University Rotterdam and the Municipal Health Service Rotterdam area. Since the data collection is still ongoing and growing, the number of collaborating research groups in and outside the Netherlands is expected to increase. Various research projects are performed as part of ongoing European or worldwide collaboration projects. The study has an open policy with regard to collaboration with other research groups. Request for collaboration can be sent to Vincent Jaddoe (v.jaddoe@erasmusmc.nl). These requests will be discussed in the Generation R Study Management Team regarding their study aims, overlap with ongoing studies, logistic consequences and related finances. After approval of a project by the Generation R Study Management Team and the Medical Ethical Committee of Erasmus MC, the collaborative research project is embedded in one of the research areas supervised by the corresponding principal investigator.

References

1. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol. 2004;18(1):61-72.
2. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23(12):801-11.
3. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25(11):823-41.
4. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van IJzendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.
5. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22(12):917-23.
6. White T, el Marroun H, Nijs I, Schmidt M, van der Lugt A, Wielopolki PA, et al. Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology. Eur J Epidemiol. 2013;28(1):99-111.
7. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, et al. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29(12):911-27.
8. Duijts L, Reiss IK, Brusselle G, de Jongste JC. Early origins of chronic obstructive lung diseases across the life course. Eur J Epidemiol. 2014;29(12):871-85.
9. Eveborn GW, Schirmer H, Lunde P, Heggelund G, Hansen JB, Rasmussen K. Assessment of risk factors for developing incident aortic stenosis: the Tromso Study. Eur J Epidemiol. 2014;29(8):567-75.
10. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol. 2015;30(11):1141-52.
11. Gunnell AS, Knuiman MW, Divitini ML, Cormie P. Leisure time physical activity and long-term cardiovascular and cancer outcomes: the Busselton Health Study. Eur J Epidemiol. 2014;29(11):851-7.
12. Horvei LD, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Hansen JB. Obesity measures and risk of venous thromboembolism and myocardial infarction. Eur J Epidemiol. 2014;29(11):821-30.
13. Jiang W, Ju C, Jiang H, Zhang D. Dairy foods intake and risk of Parkinson's disease: a dose-response meta-analysis of prospective cohort studies. Eur J Epidemiol. 2014;29(9):613-9.
14. Kunutsor SK, Burgess S, Munroe PB, Khan H. Vitamin D and high blood pressure: causal association or epiphenomenon? Eur J Epidemiol. 2014;29(1):1-14.
15. Liu XM, Liu YJ, Zhan J, He QQ. Overweight, obesity and risk of all-cause and cardiovascular mortality in patients with type 2 diabetes mellitus: a dose-response meta-analysis of prospective cohort studies. Eur J Epidemiol. 2015;30(1):35-45.
16. Luczynska A, Logan C, Nieters A, Elgizouli M, Schottker B, Brenner H, et al. Cord blood 25(OH)D levels and the subsequent risk of lower respiratory tract infections in early childhood: the Ulm birth cohort. Eur J Epidemiol. 2014;29(8):585-94.
17. Schmidt M, Botker HE, Pedersen L, Sorensen HT. Adult height and risk of ischemic heart disease, atrial fibrillation, stroke, venous thromboembolism, and premature death: a population based 36-year follow-up study. Eur J Epidemiol. 2014;29(2):111-8.
18. Sandvei MS, Lagiou P, Romundstad PR, Trichopoulos D, Vatten LJ. Size at birth and risk of breast cancer: update from a prospective population-based study. Eur J Epidemiol. 2015;30(6):485-92.
19. Aune D, Norat T, Leitzmann M, Tonstad S, Vatten LJ. Physical activity and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis. Eur J Epidemiol. 2015;30(7):529-42.
20. Etemadi A, Abnet CC, Kamangar F, Islami F, Khademi H, Pourshams A, et al. Impact of body size and physical activity during adolescence and adult life on overall and cause-specific mortality in a large cohort study from Iran. Eur J Epidemiol. 2014;29(2):95-109.
21. Ueda P, Cnattingius S, Stephansson O, Ingelsson E, Ludvigsson JF, Bonamy AK. Cerebrovascular and ischemic heart disease in young adults born preterm: a population-based Swedish cohort study. Eur J Epidemiol. 2014;29(4):253-60.

22. Engeland A, Bjorge T, Klungsoyr K, Skjaerven R, Skurtveit S, Furu K. Preeclampsia in pregnancy and later use of antihypertensive drugs. Eur J Epidemiol. 2015;30(6):501-8.
23. Vatten LJ. Dimitrios Trichopoulos and the early life origins of breast cancer. Eur J Epidemiol. 2015;30(6):463-4.
24. Lagiou P, Samoli E, Hsieh CC, Lagiou A, Xu B, Yu GP, et al. Maternal and cord blood hormones in relation to birth size. Eur J Epidemiol. 2014;29(5):343-51.
25. Rydell M, Granath F, Cnattingius S, Magnusson C, Galanti MR. In-utero exposure to maternal smoking is not linked to tobacco use in adulthood after controlling for genetic and family influences: a Swedish sibling study. Eur J Epidemiol. 2014;29(7):499-506.
26. Greenwood DC, Thatcher NJ, Ye J, Garrard L, Keogh G, King LG, et al. Caffeine intake during pregnancy and adverse birth outcomes: a systematic review and dose-response meta-analysis. Eur J Epidemiol. 2014;29(10):725-34.
27. Statistics Netherlands, Allochtonen in Nederland: Statistics Netherlands, Den Haag/Heerlen; [Available from: http://statline.cbs.nl/statweb/].
28. Troe EJ, Raat H, Jaddoe VW, Hofman A, Looman CW, Moll HA, et al. Explaining differences in birthweight between ethnic populations. The Generation R Study. BJOG. 2007;114(12):1557-65.
29. Silva LM, Jansen PW, Steegers EA, Jaddoe VW, Arends LR, Tiemeier H, et al. Mother's educational level and fetal growth: the genesis of health inequalities. Int J Epidemiol. 2010;39(5):1250-61.
30. Raat H, Wijtzes A, Jaddoe VW, Moll HA, Hofman A, Mackenbach JP. The health impact of social disadvantage in early childhood; the Generation R study. Early Hum Dev. 2011;87(11):729-33.
31. Nationaal Kompas: Volksgezondheid en zorg Nederland; [Available from: http://www.nationaalkompas.nl/bevolking/etniciteit/wat-is-etniciteit/].
32. Maritz GS, Cock ML, Louey S, Suzuki K, Harding R. Fetal growth restriction has long-term effects on postnatal lung structure in sheep. Pediatr Res. 2004;55(2):287-95.
33. Troe EJ, Raat H, Jaddoe VW, Hofman A, Steegers EA, Verhulst FC, et al. Smoking during pregnancy in ethnic populations: the Generation R study. Nicotine Tob Res. 2008;10(8):1373-84.
34. Wijtzes AI, Jansen W, Jaddoe VW, Moll HA, Tiemeier H, Verhulst FC, et al. Ethnic background and television viewing time among 4-year-old preschool children: the generation R study. J Dev Behav Pediatr. 2013;34(2):63-71.
35. Gaillard R, Jaddoe VW. Assessment of fetal growth by customized growth charts. Ann Nutr Metab. 2014;65(2-3):149-55.
36. Jaddoe VW, de Jonge LL, Hofman A, Franco OH, Steegers EA, Gaillard R. First trimester fetal growth restriction and cardiovascular risk factors in school age children: population based cohort study. BMJ. 2014;348:g14.
37. Gaillard R, de Ridder MA, Verburg BO, Witteman JC, Mackenbach JP, Moll HA, et al. Individually customised fetal weight charts derived from ultrasound measurements: the Generation R Study. Eur J Epidemiol. 2011;26(12):919-26.
38. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.
39. Verburg BO, Mulder PG, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenat Diagn. 2008;28(4):323-31.
40. Kooijman MN, Gaillard R, Reiss I, Hofman A, Steegers EA, Jaddoe VW. Influence of fetal blood flow redistribution on fetal and childhood growth and fat distribution: the Generation R Study. BJOG. 2016.
41. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring). 2013;21(5):1046-55.
42. Rurangirwa AA, Gaillard R, Steegers EA, Hofman A, Jaddoe VW. Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women; the Generation R study. Am J Hypertens. 2012;25(8):892-9.
43. Geelhoed JJ, Steegers EA, van Osch-Gevers L, Verburg BO, Hofman A, Witteman JC, et al. Cardiac structures track during the first 2 years of life and are associated with fetal growth and hemodynamics: the Generation R Study. Am Heart J. 2009;158(1):71-7.
44. Geelhoed JJ, Verburg BO, Nauta J, Lequin M, Hofman A, Moll HA, et al. Tracking and determinants of kidney size from fetal life until the age of 2 years: the Generation R Study. Am J Kidney Dis. 2009;53(2):248-58.
45. Roza SJ, Steegers EA, Verburg BO, Jaddoe VW, Moll HA, Hofman A, et al. What is spared by fetal brain-sparing? Fetal circulatory redistribution and behavioral problems in the general population. Am J Epidemiol. 2008;168(10):1145-52.

46. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
47. Roza SJ, Verburg BO, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA, et al. Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study. Eur J Neurosci. 2007;25(3):611-7.
48. Kooijman MN, Bakker H, van der Heijden AJ, Hofman A, Franco OH, Steegers EA, et al. Childhood kidney outcomes in relation to fetal blood flow and kidney size. J Am Soc Nephrol. 2014;25(11):2616-24.
49. Coolman M, de Groot CJ, Jaddoe VW, Hofman A, Raat H, Steegers EA. Medical record validation of maternally reported history of preeclampsia. J Clin Epidemiol. 2010;63(8):932-7.
50. van Batenburg-Eddes T, de Groot L, Arends L, de Vries A, Moll HA, Steegers EA, et al. Does gestational duration within the normal range predict infant neuromotor development? Early Hum Dev. 2008;84(10):659-65.
51. Rijlaarsdam J, Stevens GW, van der Ende J, Arends LR, Hofman A, Jaddoe VW, et al. A brief observational instrument for the assessment of infant home environment: development and psychometric testing. Int J Methods Psychiatr Res. 2012;21(3):195-204.
52. van Batenburg-Eddes T, Henrichs J, Schenk JJ, Sincer I, de Groot L, Hofman A, et al. Early infant neuromotor assessment is associated with language and nonverbal cognitive function in toddlers: the Generation R Study. J Dev Behav Pediatr. 2013;34(5):326-34.
53. Rijlaarsdam J, Tiemeier H, Hofman A, Jaddoe VW, Mackenbach JP, Verhulst FC, et al. Home environments of infants: relations with child development through age 3. J Epidemiol Community Health. 2013;67(1):14-20.
54. Burgmeijer RJ, Merkx JA. Pakket... en hoe pakt het uit? Ouder- en Kindzorg tussen wetenschap en praktijk. Assen, the Netherlands: van Gorcum. 1999.
55. Ay L, Hokken-Koelega AC, Mook-Kanamori DO, Hofman A, Moll HA, Mackenbach JP, et al. Tracking and determinants of subcutaneous fat mass in early childhood: the Generation R Study. Int J Obes (Lond). 2008;32(7):1050-9.
56. Durmus B, Mook-Kanamori DO, Holzhauer S, Hofman A, van der Beek EM, Boehm G, et al. Growth in foetal life and infancy is associated with abdominal adiposity at the age of 2 years: the generation R study. Clin Endocrinol (Oxf). 2010;72(5):633-40.
57. Roza SJ, Govaert PP, Vrooman HA, Lequin MH, Hofman A, Steegers EA, et al. Foetal growth determines cerebral ventricular volume in infants The Generation R Study. Neuroimage. 2008;39(4):1491-8.
58. de Jonge LL, van Osch-Gevers L, Willemsen SP, Steegers EA, Hofman A, Helbing WA, et al. Growth, obesity, and cardiac structures in early childhood: the Generation R Study. Hypertension. 2011;57(5):934-40.
59. Kok R, van IJzendoorn MH, Linting M, Bakermans-Kranenburg MJ, Tharner A, Luijk MP, et al. Attachment insecurity predicts child active resistance to parental requests in a compliance task. Child Care Health Dev. 2013;39(2):277-87.
60. Ay L, van Houten VA, Steegers EA, Hofman A, Witteman JC, Jaddoe VW, et al. Fetal and postnatal growth and body composition at 6 months of age. J Clin Endocrinol Metab. 2009;94(6):2023-30.
61. Gabriele C, Asgarali R, Jaddoe VW, Hofman A, Moll HA, de Jongste JC. Smoke exposure, airway symptoms and exhaled nitric oxide in infants: the Generation R study. Eur Respir J. 2008;32(2):307-13.
62. van Houten VA, Steegers EA, Witteman JC, Moll HA, Hofman A, Jaddoe VW. Fetal and postnatal growth and blood pressure at the age of 2 years. The Generation R Study. J Hypertens. 2009;27(6):1152-7.
63. van Houten VA, Mook-Kanamori DO, van Osch-Gevers L, Steegers EA, Hofman A, Moll HA, et al. A variant of the IGF-I gene is associated with blood pressure but not with left heart dimensions at the age of 2 years: the Generation R Study. Eur J Endocrinol. 2008;159(3):209-16.
64. Dierckx B, Kok R, Tulen JH, Jaddoe VW, Hofman A, Verhulst FC, et al. A prospective study of heart rate and externalising behaviours in young children. J Child Psychol Psychiatry. 2014;55(4):402-10.
65. Ghassabian A, Szekely E, Herba CM, Jaddoe VW, Hofman A, Oldehinkel AJ, et al. From positive emotionality to internalizing problems: the role of executive functioning in preschoolers. Eur Child Adolesc Psychiatry. 2014;23(9):729-41.
66. Szekely E, Tiemeier H, Jansen PW, Jaddoe VW, Hofman A, Verhulst FC, et al. Maternal depressive symptoms are associated with low fearfulness in preschoolers. J Clin Child Adolesc Psychol. 2014;43(5):791-8.
67. Szekely E, Lucassen N, Tiemeier H, Bakermans-Kranenburg MJ, Van IJzendoorn MH, Kok R, et al. Maternal depressive symptoms and sensitivity are related to young children's facial expression recognition: the Generation R Study. Dev Psychopathol. 2014;26(2):333-45.

68. Mileva-Seitz VR, Ghassabian A, Bakermans-Kranenburg MJ, van den Brink JD, Linting M, Jaddoe VW, et al. Are boys more sensitive to sensitivity? Parenting and executive function in preschoolers. J Exp Child Psychol. 2015;130:193-208.
69. Labout JA, Duijts L, Arends LR, Jaddoe VW, Hofman A, de Groot R, et al. Factors associated with pneumococcal carriage in healthy Dutch infants: the generation R study. J Pediatr. 2008;153(6):771-6.
70. Labout JA, Duijts L, Lebon A, de Groot R, Hofman A, Jaddoe VV, et al. Risk factors for otitis media in children with special emphasis on the role of colonization with bacterial airway pathogens: the Generation R study. Eur J Epidemiol. 2011;26(1):61-6.
71. Luijk MP, Saridjan N, Tharner A, van IJzendoorn MH, Bakermans-Kranenburg MJ, Jaddoe VW, et al. Attachment, depression, and cortisol: Deviant patterns in insecure-resistant and disorganized infants. Dev Psychobiol. 2010;52(5):441-52.
72. Toemen L, Gishti O, van Osch-Gevers L, Steegers EA, Helbing WA, Felix JF, et al. Maternal obesity, gestational weight gain and childhood cardiac outcomes: role of childhood body mass index. Int J Obes (Lond). 2016;40(7):1070-8.
73. van der Tas JT, Kragt L, Veerkamp JJ, Jaddoe VW, Moll HA, Ongkosuwito EM, et al. Ethnic Disparities in Dental Caries among Six-Year-Old Children in the Netherlands. Caries Res. 2016;50(5):489-97.
74. Ringoot AP, Tiemeier H, Jaddoe VW, So P, Hofman A, Verhulst FC, et al. Parental depression and child well-being: young children's self-reports helped addressing biases in parent reports. J Clin Epidemiol. 2015;68(8):928-38.
75. Miliku K, Bergen NE, Bakker H, Hofman A, Steegers EA, Gaillard R, et al. Associations of Maternal and Paternal Blood Pressure Patterns and Hypertensive Disorders during Pregnancy with Childhood Blood Pressure. J Am Heart Assoc. 2016;5(10).
76. Mackenbach JD, Ringoot AP, van der Ende J, Verhulst FC, Jaddoe VW, Hofman A, et al. Exploring the relation of harsh parental discipline with child emotional and behavioral problems by using multiple informants. The generation R study. PLoS One. 2014;9(8):e104793.
77. Heppe DH, Medina-Gomez C, de Jongste JC, Raat H, Steegers EA, Hofman A, et al. Fetal and childhood growth patterns associated with bone mass in school-age children: the Generation R Study. J Bone Miner Res. 2014;29(12):2584-93.
78. Gishti O, Jaddoe VW, Felix JF, Klaver CC, Hofman A, Wong TY, et al. Retinal microvasculature and cardiovascular health in childhood. Pediatrics. 2015;135(4):678-85.
79. den Dekker HT, Sonnenschein-van der Voort AM, Jaddoe VW, Reiss IK, de Jongste JC, Duijts L. Breastfeeding and asthma outcomes at the age of 6 years: The Generation R Study. Pediatr Allergy Immunol. 2016;27(5):486-92.
80. Ars CL, Nijs IM, El Marroun H, Muetzel R, Schmidt M, Steenweg-de Graaff J, et al. Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: the Generation R Study. Br J Nutr. 2016:1-9.
81. Muetzel RL, Blanken LM, Thijssen S, van der Lugt A, Jaddoe VW, Verhulst FC, et al. Resting-state networks in 6-to-10 year old children. Hum Brain Mapp. 2016.
82. Thijssen S, Ringoot AP, Wildeboer A, Bakermans-Kranenburg MJ, el Marroun H, Hofman A, et al. Brain morphology of childhood aggressive behavior: A multi-informant study in school-age children. Cogn Affect Behav Neurosci. 2015;15(3):564-77.
83. White T, Muetzel RL, Schmidt M, Langeslag SJ, Jaddoe VW, Hofman A, et al. Time of acquisition and network stability in pediatric resting-state functional magnetic resonance imaging. Brain Connect. 2014;4(6):417-27.
84. Medina-Gomez C, Felix JF, Estrada K, Peters MJ, Herrera L, Kruithof CJ, et al. Challenges in conducting genome-wide association studies in highly admixed multi-ethnic populations: the Generation R Study. Eur J Epidemiol. 2015;30(4):317-30.
85. Taal HR, St Pourcain B, Thiering E, Das S, Mook-Kanamori DO, Warrington NM, et al. Common variants at 12q15 and 12q24 are associated with infant head circumference. Nat Genet. 2012;44(5):532-8.
86. Bradfield JP, Taal HR, Timpson NJ, Scherag A, Lecoeur C, Warrington NM, et al. A genome-wide association meta-analysis identifies new childhood obesity loci. Nat Genet. 2012;44(5):526-31.
87. Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016;538(7624):248-52.
88. Freathy RM, Mook-Kanamori DO, Sovio U, Prokopenko I, Timpson NJ, Berry DJ, et al. Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. Nat Genet. 2010;42(5):430-5.

89. Parmar PG, Taal HR, Timpson NJ, Thiering E, Lehtimaki T, Marinelli M, et al. International Genome-Wide Association Study Consortium Identifies Novel Loci Associated With Blood Pressure in Children and Adolescents. Circ Cardiovasc Genet. 2016;9(3):266-78.
90. Felix JF, Bradfield JP, Monnereau C, van der Valk RJ, Stergiakouli E, Chesi A, et al. Genome-wide association analysis identifies three new susceptibility loci for childhood body mass index. Hum Mol Genet. 2016;25(2):389-403.
91. Medina-Gomez C, Kemp JP, Estrada K, Eriksson J, Liu J, Reppe S, et al. Meta-analysis of genome-wide scans for total body BMD in children and adults reveals allelic heterogeneity and age-specific effects at the WNT16 locus. PLoS Genet. 2012;8(7):e1002718.
92. Joubert BR, den Dekker HT, Felix JF, Bohlin J, Ligthart S, Beckett E, et al. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun. 2016;7:10577.
93. Rijlaarsdam J, Pappa I, Walton E, Bakermans-Kranenburg MJ, Mileva-Seitz VR, Rippe RC, et al. An epigenome-wide association meta-analysis of prenatal maternal stress in neonates: A model approach for replication. Epigenetics. 2016;11(2):140-9.
94. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. Am J Hum Genet. 2016;98(4):680-96.
95. Gruzieva O, Xu CJ, Breton CV, Annesi-Maesano I, Anto JM, Auffray C, et al. Epigenome-Wide Meta-Analysis of Methylation in Children Related to Prenatal NO2 Air Pollution Exposure. Environ Health Perspect. 2016.
96. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6.
97. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996-8.
98. Raoult D, Henrissat B. Are stool samples suitable for studying the link between gut microbiota and obesity? Eur J Epidemiol. 2014;29(5):307-9.
99. Aguirre M, Venema K. The use of fecal samples for studying human obesity. Eur J Epidemiol. 2015;30(9):1067-9.
100. Raoult D. Obesity and stools, the "emperor's new clothing" paradigm. Eur J Epidemiol. 2015;30(9):1071.
101. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.
102. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation. 2010;121(4):586-613.
103. Henry BW, Smith TJ, Ahmad S. Psychometric assessment of the Behavior and Attitudes Questionnaire for Healthy Habits: measuring parents' views on food and physical activity. Public Health Nutr. 2014;17(5):1004-12.
104. van Strien T, Frijters JE, Bergers GP, Defares PB. The Dutch Eating Behavior Questionnaire (Debq) for Assessment of Restrained, Emotional, and External Eating Behavior. Int J Eat Disorder. 1986;5(2):295-315.
105. Blumberg SJ, Bialostosky K, Hamilton WL, Briefel RR. The effectiveness of a short form of the Household Food Security Scale. Am J Public Health. 1999;89(8):1231-4.
106. Arora VS, Karanikolos M, Clair A, Reeves A, Stuckler D, McKee M. Data Resource Profile: The European Union Statistics on Income and Living Conditions (EU-SILC). Int J Epidemiol. 2015;44(2):451-61.
107. Israel AC, Roderick HA. A measure of the stability of family activities: an initial examination. Assessment. 2001;8(4):417-24.
108. Carver DJ, Chapman CA, Thomas VS, Stadnyk KJ, Rockwood K. Validity and reliability of the Medical Outcomes Study Short Form-20 questionnaire as a measure of quality of life in elderly people living at home. Age Ageing. 1999;28(2):169-74.
109. de Brock AJ, Vermulst AA, Gerris JR, Abidin RR. Nijmeegse Ouderlijke Stress Index. Lisse, Swets en Zeitlinger BV. 1992.
110. Gerris JR, Boxtel DA, Vermulst AA, Janssens JM, van Zutphen RA, Felling JA. Parenting in Dutch families, Nijmegen, the Netherlands. 1993.
111. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150:782-6.
112. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med. 1983;13(3):595-605.

113. de Beurs E. Brief Symptom Inventory: Handleiding. Pits Publishers, Leiden, the Netherlands 2004.
114. Derogatis LR, Fitzpatrick M. The SCL-90-R, the Brief Symptom Inventory (BSI) and the BSI-18. 2004.
115. Israel AC, Roderick HA, Ivanova MY. A measure of the stability of activities in a family environment. J Psychopathol Behav. 2002;24(2):85-95.
116. Epstein NB, Baldwin LM, Bishop DS. The Mcmaster Family Assessment Device. J Marital Fam Ther. 1983;9(2):171-80.
117. van Rossem L, Vogel I, Steegers EA, Moll HA, Jaddoe VW, Hofman A, et al. Breastfeeding patterns among ethnic minorities: the Generation R Study. J Epidemiol Community Health. 2010;64(12):1080-5.
118. Duijts L, Jaddoe VW, Hofman A, Moll HA. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics. 2010;126(1):e18-25.
119. Wardle J, Guthrie CA, Sanderson S, Rapoport L. Development of the Children's Eating Behaviour Questionnaire. J Child Psychol Psychiatry. 2001;42(7):963-70.
120. Landgraf JM, Maunsell E, Speechley KN, Bullinger M, Campbell S, Abetz L, et al. Canadian-French, German and UK versions of the Child Health Questionnaire: methodology and preliminary item scaling results. Qual Life Res. 1998;7(5):433-45.
121. van der Horst K, Oenema A, van de Looij-Jansen P, Brug J. The ENDORSE study: research into environmental determinants of obesity related behaviors in Rotterdam schoolchildren. BMC Public Health. 2008;8:142.
122. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. J Child Psychol Psychiatry. 2000;41(5):645-55.
123. Micali N, Simonoff E, Elberling H, Rask CU, Olsen EM, Skovgaard AM. Eating patterns in a population-based sample of children aged 5 to 7 years: association with psychopathology and parentally perceived impairment. J Dev Behav Pediatr. 2011;32(8):572-80.
124. Braet C, Van Strien T. Assessment of emotional, externally induced and restrained eating behaviour in nine to twelve-year-old obese and non-obese children. Behav Res Ther. 1997;35(9):863-73.
125. Birch LL, Fisher JO, Grimm-Thomas K, Markey CN, Sawyer R, Johnson SL. Confirmatory factor analysis of the Child Feeding Questionnaire: a measure of parental attitudes, beliefs and practices about child feeding and obesity proneness. Appetite. 2001;36(3):201-10.
126. House J, Eisler I, Simic M, Micali N. Diagnosing eating disorders in adolescents: a comparison of the eating disorder examination and the development and well-being assessment. Int J Eat Disord. 2008;41(6):535-41.
127. van Strien T, Oosterveld P. The children's DEBQ for assessment of restrained, emotional, and external eating in 7- to 12-year-old children. Int J Eat Disorder. 2008;41(1):72-81.
128. Owens J, Maxim R, McGuinn M, Nobile C, Msall M, Alario A. Television-viewing habits and sleep disturbance in school children. Pediatrics. 1999;104(3):e27.
129. Velde SJ, de Bourdeaudhuij I, Thorsdottir I, Rasmussen M, Hagstromer M, Klepp KI, et al. Patterns in sedentary and physical exercise behaviors and overweight in boys and girls. Int J Obesity. 2006;30:S20.
130. van Rooij AJ, Schoenmakers TM, van den Eijnden RJ, Vermulst AA, van de Mheen D. Video game addiction test: validity and psychometric characteristics. Cyberpsychol Behav Soc Netw. 2012;15(9):507-11.
131. Veldhuis L, Struijk MK, Kroeze W, Oenema A, Renders CM, Bulk-Bunschoten AM, et al. 'Be active, eat right', evaluation of an overweight prevention protocol among 5-year-old children: design of a cluster randomised controlled trial. BMC Public Health. 2009;9:177.
132. Raat H, Botterweck AM, Landgraf JM, Hoogeveen WC, Essink-Bot ML. Reliability and validity of the short form of the child health questionnaire for parents (CHQ-PF28) in large random school based and general population samples. J Epidemiol Community Health. 2005;59(1):75-82.
133. Raat H, Mohangoo AD, Grootenhuis MA. Pediatric health-related quality of life questionnaires in clinical trials. Curr Opin Allergy Clin Immunol. 2006;6(3):180-5.
134. Raat H, Landgraf JM, Oostenbrink R, Moll HA, Essink-Bot ML. Reliability and validity of the Infant and Toddler Quality of Life Questionnaire (ITQOL) in a general population and respiratory disease sample. Qual Life Res. 2007;16(3):445-60.
135. Raat H, van Rossem L, Jaddoe VW, Landgraf JM, Feeny D, Moll HA, et al. The Generation R study: a candidate gene study and genome-wide association study (GWAS) on health-related quality of life (HRQOL) of mothers and young children. Qual Life Res. 2010;19(10):1439-46.

136. Duijts L, Jaddoe VW, Hofman A, Steegers EA, Mackenbach JP, de Jongste JC, et al. Maternal smoking in prenatal and early postnatal life and the risk of respiratory tract infections in infancy. The Generation R study. Eur J Epidemiol. 2008;23(8):547-55.
137. Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. Int J Epidemiol. 1996;25(3):609-16.
138. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8(3):483-91.
139. Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP, et al. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. Eur Respir J. 2004;24(3):406-12.
140. Flohr C, Weinmayr G, Weiland SK, Addo-Yobo E, Annesi-Maesano I, Bjorksten B, et al. How well do questionnaires perform compared with physical examination in detecting flexural eczema? Findings from the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two. Br J Dermatol. 2009;161(4):846-53.
141. Zhang M, Qureshi AA, Fortner RT, Hankinson SE, Wei Q, Wang LE, et al. Teenage acne and cancer risk in US women: A prospective cohort study. Cancer. 2015;121(10):1681-7.
142. Clark EM, Ness AR, Tobias JH. Bone fragility contributes to the risk of fracture in children, even after moderate and severe trauma. J Bone Miner Res. 2008;23(2):173-9.
143. van Beelen ME, Beirens TM, Struijk MK, den Hertog P, Oenema A, van Beeck EF, et al. 'BeSAFE', effect-evaluation of internet-based, tailored safety information combined with personal counselling on parents' child safety behaviours: study design of a randomized controlled trial. BMC Public Health. 2010;10:466.
144. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology. 2006;130(5):1527-37.
145. Broder HL, McGrath C, Cisneros GJ. Questionnaire development: face validity and item impact testing of the Child Oral Health Impact Profile. Community Dent Oral Epidemiol. 2007;35 Suppl 1:8-19.
146. Dunlow N, Phillips C, Broder HL. Concurrent validity of the COHIP. Community Dent Oral Epidemiol. 2007;35 Suppl 1:41-9.
147. Geels LM, Hoogstraten J, Prahl-Andersen B. Confirmative factor analysis of the dimensions of the Child Oral Health Impact Profile (Dutch version). Eur J Oral Sci. 2008;116(2):148-52.
148. Wilson-Genderson M, Broder HL, Phillips C. Concordance between caregiver and child reports of children's oral health-related quality of life. Community Dent Oral. 2007;35:32-40.
149. Vogel I, Verschuure H, van der Ploeg CP, Brug J, Raat H. Estimating adolescent risk for hearing loss based on data from a large school-based survey. Am J Public Health. 2010;100(6):1095-100.
150. Stjamesroberts I. Persistent Crying in Infancy. J Child Psychol Psyc. 1989;30(2):189-95.
151. van den Berg MP, van der Ende J, Crijnen AA, Jaddoe VW, Moll HA, Mackenbach JP, et al. Paternal depressive symptoms during pregnancy are related to excessive infant crying. Pediatrics. 2009;124(1):e96-103.
152. Brouilette R, Hanson D, David R, Klemka L, Szatkowski A, Fernbach S, et al. A diagnostic approach to suspected obstructive sleep apnea in children. J Pediatr. 1984;105(1):10-4.
153. Gartstein MA, Rothbart MK. Studying infant temperament via the Revised Infant Behavior Questionnaire. Infant Behav Dev. 2003;26(1):64-86.
154. Putnam SP, Rothbart MK. Development of short and very short forms of the Children's Behavior Questionnaire. J Pers Assess. 2006;87(1):102-12.
155. Kochanska G, Devet K, Goldman M, Murray K, Putnam SP. Maternal Reports of Conscience Development and Temperament in Young-Children. Child Dev. 1994;65(3):852-68.
156. Gioia GA, Isquith PK, Guy SC, Kenworthy L, Baron IS. Test review: Behavior rating inventory of executive function. Child Neuropsychol. 2000;6(3):235-8.
157. Ireton H, Glascoe FP. Assessing children's development using parents' reports. The Child Development Inventory. Clin Pediatr (Phila). 1995;34(5):248-55.
158. James-Roberts IS, Wolke D. Differences between maternal and objective ratings of 'difficult' neonatal behavioural style: Implications for temperament research and clinical perspectives. Journal of Reproductive and Infant Psychology. 1983;1(2):53-60.

159. James-Roberts IS, Wolke D. Converges and discrepancies, among mothers' and professionals' assessments of difficult neonatal behaviour. Journal of Child Psychology and Psychiatry. 1988;29(1):21-42.
160. Verhulst FC, van der Ende J, Koot HM. Handleiding voor de cbcl/4-18. Rotterdam: Afdeling Kinder- en jeugdpsychiatrie Sophia Kinderziekenhuis. 1996.
161. Achenbach TM, Dumenci L, Rescorla LA. Ratings of relations between DSM-IV diagnostic categories and items of the CBCL/6-18, TRF, and YSR. Burlington, VT: University of Vermont. 2001.
162. Walker LS, Smith CA, Garber J, van Slyke DA. Development and validation of the pain response inventory for children. Psychol Assessment. 1997;9(4):392-405.
163. Wolff NJ, Darlington AS, Hunfeld JA, Verhulst FC, Jaddoe VW, Moll HA, et al. The Association of Parent Behaviors, Chronic Pain, and Psychological Problems With Venipuncture Distress in Infants: The Generation R Study. Health Psychol. 2009;28(5):605-13.
164. Perquin CW, Hazebroek-Kampschreur AA, Hunfeld JA, Bohnen AM, van Suijlekom-Smit LW, Passchier J, et al. Pain in children and adolescents: a common experience. Pain. 2000;87(1):51-8.
165. Fenson L, Pethick S, Renda C, Cox JL, Dale PS, Reznick JS. Short-form versions of the MacArthur Communicative Development Inventories. Appl Psycholinguist. 2000;21(1):95-115.
166. Saudino KJ, Dale PS, Oliver B, Petrill SA, Richardson V, Rutter M, et al. The validity of parent-based assessment of the cognitive abilities of 2-year-olds. Brit J Dev Psychol. 1998;16:349-63.
167. Isquith PK, Gioia GA, Espy KA. Executive function in preschool children: Examination through everyday behavior. Dev Neuropsychol. 2004;26(1):403-22.
168. Goodman R. The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry. 1997;38(5):581-6.
169. Kimonis ER, Frick PJ, Barry CT. Callous-unemotional traits and delinquent peer affiliation. J Consult Clin Psychol. 2004;72(6):956-66.
170. Pardini D, Obradovic J, Loeber R. Interpersonal callousness, hyperactivity/impulsivity, inattention, and conduct problems as precursors to delinquency persistence in boys: a comparison of three grade-based cohorts. J Clin Child Adolesc Psychol. 2006;35(1):46-59.
171. Kimonis ER, Frick PJ, Skeem JL, Marsee MA, Cruise K, Munoz LC, et al. Assessing callous-unemotional traits in adolescent offenders: validation of the Inventory of Callous-Unemotional Traits. Int J Law Psychiatry. 2008;31(3):241-52.
172. Constantino JN, Davis SA, Todd RD, Schindler MK, Gross MM, Brophy SL, et al. Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. J Autism Dev Disord. 2003;33(4):427-33.
173. James-Roberts IS, Halil T. Infant crying patterns in the first year: normal community and clinical findings. J Child Psychol Psychiatry. 1991;32(6):951-68.
174. Hoekstra RA, Vinkhuyzen AA, Wheelwright S, Bartels M, Boomsma DI, Baron-Cohen S, et al. The construction and validation of an abridged version of the autism-spectrum quotient (AQ-Short). J Autism Dev Disord. 2011;41(5):589-96.
175. Uher R, Heyman I, Mortimore C, Frampton I, Goodman R. Screening young people for obsessive compulsive disorder. Br J Psychiatry. 2007;191:353-4.
176. Widyanto L, McMurran M. The psychometric properties of the internet addiction test. Cyberpsychol Behav. 2004;7(4):443-50.
177. Young SJ. CyberPsychology & Behavior. 2009;1(3):237-44.
178. Parker JG, Asher SR. Friendship and Friendship Quality in Middle Childhood - Links with Peer Group Acceptance and Feelings of Loneliness and Social Dissatisfaction. Dev Psychol. 1993;29(4):611-21.
179. Perren S, Alsaker FD. Social behavior and peer relationships of victims, bully-victims, and bullies in kindergarten. J Child Psychol Psychiatry. 2006;47(1):45-57.
180. Wolke D, Tippett N, Dantchev S. Bullying in the family: sibling bullying. Lancet Psychiatry. 2015;2(10):917-29.
181. Tippett N, Wolke D. Aggression between siblings: Associations with the home environment and peer bullying. Aggress Behav. 2015;41(1):14-24.
182. Baber KF, Anderson J, Puzanovova M, Walker LS. Rome II versus Rome III classification of functional gastrointestinal disorders in pediatric chronic abdominal pain. J Pediatr Gastroenterol Nutr. 2008;47(3):299-302.

183. Goodman E, Adler NE, Kawachi I, Frazier AL, Huang B, Colditz GA. Adolescents' perceptions of social status: development and evaluation of a new indicator. Pediatrics. 2001;108(2):E31.
184. Zeman JL, Cassano M, Suveg C, Shipman K. Initial Validation of the Children's Worry Management Scale. J Child Fam Stud. 2010;19(4):381-92.
185. Muris P, Meesters C, Eijkelenboom A, Vincken M. The self-report version of the Strengths and Difficulties Questionnaire: its psychometric properties in 8- to 13-year-old non-clinical children. Br J Clin Psychol. 2004;43(Pt 4):437-48.
186. Sonneville KR, Calzo JP, Horton NJ, Field AE, Crosby RD, Solmi F, et al. Childhood hyperactivity/inattention and eating disturbances predict binge eating in adolescence. Psychol Med. 2015;45(12):2511-20.
187. de Lauzon B, Romon M, Deschamps V, Lafay L, Borys JM, Karlsson J, et al. The Three-Factor Eating Questionnaire-R18 is able to distinguish among different eating patterns in a general population. J Nutr. 2004;134(9):2372-80.
188. Cappelleri JC, Bushmakin AG, Gerber RA, Leidy NK, Sexton CC, Lowe MR, et al. Psychometric analysis of the Three-Factor Eating Questionnaire-R21: results from a large diverse sample of obese and non-obese participants. Int J Obes (Lond). 2009;33(6):611-20.
189. Thompson JK, Cattarin J, Fowler B, Fisher E. The Perception of Teasing Scale (POTS): a revision and extension of the Physical Appearance Related Teasing Scale (PARTS). J Pers Assess. 1995;65(1):146-57.
190. Wijtzes AI, Jansen W, Kamphuis CB, Jaddoe VW, Moll HA, Tiemeier H, et al. Increased risk of exceeding entertainment-media guidelines in preschool children from low socioeconomic background: the Generation R Study. Prev Med. 2012;55(4):325-9.
191. Roberts C, Freeman J, Samdal O, Schnohr CW, de Looze ME, Nic Gabhainn S, et al. The Health Behaviour in School-aged Children (HBSC) study: methodological developments and current tensions. Int J Public Health. 2009;54 Suppl 2:140-50.
192. Ellis LK, Rothbart MK. Revision of the Early Adolescent Temperament Questionnaire. Poster presented at the 2001 Biennial Meeting of the Society for Research in Child Development, Minneaopolis, Minnesota. 2001.
193. Snyder HR, Gulley LD, Bijttebier P, Hartman CA, Oldehinkel AJ, Mezulis A, et al. Adolescent emotionality and effortful control: Core latent constructs and links to psychopathology and functioning. J Pers Soc Psychol. 2015;109(6):1132-49.
194. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36(7):980-8.
195. Adriaanse M, van Domburgh L, Hoek HW, Susser E, Doreleijers TAH, Veling W. Prevalence, impact and cultural context of psychotic experiences among ethnic minority youth. Psychological Medicine. 2015;45(3):637-46.
196. Truby H, Paxton SJ. Development of the Children's Body Image Scale. Br J Clin Psychol. 2002;41(2):185-203.
197. Saxton J, Hill C, Chadwick P, Wardle J. Weight status and perceived body size in children. Arch Dis Child. 2009;94(12):944-9.
198. Collins ME. Body Figure Perceptions and Preferences among Preadolescent Children. Int J Eat Disorder. 1991;10(2):199-208.
199. Veerman JW, Straathof MA, Treffers PD, Bergh BR, van den Brink LT. Handleiding bij de competentiebelevingsschaal voor kinderen, CBSK : handleiding (manual of the self perception profile for children - Dutch version). Lise: Swets & Zeitlinger. 1997.
200. Harter S. Manual for the Self-Perception Profile for Children. Denver: University of Denver. 1985.
201. Wolfson AR, Carskadon MA, Acebo C, Seifer R, Fallone G, Labyak SE, et al. Evidence for the validity of a sleep habits survey for adolescents. Sleep. 2003;26(2):213-6.
202. Drake C, Nickel C, Burduvali E, Roth T, Jefferson C, Pietro B. The pediatric daytime sleepiness scale (PDSS): sleep habits and school outcomes in middle-school children. Sleep. 2003;26(4):455-8.
203. Carskadon MA, Vieira C, Acebo C. Association between puberty and delayed phase preference. Sleep. 1993;16(3):258-62.
204. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. Sleep. 2000;23(8):1043-51.
205. Carskadon MA, Acebo C. A self-administered rating scale for pubertal development. J Adolesc Health. 1993;14(3):190

Chapter 2

Maternal determinants of placental and fetal hemodynamic alterations

Chapter 2

Associations of maternal metabolic profile with placental, and fetal cerebral and cardiac hemodynamics

Kooijman MN, Jaddoe VWV, Steegers EAP, Gaillard R

Submitted

Abstract

Background Maternal obesity and metabolic health affect pregnancy outcomes. We examined whether maternal metabolic profiles are associated with placental and fetal hemodynamics.

Methods In a population-based prospective cohort study among 1,175 women we examined the associations of an adverse metabolic profile in early pregnancy with placental, fetal cerebral and cardiac hemodynamic development. We obtained maternal pre-pregnancy BMI by questionnaire and measured blood pressure, cholesterol, triglycerides and glucose concentrations at a median gestational age of 12.6 (95% range 9.6-17.1) weeks. An adverse metabolic profile was defined as >=4 risk factors. Placental and fetal hemodynamics were measured by pulsed-wave-Doppler at a median gestational age of 30.3 (95% range 28.8–32.3) weeks.

Results An adverse metabolic profile was associated with a 0.29 Z-score higher (95%CI 0.08-0.50) fetal cerebral middle artery pulsatility index (PI), but not with placental or fetal cardiac hemodynamic patterns. When the individual components of an adverse metabolic profile were assessed, we observed that higher total cholesterol and triglyceride concentrations were associated with a higher cerebral middle artery PI (Z-score, 0.09 (95%CI 0.02-0.15), 0.09 (95%CI 0.03-0.15) per Z-score increase). Higher total cholesterol concentrations were also associated with a higher aorta ascendens peak systolic velocity Z-score, 0.08 (95%CI 0.01-0.14)), and a larger left cardiac output (Z-score, 0.08 (95%CI 0.00-0.15)).

Conclusion An adverse metabolic profile, especially higher cholesterol and triglycerides concentrations, are associated with increased fetal cerebral vascular resistance and increased fetal cardiac hemodynamics, but not with placental vascular resistance indices. Further studies are needed to identify long-term consequences of the observed associations.

Introduction

Maternal pre-pregnancy obesity is strongly related to metabolic disturbances during pregnancy, including insulin resistance, an adverse cholesterol profile and high triglycerides concentrations.[1, 2] Both maternal pre-pregnancy obesity and these subsequent metabolic disturbances are major risk factors for pregnancy complications and adverse cardiovascular outcomes in offspring.[3] The mechanisms by which maternal pre-pregnancy obesity leads to adverse fetal and childhood outcomes might involve suboptimal early placental development leading to placental and fetal hemodynamic alterations.[4]

Several studies have shown that maternal pre-pregnancy obesity, gestational diabetes and hyperlipidemia are associated with larger placental weight at birth.[5-7] An adverse maternal metabolic profile may also lead to a pro-inflammatory state leading to reduced placental vascularization, placental infarction and reduced placenta growth.[8] Placental weight is only a crude measure of placental development and function during pregnancy. Utero-placental and feto-placental peripheral vascular resistance can be assessed by Doppler ultrasound of the umbilical and uterine arteries throughout pregnancy. Altered vascular resistance in the main placental arteries may subsequently lead to changes in blood circulation of the brain and heart of the fetus.

We hypothesized that an adverse maternal early-pregnancy metabolic profile affects early placental development leading to subsequent adaptations in the placental, fetal cerebral and cardiac circulation. We examined in a population-based prospective cohort study among 1,175 mothers and their children, the associations of maternal early pregnancy metabolic profile and it's separate components with placental, fetal cerebral and cardiac hemodynamics.

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[9, 10] The Medical Ethics Committee of the Erasmus MC, University Medical Center, Rotterdam, has approved the study. All children were born between April 2002 and January 2006. Detailed assessments of fetal and childhood growth and development were conducted in a random subgroup of 1,232 Dutch mothers and children.[11] For the current analyses, twin pregnancies (n = 15), and pregnancies leading to perinatal death (n = 2) were excluded from this analyses, resulting in 1,215 singleton live born children. First trimester maternal metabolic profile measurements and third trimester placental and fetal hemodynamic patterns were available in 1,175 mothers and their children (**Figure S2.1**).

Maternal metabolic profile

At enrollment, we measured maternal height (cm) without shoes and clothing. Information about maternal weight just before pregnancy was obtained by questionnaire. We calculated BMI (kg/m^2). First trimester blood pressure and blood samples at a median gestational age of 12.6 (95% range 9.6-17.1) weeks, as described in detail.[12, 13] Briefly, blood pressure measurements were performed when participants were seated in upright position with back support and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32–42 cm) was used. The mean value of two BP readings over a 60-second interval was documented for each participant.[14, 15] All non-fasting blood samples were transported to a dedicated laboratory facility in Rotterdam, the Netherlands (STAR-MDC). Processing was aimed to finish within a maximum of 3 hours after sampling and stored at -80°C. Total cholesterol, HDL-cholesterol, triglycerides and glucose concentrations are enzymatic assays and were measured with c702 module on the Cobas 8000 analyzer.[13] As a measure of a metabolic syndrome like phenotype, we defined an adverse maternal metabolic profile as >= 4 of the following risk factors; BMI higher than 25.0, blood pressure, total cholesterol, triglycerides and glucose concentrations belonging to the highest 25% of our study population or HDL-cholesterol concentrations belonging to the lowest 25% of our study population.[16]

Third trimester placental and fetal hemodynamic characteristics

Utero-placental and feto-placental peripheral vascular resistance were assessed by pulsed-wave Doppler at a median gestational age of 30.3 (95% range 28.8–32.3) weeks, as described previously.[17, 18]

Uterine artery resistance index (RI) was measured in the uterine arteries near the crossover with the external iliac artery. Umbilical artery pulsatility index (PI) was determined in a free-floating loop of the umbilical cord. A higher uterine resistance index and umbilical artery PI indicated a higher peripheral vascular resistance.[19, 20] Middle cerebral artery Doppler measurements were obtained in the proximal part of the cerebral arteries. The middle cerebral artery PI quantifies the redistribution of blood flow, and when lower, in favor of the fetal brain. Reductions in middle cerebral artery PI is a valid indicator of fetal redistribution.[21, 22] An indicator of the 'brain-sparing effect' is a raised ratio between the umbilical artery PI and the cerebral artery PI (U/C ratio).[23] Cardiac flow-velocity waveforms at the level of the mitral valves were recorded from the apical 4-champer view of the fetal heart. Peak velocities of the E wave and the A wave, were recorded. The E/A ratio, which is an index for ventricular diastolic function and expresses both cardiac compliance and preload conditions, was calculated.[18] Cardiac outflow flow-velocity waveforms from the aorta were recorded from the 5-chamber view and the short-axis view of the fetal heart just above the semi-lunar valves, respectively. Peak systolic velocity (PSV) and the inner diameter during systole were recorded. Left cardiac output was calculated in milliliters per minute by multiplying the vessel area by the time-velocity integral by fetal heart rate. All ultrasound examinations were performed with an ATL-Philips model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0-MHz high-frequency, curved-array transducer.

Covariates

We obtained information on maternal educational level, parity, and smoking during pregnancy from multiple questionnaires during pregnancy by the mother. Third trimester estimated fetal weight was obtained during ultrasound.24 Infant sex was obtained from midwife and hospital registries.

Statistical analyses

First, we assessed the associations of an adverse maternal early-pregnancy metabolic profile with placental, fetal cerebral and cardiac hemodynamics using multiple linear regression models. The models were adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age, estimated fetal weight and child sex. Next, we examined the associations of each of the individual components of an adverse maternal early-pregnancy metabolic profile with placental, fetal cerebral and cardiac hemodynamics using similar models. For the associations of maternal early-pregnancy blood pressure, cholesterol, triglycerides and glucose concentrations with placental and fetal hemodynamic measures, we further explored whether the associations were explained by maternal pre-pregnancy BMI. We also tested potential interaction between maternal metabolic factors and BMI for all of our analyses. No significant interactions were present and no further stratified analyses were performed. The percentages of missing covariate values within the population for analyses was lower than 13%. Missing covariate data were imputed using the multiple imputations procedure (n = 5 imputations) and the imputed datasets were analyzed together. No major differences in the effect estimates were observed between analyses with imputed missing data and complete cases only (data not shown). All measures of associations are presented within their 95% confidence intervals (CI). Statistical analyses were performed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Participants characteristics

Table 2.1 shows the population characteristics. 22.5% of the mothers were overweight or obese at the start of pregnancy and 9.2% of the mothers had an adverse metabolic profile at the start of their pregnancy. **Table S2.1** shows the participant characteristics before multiple imputation.

TABLE 2.1 | Characteristics of mothers and their children after multiple imputation (n=1,175).

Maternal characteristics	
Maternal age	31.9 (22.0 – 39.1)
Education (%)	
Low (no, primary, secondary education)	36.9 (434)
High (higher education)	63.1 (741)
Pre-pregnancy body mass index (kg/m²)	23.5 (4.0)
BMI > 25.0	22.4 (263)
Parity (%)	
Nullipara	60.8 (714)
Multipara	39.2 (461)
Smoking during pregnancy (%)	
No smoking throughout pregnancy	76.1 (894)
Yes	23.9 (281)
First trimester maternal characteristics	
Gestational age at measurement, weeks	12.6 (9.6 - 17.1)
Systolic blood pressure (mmHg)	119 (13)
Diastolic blood pressure (mmHg)	70 (10)
Total cholesterol, mmol/L	4.9 (0.9)
HDL-cholesterol, mmol/L	1.8 (0.3)
Triglycerides, mmol/L	1.3 (0.5)
Glucose mmol/L	4.4 (0.8)
Adverse metabolic profile (%)	9.2 (108)
Third trimester fetal characteristics	
Sex	
Male	52.4 (616)
Female	47.6 (559)
Gestational age at measurement, weeks	30.3 (27.4 – 32.6)
Estimated fetal weight, grams	1628 (268)
Third trimester feto-placental hemodynamics	
Uterine artery RI	0.49 (0.08)
Umbilical artery PI	0.97 (0.17)
Third trimester fetal cerebral hemodynamics	
Middle cerebral artery PI	1.97 (0.33)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Third trimester fetal cardiac hemodynamics	
Aorta ascendens diameter (cm)	0.64 (0.07)
Aorta ascendens PSV (cm/s)	91.3 (12.4)
Left cardiac output (ml/min)	606 (173)
Mitral valve E/A ratio	0.78 (0.10)

Values are means (standard deviation), medians (95% range) or valid percentages (absolute numbers). RI: resistance index, PI: pulsatility index, PSV: Peak Systolic Volume

Third trimester placental hemodynamics

Figure 2.1a shows no associations were present of an adverse maternal metabolic profile with uterine artery RI and umbilical artery PI. Similarly, none of the individual components of an adverse maternal metabolic profile were associated with placental hemodynamics (**Table 2.2**).

Third trimester fetal cerebral hemodynamics

Mothers with an adverse metabolic profile had a 0.29 (95% CI 0.08, 0.50) Z-score increase in the fetal cerebral middle artery PI compared to mothers without an adverse metabolic profile (**Figure 2.1b**). When we assessed the individual components of an adverse maternal metabolic profile separately, higher total cholesterol and triglyceride concentrations were associated with a higher cerebral middle artery PI (**Table 2.3**, Z-score, 0.09 (95% CI 0.02, 0.15), 0.09 (95% CI 0.03, 0.15) per Z-score increase in total cholesterol and triglyceride concentrations, respectively). These associations were not explained by maternal BMI. No associations of maternal pre-pregnancy BMI, blood pressure, HDL-cholesterol and glucose concentrations with fetal cerebral hemodynamics were present. The associations with the fetal U/C ratio artery PI were similar (**Figure S2.2**, **Table S2.2**).

Third trimester fetal cardiac hemodynamics

An adverse maternal metabolic profile was not associated with fetal cardiac hemodynamics (**Figure 2.1c**). When we assessed the associations of individual components of an adverse maternal metabolic profile with fetal cardiac hemodynamics, a higher maternal diastolic blood pressure, total and HDL-cholesterol concentrations were associated with a higher fetal left cardiac output (**Table 2.4**, Z-score 0.08 (95% CI 0.02, 0.15), 0.07 (95% CI 0.01, 0.13), 0.11 (95% CI 0.05, 0.18) per Z-score increase in diastolic blood pressure, total and HDL-cholesterol concentrations, respectively). Higher HDL-cholesterol concentrations were associated with a larger aorta ascendens diameter and aorta ascendens PSV (Z-score 0.10 (95% CI 0.04, 0.17), 0.08 (95% CI 0.01, 0.14) per Z-score increase in HDL-cholesterol concentrations). Total cholesterol concentrations were also associated with a higher aorta ascendens PSV (Z-score 0.08 (95% CI 0.01, 0.14) per Z-score increase in total cholesterol concentrations). These associations were not explained by adjustment for maternal pre-pregnancy BMI. No associations of triglyceride or glucose concentrations with fetal cardiac hemodynamics were present.

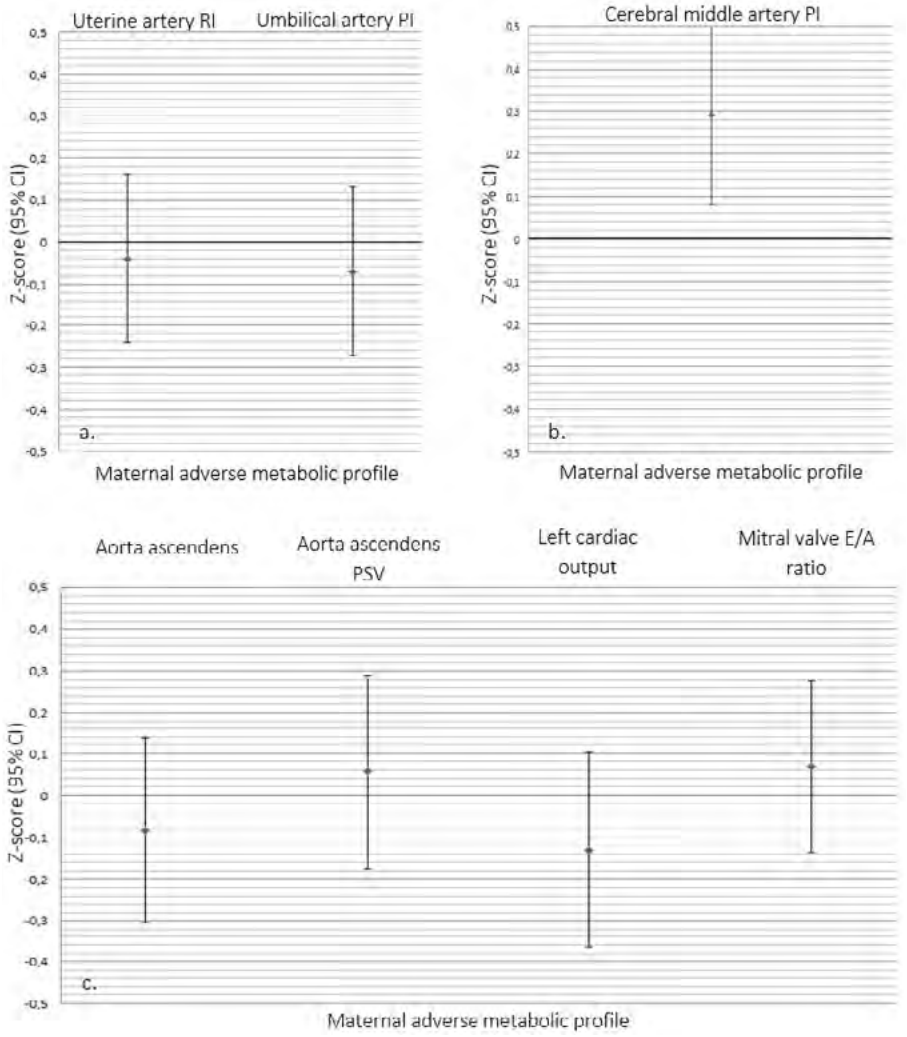

FIGURE 2.1 | Associations of maternal adverse metabolic profile with third trimester placental (a), fetal cerebral (b) and fetal cardiac hemodynamics (c). Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of fetal hemodynamics of mothers with overweight and an adverse metabolic profile compared to mothers without overweight and adverse metabolic profile. The model is adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age, estimated fetal weight, and child sex. RI: resistance index, PI: pulsatility index, PSV: Peak Systolic Volume

TABLE 2.2 | Associations of early pregnancy BMI, blood pressure and first trimester metabolic concentrations with third trimester utero-placental and feto-placental hemodynamics (n=1,175).

	Uterine artery RI Z-score (95% CI)	**Umbilical artery PI Z-score (95% CI)**
Pre-pregnancy BMI (Z-score)	0.06 (-0.00, 0.12)	0.06 (-0.01, 0.12)
Systolic blood pressure (Z-score)	0.07 (-0.01, 0.16)	0.00 (-0.06, 0.06)
Diastolic blood pressure (Z-score)	0.01 (-0.05, 0.08)	-0.01 (-0.07, 0.06)
Total cholesterol (Z-score)	-0.03 (-0.09, 0.03)	0.00 (-0.06, 0.06)
HDL-cholesterol (Z-score)	-0.00 (-0.07, 0.06)	-0.05 (-0.11, 0.01)
Triglycerides (Z-score)	-0.01 (-0.07, 0.05)	0.05 (-0.02, 0.11)
Glucose (Z-score)	0.06 (-0.01, 0.12)	-0.01 (-0.07, 0.06)

Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of placental indexes per Z-score change in maternal BMI, blood pressure and metabolic concentrations. The models are adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age and estimated fetal weight, and child sex. RI: resistance index, PI: pulsatility index

TABLE 2.3 | Associations of early pregnancy BMI, blood pressure, and first trimester metabolic concentrations with third trimester fetal cerebral hemodynamics (n=1,175).

	Cerebral middle artery PI Z-score (95% CI)
Pre-pregnancy BMI (Z-score)	0.05 (-0.01, 0.12)
Systolic blood pressure (Z-score)	0.05 (-0.01, 0.12)
Diastolic blood pressure (Z-score)	0.05 (-0.02, 0.11)
Total cholesterol (Z-score)	0.09 (0.02, 0.15)†
+pre-pregnancy BMI	0.08 (0.02, 0.15)*
HDL-cholesterol (Z-score)	-0.04 (-0.10, 0.03)
Triglycerides (Z-score)	0.09 (0.03, 0.15)†
+pre-pregnancy BMI	0.09 (0.02, 0.15)†
Glucose (Z-score)	0.02 (-0.04, 0.09)

Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of fetal cerebral indexes per Z-score change in maternal BMI, blood pressure and metabolic concentrations. The models are adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age and estimated fetal weight, and child sex. PI: pulsatility index. *=P<0.05, †=P<0.01

TABLE 2.4 | Associations of early pregnancy BMI, blood pressure and first trimester metabolic concentrations with third trimester fetal cardiac hemodynamics (n=1,175).

	Aorta ascendens diameter Z-score (95% CI)	Aorta ascendens PSV Z-score (95% CI)	Left cardiac output Z-score (95% CI)	Mitral valve E/A ratio Z-score (95% CI)
Pre-pregnancy BMI (Z-score)	-0.02 (-0.09, 0.04)	0.00 (-0.07, 0.07)	-0.03 (-0.10, 0.04)	0.01 (-0.05, 0.08)
Systolic blood pressure (Z-score)	0.01 (-0.06, 0.07)	0.00 (-0.07, 0.07)	0.01 (-0.06, 0.07)	0.03 (-0.03, 0.09)
Diastolic blood pressure (Z-score)	0.04 (-0.02, 0.10)	0.03(-0.04, 0.09)	0.08 (0.02, 0.15)†	0.01 (-0.05, 0.07)
+pre-pregnancy BMI			0.12 (0.05, 0.20)†	
Total cholesterol (Z-score)	0.02 (-0.04, 0.08)	0.08 (0.01, 0.14)*	0.07 (0.01, 0.13)*	-0.01 (-0.08, 0.05)
+pre-pregnancy BMI		0.08 (0.01, 0.15)*	0.07 (0.01, 0.14)*	
HDL-cholesterol (Z-score)	0.10 (0.04, 0.17)†	0.08 (0.01, 0.14)*	0.11 (0.05, 0.18)†	-0.05 (-0.11, 0.02)
+pre-pregnancy BMI	0.10 (0.04, 0.16)†	0.07 (0.00, 0.14)*	0.11 (0.04, 0.17)†	
Triglycerides (Z-score)	-0.03 (-0.10, 0.03)	0.04 (-0.03, 0.10)	0.01 (-0.06, 0.07)	0.00 (-0.06, 0.07)
Glucose (Z-score)	-0.01 (-0.07, 0.05)	0.05 (-0.02, 0.11)	-0.02 (-0.08, 0.05)	-0.05 (-0.12, 0.02)

Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of fetal cardiac hemodynamics per Z-score change in maternal BMI, blood pressure and metabolic concentrations. The models are adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age and estimated fetal weight, and child sex. PSV: Peak Systolic Volume. *=P<0.05, †=P<0.01

Discussion

Main findings

In this population prospective cohort study we observed that an adverse maternal early-pregnancy metabolic profile, especially higher maternal cholesterol and triglycerides concentrations, were associated with increased fetal cerebral vascular resistance and increased fetal cardiac hemodynamics, but not with placental vascular resistance indices. These associations were not explained by maternal BMI.

Interpretation

Maternal pre-pregnancy obesity is strongly related to metabolic disturbances during pregnancy.[1,2] Both maternal pre-pregnancy obesity and these subsequent metabolic disturbances are major risk factors for pregnancy complications and adverse birth outcomes.[3] The underlying mechanisms are not known, but might be related to impaired placental growth and function.[4] The placenta can be considered as the interface between the maternal and fetal environment and the major regulator of fetal nutrition, growth and cardiovascular development.[25] Multiple studies have shown that maternal obesity is related to larger placental weight.[26] Several studies also showed that individual components of an adverse metabolic profile, such as high blood pressure, high triglycerides, adverse cholesterol profile and high glucose concentrations are associated with both low and high placental weight at birth [27-29], which suggest various mechanisms may be involved. An adverse maternal metabolic profile may lead to a pro-inflammatory state leading to

reduced placental vascularization, placental infarction and reduced placental growth, whereas an adverse maternal metabolic profile may also lead to increased nutrient transfer to the placental, larger placental growth and accelerate fetal growth.[8]

Placental weight is only a crude measurement of placental development and function during pregnancy. More detailed measures of placental function can be assessed by Doppler ultrasound of the umbilical and uterine arteries during pregnancy. We observed no associations of maternal metabolic profile with the uterine and umbilical artery vascular resistance. A few other studies explored the associations of individual components of an adverse maternal metabolic profile with uterine and umbilical artery vascular resistance in pregnancy. A previous prospective study focused on 231 women affected by hypertensive disorders showed a higher uterine artery PI compared with normative pregnancies.[30] Among 10 women with familial hypercholesterolemia, it was observed that the PI of the uterine arteries was similar at 24 weeks of gestation, and remained unaltered at 36 weeks of gestation, in contrast to a decrease in de reference group.[31] An intervention study among 290 pregnant women demonstrate a more pronounced gestational decrease in the umbilical artery PI between 24 and 30 weeks of pregnancy after following a low-cholesterol low-saturated fat diet.[32] Two studies among Chilean women showed a correlation of total- and LDL-cholesterol concentrations, but not triglyceride concentrations, with lower sensitivity of the umbilical vein rings and reactivity, a phenomena that is likely due to endothelial dysfunction.[33, 34] Differences between our study and previous studies may be explained by differences in study population. We studied women with relatively healthy pregnancies and assessed associations across the full range of maternal metabolic factors, whereas many of the previous studies focused on high-risk populations and women with clinically abnormal metabolic parameters, such as clinical hypercholesterolemia. This may suggest that associations with uterine and umbilical vascular resistance are only present at the extremes of these adverse maternal metabolic factors, but not across the full range.

Consequences of impaired placentation might lead to redistribution of blood flow, with increased fetal blood to the brain and heart of the fetus due to reduced oxygen supply and its influence on fetal vascular development. We observed that an adverse maternal early-pregnancy metabolic profile was associated with increased fetal cerebral vascular resistance. These associations were mainly present for high cholesterol and triglyceride concentrations. Higher cholesterol concentrations were also associated with a larger fetal left cardiac output, larger aorta ascendens diameter and larger aorta ascendens PSV. Our findings thus suggest that increased maternal cholesterol and triglycerides levels, still within the normal range, lead to increased fetal cerebral resistance indexes and aorta ascendens diameter,- PSV and cardiac output suggesting a 'reverse' redistribution in favor of increased cardiac growth. The findings were not explained by maternal BMI, suggesting effects of maternal cholesterol and triglyceride concentrations within the normal range were independent of BMI. No other previous studies explored the associations of maternal cholesterol or triglycerides concentrations with fetal cerebral vascular resistance or fetal cardiac hemodynamics. However, the effect estimates are small and are mainly relevant on a population level providing further insight into pathophysiological mechanisms. Further studies

are needed to replicate our findings, to explore these associations throughout pregnancy and to assess the consequences for both birth outcomes and long-term offspring outcomes.

Strengths and limitations

The main strength of this study is the large population-based cohort studied. To our knowledge, this is the largest study, which examined the effects of maternal metabolic factors on placental and fetal hemodynamics. The population-based setting enabled us to assess maternal metabolic factors and placental and fetal hemodynamics measures across the full range, rather than only in mothers or fetuses with complications. However, because of our relatively healthy population, it should be further studied whether the observed associations are generalizable to high-risk populations. In the present study, we evaluated multiple associations; this might have led to chance findings due to multiple testing. However, because of the correlations between the fetal hemodynamic measures we did not correct for multiple testing. Missing fetal hemodynamic measurements could lead to selection bias and loss of power. Our results would be biased if the associations between maternal metabolic factors and fetal hemodynamics differ between those included and those not included in the study. Although this seems unlikely, it cannot be excluded. Finally, although we had information about a large number of confounders, the influence of residual confounding should be considered, as in any observational study.

Conclusions

An adverse maternal metabolic profile, especially higher maternal cholesterol and triglycerides concentrations still within the normal range, were associated with increased fetal cerebral vascular resistance and increased fetal cardiac hemodynamics, but not with placental vascular resistance indices.

References

1. Martin KA, Mani MV, Mani A. New targets to treat obesity and the metabolic syndrome. Eur J Pharmacol. 2015;763(Pt A):64-74.
2. Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am. 2014;43(1):1-23.
3. Ramachenderan J, Bradford J, McLean M. Maternal obesity and pregnancy complications: a review. Aust N Z J Obstet Gynaecol. 2008;48(3):228-35.
4. Longtine MS, Nelson DM. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. Semin Reprod Med. 2011;29(3):187-96.
5. Wallace JM, Horgan GW, Bhattacharya S. Placental weight and efficiency in relation to maternal body mass index and the risk of pregnancy complications in women delivering singleton babies. Placenta. 2012;33(8):611-8.
6. Macdonald EM, Natale R, Regnault TR, Koval JJ, Campbell MK. Obstetric conditions and the placental weight ratio. Placenta. 2014;35(8):582-6.
7. Reijnders IF, Mulders A, van der Windt M, Steegers EAP, Steegers-Theunissen RPM. The impact of periconceptional maternal lifestyle on clinical features and biomarkers of placental development and function: a systematic review. Hum Reprod Update. 2019;25(1):72-94.
8. Howell KR, Powell TL. Effects of maternal obesity on placental function and fetal development. Reproduction. 2017;153(3):R97-R108.
9. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
10. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, et al. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29(12):911-27.
11. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.
12. Benschop L, Bergen NE, Schalekamp-Timmermans S, Jaddoe VWV, Mulder MT, Steegers EAP, et al. Maternal lipid profile 6 years after a gestational hypertensive disorder. J Clin Lipidol. 2018;12(2):428-36 e4.
13. Adank MC, Benschop L, Peterbroers KR, Smak Gregoor AM, Kors AW, Mulder MT, et al. Is maternal lipid profile in early pregnancy associated with pregnancy complications and blood pressure in pregnancy and long term postpartum? Am J Obstet Gynecol. 2019;221(2):150 e1- e13.
14. Silva LM, Steegers EA, Burdorf A, Jaddoe VW, Arends LR, Hofman A, et al. No midpregnancy fall in diastolic blood pressure in women with a low educational level: the Generation R Study. Hypertension. 2008;52(4):645-51.
15. Bakker R, Steegers EA, Mackenbach JP, Hofman A, Jaddoe VW. Maternal smoking and blood pressure in different trimesters of pregnancy: the Generation R study. J Hypertens. 2010;28(11):2210-8.
16. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
17. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.
18. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
19. Albaiges G, Missfelder-Lobos H, Parra M, Lees C, Cooper D, Nicolaides KH. Comparison of color Doppler uterine artery indices in a population at high risk for adverse outcome at 24 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21(2):170-3.
20. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
21. van den Wijngaard JA, Groenenberg IA, Wladimiroff JW, Hop WC. Cerebral Doppler ultrasound of the human fetus. Br J Obstet Gynaecol. 1989;96(7):845-9.
22. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
23. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: A pulsed Doppler study. J PERINAT MED. 1992;20(1):79-82.

24. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.
25. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015;370(1663):20140066.
26. Tarrade A, Panchenko P, Junien C, Gabory A. Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. J Exp Biol. 2015;218(Pt 1):50-8.
27. Valero De Bernabe J, Soriano T, Albaladejo R, Juarranz M, Calle ME, Martinez D, et al. Risk factors for low birth weight: a review. Eur J Obstet Gynecol Reprod Biol. 2004;116(1):3-15.
28. Panaitescu AM, Syngelaki A, Prodan N, Akolekar R, Nicolaides KH. Chronic hypertension and adverse pregnancy outcome: a cohort study. Ultrasound Obstet Gynecol. 2017;50(2):228-35.
29. Grieger JA, Bianco-Miotto T, Grzeskowiak LE, Leemaqz SY, Poston L, McCowan LM, et al. Metabolic syndrome in pregnancy and risk for adverse pregnancy outcomes: A prospective cohort of nulliparous women. PLoS Med. 2018;15(12):e1002710.
30. Perry H, Lehmann H, Mantovani E, Thilaganathan B, Khalil A. Correlation between central and uterine hemodynamics in hypertensive disorders of pregnancy. Ultrasound Obstet Gynecol. 2019;54(1):58-63.
31. Khoury J, Amundsen AL, Tonstad S, Henriksen T, Ose L, Retterstol K, et al. Evidence for impaired physiological decrease in the uteroplacental vascular resistance in pregnant women with familial hypercholesterolemia. Acta Obstet Gynecol Scand. 2009;88(2):222-6.
32. Khoury J, Haugen G, Tonstad S, Froslie KF, Henriksen T. Effect of a cholesterol-lowering diet during pregnancy on maternal and fetal Doppler velocimetry: the CARRDIP study. Am J Obstet Gynecol. 2007;196(6):549 e1-7.
33. Leiva A, de Medina CD, Salsoso R, Saez T, San Martin S, Abarzua F, et al. Maternal hypercholesterolemia in pregnancy associates with umbilical vein endothelial dysfunction: role of endothelial nitric oxide synthase and arginase II. Arterioscler Thromb Vasc Biol. 2013;33(10):2444-53.
34. Leiva A, Salsoso R, Saez T, Sanhueza C, Pardo F, Sobrevia L. Cross-sectional and longitudinal lipid determination studies in pregnant women reveal an association between increased maternal LDL cholesterol concentrations and reduced human umbilical vein relaxation. Placenta. 2015;36(8):895-902.

Supplementary Material

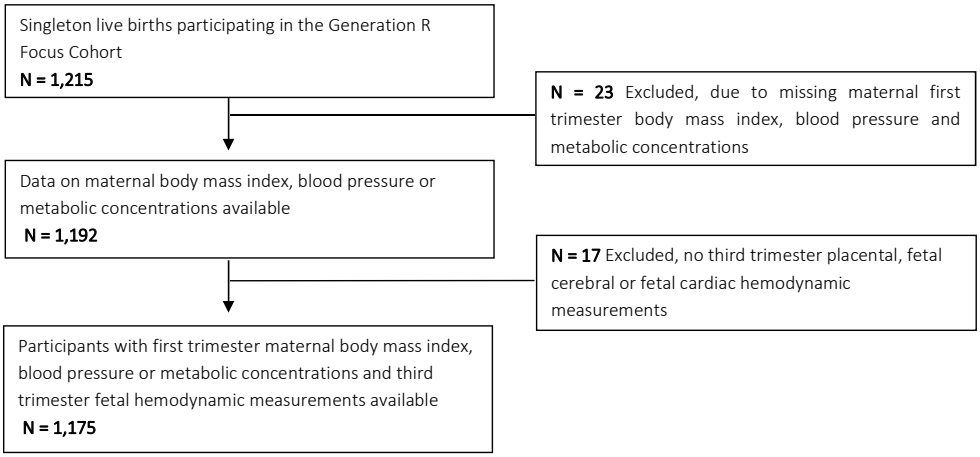

FIGURE S2.1 | Flow chart of participants included in the analysis.

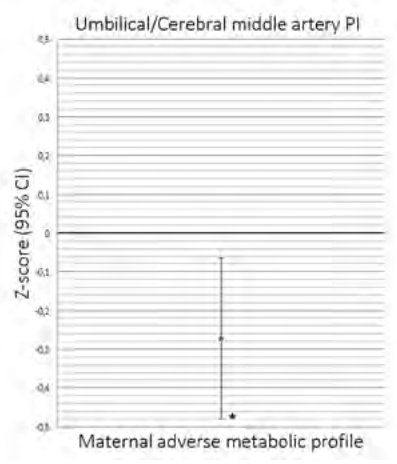

FIGURE S2.2 | Associations of maternal adverse metabolic profile with third trimester fetal umbilical/cerebral middle artery PI ratio.

Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of fetal hemodynamics of mothers with an adverse metabolic profile compared to mothers without an adverse metabolic profile. The model is adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age, estimated fetal weight, and child sex. PI: pulsatility index. *=P<0.05

TABLE S2.1 | Characteristics of mothers and their children (n=1,175) – non imputed data.

Maternal characteristics	
Maternal age	31.9 (22.0 – 39.1)
Missing (%)	-
Education (%)	
Low (no, primary, secondary education)	36.4 (426)
High (higher education)	62.4 (733)
Missing	1.3 (16)
Pre-pregnancy body mass index (kg/m²)	23.5 (4.0)
Missing (%)	155 (13)
BMI > 25.0	22.4 (263)
Parity (%)	
Nullipara	60.8 (711)
Multipara	39.1 (459)
Missing	0.2 (2)
Smoking during pregnancy (%)	
No smoking throughout pregnancy	68.9 (809)
Yes	22.3 (262)
Missing	8.9 (104)
First trimester maternal characteristics	
Gestational age at measurement, weeks	12.6 (9.6 - 17.1)
Missing (%)	183 (16)
Systolic blood pressure (mmHg)	119 (13)
Missing (%)	130 (12)
Diastolic blood pressure (mmHg)	70 (10)
Missing (%)	130 (12)
Total cholesterol, mmol/L	4.9 (0.9)
Missing (%)	171 (15)
HDL-cholesterol, mmol/L	1.8 (0.3)
Missing (%)	172 (15)
Triglycerides, mmol/L	1.3 (0.5)
Missing (%)	173 (15)
Glucose mmol/L	4.4 (0.8)
Missing (%)	186 (16)
Adverse metabolic profile (%)	9.2 (108)
Third trimester fetal characteristics	
Sex	
Male	52.4 (616)
Female	47.6 (559)
Missing	-
Gestational age at measurement, weeks	30.3 (28.5 – 32.6)
Missing	-
Estimated fetal weight, grams	1630 (267)
Missing (%)	9 (0.8)
Third trimester feto-placental hemodynamics	
Uterine artery RI	0.49 (0.08)
Missing (%)	64 (5)
Umbilical artery PI	0.97 (0.17)
Missing (%)	23 (2)
Third trimester fetal cerebral hemodynamics	
Middle cerebral artery PI	1.97 (0.33)
Missing (%)	34 (3)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Missing (%)	58 (5)

Third trimester fetal cardiac hemodynamics	
Aorta ascendens diameter (cm)	0.64 (0.07)
Missing (%)	111 (9)
Aorta ascendens PSV (cm/s)	91.3 (12.4)
Missing (%)	131 (11.2)
Left cardiac output (ml/min)	606 (172)
Missing (%)	157 (13)
Mitral valve E/A ratio	0.78 (0.10)
Missing (%)	42 (4)

Values are means (standard deviation), medians (95% range) or valid percentages (absolute numbers). RI: resistance index, PI: pulsatility index, PSV: Peak Systolic Volume

TABLE S2.2 | Associations of maternal pre-pregnancy BMI and first trimester metabolic concentrations with third trimester fetal umbilical/cerebral middle artery PI ratio (n=1,175).

	Umbilical/Cerebral middle artery PI ratio Z-score (95% CI)
Pre-pregnancy BMI (Z-score)	-0.01 (-0.07, 0.06)
Systolic blood pressure (Z-score)	-0.03 (-0.09, 0.03)
Diastolic blood pressure (Z-score)	-0.03 (-0.09, 0.04)
Total cholesterol (Z-score)	-0.07 (-0.13, -0.01)*
+pre-pregnancy BMI	-0.07 (-0.13, -0.01)*
HDL-cholesterol (Z-score)	-0.02 (-0.09, 0.04)
Triglycerides (Z-score)	-0.03 (-0.10, 0.03)
Glucose (Z-score)	-0.02 (-0.09, 0.04)

Values are regression coefficients (95% confidence intervals) and reflect the change in Z-score of fetal umbilical/cerebral middle artery PI ratio per Z-score change in maternal BMI, blood pressure and metabolic concentrations. The models are adjusted for maternal age, educational level, parity, smoking status during pregnancy, third trimester gestational age and estimated fetal weight, and child sex. PI: pulsatility index. *=P<0.05

Chapter 3

Fetal blood flow redistribution and childhood growth and development

Chapter 3

Chapter 3.1

Influence of fetal blood flow redistribution on fetal and childhood growth and fat distribution

Kooijman MN, Gaillard R, Reiss IKM, Hofman A, Steegers EAP, Jaddoe VWV

Adapted from BJOG, 2016;123(13):2104-2112

Abstract

Objective A suboptimal intra-uterine environment leads to fetal blood flow redistribution and fetal growth restriction. Not much is known about childhood growth consequences. We examined the associations of fetal blood flow redistribution with birth outcomes, repeatedly measured fetal and childhood growth and fat mass measures.

Design Prospective cohort study.

Setting Population-based.

Population 1,195 pregnant women and their children.

Methods We measured umbilical and cerebral artery blood flow at a gestational age of 30.3 weeks (95%range 28.5–32.6). A higher umbilical/cerebral (U/C) pulsatility index ratio is an indicator of preferential blood flow to the brain cerebral circulation at expense of the lower body parts.

Main Outcome Measures Fetal and childhood growth were repeatedly measured from third trimester until childhood. We measured total body fat mass, lean fat mass and android/gynoid fat mass ratio by dual-energy x-ray absorptiometry and preperitoneal fat by ultrasound at 6 years.

Results A higher fetal U/C ratio was associated with increased risks of preterm birth and small size for gestational age at birth (Odds Ratios 1.41 (95%CI 1.08 to 1.85) and 1.63 (1.21 to 2.19), respectively per SD increase in U/C ratio). Longitudinal growth analyses showed that a higher fetal U/C ratio was associated with persistently lower head circumference, length, and weight from third trimester fetal life until childhood (all p-values <0.05). Fetal U/C ratio was not associated with total body and abdominal fat measures at 6 years.

Conclusion Our results suggest that fetal blood flow redistribution affects fetal development and has persistent consequences for childhood growth.

Introduction

Fetal growth and development largely depends on an adequate placental and fetal circulation.[1] An adverse intra-uterine environment leads to fetal blood flow redistribution with a preferential fetal blood flow to the brain cerebral circulation at the expense of lower body parts.[2,3] This fetal blood flow distribution leads to relatively higher blood and oxygen supply to the brains and heart.[4,5] Fetal blood flow redistribution is caused by higher peripheral and lower cerebral vascular resistance, and can be measured as a higher umbilical artery pulsatility index (PI) and a lower cerebral PI.[2] This combination leads to a higher umbilical/cerebral (U/C) ratio.[6] Previous studies have shown that fetal blood flow redistribution in late pregnancy might be accompanied with asymmetrical fetal growth restriction characterized by a relatively larger head size than body size at birth.[7-9] Subsequently, this can lead to a different body composition and increased percentages of body fat in later life.[10] Previously, we observed that third trimester uterine, umbilical and cerebral blood flow measures are correlated with a lower estimated fetal weight.[11] We also observed that an increased third trimester umbilical artery vascular resistance is associated with lower fetal length and weight growth in third trimester resulting in smaller size at birth.[12] These differences in length and weight growth characteristics became smaller from the age of 6 months, but were still present at the age of 6 years.[12] Thus far, it is not known whether only umbilical artery flow changes or also cerebral artery flow changes affect childhood growth and body fat distribution.

We examined in a population-based prospective cohort study among 1,195 pregnant women and their children, the associations of third trimester fetal blood flow redistribution with repeatedly measured fetal and childhood growth characteristics and fat distribution outcomes.

Methods

Design and study population

The current study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[13, 14] The Medical Ethics Committee of the Erasmus MC, University Medical Center, Rotterdam, has approved the study.

The study has been described in detail previously. Briefly, all participating were born between April 2002 and January 2006. Enrollment was aimed at early pregnancy but allowed until birth of the child. In total, 9,778 mothers and their children were enrolled in the study. More detailed assessments of fetal and childhood growth and development were conducted in a random subgroup of 1,232 Dutch mothers and children.[13] For the present analysis, twin pregnancies (n=15) and pregnancies leading to perinatal death (n=2) were excluded from this analyses, resulting in 1,215 singleton live born children. Third trimester fetal blood flow distribution measurements were available in 1,195 children (**Supplementary Material Figure S3.1.1**). Of all of these children, at least one growth measure was available.

Third trimester fetal blood flow distribution

In the Netherlands, routinely ultrasound scans are performed at 12 and 20 weeks of gestation. For this study, third trimester fetal ultrasound examinations were performed at a median gestational age of 30.3 (95% range 28.5 – 32.6) weeks. Gestational age was established by first trimester ultrasound measurements.[15] Fetal blood flow distribution was measured as inverse of the corresponding resistance indices in the umbilical and cerebral artery by pulsed-wave Doppler, as described previously.[16-18] A higher pulsatility index (PI) in a fetal artery might be an indication of a lower blood flow and an increased vascular resistance.[19] For all Doppler measurements, colour imaging was used to optimize placement of the pulsed-was Doppler gate. The mean of three measurements was used for analyses. The redistribution of blood flow in favour of the fetal brain was quantified by the middle cerebral artey PI.[20] Middle cerebral artery Doppler measurements were performed with colour Doppler visualization of the circle of Willis in the fetal brain, and flow-velocity waveforms were obtained in the proximal part of the cerebral arteries. Fetal blood flow redistribution in favour to the brain at expense of the trunk, including the abdominal organs, is indicated by an increased ratio between the umbilical artery PI and the cerebral artery PI (higher U/C ratio).[6] Intra- and interobserver analyses showed good reproducibility for all Doppler measurements, as described previously (all intra-class correlation coefficients > 0.80).[11] The mean and SD of the umbilical and cerebral artery PI observed in our study were in line with a previous longitudinal study focused on serial measurements.[21] Variables were normally distributed, as shown in **Supplementary Material Figure S3.1.6**.

Fetal and childhood growth

During the same visit in third trimester, we measured fetal head circumference, abdominal circumference, femur length and calculated estimated fetal weight.15, 22 Gestational age adjusted standard deviation scores were constructed for all fetal growth measurements23. We obtained information on date of birth, birth anthropometrics (head circumference, length, weight) and offspring sex from community midwife and hospital registries. Preterm birth was defined as a gestational age of <37 weeks at birth. Small size for gestational age at birth was defined as gestational age corrected birth weight below the fifth centile in the study cohort. Since head circumference and length at birth were not routinely measured at birth, missing birth measures were completed with data from the first month visit at the child health center. Well-trained staff obtained postnatal growth characteristics (head circumference, height, weight) using standardized procedures. Visits were scheduled for age 6 months (median: 6.2, 95% range: 5.2 – 7.8), 12 months (median: 11.0, 95% range: 10.1 – 12.6), 24 months (median: 24.9, 95% range: 23.4 – 28.2), 36 months (median: 37.8, 95% range: 35.4 – 40.7), 48 months (median: 45.8, 95% range: 44.5 – 48.4) and 72 (95% range 68.4 – 79.2) months. Head circumference was measured by a measuring tape (SECA). We measured length in infants in supine position to the nearest 0.1cm by a neonanometer (Holtain Limited) and after this period by a Harpenden stadiometer (Holtain Limited) in standing position. Weight

was measured in naked infants at the age of 6 months to the nearest grams by using an electronic infant scale (SECA) and from 24 months onwards by a mechanical personal scale (SECA). We calculated body mass index (kg/m2). For postnatal growth characteristics, we calculated standard deviation scores using Dutch reference growth curves (Growth Analyzer 3.0, Dutch Growth Research Foundation, Rotterdam, the Netherlands).24

Childhood adiposity outcomes

We measured total body and regional fat mass using a Dual energy X-ray absorptiometry (DXA) scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA) and analysed with the enCORE software v.12.6.[25] DXA can accurately detect whole-body fat mass within less than 0.25% coefficient of variation. Children were placed without shoes, heavy clothing and metal objects in supine position on the DXA table. Total fat mass (kg) and lean mass were calculated as percentage of total body weight (kg) measured by DXA. The android/gynoid fat mass ratio was calculated. The android/gynoid fat ratio reflects the central body fat distribution in the abdomen and hip regions, respectively and was used as a marker of waist/hip fat distribution.[26]

Abdominal ultrasound examinations were performed with ultrasound, as described in detail.[27,28] Briefly, preperitoneal fat thicknesses were measured with a linear transducer[27], which was placed perpendicular to the skin surface on the median upper abdomen. We scanned longitudinally just below the xiphoid process to the navel along the midline (linea alba). All measurements were performed off-line. Preperitoneal fat mass distance was measured as distance of the linea alba to the peritoneum on top of the liver. Preperitoneal fat mass area was measured as area of 2 cm length along the midline starting from the maximum preperitoneal distance in direction of the area. We measured three times the areas of 2 cm length along midline, and we used the mean value of these measures. The intra-observer reproducibility and the intra-class correlation coefficients showed good reproducibility (all intra-class correlation coefficients > 0.92).[29]

Covariates

We obtained information on maternal age, prepregnancy weight, parity, educational level, smoking during pregnancy, folic acid use, gestational hypertensive disorders (gestational hypertension/ preeclampsia) and gestational diabetes by questionnaires and medical records. We measured maternal height without shoes and we calculated prepregnancy body mass index (kg/m^2).

Statistical analysis

First, we used multivariate logistic regression models to examine the associations of third trimester fetal blood flow distribution (umbilical artery PI, middle cerebral artery PI and U/C ratio) with the risks of preterm birth and small size for gestational age at birth. The models were first adjusted for gestational age at third trimester measurement and child sex (basic model). Additionally, we included potential confounders including maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, folic acid supplementation

use, and pregnancy complications (confounder model). Second, the associations of third trimester fetal blood flow redistribution (umbilical artery PI, middle cerebral artery PI and U/C ratio) were analysed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the subject into account and allow for incomplete outcome data.[30, 31] We created fetal and childhood models in which gestational or childhood age were included as continuous variables. From the constructed models, we obtained effect estimates at specific time points of interest. The fetal flow measurements were included in the models as intercept, which reflects the mean SDS for each growth characteristics per SD increase in U/C ratio, and as interaction term with gestational/postnatal age. This interaction term reflects the difference in change in growth characteristics over time per SD increase in U/C ratio. We used a compound symmetry covariance structure. The models are described in more detail in the **Supplementary Material**. Furthermore, we performed similar analyses focused on the associations of U/C ratio with childhood growth in groups of children with the lowest third trimester estimated fetal weight (<10% of EFW) and highest third trimester estimated fetal weight (> 90% of EFW). Third, we used multivariate linear regression models to examine the associations of fetal blood flow distribution (umbilical artery PI, middle cerebral artery PI and U/C ratio) with childhood fat measures. As preperitoneal fat area had a skewed distribution, we applied natural log-transformation. The models were first adjusted for gestational age and estimated fetal weight at third trimester measurement, child sex and current age. Additionally, we included same potential confounders as for the birth outcome models. The models with childhood fat and lean mass as outcome were also adjusted for height at time of the fat measurements. Covariates were included in the models based on their association with fetal blood flow distribution and growth measurements as shown in previous studies.[12] The confounder models were considered as the main models. We tested potential interactions between fetal blood flow distribution and sex for all our analyses. Significant interactions were present for the repeated measured head circumference in pre- and postnatal models and prenatal measured length ($p<0.05$). All these analyses were performed for total group, and for boys and girls separately. All effect estimates were given per standard deviation (SD) change in fetal blood flow distribution measures. To reduce the possibility of potential bias associated with missing data (less than 18%), missing values were imputed using the multiple imputations procedure. In the imputation model we included all covariates. Five imputed datasets were created and analyzed together. Further information about the methods of multiple imputations are given in the **Supplementary Material**. The repeated measurement analyses were performed using the Statistical Analysis System version 9.3 (SAS, Institute Inc. Cary NC). All other analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

Participant characteristics

Table 1 showed a mean umbilical artery PI of 0.97 (0.16), a middle cerebral artery PI of 1.97 (0.16), and an U/C ratio of 0.50 (0.11). At age 6 years, children had a mean body length of 119.0 (5.2) cm, weight of 22.6 (3.2) kg and the mean total fat mass was 23.8 (4.5)%. Descriptive data of the participants without multiple imputation are shown in **Supplementary Material Table S3.1.1**. **Supplementary Material Table S3.1.2** shows all infant growth characteristics, **Table S3.1.3** shows maternal characteristics.

TABLE 3.1.1 | Subject characteristics (N=1,195).

Third trimester fetal characteristics	
Gestational age at measurement, wk	30.3 (28.5, 32.6)
Umbilical artery pulsatility index	0.97 (0.16)
Middle cerebral artery pulsatility index	1.97 (0.33)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Fetal head circumference, cm	28.6 (1.2)
Fetal femur length, cm	5.7 (0.3)
Estimated fetal weight, g	1630 (268)
Birth characteristics	
Gestational age at birth, wk	40.3 (35.9, 42.4)
Head circumference, cm	34.0 (1.6)
Birth weight, g	3514 (540)
Birth length, cm	50.5 (2.3)
Preterm, %	4.7 (56)
Small for gestational age, %	3.7 (44)
Boys, %	52.1 (622)
Childhood characteristics	
Age at follow up, y	5.9 (5.7, 6.6)
Head circumference, cm	51.4 (1.4)
Height, cm	119.0 (5.2)
Weight, kg	22.6 (3.2)
Body mass index, kg/m^2	15.9 (1.4)
Total fat mass, %	23.8 (4.5)
Lean mass, %	72.6 (4.4)
Android/gynoid fat mass ratio, %	23.7 (16.1, 37.8)
Preperitoneal fat area, cm^2	0.37 (0.17, 0.89)

Values are means (standard deviation), medians (95% range), or numbers (%). Non-imputed subject characteristics can be found in **Supplementary Material Table S3.1.1**, infant growth characteristics in **Table S3.1.2** and maternal characteristics in **Table S3.1.3**.

Third trimester fetal flow distribution and the risks of adverse birth outcomes

Table 3.1.2 shows that a higher third trimester fetal U/C ratio was associated with increased risk of preterm birth (OR 1.41 (95% CI 1.08 to1.85) per SD increase in fetal U/C ratio) and small for gestational age (OR 1.63 (95% CI 1.21, 2.19) per SD increase in fetal U/C ratio).

TABLE 3.1.2 | Associations of third trimester fetal umbilical and cerebral blood flow with risks of adverse birth outcomes (N = 1,195).

	Preterm Odds Ratios (95% CI)	**Small for gestational age** Odds Ratios (95% CI)
Basic model		
Umbilical artery pulsatility index	1.06 (0.80, 1.41)	2.25 (1.65, 3.06)†
Middle cerebral artery pulsatility index	0.66 (0.50, 0.87)†	1.01 (0.74, 1.37)
Umbilical/Middle cerebral artery ratio	1.42 (1.09, 1.83)†	1.62(1.22, 2.14)†
Confounder model		
Umbilical artery pulsatility index	1.02 (0.77, 1.37)	2.18 (1.59, 2.99)†
Middle cerebral artery pulsatility index	0.65 (0.49, 0.87)†	1.01 (0.74, 1.38)
Umbilical/Middle cerebral artery ratio	1.41 (1.08, 1.85)†	1.63 (1.21, 2.19)†

Values are Odds Ratios (95% Confidence Interval) from logistic regression models that reflect the risk of preterm and small for gestational age at birth per SDS change in third trimester fetal umbilical and cerebral blood flow. Basic model is adjusted for gestational age at third trimester and child's sex. Confounder model is additionally adjusted for maternal age, parity, educational level, body mass index, smoking, folic acid supplementation use and pregnancy complications. †P-value<0.01

Third trimester fetal flow distribution and longitudinal growth measurements

Figure 3.1.1 shows the associations of third trimester fetal U/C ratio with longitudinal fetal and childhood growth patterns. A higher third trimester fetal U/C ratio was associated with a smaller fetal head circumference, length and weight from third trimester onwards, leading to a smaller size at birth (differences for birth head circumference -0.08 SD (95% CI -0.15 to -0.01); birth length -0.09 SD (95%CI -0.16 to -0.02) and birth weight (-0.18 SD (95% CI -0.24 to -0.12) per SD increase in fetal U/C ratio). The differences were smaller during childhood but remained significant. At the age of 6, a higher third trimester fetal U/C ratio was associated with smaller head circumference (difference -0.05 SD (95% CI -0.11 to 0), height (difference -0.12 SD (95%CI -0.17 to -0.06), weight (difference -0.12 SDS (95% CI -0.17 to -0.06)) and body mass index (difference -0.06 SDS (95% CI -0.12 to -0.01). We observed significant interactions between sex and repeated measured head circumference in fetal - and childhood models and fetal length (P<0.05). The differences tended to be stronger in boys than in girls. The associations of fetal blood flow redistribution with growth patterns remain relatively constant over time. We did observe an overall association of fetal blood flow redistribution with growth, but no significant interaction between fetal flow and (gestational) age were present. The associations of third trimester fetal umbilical artery PI and cerebral artery PI separately with longitudinal fetal and childhood growth patterns are given in the **Supplementary Material** (**Figure S3.1.2**). The associations of third trimester fetal umbilical artery PI with longitudinal growth patterns tended to be similar as those for the fetal U/C ratio (**Figure S3.1.2**). The associations of third trimester cerebral artery PI with longitudinal growth patterns are given in **Figure S3.1.3** and showed a non-significant tendency for a positive association of third trimester higher cerebral artery PI with head circumference, height and weight growth during fetal life and childhood, with smaller differences at older ages (**Figure S3.1.3**). The associations of third trimester fetal U/C ratio with childhood growth patterns in the groups of

children in the lowest and highest third trimester estimated fetal weight categories are given in the **Supplementary Material** (**Figures S3.1.4** and **S3.1.5**). Children in the lowest 10 percent of third trimester estimated fetal weight group tended to have a stronger inverse association of third trimester fetal U/C ratio with head circumference, height and weight growth during fetal life and childhood, with larger differences at older ages compared with growth patterns in total group. Children in highest 10 percent of third trimester estimated fetal weight group showed similar longitudinal growth patterns compared the total group.

Third trimester fetal flow distribution and childhood adiposity outcomes

Table 3.1.3 showed that third trimester fetal umbilical artery PI, middle cerebral artery PI or the U/C ratio were not associated with, total fat mass, lean mass, android/gynoid fat mass ratio or preperitoneal fat area at the age of 6.

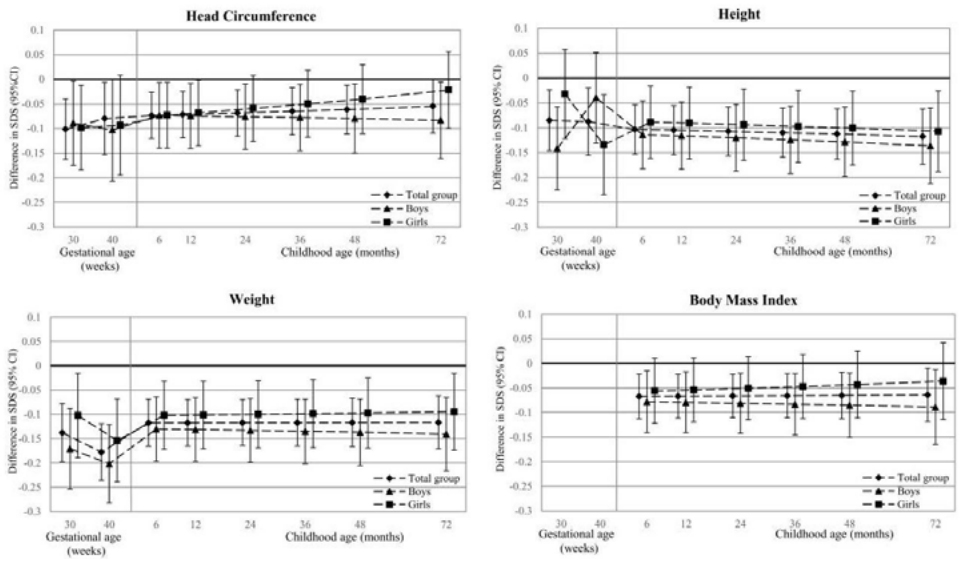

FIGURE 3.1.1 | Associations of third trimester fetal flow redistribution with growth characteristics.
Associations of third trimester fetal U/C ratio with fetal and childhood growth characteristics. Values reflect regression coefficients (95% Confidence Interval) and reflect differences in (gestational) age adjusted SDS of growth characteristics per SDS change in U/C ratio. Models are adjusted for age at third trimester. Total group analyses were additionally adjusted for child's sex. P-value of the interaction term of fetal flow with (gestational) age was not significant. P-value for sex interaction <0.01 for model focused on head circumference and prenatal length.

TABLE 3.1.3 | Associations of third trimester fetal umbilical and cerebral blood flow with body fat distribution at the age of 6 (N = 923).

	Fat mass, %	Lean mass, %	Android/gynoid fat mass ratio	Preperitoneal fat area (cm²)
Basic model				
Umbilical artery pulsatility index	0.26 (-0.02, 0.53)	-0.26 (-0.53, 0.01)	-0.01 (-0.39, 0.37)	0.01 (-0.03, 0.04)
Middle cerebral artery pulsatility index	0.18 (-0.09, 0.45)	-0.19 (-0.44, 0.07)	-0.05 (-0.41, 0.32)	0.01 (-0.02, 0.04)
Umbilical/Middle cerebral artery ratio	-0.00 (-0.28, 0.28)	0.00 (-0.27, -0.27)	-0.02 (-0.37, 0.39)	-0.01 (-0.04, 0.02)
Confounder model				
Umbilical artery pulsatility index	0.20 (-0.07, 0.47)	-0.20 (-0.46, 0.05)	-0.11 (-0.49, 0.27)	-0.00 (-0.04, 0.03)
Middle cerebral artery pulsatility index	0.11 (-0.15, 0.37)	-0.12 (-0.37, 0.13)	-0.13 (-0.49, 0.24)	0.01 (-0.02, 0.04)
Umbilical/Middle cerebral artery ratio	0.05 (-0.23, 0.32)	-0.04 (-0.31, 0.22)	0.03 (-0.35, 0.41)	-0.01 (-0.04, 0.02)

Values are regression coefficients (95% Confidence Interval) from linear regression models that reflect differences in childhood body fat distribution per SDS change in third trimester fetal umbilical and cerebral blood flow. Basic model is adjusted for gestational age at third trimester, estimated fetal weight, child's sex and current age. Confounder model is additionally adjusted maternal age, parity, educational level, body mass index, smoking, folic acid supplementation use and pregnancy complications. Preperitoneal fat area was log transformed. Models with fat mass and lean mass were additionally adjusted for length of the child at age 6.

Discussion

Main findings

In this population-based prospective cohort study we observed that third trimester fetal blood flow redistribution, with a preferential fetal blood flow to brain cerebral circulation at the expense of lower body parts, was associated with increased risks of adverse birth outcomes. Also, fetal blood flow redistribution was associated with a smaller third trimester fetal head circumference, length, and weight growth from third trimester onwards with persistent effects until the age of 6 years. We did not observe associations of third trimester fetal blood flow redistribution with childhood total body and abdominal fat measures.

Strengths and limitations

The main strength of this study is the large population-based cohort studied from fetal life onwards. To our knowledge, thus far this is the largest study, which examined the effects of fetal blood flow distribution on early growth trajectories. The population-based setting enabled us to assess the fetal blood flow measures across the full range, rather than only in fetuses with growth restriction or other complications. Follow-up measurements at the age of 6 were available in 75% of the children. Missing growth and fat distribution measurements could lead to selection bias and loss of power. Our results would be biased if the associations between fetal blood flow distribution and growth outcomes differ between those included and those not included in the study. Although this seems unlikely, it cannot be excluded. Finally, although we had

information about a large number of confounders, the influence of residual confounding should be considered, as in any observational study.

Interpretation

Fetal growth and development largely depends on an adequate placental and fetal circulation.[1] An adverse intra-uterine environment with reduced oxygenation or nutrition levels in the developing fetus leads to fetal blood flow redistribution, an important fetal adaptation mechanism to ensure blood transport to the most important organs.[2, 3] The first signs of a suboptimal intra-uterine environment can be detected in a decline in umbilical vein blood flow. A compensatory increase in ductus venous diameter increases the blood flow to the heart.[4, 5] This will be followed by an increase in the umbilical artery blood flow resistance and a decrease in cerebral artery resistance.[32] Both the umbilical and uterine artery blood flow parameters provide information about the placental circulation, but assessment of vascular resistance in the umbilical artery may be more closely related to the fetal condition. Umbilical artery Doppler resistance indices were related to fetal levels of glucose and amino acids[33], and therefore the umbilical artery PI could be used as substitute measurement of placenta function.[34] In line with previous studies from the same cohort[17, 18] we used the U/C ratio, and not the Cerebrum Placental ratio (CPR) which reflects cerebral artery PI/umbilical artery PI ratio. These measurements have an inverse 1:1 correlation.

Several studies have shown that placental insufficiency is related to increased risks of adverse birth outcomes.[35-37] However, Akolekar et al. showed that at 35-37 weeks of gestation the association of a low CPR with adverse birth outcomes is weak.[38] Mechanisms that cause preterm birth were not clearly defined.[39] We hypothesized that it might be that a suboptimal fetal circulation causes fetal undernutrition and subsequently results in preterm delivery.[40] In the current study, we observed that small variations in third trimester fetal umbilical artery PI, middle cerebral artery PI and the U/C ratio, in a low risk population, were associated with increased risks of preterm birth and small-size for gestational age at birth. These observations suggest measuring the middle cerebral artery PI and the U/C ratio, in addition to placental pulsatility indices, may be useful for identifying fetuses at risk of adverse birth outcomes.

Studies have shown that fetal blood flow redistribution in late pregnancy results in asymmetrical fetal growth restriction characterized by a relatively larger head size than body size at birth.[7-9] Previously, we observed that third trimester uterine, fetal umbilical and cerebral blood flow measures are correlated with a lower estimated fetal weight.[11] However, not much is known about fetal blood flow redistribution and the consequences on childhood growth. We have previously shown that an increased third trimester umbilical artery vascular resistance is associated with lower fetal, infant and childhood growth.[12] Thus far, it is not known whether only umbilical artery flow changes or also cerebral artery flow changes affect childhood growth and body fat distribution. Results from the longitudinal growth analyses in the current study showed that a higher third trimester U/C ratio and umbilical artery PI were associated with persistently lower head circumference, length, and weight growth from third trimester until 6 years. The

associations of third trimester cerebral artery PI with longitudinal growth patterns tended to have smaller effect estimates compared with third trimester umbilical artery PI and the U/C ratio. Our results among children with low third trimester estimated fetal weight and high third trimester estimated fetal weight suggest that the effects of third trimester U/C ratio on childhood growth patterns may be stronger among children with a lower third trimester estimated fetal weight. However, the confidence intervals were wide and the observed differences in effect estimates were not significant. Further studies are needed to explore whether combining festal growth and U/C ratio may help to identify children at risk for long term health outcomes.

In our study, we did not find evidence for full catch up growth following small-size at birth in response to fetal blood flow distribution. These findings suggest that fetal blood flow distribution leads to persistently smaller size during fetal life and childhood. Previous studies have shown that a low birth weight is associated with increased fat measures at different ages.[10, 41, 42] The underlying pathways may include differences in body proportions, which subsequently predispose individuals to increased risks of cardiovascular disease in later life.[35, 43-45] Previously, in a larger group of our cohort, we observed small associations of higher umbilical placental PI with increased total fat mass and the android/gynoid fat mass ratio in childhood.[12] However, our study did not suggest any effect of fetal blood flow redistribution on childhood overweight and fat mass measures, which might be due to the smaller study sample. Also, it might be that the consequences of a suboptimal fetal environment might not be detectable in early childhood, but might become more evident in later life. Further studies are needed to identify the long-term consequences of fetal blood flow redistribution on growth patterns and adiposity measures in young adulthood.

The umbilical artery PI reflects the fetal-placental connection, whereas the middle cerebral artery PI reflects the fetal response. The U/C ratio detects the redistribution of fetal blood flow due to adaptions in these two vascular units.[34] Animal models have demonstrated that the ratio is better correlated with fetal hypoxia than the umbilical or the cerebral component by itself.[46] It has also been shown that using the U/C ratio, the MCA/uterine artery or the MCA/umbilical artery, improves the prediction of adverse pregnancy outcomes compared to its individual components.[47] In line with these studies, we observed the strongest associations of the U/C ratio with longitudinal growth analyses from third trimester fetal life until the age of 6 years. However, although our findings suggest that fetal blood flow redistribution increased the risks of adverse birth outcomes and affects a child's growth trajectory, the effect estimates were small and likely to be without direct clinical consequences for individuals.

Conclusion

In this study we observed that third trimester fetal blood flow redistribution with a preferential fetal blood flow to the brain cerebral circulation at the expense of lower body parts, is associated with increased risks of adverse birth outcomes and growth restriction from fetal life onwards.

Direct clinical consequences are limited, however our findings are important from an etiological point of view. Follow-up studies to health consequences in adulthood of suboptimal fetal blood flow circulation are needed.

References

1. Marconi AM, Paolini CL. Nutrient transport across the intrauterine growth-restricted placenta. Semin Perinatol. 2008;32(3):178-81.
2. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
3. van den Wijngaard JA, Groenenberg IA, Wladimiroff JW, Hop WC. Cerebral Doppler ultrasound of the human fetus. Br J Obstet Gynaecol. 1989;96(7):845-9.
4. Bellotti M, Pennati G, De Gasperi C, Bozzo M, Battaglia FC, Ferrazzi E. Simultaneous measurements of umbilical venous, fetal hepatic, and ductus venosus blood flow in growth-restricted human fetuses. Am J Obstet Gynecol. 2004;190(5):1347-58.
5. Kiserud T, Kessler J, Ebbing C, Rasmussen S. Ductus venosus shunting in growth-restricted fetuses and the effect of umbilical circulatory compromise. Ultrasound Obstet Gynecol. 2006;28(2):143-9.
6. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: a pulsed Doppler study. J Perinat Med. 1992;20(1):79-82.
7. Bocca-Tjeertes I, Bos A, Kerstjens J, de Winter A, Reijneveld S. Symmetrical and asymmetrical growth restriction in preterm-born children. Pediatrics. 2014;133(3):e650-6.
8. Saleem T, Sajjad N, Fatima S, Habib N, Ali SR, Qadir M. Intrauterine growth retardation--small events, big consequences. Ital J Pediatr. 2011;37:41.
9. Vandenbosche RC, Kirchner JT. Intrauterine growth retardation. Am Fam Physician. 1998;58(6):1384-90, 93-4.
10. Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M, et al. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am J Clin Nutr. 2005;82(5):980-7.
11. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
12. Gaillard R, Steegers EA, Tiemeier H, Hofman A, Jaddoe VW. Placental vascular dysfunction, fetal and childhood growth, and cardiovascular development: the generation R study. Circulation. 2013;128(20):2202-10.
13. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.
14. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, et al. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29(12):911-27.
15. Gaillard R, Steegers EA, de Jongste JC, Hofman A, Jaddoe VW. Tracking of fetal growth characteristics during different trimesters and the risks of adverse birth outcomes. Int J Epidemiol. 2014;43(4):1140-53.
16. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.
17. Kooijman MN, Bakker H, van der Heijden AJ, Hofman A, Franco OH, Steegers EA, et al. Childhood Kidney Outcomes in Relation to Fetal Blood Flow and Kidney Size. J Am Soc Nephrol. 2014.
18. Kooijman MN, de Jonge LL, Steegers EA, van Osch-Gevers L, Verburg BO, Hofman A, et al. Third trimester fetal hemodynamics and cardiovascular outcomes in childhood: the Generation R study. J Hypertens. 2014;32(6):1275-82.
19. Baschat AA. Fetal responses to placental insufficiency: an update. BJOG. 2004;111(10):1031-41.
20. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
21. Ebbing C, Rasmussen S, Kiserud T. Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements. Ultrasound

Obstet Gynecol. 2007;30(3):287-96.
22. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535-40.
23. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.
24. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res. 2000;47(3):316-23.
25. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, et al. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring). 2012;20(6):1313-8.
26. Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy X-ray absorptiometry. Pediatr Radiol. 2009;39(7):647-56.
27. Suzuki R, Watanabe S, Hirai Y, Akiyama K, Nishide T, Matsushima Y, et al. Abdominal wall fat index, estimated by ultrasonography, for assessment of the ratio of visceral fat to subcutaneous fat in the abdomen. Am J Med. 1993;95(3):309-14.
28. Mook-Kanamori DO, Holzhauer S, Hollestein LM, Durmus B, Manniesing R, Koek M, et al. Abdominal fat in children measured by ultrasound and computed tomography. Ultrasound Med Biol. 2009;35(12):1938-46.
29. Gishti O, Gaillard R, Manniesing R, Abrahamse-Berkeveld M, van der Beek EM, Heppe DH, et al. Fetal and infant growth patterns associated with total and abdominal fat distribution in school-age children. J Clin Endocrinol Metab. 2014;99(7):2557-66.
30. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28(5):964-74.
31. H. G. Multilevel statistical methods. 2nd ed. . Edward Arnold: London. 1995.
32. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. Obstet Gynecol. 1992;79(3):416-20.
33. Karsdorp VH, van Vugt JM, Jakobs C, Dekker GA, van Geijn HP. Amino acids, glucose and lactate concentrations in umbilical cord blood in relation to umbilical artery flow patterns. Eur J Obstet Gynecol Reprod Biol. 1994;57(2):117-22.
34. Cruz-Martinez R, Figueras F. The role of Doppler and placental screening. Best Pract Res Clin Obstet Gynaecol. 2009;23(6):845-55.
35. Zhang S, Regnault TR, Barker PL, Botting KJ, McMillen IC, McMillan CM, et al. Placental Adaptations in Growth Restriction. Nutrients. 2015;7(1):360-89.
36. Bamfo JE, Odibo AO. Diagnosis and management of fetal growth restriction. J Pregnancy. 2011;2011:640715.
37. Flood K, Unterscheider J, Daly S, Geary MP, Kennelly MM, McAuliffe FM, et al. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multicenter PORTO Study. Am J Obstet Gynecol. 2014;211(3):288 e1-5.
38. Akolekar R, Syngelaki A, Gallo DM, Poon LC, Nicolaides KH. Umbilical and fetal middle cerebral artery Doppler at 35-37 weeks' gestation in the prediction of adverse perinatal outcome. Ultrasound Obstet Gynecol. 2015;46(1):82-92.
39. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
40. Cooper NA, Moores R, East London Preterm Prevention C. A review of the literature regarding nutritional supplements and their effect on vaginal flora and preterm birth. Curr Opin Obstet Gynecol. 2014;26(6):487-92.
41. Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C. Consequences of being born small for gestational age on body composition: an 8-year follow-up study. J Clin Endocrinol Metab. 2008;93(10):3804-9.
42. Ibanez L, Suarez L, Lopez-Bermejo A, Diaz M, Valls C, de Zegher F. Early development of visceral fat excess after spontaneous catch-up growth in children with low birth weight. J Clin Endocrinol Metab. 2008;93(3):925-8.
43. Jansson T, Powell TL. Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. Clin Sci (Lond). 2007;113(1):1-13.
44. Barker DJ, Gelow J, Thornburg K, Osmond C, Kajantie E, Eriksson JG. The early origins of chronic heart failure: impaired placental growth and initiation of insulin resistance in childhood. Eur J Heart Fail. 2010;12(8):819-25.

45. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
46. Arbeille P, Maulik D, Fignon A, Stale H, Berson M, Bodard S, et al. Assessment of the fetal PO2 changes by cerebral and umbilical Doppler on lamb fetuses during acute hypoxia. Ultrasound Med Biol. 1995;21(7):861-70.
47. Simanaviciute D, Gudmundsson S. Fetal middle cerebral to uterine artery pulsatility index ratios in normal and pre-eclamptic pregnancies. Ultrasound Obstet Gynecol. 2006;28(6):794-801.

Supplementary Material

Longitudinal growth models

The models can be written as

Fetal:

Head circumference = $B_0 + B_1$* fetalflow + B_2* gestational age + B_3* fetalflow * gestational age

Length = $B_0 + B_1$* fetalflow + B_2* gestational age + B_3* fetalflow * gestational age

Weight = $B_0 + B_1$* fetalflow + B_2* gestational age + B_3* fetalflow * gestational age

Childhood:

Head circumference = $B_0 + B_1$* fetalflow + B_2* age + B_3* fetalflow * age

Heigth = $B_0 + B_1$* fetalflow + B_2* age + B_3* fetalflow * age

Weight = $B_0 + B_1$* fetalflow + B_2* age + B_3* fetalflow * age

BMI = $B_0 + B_1$* fetalflow + B_2* age + B_3* fetalflow * age

Multiple imputation procedure

To reduce the possibility of potential bias associated with missing data (less than 18%), missing values for covariates were imputed using the multiple imputations procedure. For the multiple imputations, we used Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach. For each variable, the fully conditional specification method fits a model using all other available variables in the model as predictors, and then imputes missing values for the specific variable being fit. In the imputation model, we included all determinants, outcomes and covariates plus educational level of the father. The determinants and outcomes were only used as predictors in the imputation model, and not imputed themselves. Five imputed datasets were created and analyzed together.[1]

[1] *Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009 Jun 29;338:b2393.*

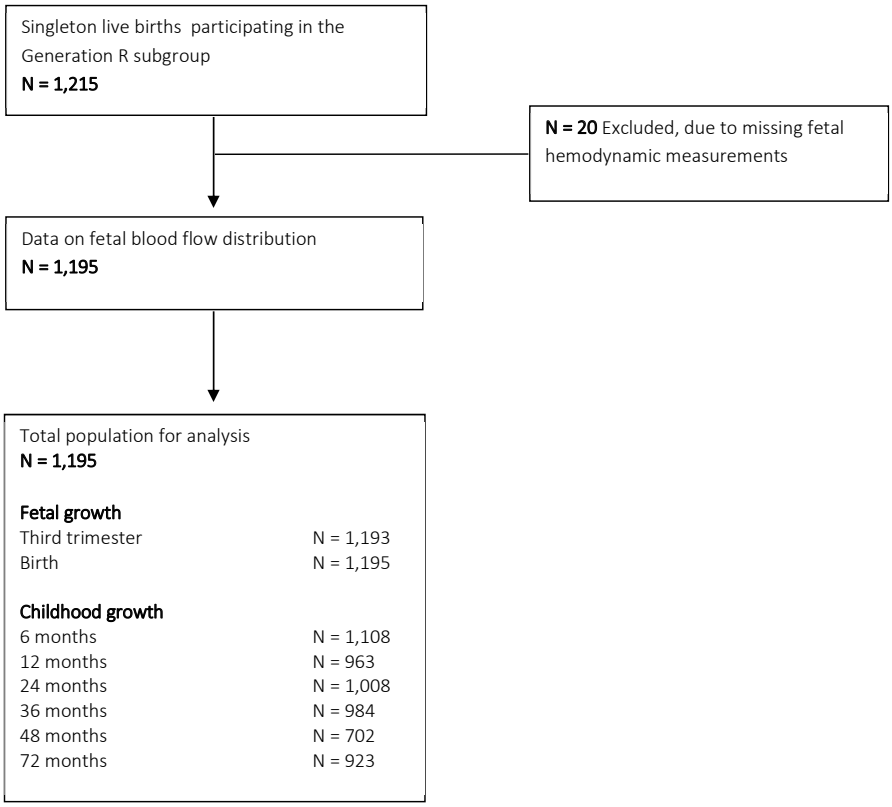

FIGURE S3.1.1 | Flow chart of participants included in the analysis.

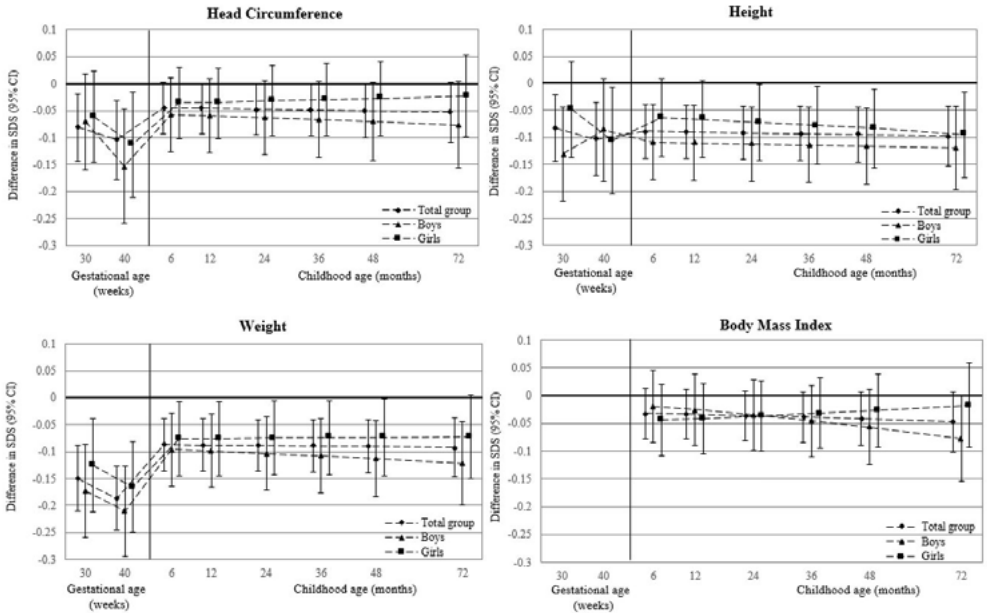

FIGURE S3.1.2 | Associations of third trimester fetal umbilical artery PI with growth characteristics.

Associations of third trimester fetal umbilical artery PI with fetal and childhood growth characteristics. Values reflect regression coefficients (95% Confidence Interval) and reflect differences in (gestational) age adjusted SDS of growth characteristics per SDS change in umbilical artery PI. Models are adjusted for age at third trimester. Total group analyses were additionally adjusted for child's sex. P-value for sex interaction <0.01 for model focused on head circumference and prenatal length and weight.

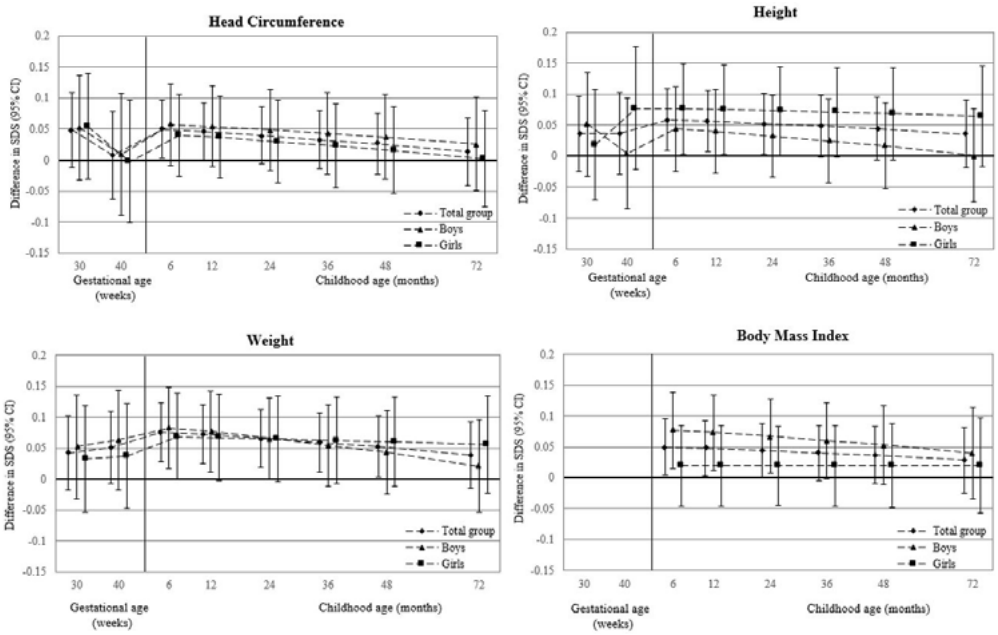

FIGURE S3.1.3 | Associations of third trimester fetal cerebral artery PI with growth characteristics.

Associations of third trimester fetal cerebral artery PI with fetal and childhood growth characteristics. Values reflect regression coefficients (95% Confidence Interval) and reflect differences in (gestational) age adjusted SDS of growth characteristics per SDS change in cerebral artery PI. Models are adjusted for age at third trimester. Total group analyses were additionally adjusted for child's sex. P-value for sex interaction <0.01 for model focused on head circumference and prenatal length.

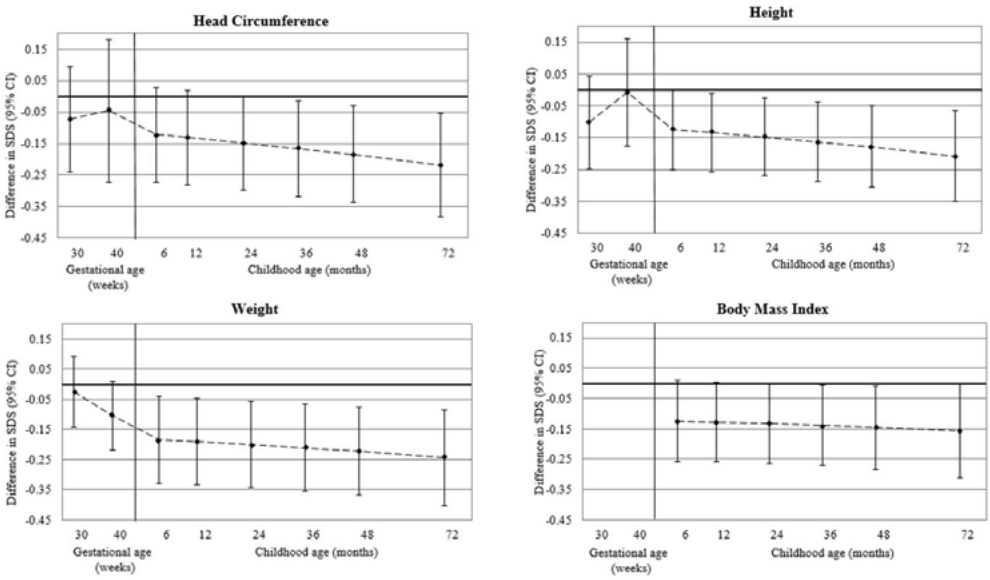

FIGURE S3.1.4 | Associations of third trimester fetal flow redistribution with growth characteristics. Estimated fetal weight < 10th percentile.

Associations of third trimester fetal U/C ratio with fetal and childhood growth characteristics, in the group with the lowest third trimester estimated fetal weight (<10 percentile EFW) children. Values reflect regression coefficients (95% Confidence Interval) and reflect difference in (gestational) age adjusted SDS of growth characteristics per SDS change in U/C ratio. Models are adjusted for age at third trimester and child's sex. P-value of the interaction term of fetal flow with (gestational) age was not significant.

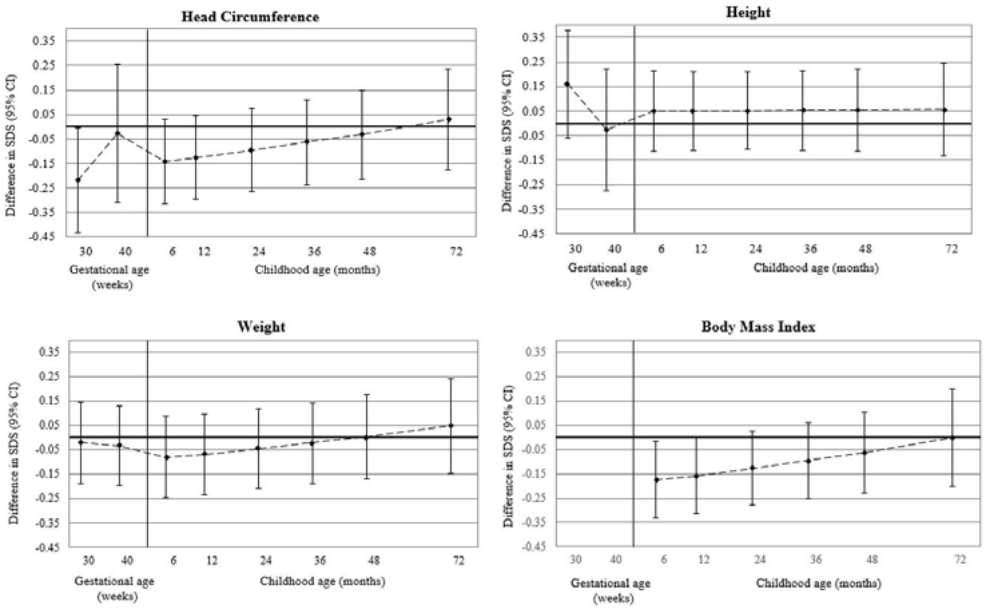

FIGURE S3.1.5 | Associations of third trimester fetal flow redistribution with growth characteristics. Estimated fetal weight > 90th percentile.

Associations of third trimester fetal U/C ratio with fetal and childhood growth characteristics, in the group with the highest third trimester estimated fetal weight (>90 percentile EFW) children. Values reflect regression coefficients (95% Confidence Interval) and reflect difference in (gestational) age adjusted SDS of growth characteristics per SDS change in U/C ratio. Models are adjusted for age at third trimester and child's sex. P-value of the interaction term of fetal flow with (gestational) age was not significant.

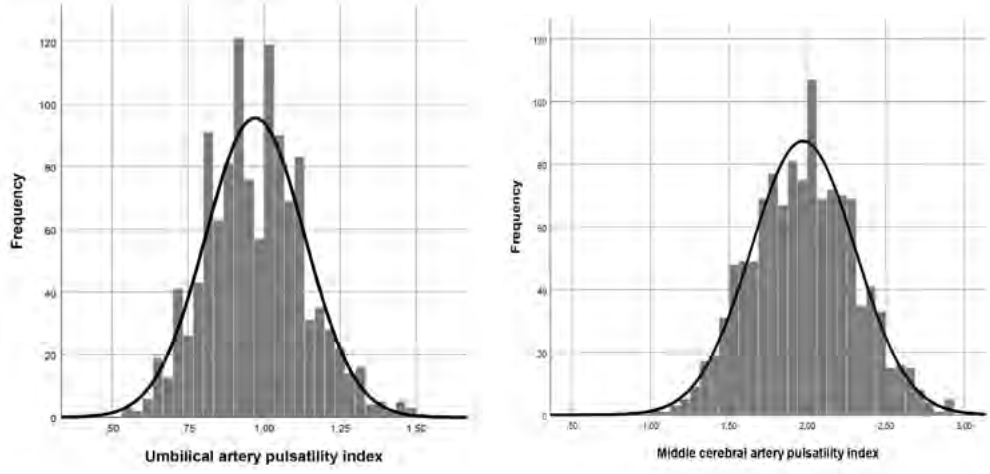

FIGURE S3.1.6 | Histograms of fetal flow measurements.

TABLE S3.1.1 | Subject characteristics (N = 1,195) – non imputed data.

Maternal characteristics	
Age, y	31.9 (21.8, 39.1)
Gestational age at enrolment, wk	12.9 (9.9, 21.1)
Height, cm	170.9 (6.3)
Prepregnancy weight, kg	68.8 (12.7)
Prepregnancy body mass index, kg/m^2	23.5 (4.1)
Parity ≥1, %	469 (39.2)
Missing	3 (0.3)
Educational level, %	
Primary/secondary	434 (36.3)
Secondary or higher	744 (62.1)
Missing	19 (1.6)
Folic acid supplement use, %	
No	93 (7.8)
Start in first 10 weeks of pregnancy	288 (24.1)
Start periconceptional	608 (50.9)
Missing	206 (17.2)
Smoking during pregnancy, %	
No	822 (68.6)
Yes	267 (22.3)
Missing	109 (9.1)
Pregnancy complications	
Gestational hypertension, %	
No	1073 (89.8)
Yes	55 (4.6)
Missing	67 (5.6)
Pre-eclampsia, %	
No	1073 (89.8)
Yes	28 (2.3)
Missing	94 (7.9)
Diabetes gravidarum, %	
No	1153 (96.5)
Yes	11 (0.9)
Missing	31 (2.6)
Third trimester fetal characteristics	
Gestational age at measurement, wk	30.3 (28.5, 32.6)
Umbilical artery pulsatility index	0.97 (0.16)
Middle cerebral artery pulsatility index	1.97 (0.33)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Head circumference, cm	28.6 (1.2)
Femur length, cm	5.7 (0.3)
Estimated fetal weight, g	1630 (268)
Birth characteristics	
Gestational age at birth, wk	40.3 (35.9, 42.4)
Head circumference, cm	34.0 (1.6)
Birth weight, g	3514 (540)
Birth length, cm	50.5 (2.3)
Preterm, %	4.7 (56)
Small for gestational age, %	3.7 (44)
Boys, %	52.1 (622)

Childhood characteristics	
Age at follow up, y	5.9 (5.7, 6.6)
Head circumference, cm	51.4 (1.4)
Height, cm	119.0 (5.2)
Weight, kg	22.6 (3.2)
Body mass index, kg/m^2	15.9 (1.4)
Total fat mass, %	23.8 (4.5)
Lean mass, %	72.6 (4.4)
Android/gynoid fat mass ratio, mean (SD), %	23.7 (16.1, 37.8)
Preperitoneal fat area, cm^2	0.37 (0.17, 0.89)

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

TABLE S3.1.2 | Infant growth characteristics (N = 1,195).

Infant growth characteristics	
6 months	
Age, months	6.2 (5.2 – 7.8)
Head circumference, cm	43.6 (1.4)
Weight, kg	7.8 (0.9)
Length, cm	67.8 (2.6)
Body mass index, kg/m^2	17.1 (1.3)
12 months	
Age, months	11.0 (10.1 – 12.6)
Head circumference, cm	46.1 (1.4)
Weight, g	9.6 (1.0)
Height, cm	74.4 (2.5)
Body mass index, kg/m^2	17.4 (1.3)
24 months	
Age, months	24.9 (23.4 – 28.2)
Head circumference, cm	49.3 (1.5)
Weight, g	12.9 (1.4)
Height, cm	88.4 (3.3)
Body mass index, kg/m^2	16.5 (1.3)
36 months	
Age, months	366.8 (35.4 – 40.7)
Weight, g	15.3 (1.7)
Height, cm	97.7 (3.8)
Body mass index, kg/m^2	16.0 (1.2)
48 months	
Age, months	45.8 (44.5 – 48.4)
Weight, g	17.0 (2.0)
Height, cm	103.7 (4.1)
Body mass index, kg/m^2	15.8 (1.2)

Values are means (standard deviation), medians (95% range)

TABLE S3.1.3 | Maternal characteristics (N = 1,195).

Maternal characteristics	
Age, y	31.9 (21.8, 39.1)
Gestational age at enrolment, wk	12.9 (9.9, 21.1)
Height, cm	170.9 (6.3)
Prepregnancy weight, kg	68.7 (12.1)
Prepregnancy body mass index, kg/m^2	23.5 (3.9)
Parity ≥1, %	470 (39.3)
Educational level, %	
Primary/secondary	445 (37.2)
Secondary or higher	750 (62.8)
Folic acid supplement use, %	
No	122 (10.2)
Start in first 10 weeks of pregnancy	356 (29.8)
Start periconceptional	717 (60)
Smoking during pregnancy, %	
No	882 (73.8)
Yes	313 (26.2)
Pregnancy complications	
Gestational hypertension, %	
No	1123 (94.0)
Yes	72 (6.0)
Pre-eclampsia, %	
No	1160 (97.1)
Yes	35 (2.9)
Diabetes gravidarum, %	
No	1176 (98.4)
Yes	19 (1.6)

Values are means (standard deviation), medians (95% range)

Chapter 3

Chapter 3.2

Third trimester fetal hemodynamics and cardiovascular outcomes in childhood

Kooijman MN, de Jonge LL, Steegers EAP, van Osch-Gevers L, Verburg BO, Hofman A, Helbing WA, Jaddoe VWV

Adapted from: J Hypertens. 2014;32(6):1275-82

Abstract

Objective Low birth weight is associated with cardiovascular disease in adulthood. Hemodynamic adaptations related to fetal growth restriction may underlie these associations, through persistent influences on cardiovascular development. We examined the associations of third trimester fetal hemodynamics with cardiovascular outcomes in childhood.

Methods In a prospective cohort study among 917 pregnant women and their children, we measured fetal growth, and fetal arterial and cardiac hemodynamic variables with ultrasound and Doppler examinations at a gestational age of 30.3 (95% range 28.8 – 32.3) weeks. At the age of 6 years, we measured blood pressure, carotid-femoral pulse wave velocity, and left cardiac structures and function.

Results We observed that fetal hemodynamics were not associated with childhood blood pressure and carotid-femoral pulse wave velocity. The fetal aorta ascendens diameter and left cardiac output were positively associated with childhood aortic root diameter (0.14 SDS, 95% CI 0.07, 0.22 and 0.08 SDS, 95% CI 0.01, 0.15 per SDS change in diameter and output, respectively). Fetal left ventricular diastolic filling pattern was inversely associated with aortic root diameter (-0.07 SDS, 95% CI -0.13, 0.00 per SDS change in E/A ratio) at 6 years. Analyses adjusted and stratified for estimated fetal weight showed no differences in results.

Conclusion Our results suggest that third trimester fetal vascular resistance parameters do not affect blood pressure or arterial stiffness in childhood. Fetal cardiac functional and structural measures are associated with cardiac outcomes in childhood. Whether these early adaptations lead to greater risks of cardiovascular disease should be further studied.

Introduction

Low birth weight is associated with higher risks of cardiovascular disease in later life.[1] The mechanisms underlying these associations are not known, but might include hemodynamic adaptations in the fetal circulation related to fetal growth restriction.[2] These early fetal hemodynamic adaptations may have persistent influences on cardiovascular structure and function development[3], which may be without clinical consequences on the short term, but lead to cardiovascular diseases in later life. This hypothesis is supported by previous studies showing that fetal growth restriction is associated with changes in fetal blood flow patterns and redistribution with preferential supply of oxygen and nutrients to the brain and heart instead of other organs.[4, 5] Previously, we observed that even within the normal range of fetal growth, fetal growth variation is related with fetal hemodynamic adaptions, including changes in cardiac output and cardiac compliance.[6] Children with fetal growth retardation showed changes in cardiac morphology at the age of 5 years, which increased with the severity of growth restriction.[7] Also, low birth weight seems to be associated with structural and functional cardiovascular outcomes such as higher arterial stiffness and blood pressure in childhood and adulthood[8, 9], and smaller diameters of the coronary arteries, aortic root, and left ventricular outflow tract in children.[10] Although many studies examined the associations of birth weight with cardiovascular outcomes in childhood, the associations of fetal hemodynamics with development of cardiovascular structures and function in later life are not known. Previously we observed in a large scale cohort that third trimester umbilical artery resistance is associated with childhood systolic blood pressure and left ventricular mass.[11] In a subsample of this latter study we explored whether detailed fetal vascular and cardiac hemodynamics affect childhood cardiovascular outcomes. With this study we aim to give insight in the associations of fetal life blood flow profiles with cardiovascular outcomes in healthy, low risk children.

Therefore, we examined in a a population-based, prospective cohort study from fetal life onwards among 917 children the associations of third trimester fetal hemodynamics with cardiovascular outcomes in 6 year old children.

Methods

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onward among 9,778 mothers and children in Rotterdam, the Netherlands[12]. All children were born between April 2002 and January 2006. Enrollment was aimed at early pregnancy but was allowed until birth of the child. Detailed third trimester assessments were conducted in a random subgroup of 1,232 Dutch pregnant women and their children. In this group, third trimester fetal hemodynamics were available in 1,201 singleton live born children, of whom 925 children (77%) visited the research center at the median age of 5.9 (95% range 5.7 – 6.4)

years. Blood pressure, carotid-femoral pulse wave velocity, or cardiac ultrasound measurements were performed in 918 children. One child with echocardiographic evidence of heart disease was excluded from the study, leaving 917 children for the current analyses (**Figure S3.2.1**). Written informed consent was obtained from all participants. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

Third trimester fetal growth and hemodynamic characteristics

Gestational age was established by first trimester ultrasound measurements. In third trimester, head circumference, abdominal circumference, and femur length were measured and estimated fetal weight was calculated using the formula by Hadlock et al.[13]

Fetal hemodynamics were assessed by pulsed-wave Doppler at a median gestational age of 30.3 (95% range 28.8 – 32.3) weeks, as described previously.[6] For each measurement 3 consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, and the mean of 3 measurements was used for further analysis. Feto-placental vascular resistance was evaluated with recorded flow-velocity waveforms from the umbilical artery. A raised umbilical artery pulsatility index (PI) indicate increased resistance.[14, 15] Umbilical artery PI was determined in a free-floating loop of the umbilical cord.

Middle cerebral artery Doppler measurements were performed with color Doppler visualization of the circle of Willis in the fetal brain, and flow-velocity waveforms were obtained in the proximal part of the cerebral arteries. The middle cerebral artery PI quantify the redistribution of blood flow in favour of the fetal brain. Reductions in middle cerebral artery PI are valid indicators of the brain-sparing effect and fetal redistribution.[16, 17] An indicator of the 'brain-sparing effect' is a raised ratio between the umbilical artery PI and the cerebral artery PI (U/C ratio).[18] We calculated the U/C ratio by dividing the PI of the umbilical artery by the PI of the middle cerebral artery.

Cardiac flow-velocity waveforms at the level of the mitral valves were recorded from the apical 4-champer view of the fetal heart. Peak velocities of the E wave, which represents early passive ventricular filling, and the A wave, which represents active atrial contraction filling, were recorded. The E/A ratio, which is an index for ventricular diastolic function and expresses both cardiac compliance and preload conditions, was calculated[6]. A higher E/A ratio indicates a stiffer left ventricle.

Cardiac outflow flow-velocity waveforms from the aorta were recorded from the 5-chamber view and the short-axis view of the fetal heart just above the semi-lunar valves, respectively. Peak systolic velocity (PSV), time-velocity integral, fetal heart rate, and the inner diameter during systole were recorded. Left cardiac output was calculated in millilitres per minute by multiplying the vessel area by the time-velocity integral by fetal heart rate.

To assess reproducibility of ultrasound measurements, the interobserver and intraobserver intra-class correlation coefficient and coefficient of variation were calculated previously in 12 subjects for various Doppler measurements.[6] High intraclass correlation coefficient values (>0.80) with corresponding low coefficient of variation values (<10%) have been reported, indicating

adequate reproducibility for all assessed Doppler measurements. All ultrasound examinations were performed with an ATL-Philips model HDI 5000 (Seattle, Wash) equipped with a 5.0-MHz high-frequency, curved-array transducer.

Childhood cardiovascular structures and function

We measured blood pressure with the child in supine position. Systolic and diastolic blood pressure was measured at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus™ (Paramus, NJ, USA)[19]. A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference. More than 90% of the children who visited the research center had four successful blood pressure measurements available. Hypertension was defined as systolic and diastolic blood pressure above the 95th percentile, high normal blood pressure as systolic and diastolic blood pressure were between the 90th and 95th percentile.[20]

Carotid-femoral pulse wave velocity, the reference method to assess aortic stiffness[21], was assessed using the automatic Complior SP device (Complior; Artech Medical, Pantin, France) with participants in supine position. The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured over the surface of the body to the nearest centimeter. Through piezoelectric sensors placed on the skin, the device collected signals to assess the time delay between the upstroke of carotid and femoral waveforms. Carotid-femoral pulse wave velocity was calculated as the ratio of the distance travelled by the pulse wave and the time delay between the waveforms, as expressed in meters per second.[22] To cover a complete respiratory cycle, the mean of at least 10 consecutive pressure waveforms was used in the analyses. Pulse wave velocity can be measured reliably, with good reproducibility in large pediatric population-based cohorts.[23] Two-dimensional M-mode echocardiographic measurements were performed using the ATL-Philips Model HDI 5000 (Seattle, WA, USA) or the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA) devices. Quality checks were frequently carried out and feedback was provided regularly, to minimise interobserver differences. The children were examined in a quiet room with the child awake in supine position. Missing echocardiograms were mainly due to restlessness of the child or unavailability of equipment or sonographer. Aortic root diameter, interventricular end-diastolic septal thickness (IVSTD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-diastolic posterior wall thickness (LVPWTD) and fractional shortening were measured using methods recommended by the American Society of Echocardiography.[24] Left ventricular mass (LV mass) was calculated using the formula derived by Devereux et al[25]: LV mass = 0.80 × 1.04((IVSTD + LVEDD + LVPWTD)^3 – (LVEDD)^3)+ 0.6. To assess reproducibility of ultrasound measurements, the intraobserver and interobserver intra-class correlation coefficients were calculated previously for left atrial diameter, aortic root diameter, IVSTD, LVEDD and LVPWTD in 28 subjects (median age 7.5 years, inter-quartile range 3.0 – 11.0) and varied between 0.91 to 0.99 and 0.78 to 0.96, respectively.[26]

Covariates

Information on maternal age, pre-pregnancy weight, parity, educational level, smoking status during pregnancy (yes/no) and folic acid use (no, start in first 10 weeks of pregnancy, start periconceptional) was obtained by questionnaires. Maternal height was measured without shoes and pre-pregnancy body mass index (BMI) was calculated (kg/m^2). Date of birth, infant sex and birth weight were obtained from midwife and hospital registries. Breastfeeding (yes/no) was assessed using questionnaires. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and BMI was calculated.

Statistical analysis

First, we assessed the differences in subject characteristics between boys and girls by independent sample t-test for continuous variables, and chi-square test for categorical variables. Second, we assessed the associations of third trimester vascular resistance parameters with cardiovascular outcomes at the age of 6 years using multiple linear regression models. These models were first only adjusted for gestational age at third trimester measurement, child sex and current age, and second, additionally adjusted for maternal age, parity, educational level, pre-pregnancy BMI, smoking status during pregnancy and folic acid use, fetal heart rate, gestational age at birth, third trimester estimated fetal weight, birth weight, breastfeeding status and current BMI. Covariates were included in the models based on their associations with fetal blood flow, cardiac structures and function as shown in previous studies. All models with echocardiographic outcomes were additionally adjusted for ultrasound device and performing sonographer. Third, we investigated the associations of fetal cardiac hemodynamics with childhood left cardiac structures and fractional shortening, using similar models. To explore whether any associations of vascular resistance indices or fetal hemodynamics with cardiovascular outcome were only present in fetal growth restricted subjects, we also performed these analyses in quintiles of third trimester estimated fetal weight. Since we measured blood pressure four times, we applied linear mixed effects models[27], that fit the four blood pressure measurements within the same child as repeated outcome measures. The major advantage of this approach is that subjects with the maximum number of blood pressure measurements available and the least individual variability in their blood pressure measurements are assigned the highest weight in the analysis.[28]

All cardiovascular outcome measurements were converted to the standard deviation score (SDS) for comparison of effect estimates. We did not construct age adjusted SDS because of the small age range in these measurements. The percentages of missing covariate values within the population for analysis were lower than 17%. Missing covariate data were imputed using the multiple imputations procedure (N = 5 imputations) and the imputed datasets were analyzed together. All measures of association are presented with their 95% confidence intervals (CI). The mixed-models were fitted using the Statistical Analysis System version 9.2 (SAS, Institute Inc., Gary, NC, USA). All other statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

The study population consisted of 462 boys and 455 girls (**Table 3.2.1**), with a median age of 5.9 years (95% range 5.7 – 6.4). Boys had a lower fetal heart rate and lower umbilical artery PI than girls. The fetal aorta ascendens diameter was larger in boys. At the age of 6 years left atrial diameter, aortic root diameter and the left ventricular mass were larger in boys than in girls, while girls had a higher blood pressure. In total, 7 (0.8%) children had hypertension, and 21 (2.5%) children had a high normal blood pressure using age and height specific cut-points.[20] None of the children had known clinical heart disease.

Third trimester fetal vascular resistance parameters and childhood cardiovascular outcomes

Results from the models focused on the associations of placental and fetal vascular resistance indices with cardiovascular outcomes only adjusted for gestational age, child's sex and current age are given in the **supplementary material** (**Table S3.2.1**). These models showed no associations of placental and fetal vascular resistance indices with blood pressure or carotid-femoral pulse wave velocity at 6 years. Third trimester umbilical artery PI and the U/C ratio of the middle cerebral artery were inversely associated with aortic root diameter and left ventricular mass at age 6 years. However, after additional adjustment for maternal and childhood variables, **Table 3.2.2** shows no consistent associations of fetal vascular resistance indices with cardiovascular structures or function in childhood. However, the umbilical artery PI was inversely associated with left ventricular mass at age 6 years (differences -0.07 SDS, 95% CI -0.13, 0.00 ($p<0.05$)). Also, a higher U/C ratio, which indicates a preferential blood flow to the brain at expense of the trunk, tended to be associated with a lower aortic root diameter and left ventricular mass at age 6 years, but these associations were borderline significant.

Third trimester fetal cardiac hemodynamics and childhood cardiovascular outcomes

In the fully adjusted models (**Table 3.2.3**), third trimester fetal aorta ascendens diameter and fetal left cardiac output were positively associated with childhood aortic root diameter (differences 0.14 SDS 95% CI 0.07, 0.22 ($p<0.01$) and 0.08 SDS , 95% CI 0.01, 0.15 ($p<0.05$), respectively) . The fetal mitral valve E wave and the E/A ratio were inversely associated with childhood aortic root diameter (differences -0.08 SDS, 95% CI -0.15, -0.02 ($p<0.05$) and -0.07 SDS, 95% CI -0.13, 0.00 ($p<0.05$), respectively). Fetal aorta ascendens diameter was also positively associated with childhood left ventricular mass (difference 0.08 SDS, 95% CI 0.02, 0.15 ($p<0.05$)). The fetal mitral valve E was inversely associated with left ventricular mass at the age of 6 (differences -0.07 SDS, 95% CI -0.13,- 0.01 ($p<0.05$)). The effect estimates for associations of third trimester fetal cardiovascular measurements with childhood cardiovascular outcomes only

adjusted for gestational age at third trimester measurement, child sex and current age were only slightly different (**Table S3.2.2**). The analyses stratified for estimated fetal weight showed no differences in effect estimates for the associations of fetal hemodynamics with cardiovascular outcomes between the estimated fetal weight quintiles (data not shown).

TABLE 3.2.1 | Subject characteristics (n=917).

	Boys (N = 462)	Girls (N = 455)	P-value
Maternal characteristics			
Age, y	32.1 (23.3 -39.4)	32.3 (23.9 -39.8)	0.25
Prepregnancy body mass index, kg/m²	23.4 (4.2)	23.8 (4.2)	0.23
Parity ≥1, %	173 (37.4)	174 (38.4)	0.76
Smoking during pregnancy, %			0.61
No	328 (71.0)	322 (70.8)	
Yes	85 (18.4)	91 (20.0)	
Missing	49 (10.6)	42 (9.2)	
Educational level, %			0.81
Primary/secondary	155 (33.5)	155 (34.1)	
Secondary or higher	304 (65.8)	294 (64.6)	
Missing	3 (0.6)	6 (1.3)	
Folic acid, %			0.79
No	27 (5.8)	32 (7.0)	
Start in first 10 weeks of pregnancy	105 (22.7)	113 (24.8)	
Start periconceptional	242 (52.4)	243 (53.4)	
Missing	88 (19.0)	67 (14.7)	
Third trimester fetal characteristics			
General			
Gestational age at measurement, wk	30.4 (28.8-32.8)	30.3 (28.4-32.5)	0.07
Estimated fetal weight, g	1643 (260)	1626 (265)	0.34
Fetal aortic heart rate, bpm	137 (9)	139 (9)	0.02
Vascular resistance parameters			
Umbilical artery PI	0.95 (0.16)	0.99 (0.17)	<0.01
U/C ratio of middle cerebral artery	0.50 (0.12)	0.51 (0.11)	0.49
Cardiac hemodynamics			
Aorta ascendens diameter, cm	0.65 (0.1)	0.64 (0.1)	0.01
Aorta ascendens PSV, cm/s	90.6 (12.2)	92.0 (12.6)	0.12
Aorta ascendens TVI	13.2 (2.1)	13.4 (2.1)	0.28
Left cardiac output, ml/min	588 (327 – 1,048)	580 (334 - 971)	0.30
Mitral valve E wave, cm/s	39.6 (6.4)	40.7 (6.2)	0.01
Mitral valve A wave, cm/s	51.6 (7.8)	52.2 (8.3)	0.30
Mitral valve E/A ratio	0.8 (0.1)	0.8 (0.1)	0.04
Birth characteristics			
Gestational age at birth, wk	40.4 (36.9 - 42.4)	40.3 (35.6 - 42.4)	0.10
Birth weight, g	3589 (477)	3480 (532)	<0.01
Breastfeeding, %			0.88
No	41 (8.9)	39 (8.6)	
Yes	408 (88.3)	402 (88.4)	
Missing	13 (2.8)	14 (3.1)	

	Boys (N = 462)	Girls (N = 455)	P-value
Childhood characteristics			
Age at follow up, y	5.9 (5.7-6.7)	5.9 (5.7-6.6)	0.82
Length, cm	119.4 (5.1)	118.7 (5.2)	0.03
Weight, kg	22.7 (3.1)	22.5 (3.4)	0.33
Body mass index, kg/m^2	15.9 (1.3)	15.9 (1.5)	0.67
Systolic blood pressure, mmHg	101.6 (7.4)	102.8 (7.9)	0.03
Diastolic blood pressure, mmHg	59.6 (6.6)	60.6 (5.9)	0.02
Carotid-femoral PWV, m/s	5.4 (1.1)	5.5 (0.9)	0.33
Left atrial diameter, mm	25.3 (2.6)	24.6 (2.8)	<0.01
Aortic root diameter, mm	19.7 (1.9)	18.6 (1.6)	<0.01
Left ventricular mass, g	55.7 (11.6)	51.8 (10.5)	<0.01
Fractional shortening , %	35.4 (4.5)	35.1 (4.5)	P=0.34

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

TABLE 3.2.2 | Associations of third trimester fetal vascular resistance parameters with cardiovascular structures and function at the age of 6 years (n=917).

	Systolic blood pressure (SD = 7.8 mmHg)	Diastolic blood pressure (SD = 6.4 mmHg)	Pulse wave velocity (SD = 1.0 m/s)	Aortic root diameter (SD = 1.8 mm)	Left ventricular mass (SD = 11.3 g)	Fractional shortening (SD = 4.5 %)
Umbilical artery PI	0.00	-0.01	0.03	-0.04	-0.07	0.04
(SD = 0.16)	(-0.06, 0.07)	(-0.06, 0.04)	(-0.06, 0.12)	(-0.11, 0.03)	(-0.13, 0.00)*	(-0.03, 0.12)
U/C ratio (middle cerebral artery)	0.02	0.00	0.07	-0.05	-0.06	0.02
(SD = 0.12)	(-0.04, 0.08)	(-0.06, 0.05)	(-0.02, 0.16)	(-0.12, 0.02)	(-0.12, 0.01)	(-0.06, 0.09)

Estimates are based on multiple imputed data. Values are regression coefficients (95% confidence intervals) and reflect the change in SD score of blood pressure, carotid-femoral pulse wave velocity, left cardiac structures and fractional shortening per change in SD score of vascular resistance parameters. Models are adjusted for maternal age, parity, educational level, pre-pregnancy BMI, smoking status during pregnancy and folic acid use, fetal heart rate, gestational age at third trimester measurement and at birth, third trimester estimated fetal weight, birth weight, child sex, breastfeeding status, current age and BMI. Models with ultrasound outcomes are additionally adjusted for ultrasound device and performing sonographer.

SD = standard deviation score

* P<0.05

TABLE 3.2.3 | Associations of third trimester fetal cardiac hemodynamics with cardiovascular structures and function at the age of 6 years (n=917).

	Aortic root diameter (SD = 1.8 mm)	**Left ventricular mass (SD = 11.3 g)**	**Fractional shortening (SD = 4.5 %)**
Aorta ascendens diameter	0.14	0.08	-0.07
(SD = 7.0mm)	(0.07, 0.22)†	(0.02, 0.15)*	(-0.15, 0.01)
Aorta ascendens PSV	-0.06	0.00	0.01
(SD = 12.4 cm/s)	(-0.12, 0.01)	(-0.06, 0.06)	(-0.06, 0.08)
Aorta ascendens TVI	-0.03	-0.01	0.00
(SD = 2.1)	(-0.10, 0.03)	(-0.07, 0.05)	(-0.07, 0.07)
Left cardiac output	0.08	0.06	-0.05
(SD = 175.4 ml/min)	(0.01, 0.15)*	(-0.01, 0.12)	(-0.13, 0.03)
Mitral valve E wave	-0.09	-0.07	0.01
(SD = 6.3 cm/s)	(-0.15, -0.02)*	(-0.13, -0.01)*	(-0.06, 0.08)
Mitral valve A wave	-0.03	-0.06	0.02
(SD = 8.0 cm/s)	(-0.09, 0.04)	(-0.12, 0.01)	(-0.05, 0.09)
Mitral valve E/A ratio	-0.07	-0.02	-0.02
(SD = 0.1)	(-0.13, 0.00)*	(-0.08, 0.04)	(-0.09, 0.05)

Estimates are based on multiple imputed data. Values are regression coefficients (95% confidence intervals) and reflect the change in SD score of left cardiac structures and fractional shortening per change in SD score of fetal cardiac hemodynamic parameters. Models are adjusted for maternal age, parity, educational level, pre-pregnancy BMI, smoking status during pregnancy and folic acid use, fetal heart rate, gestational age at third trimester measurement and at birth, third trimester estimated fetal weight, birth weight, child sex, breastfeeding status, current age and BMI, ultrasound device and performing sonographer.

SD = standard deviation

* P<0.05, † P<0.01

Discussion

In this study we observed that third trimester vascular resistance parameters are not associated with blood pressure or arterial stiffness at the child age of 6 years. Third trimester fetal cardiac hemodynamics were associated with cardiac structural outcomes in childhood. These results suggest that fetal cardiac adaptations influence cardiovascular structures in childhood. The observed effect estimates were small and likely to be without clinical consequences in childhood. However, they are important from an etiological point of view. Whether third trimester fetal hemodynamics affect the risk of cardiovascular disease in later life should be further studied.

Previous studies have shown associations of low birth weight with higher risks of cardiovascular disease in later life.[1] Hemodynamic adaptations related to fetal growth restriction may partly underlie these associations, through persistent influences on cardiovascular development. Cardiovascular development may be affected in response to changes in intrauterine blood flow patterns and impaired perfusion.[3] These hemodynamic adaptations may subsequently lead to cardiovascular dysfunction in early life, and diseases in later life.

Studies in animals showed that placental insufficiency was related to hypertension in offspring.[29] A study in human twins with the twin-twin transfusion syndrome showed that the recipient fetuses had increased aortic and pulmonary velocities compared with the donor co-twins, however no hemodynamic differences were found between the heavier and lighter twins in uncomplicated monochorionic diamniotic pregnancies.[30] No differences were seen in pulse wave velocity in the brachioradial artery between the heavier and lighter uncomplicated monochorionic or dichorionic twins, while in the twin-twin transfusion syndrome, pulse wave velocity was higher in the donor twin.[31] These results suggest that adaptations of the twin fetuses to hemodynamic imbalance may have persistent influences on their cardiovascular properties.[30, 31] We previously observed that cardiac output, peak systolic velocity of the outflow tracts and cardiac compliance gradually reduced with diminished fetal growth.[6] A follow-up study among 727 in children aged 2 years, showed an inverse association of umbilical artery PI and aortic root diameter, whereas fetal aorta ascendens diameter and left cardiac output were positively associated with left arterial diameter at 2 years of age.[32] In the current study, we did not observe associations of third trimester placental and fetal vascular resistance indices with childhood blood pressure or arterial stiffness at the age of 6 years. We found an inverse association of the umbilical artery PI, an indicator of increased placental resistance, with childhood left ventricular mass. This might suggest these fetuses, in case of increased placental resistance, do not have the ability to adequately increase their heart structures during development. However, it has also been suggested that fetal development under hypoxic conditions may influence ventricular wall mass, by an increase in afterload, or elevation in pressure required to eject blood to systemic tissues, resulting in compensatory growth of the myocardial wall.[3] In addition, we did not find effects of feto-placental vascular resistance on other left cardiac structures, which indicates that the observed association might be a chance finding. Brodzski et al. performed a study among 44 children followed from birth until adolescence and observed smaller diameters of the abdominal and popliteal artery in subjects with abnormal fetal aortic blood flow and growth restriction.[33] In our study, fetal aorta ascendens diameter and left cardiac output were positively associated with aorta root diameter at the age of 6. In addition, we observed that fetuses with higher mitral E/A ratio have a lower aortic root diameter at the age of 6. In human fetuses with normal growth the majority of ventricular filling occurs late in diastole. During fetal life, the E/A ratio significantly increases with advancing gestational age, suggesting a change from late to early diastolic filling 'predominance'.[34] In healthy adults the majority of ventricular filling occurs early in diastole. A higher E/A ratio reflects a more compliant left ventricle. Our results may indicate that an 'adult-type' third trimester filling pattern is associated with smaller vascular properties in childhood.

An adverse fetal environment leads to a redistribution of blood flow preferential to the brain and heart, to maintain oxygen supply to these vital organs.[4,5] Studies showed that Doppler signs of fetal circulatory redistribution in favor of the brain were associated with perinatal complications such as prematurity, low birth weight, severe morbidity or behavioral problems.[35-37] We observed that a raised ratio between the umbilical artery PI and the middle or anterior cerebral artery PI,

an indicator of brain sparing, tended to be inversely associated with aortic root diameter and left ventricular mass at age 6 years, suggesting that preferential blood flow to the brain at expense of the trunk might be associated with smaller left cardiac structures in childhood.

We observed sex differences in childhood blood pressure, left atrial diameter, aortic root diameter and left ventricular mass. Further studies focused on factors explaining sex differences in cardiovascular outcomes in childhood are needed. However, in a previous study from the same cohort, we observed that a higher third trimester umbilical artery resistance was associated with childhood cardiovascular adaptations, with stronger effects among girls than boys. These sex-specific differences were only partly explained by differences in fat mass, growth and birth weight.[11] Only a small percentage of our population had a high blood pressure. High blood pressure was defined on cohort specific percentiles. It is unlikely that inclusion of this group affected our results extensively.

We explored whether the associations of fetal hemodynamic adaptations and childhood cardiovascular outcomes were explained or only present in fetal growth restricted subjects. Analyses stratified for estimated fetal weight showed no differences in effect estimates, which suggest no stronger effects of fetal hemodynamic adaptations in smaller fetuses on childhood cardiovascular outcomes.

Strengths and limitations

The main strength of this study is the prospective design from fetal life onwards within a large population-based cohort. To our knowledge this is the largest study, which examined the effects of placental and fetal hemodynamics on cardiovascular outcomes in childhood. The population-based setting enabled us to assess these hemodynamics across the full range, rather than only in both fetuses with growth restriction or other complications. A limitation of this study is that we had no information about fetal growth and hemodynamics after 32 weeks, although we adjusted our analyses for size at birth. It might be that fetal growth and hemodynamics after 32 weeks may also influence cardiovascular structures at the age of 6. Another limitation is that the cardiovascular measurements at the age of 6 years were only obtained in 75% of the children of the subgroup. In the present study we evaluated multiple associations, this might have led to chance findings due to multiple testing. However, because of the correlations between the vascular and cardiac outcomes, we did not correct for multiple testing. Missing cardiovascular outcome measurements could lead to selection bias and loss of power. Our results would be biased if the associations among placental and fetal hemodynamics and cardiac structures and function differ between those included and those not included in the study. Although this seems unlikely, it cannot be excluded. Young children have limited factors related to the risk of cardiovascular disease, and therefore it is expected that the potential confounding effect of these factors are restricted. However, the influence of residual confounding should be considered, as in all observational studies.

Conclusions

In this study, we observed that third trimester fetal vascular resistance parameters did not affect blood pressure or arterial stiffness in childhood. Fetal cardiac hemodynamics were associated with left cardiac structures in childhood. These findings suggest that fetal cardiac hemodynamics adaptations have long-term consequences. However, the effect estimates were small and may not clinically relevant. Furthermore, it is unknown whether these developmental adaptations in childhood predict a greater risk of cardiovascular disease in later life. Therefore, further follow-up studies are needed.

References

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
2. Martyn CN, Greenwald SE. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Lancet. 1997;350(9082):953-5.
3. Patterson AJ, Zhang L. Hypoxia and fetal heart development. Curr Mol Med. 2010;10(7):653-66.
4. Baschat AA. Examination of the fetal cardiovascular system. Semin Fetal Neonatal Med. 2011;16(1):2-12.
5. Uerpairojkit B, Manotaya S, Tanawattanacharoen S, Wuttikonsammakit P, Charoenvidhya D. Are the cardiac dimensions spared in growth-restricted fetuses resulting from uteroplacental insufficiency? J Obstet Gynaecol Res. 2012;38(2):390-5.
6. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
7. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F, et al. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation. 2010;121(22):2427-36.
8. Cheung YF, Wong KY, Lam BC, Tsoi NS. Relation of arterial stiffness with gestational age and birth weight. Arch Dis Child. 2004;89(3):217-21.
9. Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. Br Heart J. 1995;73(2):116-21.
10. Jiang B, Godfrey KM, Martyn CN, Gale CR. Birth weight and cardiac structure in children. Pediatrics. 2006;117(2):e257-61.
11. Gaillard R, Steegers EA, Tiemeier H, Hofman A, Jaddoe VW. Placental Vascular Dysfunction, Fetal and Childhood Growth and Cardiovascular Development: The Generation R Study. Circulation. 2013;128(20):2202-10.
12. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.
13. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535-40.
14. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
15. Albaiges G, Missfelder-Lobos H, Parra M, Lees C, Cooper D, Nicolaides KH. Comparison of color Doppler uterine artery indices in a population at high risk for adverse outcome at 24 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21(2):170-3.
16. van den Wijngaard JA, Groenenberg IA, Wladimiroff JW, Hop WC. Cerebral Doppler ultrasound of the human fetus. Br J Obstet Gynaecol. 1989;96(7):845-9.
17. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.

18. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: a pulsed Doppler study. J Perinat Med. 1992;20(1):79-82.
19. Wong SN, Tz Sung RY, Leung LC. Validation of three oscillometric blood pressure devices against auscultatory mercury sphygmomanometer in children. Blood Press Monit. 2006;11(5):281-91.
20. National High Blood Pressure Education Program Working Group on High Blood Pressure in C, Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(2 Suppl 4th Report):555-76.
21. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.
22. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. 1995;26(3):485-90.
23. Donald AE, Charakida M, Falaschetti E, Lawlor DA, Halcox JP, Golding J, et al. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). Eur Heart J. 2010;31(12):1502-10.
24. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr. 1989;2(5):358-67.
25. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol. 1986;57(6):450-8.
26. Geelhoed MJ, Snijders SP, Kleyburg-Linkers VE, Steegers EA, van Osch-Gevers L, Jaddoe VW. Reliability of echocardiographic measurements of left cardiac structures in healthy children. Cardiol Young. 2009;19(5):494-500.
27. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982;38(4):963-74.
28. Gillman MW, Cook NR. Blood pressure measurement in childhood epidemiological studies. Circulation. 1995;92(4):1049-57.
29. Anderson CM, Lopez F, Zimmer A, Benoit JN. Placental insufficiency leads to developmental hypertension and mesenteric artery dysfunction in two generations of Sprague-Dawley rat offspring. Biol Reprod. 2006;74(3):538-44.
30. Karatza AA, Wolfenden JL, Taylor MJ, Wee L, Fisk NM, Gardiner HM. Influence of twin-twin transfusion syndrome on fetal cardiovascular structure and function: prospective case-control study of 136 monochorionic twin pregnancies. Heart. 2002;88(3):271-7.
31. Cheung YF, Taylor MJ, Fisk NM, Redington AN, Gardiner HM. Fetal origins of reduced arterial distensibility in the donor twin in twin-twin transfusion syndrome. Lancet. 2000;355(9210):1157-8.
32. Geelhoed JJ, El Marroun H, Verburg BO, van Osch-Gevers L, Hofman A, Huizink AC, et al. Maternal smoking during pregnancy, fetal arterial resistance adaptations and cardiovascular function in childhood. BJOG. 2011;118(6):755-62.
33. Brodszki J, Lanne T, Marsal K, Ley D. Impaired vascular growth in late adolescence after intrauterine growth restriction. Circulation. 2005;111(20):2623-8.
34. Veille JC, Smith N, Zaccaro D. Ventricular filling patterns of the right and left ventricles in normally grown fetuses: a longitudinal follow-up study from early intrauterine life to age 1 year. Am J Obstet Gynecol. 1999;180(4):849-58.
35. Roza SJ, Steegers EA, Verburg BO, Jaddoe VW, Moll HA, Hofman A, et al. What is spared by fetal brain-sparing? Fetal circulatory redistribution and behavioral problems in the general population. Am J Epidemiol. 2008;168(10):1145-52.
36. Sterne G, Shields LE, Dubinsky TJ. Abnormal fetal cerebral and umbilical Doppler measurements in fetuses with intrauterine growth restriction predicts the severity of perinatal morbidity. J Clin Ultrasound. 2001;29(3):146-51.
37. Vergani P, Roncaglia N, Locatelli A, Andreotti C, Crippa I, Pezzullo JC, et al. Antenatal predictors of neonatal outcome in fetal growth restriction with absent end-diastolic flow in the umbilical artery. Am J Obstet Gynecol. 2005;193(3 Pt 2):1213-8.

Supplementary Material

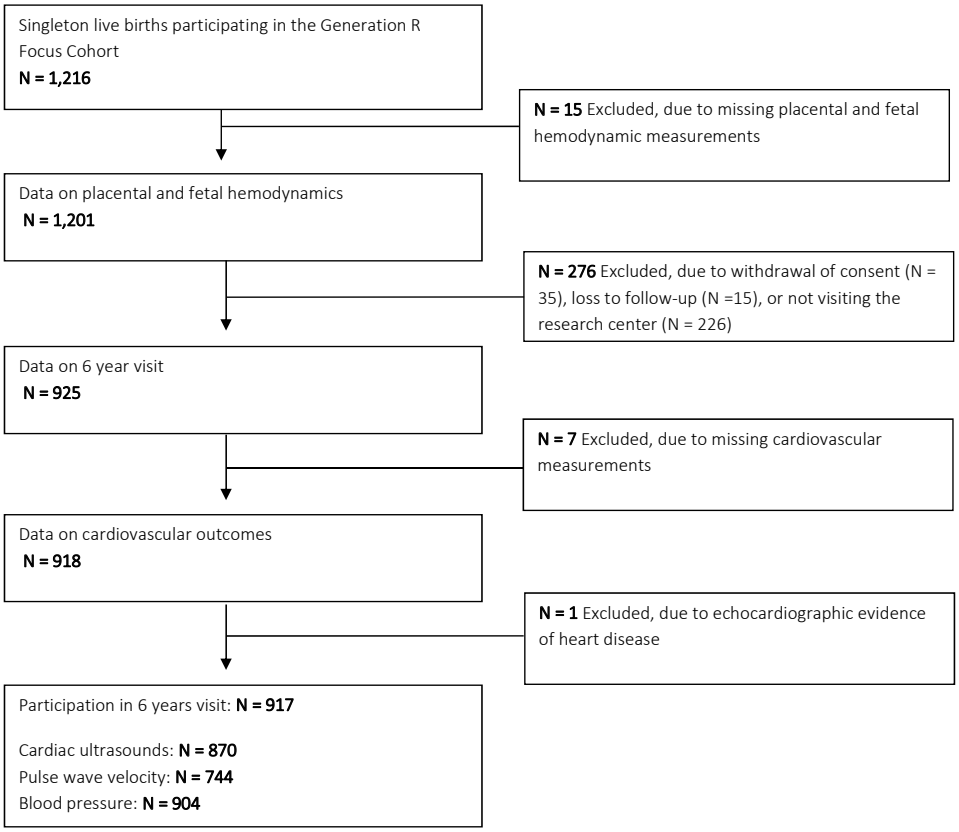

FIGURE S3.2.1 | Flow chart of participants included in the analysis.

TABLE S3.2.1 | Associations of fetal third trimester vascular resistance parameters with cardiovascular structures and function at the age of 6 years (n=917).

	Systolic blood pressure (SD = 7.8 mmHg)	Diastolic blood pressure (SD = 6.4 mmHg)	Pulse wave velocity (SD = 1.0 m/s)	Aortic root diameter (SD = 1.8 mm)	Left ventricular mass (SD = 11.3 g)	Fractional shortening (SD = 4.5 %)
Umbilical artery PI	0.00	-0.02	0.03	-0.06	-0.07	0.07
(SD = 0.16)	(-0.05, 0.06)	(-0.07, 0.03)	(-0.05, 0.11)	(-0.13, 0.00)	(-0.13, -0.01)*	(0.00, 0.14)
U/C ratio (middle cerebral artery)	0.01	-0.02	0.06	-0.08	-0.06	0.02
(SD = 0.12)	(-0.05, 0.06)	(-0.07, 0.03)	(-0.02, 0.15)	(-0.15, -0.02)*	(-0.13, 0.00)*	(-0.04, 0.09)

Values are regression coefficients (95% confidence intervals) and reflect the change in SD score of blood pressure, carotid-femoral pulse wave velocity, left cardiac structures and fractional shortening per change in SD score of vascular resistance parameters. Models are adjusted for gestational age at third trimester measurement, child sex and current age. Models with ultrasound outcomes are additionally adjusted for ultrasound device and performing sonographer.
SD = standard deviation
* P<0.05

TABLE S3.2.2 | Associations of third trimester fetal cardiac hemodynamics with cardiovascular structures and function at the age of 6 years (n=917).

	Aortic root diameter (SD = 1.8 mm)	Left ventricular mass (SD = 11.3 g)	Fractional shortening (SD = 4.5 %)
Aorta ascendens diameter	0.17	0.11	-0.05
(SD = 7.0 mm)	(0.09, 0.24)†	(0.04, 0.17)†	(-0.13, 0.03)
Aorta ascendens PSV	-0.04	0.01	0.01
(SD = 12.4 cm/s)	(-0.10, 0.03)	(-0.05, 0.08)	(-0.06, 0.08)
Aorta ascendens TVI	-0.01	0.02	-0.01
(SD = 2.1)	(-0.08, 0.05)	(-0.04, 0.08)	(-0.08, 0.06)
Left cardiac output	0.12	0.08	-0.04
(SD = 175.4 ml/min)	(0.05, 0.19)†	(0.01, 0.15)*	(-0.12, 0.03)
Mitral valve E wave	-0.06	-0.04	0.02
(SD = 6.3 cm/s)	(-0.12, 0.00)	(-0.10, 0.02)	(-0.05, 0.08)
Mitral valve A wave	0.02	-0.02	0.03
(SD = 8.0 cm/s)	(-0.05, 0.08)	(-0.08, 0.04)	(-0.04, 0.09)
Mitral valve E/A ratio	-0.10	-0.04	-0.01
(SD = 0.1)	(-0.16, -0.04)†	(-0.10, 0.02)	(-0.08, 0.06)

Values are regression coefficients (95% confidence intervals) and reflect the change in SD score of left cardiac structures and fractional shortening per change in SD score of fetal cardiac hemodynamic parameters. Models are adjusted for gestational age at third trimester measurement, child sex and current age, ultrasound device and performing sonographer.
SD = standard deviation
* P<0.05, † P<0.01

Chapter 3

Chapter 3.3

Fetal umbilical, cerebral and pulmonary blood flow patterns in relation to lung function and asthma in childhood

Kooijman MN, van Meel ER, Steegers EAP, Reiss IKM, de Jongste JC, Jaddoe VWV, Duijts L

Adapted from: Pediatr Allergy Immunol. 2019;30(4):443-450

Abstract

Background Fetal growth restriction is associated with higher risks of childhood respiratory morbidity. Fetal blood flow adaptations might contribute to these associations. We examined the associations of fetal umbilical, cerebral and pulmonary blood flow with wheezing patterns, lung function and asthma in childhood.

Methods In a population-based prospective cohort study among 903 children we measured fetal umbilical, cerebral and pulmonary blood flow by pulsed-wave-Doppler at a median gestational age of 30.3 (95% range 28.8–32.3) weeks. We obtained information about wheezing patterns until the age of 6-years by questionnaires. Lung function was measured by spirometry and information about current asthma was obtained by questionnaire at the age of 10-years.

Results Results showed a non–significant relationship between a higher umbilical artery pulsatility index (PI) and umbilical artery PI/cerebral artery PI ratio, indicating fetal blood flow redistribution at expense of the trunk, with higher risks of early wheezing (OR (95%CI): 2.07 (0.70-6.10) and 2.74 (0.60, 12.62) per unit increase, respectively). A higher pulmonary artery time velocity integral, indicating higher pulmonary vascular resistance, was associated with a higher risk of late/persistent wheezing (1.14 (1.01-1.29) per unit increase). A higher middle cerebral artery PI was associated with a higher FEV_1/FVC (Z-score (95%CI): 0.21 (0.01-0.42)). Results did not materially change after additionally adjustment for birth and growth characteristics.

Conclusion Third trimester fetal blood flow patterns might be related to childhood respiratory health. These findings should be considered as hypothesis generating and need further replication.

Introduction

Pregnancy is a critical period for fetal lung development. In late fetal life, the small airways and alveoli are formed.[1] An adverse intrauterine environment in this period seems to have persistent effects on respiratory health and disease across the life course.[2] Fetal blood flow adaptations are important mechanisms by which the fetus protects the most important organs such as the brain and heart from an adverse fetal environment.[3] A preferential fetal blood flow to the brain at the expense of the trunk is characterized by changes in fetal blood flow including a higher umbilical and lower cerebral arterial resistance.[4,5] This redistribution of fetal blood flow may be beneficial for short-term survival but may lead to a lower delivery of oxygen and nutrients to the trunk, including the lungs and airways.[6,7] A potential consequence of fetal blood flow redistribution is a reduction in number and metabolism of alveolar type-II cells, fewer but larger alveoli and impaired growth and maturation of the airways and lungs.[8,9] Impaired fetal development of the airways and lungs could predispose individuals to a higher risk of lung disease in later life.[8] Previous studies reported associations of fetal growth restriction with impaired lung function and respiratory diseases in later life.[10,11] We previously showed that fetal growth restriction and being born small for gestational age were associated with higher airway resistance and lower lung function in childhood.[12] Fetal blood flow adaptations related to fetal growth restriction may underlie these associations. Although the effects of fetal umbilical and cerebral blood flow adaptations on fetal and childhood growth are well-known, it is unknown whether fetal blood flow adaptations affect childhood respiratory morbidity. Also, the role of a suboptimal fetal pulmonary blood flow on the development of respiratory morbidity is unclear.

Therefore, we examined in a population-based prospective cohort study among 903 children the associations of fetal umbilical, cerebral and pulmonary blood flow with wheezing at age 6-years, and lung function and asthma in children aged 10-years. We also explored whether birth weight, gestational age at birth or childhood growth mediated these associations.

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[13] The Medical Ethics Committee of the Erasmus MC, University Medical Center, Rotterdam, has approved the study. Written informed consent was obtained from all participants. Detailed assessments of fetal growth and development were conducted in a random subgroup of 1,232 Dutch mothers and children born between April 2002 and January 2006.[4] Present analyses were performed on 903 children (**Figure 3.3.1**).

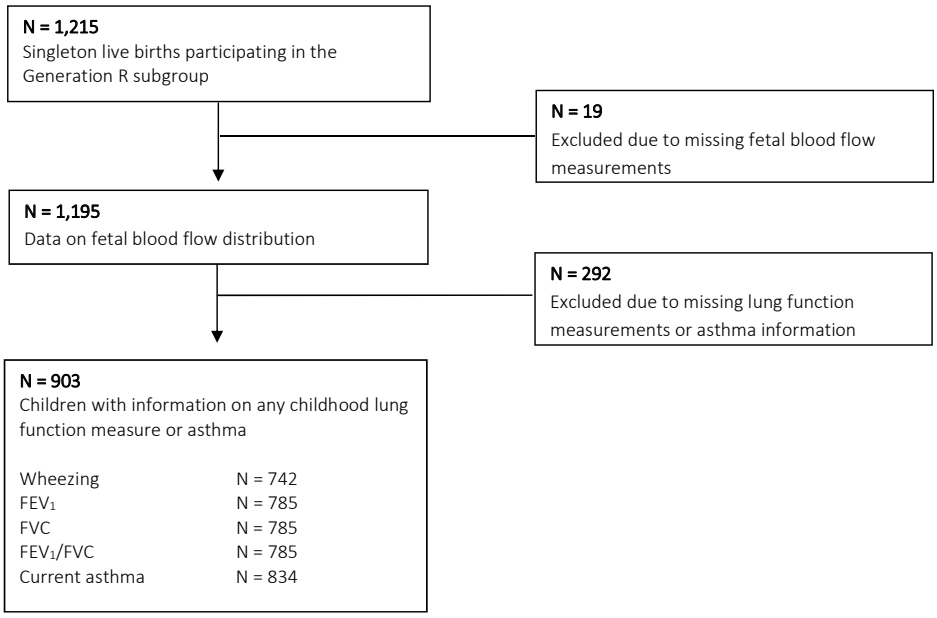

FIGURE 3.3.1 | Flow chart of participants included in the analysis.

Third trimester fetal blood flow

Fetal blood flow measures were assessed by pulsed-wave Doppler at a median gestational age of 30.3 (95% range 28.8–32.3) weeks.

Feto-placental vascular resistance was evaluated with recorded flow-velocity waveforms from the umbilical artery. Umbilical artery pulsatility index (PI) was determined in a free-floating loop of the umbilical cord. A higher umbilical artery PI indicates a higher peripheral vascular resistance.[14] Middle cerebral artery Doppler measurements were performed with visualization of the circle of Willis in the fetal brain, and flow-velocity waveforms were obtained in the proximal part of the cerebral arteries. The middle cerebral artery PI quantifies the redistribution of blood flow, and when lower, in favor of the fetal brain. Reductions in middle cerebral artery PI are valid indicators of the brain-sparing effect and fetal redistribution.[15] An indicator of the 'brain-sparing effect' is a raised ratio between the umbilical artery PI and the cerebral artery PI (U/C ratio).[5]

Pulmonary outflow flow-velocity waveforms from the aorta were recorded from the five-chamber view and the short-axis view of the fetal heart just above the semi-lunar valves. Time velocity integral (TVI) during systole was recorded. A higher pulmonary artery TVI indicated higher pulmonary vascular resistance.[16]

Reproducibility of ultrasound measurements were adequate with high intraclass correlation coefficient values (>0.80) with corresponding low coefficient of variation values (<10%).[4] All ultrasound examinations were performed with an ATL-Philips model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0-MHz high-frequency, curved-array transducer.

Childhood lung function and asthma

Information about wheezing was obtained by questionnaires until 6-years. We constructed wheezing patterns based on time of onset and subsequent absence or persistence ('never'; 'early' (≤3-years only); 'late' (>3-6 years) and 'persistent wheezing' in children with information on wheezing for at least two time points.[17] Children visited the research center at a median age of 9.7-years (range 8.5–12.0 years) and we performed spirometry: forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and FEV_1/FVC. Spirometry was performed according to the American Thoracic Society and European Respiratory Society recommendations.[18] All spirometry variables were converted into sex-, age-, height- and ethnicity-adjusted z-scores.[19] Current asthma (no; yes) was defined as physician-diagnosed asthma ever, with either wheezing or the use of (airway) medication in the past 12-months by questionnaires at age 10-years.

Covariates

We obtained information on maternal educational level, pre-pregnancy weight, parity, smoking during pregnancy, folic acid use during pregnancy, history of asthma or atopy, from questionnaires. Maternal height was measured and pre-pregnancy BMI was calculated (kg/m^2). Estimated fetal weight in third trimester was calculated.[20] Information on gestational hypertensive disorders, child's sex, gestational age at birth and birth weight were obtained by midwife and hospital registries. At age 10-years, information on ever diagnosis of eczema was obtained by questionnaire. During the research center visit at age 10-years, child height and weight were measured using Standard Operating Procedures. We measured the children without shoes and heavy clothing, and BMI was calculated. Allergic sensitization for the most common inhalant allergens (house dust mite, grass, birch, cat and dog; ALK-Abelló B.V., Almere, The Netherlands) was determined by a skin prick test using the "scanned-area-method".[21]

Statistical analysis

First, we performed a non-response analysis by assessing the differences in characteristics of participants included and not included in the study. Second, we assessed the associations of fetal blood flow with wheezing patterns, lung function and asthma using multivariate logistic or linear regression models. These models were first adjusted for gestational age at third trimester fetal blood flow measurement and estimated fetal weight at third trimester fetal blood flow measurement or child sex only (basic model), and secondly, additionally adjusted for maternal educational level, pre-pregnancy BMI, parity, smoking, folic acid use and gestational hypertensive disorders (adjusted model). We used generalized estimating equations (GEEs) to examine longitudinal effects of fetal blood flow with the risk of overall wheezing until age 6-years. These models take into account the correlations between repeated measurements within the same subject. An unstructured correlation matrix was used. Third, we examined if any association of fetal blood flow with wheezing patterns, lung function or asthma were mediated by birth weight or gestational age at birth or child's BMI at age 10-years by adding them additionally in our

models (mediation model). Furthermore, we examined whether maternal history of asthma or atopy, child's eczema or inhalant allergic sensitization modified any association by analyzing the statistical interaction between these variables and the fetal blood flow related exposures (effect modification model). The percentages of missing covariate values within the population for analysis was lower than 19%. Missing covariate data were imputed using the multiple imputations procedure (n=5 imputations) and the imputed datasets were analyzed together. All measures of associations are presented with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Participants characteristics

Table 3.3.1 shows the characteristics of the mothers and children included in the current study. **Table S3.3.1** shows the participant characteristics before multiple imputation. Non-response analyses showed that mothers of children not included in the analysis were more frequently lower educated, had a higher prevalence of multiparity, smoked more often and used less often folium acid supplement during pregnancy. Their children were born at a younger gestational age (**Table S3.3.2**).

TABLE 3.3.1 | Characteristics of children and their mothers after multiple imputation (n = 903).

Maternal characteristics	
Education (%)	
Low (no, primary, secondary education)	32.9 (297)
High (higher education)	67.1 (606)
Pre-pregnancy body mass index (kg/m^2)	23.6 (3.9)
Parity (%)	
Nullipara	62.8 (567)
Multipara	37.2 (336)
History of asthma or atopy (%)	
No	61.7 (557)
Yes	38.2 (346)
Smoking during pregnancy (%)	
No smoking throughout pregnancy	79.1 (714)
Yes	20.9 (189)
Folic acid supplement use (%)	
No use	8.2 (74)
Start within first 10 weeks of pregnancy	29.3 (265)
Preconceptional start	62.5 (564)
Pregnancy-induced complications (gestational hypertension/pre-eclampsia) (%)	
No	91.7 (828)
Yes	8.3 (75)

Third trimester fetal characteristics	
Gestational age at measurement, weeks	30.4 (28.5 – 32.7)
Estimated fetal weight, grams	1632 (266)
Umbilical artery PI	0.97 (0.16)
Middle cerebral artery PI	1.97 (0.33)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Pulmonary artery time velocity integral	12.04 (1.83)
Birth characteristics	
Gestational age at birth, weeks	40.3 (36.7 – 42.4)
Birth weight, grams	3528 (509)
Sex	
Male	50.6 (457)
Female	49.4 (446)
Childhood characteristics	
Wheezing patterns until 6 years (%)	
Never	45.8 (414)
Early	23.0 (208)
Late/Persistent	13.3 (120)
Missing	17.8 (161)
Age at follow up, y	9.8 (9.1 – 10.5)
Body Mass Index at age 10-years (kg/m^2)	17.1 (2.2)
Ever eczema at age 10-years (%)	
No	73.9 (667)
Yes	26.1 (236)
Inhalant allergic sensitization at age 10-years (%)	
No	67.7 (611)
Yes	32.3 (292)
Current Asthma (%)	
No	88.7 (801)
Yes	3.7 (33)
Missing	7.6 (69)
FEV_1 (L/s)	2.05 (0.29)
FVC (L)	2.39 (0.35)
FEV_1/FVC	0.86 (0.06)

Values are means (standard deviation), medians (95% range) or valid percentages (absolute numbers). PI: pulsatility index, FEV_1: forced expiratory volume in the 1st second, FVC: forced vital capacity. NI: not imputed. Data were not imputed for third trimester, birth characteristics, and respiratory outcomes.

Fetal blood flow and wheezing patterns until age 6-years

Table 3.3.2 shows, in the adjusted models, a non-significant relationship between a higher umbilical artery PI with higher risks for early and late/persistent wheezing in children (Odds Ratio (OR) 2.07, (95% CI, 0.70, 6.10) and 1.68 (0.34, 6.50) per unit increase in umbilical artery PI, respectively). Similarly, a higher U/C ratio, which indicates redistribution of blood flow in favor of the fetal brain, tended to be associated with a higher risk for early wheezing (OR, 2.74 (0.60, 12.62) per unit increase in U/C ratio). None of these associations were statistically significant. Middle cerebral artery PI was not associated with wheezing patterns. A higher pulmonary artery TVI, which indicates higher pulmonary vascular resistance, was associated with a higher risk of late or persistent wheezing (OR, 1.14 (1.01, 1.29) per unit increase in pulmonary artery TVI). The effect

estimates for the associations of fetal umbilical, cerebral and pulmonary blood flow adaptations with wheezing patterns adjusted for gestational age at third trimester, estimated fetal weight and child sex only (basic model) were similar as for the adjusted models, and presented in **Table S3.3.3**.

TABLE 3.3.2 | Associations of third trimester fetal blood flow with wheezing patterns until the age of 6 years (adjusted model).

	Never wheezing	**Early wheezing**	**Late or persistent wheezing**	**Overall wheezing**
	Odds Ratio (95% CI) (n = 414)	**Odds Ratio (95% CI)** (n = 208)	**Odds Ratio (95% CI)** (n = 120)	**Odds Ratio (95% CI)** (n = 386)
Umbilical artery PI (n = 884)	*Reference*	2.07 (0.70, 6.10)	1.68 (0.34, 6.50)	1.61 (0.92, 2,83)
Middle cerebral artery PI (n = 877)	*Reference*	0.77 (0.46, 1.31)	1.08 (0.56, 2.05)	1.06 (0.81, 1.39)
Umbilical/Middle cerebral artery ratio (n = 858)	*Reference*	2.74 (0.60, 12.62)	0.96 (0.14, 6.80)	1.07 (0.48, 2.41)
Pulmonary artery time velocity integral (n = 788)	*Reference*	1.01 (0.91, 1.12)	1.14 (1.01, 1.29)*	1.03 (0.98, 1.08)

Values are odds ratios (95% confidence intervals) from logistic regression models and generalized estimating equation models (overall wheezing; wheezing on at least one time point). "n" represent number of cases. Models were adjusted for maternal educational level, parity, body mass index, smoking, folic acid use, pregnancy complications and gestational age at third trimester, estimated fetal weight and child sex. *P<0.05

Fetal blood flow and lung function and asthma at age 10-years

Table 3.3.3 shows that in the adjusted models, a higher third trimester fetal middle cerebral artery PI was associated with a higher FEV_1/FVC only (Z-score, 0.21 (95%CI, 0.01, 0.42) per unit increase middle cerebral artery PI). We observed no consistent associations of fetal blood flow with other lung function measures. Results from the models focused on these associations adjusted for gestational age at third trimester, estimated fetal weight and child's sex only (basic model) showed no associations of fetal umbilical, cerebral and pulmonary blood flow with lung function or asthma at age 10-years (**Table S3.3.4**).

Results of the associations of fetal umbilical, cerebral and pulmonary blood flow with wheezing patterns, lung function or asthma did not materially change after adding the mediators birth weight, gestational age at birth, or BMI at age 10-years (mediation model, data not shown). The associations were not modified by maternal history of asthma or atopy or child's eczema or inhalant allergic sensitization (effect modification model, all p-values for interaction >0.05).

TABLE 3.3.3 | Associations of third trimester fetal blood flow with lung function and asthma at age 10 years (adjusted model).

	FEV_1 Z-score (95% CI) (n = 785)	FVC Z-score (95% CI) (n = 785)	FEV_1/FVC Z-score (95% CI) (n = 785)	Current asthma Odds Ratio (95% CI) (n = 834)
Umbilical artery PI (n = 884)	-0.06 (-0.46, 0.33)	-0.23 (-0.61, 0.14)	0.32 (-0.09, 0.74)	2.54 (0.26, 24.59)
Middle cerebral artery PI (n = 877)	0.01 (-0.18, 0.20)	-0.10 (-0.28, 0.08)	0.21 (0.01, 0.42)*	0.83 (0.28, 2.44)
Umbilical/Middle cerebral artery ratio (n = 858)	-0.16 (-0.73, 0.41)	-0.20 (-0.74, 0.34)	0.09 (-0.52, 0.69)	0.84 (0.03, 23.28)
Pulmonary artery time velocity integral (n = 788)	0.01 (-0.03, 0.05)	0.02 (-0.01, 0.06)	-0.04 (-0.08, 0.01)	1.08 (0.88, 1.34)

Values are z-score differences or odds ratios (95% confidence intervals) and reflect the change in lung function or risk for asthma per change in fetal blood flow. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV_1: forced expiratory volume in the 1st second, FVC: forced vital capacity. Models were adjusted for maternal educational level, parity, body mass index, smoking, folic acid use, pregnancy complications and gestational age at third trimester, estimated fetal weight and child sex. *P<0.05

Discussion

We observed a non-significant relationship between a higher umbilical artery PI and U/C ratio with higher risk of early and late/persistent wheezing. A higher pulmonary artery TVI was associated with a higher risk of late/persistent wheezing. We found that a higher middle cerebral artery PI was associated with a higher FEV_1/FVC. Associations were not explained by birth parameters, current BMI or allergic predisposition. No other consistent associations of changes in fetal umbilical, cerebral or pulmonary blood flow with wheezing patterns until age 6-years, or lung function and asthma at age 10-years were found.

Interpretation and comparison with previous studies

An adverse intrauterine environment in the developing fetus leads to fetal blood flow adaptations. These adaptations may be beneficial for short term survival but may lead to a lower delivery of oxygen and nutrients to the trunk.[6] Fetal blood flow adaptations can be detected by umbilical vein blood flow. A compensatory increase in ductus venous diameter increases the blood flow to the heart.[22] This is eventually followed by a higher umbilical artery blood flow resistance and a decrease in cerebral artery resistance.[23] Subsequently, changes in the pulmonary arteries can be observed, such as a higher pulmonary TVI.[24] For the current study, we hypothesized that fetal umbilical, cerebral and pulmonary blood flow adaptations may affect growth and maturation of the airways and lungs, which predispose individuals to lung disease.[8, 9]

Fetal blood flow adaptations are related to fetal growth restriction or low birth weight with further consequences for childhood respiratory health.[12] Umbilical placental embolization in sheep was associated with structural alterations in the lungs, such as fewer but larger alveoli and a 10% reduction in the internal surface area.[9] Another animal study did not find differences in lung

growth after umbilical placental embolization, but did find higher pulmonary deoxyribonucleic acid and plasma cortisol levels suggesting that the offspring lungs remain underdeveloped during life.[25] Children born with a very low birth weight or preterm are at higher risks for severe chronic respiratory diseases, such as bronchopulmonary dysplasia (BPD).[26] Intrauterine conditions, such as abnormal placental flow or suboptimal development of the placenta may lead to an increased expression of angiogenic factors, such as soluble fms-like tyrosine kinase-1 and vascular endothelial growth factor, which increases the risk of chronic respiratory diseases in childhood.[27, 28] Also, an elevated inflammation status, found in growth restricted fetuses, could lead to reduced lung function later in life.[29] Impaired vasculogenesis and angiogenesis, found in children with intrauterine growth restriction, might be other mechanisms than structural lung growth alterations leading to a suboptimal lung development.[30]Children with fetal growth restriction might be vulnerable to more adaptive processes and have increased risk of respiratory morbidity in later life. Furthermore, maternal hypertensive disorders during pregnancy could have an effect on respiratory morbidity through multiple underlying mechanisms such as a disturbed placental blood flow and an altered angiogenic status. Previous published studies have shown that hypertensive disorders in pregnancy might be related to lower lung function in newborn infants or increased risk of wheezing.[31-33] A recent study from the same cohort as the current study reported associations for blood pressure across the full range in different trimesters with asthma related outcomes in childhood, but not for maternal hypertensive disorders with these outcomes.[34] The role of fetal blood flow patterns for these associations is not clear. Our observed associations were not mediated through birth weight or gestational age at birth, but a possible role of the placenta or inflammatory status of the newborn warrants further studies.

Our study resulted mainly in negative findings, with two exceptions. First, our results showed that an increase in TVI was related to late/persistent wheezing. An increased TVI might be a sign of underdevelopment of the fetal airways, such as fewer but larger alveoli and impaired growth of the airways and lungs.[8, 9] This finding might suggest that pulmonary blood flow in fetal life might be related to an increased risk of late/persistent wheezing in later life. A higher middle cerebral artery PI was associated with a higher FEV_1/FVC. Our results showed a non-significant inverse relation of the middle cerebral artery PI with FVC, but no associations was observed for FEV_1. We speculate that flow patterns related to fetal brain sparing might have consequences on the growth of the lungs. However, as no other effects of fetal blood flow measures on lung function or asthma in childhood were found. the observed association might be a chance finding.

Strengths and limitations

The main strength of this study was the large population-based cohort examined from fetal life onwards. To our knowledge, this is the first study to examine the effects of fetal blood flow on respiratory outcomes. The population-based setting enabled us to assess the fetal blood flow across the full range, rather than only in fetuses with growth restriction or other complications. Follow-up measurements at the age of 10-years were available in 74% of the children. Missing

information about wheezing, lung function or asthma could lead to selection bias and loss of power. Our results would be biased if the associations between fetal blood flow and wheezing, lung function or asthma differed between those included and those not included in the study. Although this seems unlikely, it cannot be excluded. In the present study we evaluated multiple associations. However, because of the correlations between the outcome measures, we did not correct for multiple testing. Our results were inconsistent and the associations might be a chance finding. In this study there might have occurred some bias towards a more affluent and healthy population due to differences in characteristics between those lost to follow-up and included in the study.[13] Information on wheezing patterns, asthma and eczema were obtained by questionnaires, adapted from the ISAAC-Core questionnaires. These have been validated and shown adequate for epidemiological studies.[35] Although misclassification due to under- or over reporting cannot be excluded. Finally, although we had information about a large number of confounders, the influence of residual confounding should be considered, as in any observational study.

Conclusion and perspectives

The results of our study are important from an etiological perspective. Our findings suggest that adaptations in fetal blood flow might contribute to the risk of wheezing and lung function in childhood. However, the observed effects were small or non-significant and may reflect subclinical changes only. These findings should be considered as hypothesis generating and need further replication.

References

1. Maritz GS, Morley CJ, Harding R. Early developmental origins of impaired lung structure and function. Early Hum Dev. 2005;81(9):763-71.
2. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med. 2013;1(9):728-42.
3. Degani S. Fetal cerebrovascular circulation: a review of prenatal ultrasound assessment. Gynecol Obstet Invest. 2008;66(3):184-96.
4. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
5. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: a pulsed Doppler study. J Perinat Med. 1992;20(1):79-82.
6. Zhang S, Regnault TR, Barker PL, Botting KJ, McMillen IC, McMillan CM, et al. Placental adaptations in growth restriction. Nutrients. 2015;7(1):360-89.
7. Haworth SG, Hislop AA. Lung development-the effects of chronic hypoxia. Semin Neonatol. 2003;8(1):1-8.
8. Duijts L. Fetal and infant origins of asthma. Eur J Epidemiol. 2012;27(1):5-14.
9. Maritz GS, Cock ML, Louey S, Suzuki K, Harding R. Fetal growth restriction has long-term effects on postnatal lung structure in sheep. Pediatr Res. 2004;55(2):287-95.
10. Pike K, Jane Pillow J, Lucas JS. Long term respiratory consequences of intrauterine growth restriction. Semin Fetal Neonatal Med. 2012;17(2):92-8.

11. Turner S, Prabhu N, Danielian P, McNeill G, Craig L, Allan K, et al. First- and Second-Trimester Fetal Size and Asthma Outcomes at Age 10 Years. Am J Resp Crit Care. 2011;184(4):407-13.
12. den Dekker HT, Sonnenschein-van der Voort AM, de Jongste JC, Anessi-Maesano I, Arshad SH, Barros H, et al. Early growth characteristics and the risk of reduced lung function and asthma: A meta-analysis of 25,000 children. J Allergy Clin Immunol. 2016;137(4):1026-35.
13. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
14. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
15. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
16. Roule V, Labombarda F, Pellissier A, Sabatier R, Lognone T, Gomes S, et al. Echocardiographic assessment of pulmonary vascular resistance in pulmonary arterial hypertension. Cardiovasc Ultrasound. 2010;8:21.
17. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332(3):133-8.
18. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
19. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.
20. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535-40.
21. van der Valk JP, Gerth van Wijk R, Hoorn E, Groenendijk L, Groenendijk IM, de Jong NW. Measurement and interpretation of skin prick test results. Clin Transl Allergy. 2015;6:8.
22. Kiserud T, Kessler J, Ebbing C, Rasmussen S. Ductus venosus shunting in growth-restricted fetuses and the effect of umbilical circulatory compromise. Ultrasound Obstet Gynecol. 2006;28(2):143-9.
23. Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A. Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome. Obstet Gynecol. 1992;79(3):416-20.
24. Mielke G, Benda N. Blood flow velocity waveforms of the fetal pulmonary artery and the ductus arteriosus: reference ranges from 13 weeks to term. Ultrasound Obstet Gynecol. 2000;15(3):213-8.
25. Cock ML, Albuquerque CA, Joyce BJ, Hooper SB, Harding R. Effects of intrauterine growth restriction on lung liquid dynamics and lung development in fetal sheep. Am J Obstet Gynecol. 2001;184(2):209-16.
26. Reiss I, Landmann E, Heckmann M, Misselwitz B, Gortner L. Increased risk of bronchopulmonary dysplasia and increased mortality in very preterm infants being small for gestational age. Arch Gynecol Obstet. 2003;269(1):40-4.
27. Wallace B, Peisl A, Seedorf G, Nowlin T, Kim C, Bosco J, et al. Anti-sFlt-1 Therapy Preserves Lung Alveolar and Vascular Growth in Antenatal Models of Bronchopulmonary Dysplasia. Am J Respir Crit Care Med. 2018;197(6):776-87.
28. Collins JJP, Tibboel D, de Kleer IM, Reiss IKM, Rottier RJ. The Future of Bronchopulmonary Dysplasia: Emerging Pathophysiological Concepts and Potential New Avenues of Treatment. Front Med (Lausanne). 2017;4:61.
29. McElrath TF, Allred EN, Van Marter L, Fichorova RN, Leviton A, Investigators ES. Perinatal systemic inflammatory responses of growth-restricted preterm newborns. Acta Paediatr. 2013;102(10):e439-42.
30. Bolehovska P, Sehnal B, Driak D, Halaska M, Magner M, Novotny J, et al. Changes in placental angiogenesis and their correlation with foetal intrauterine restriction. Ceska Gynekol. 2015;80(2):144-50.
31. Stick SM, Burton PR, Gurrin L, Sly PD, LeSouef PN. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. Lancet. 1996;348(9034):1060-4.
32. Zugna D, Galassi C, Annesi-Maesano I, Baiz N, Barros H, Basterrechea M, et al. Maternal complications in pregnancy and wheezing in early childhood: a pooled analysis of 14 birth cohorts. Int J Epidemiol. 2015;44(1):199-208.
33. Shaheen SO, Macdonald-Wallis C, Lawlor DA, Henderson AJ. Hypertensive disorders of pregnancy, respiratory outcomes and atopy in childhood. Eur Respir J. 2016;47(1):156-65.
34. Wilmink FA, den Dekker HT, de Jongste JC, Reiss IKM, Jaddoe VWV, Steegers EA, et al. Maternal blood pressure and hypertensive disorders during pregnancy and childhood respiratory morbidity: the Generation R Study. Eur Respir J. 2018;52(5).
35. Silverberg JI, Patel N, Immaneni S, Rusniak B, Silverberg NB, Debashis R, et al. Assessment of atopic dermatitis using self-report and caregiver report: a multicentre validation study. Br J Dermatol. 2015;173(6):1400-4.

Supplementary Material

TABLE S3.3.1 | Characteristics of children and their mothers (n = 903) – non imputed data.

Maternal characteristics	
Education (%)	
Low (no, primary, secondary education)	32.3 (292)
High (higher education)	66.7 (602)
Missing	1.0 (9)
Pre-pregnancy body mass index (kg/m^2)	23.5 (4.0)
Missing (%)	14.7 (131)
Parity (%)	
Nullipara	62.7 (566)
Multipara	37.1 (335)
Missing	0.2 (2)
History of asthma or atopy (%)	
No	55.3 (499)
Yes	33.9 (306)
Missing	10.9 (98)
Smoking during pregnancy (%)	
No smoking throughout pregnancy	71.1 (642)
Yes	18.9 (171)
Missing	10.0 (90)
Folic acid supplement use (%)	
No use	6.0 (54)
Start within first 10 weeks of pregnancy	23.8 (215)
Preconceptional start	52.7 (476)
Missing	17.5 (158)
Pregnancy-induced complications (gestational hypertension/pre-eclampsia) (%)	
No	88.3 (797)
Yes	7.4 (67)
Missing	4.3 (39)
Third trimester fetal characteristics	
Gestational age at measurement, weeks	30.4 (28.5 – 32.7)
Missing	-
Estimated fetal weight, grams	1632 (266)
Missing (%)	0.7 (6)
Umbilical artery PI	0.97 (0.16)
Missing (%)	2.1 (19)
Middle cerebral artery PI	1.97 (0.33)
Missing (%)	2.8 (26)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)
Missing (%)	5.0 (45)
Pulmonary artery time velocity integral	12.04 (1.83)
Missing (%)	12.8 (116)
Birth characteristics	
Gestational age at birth, weeks	40.3 (36.7 – 42.4)
Missing (%)	-
Birth weight, grams	3528 (509)
Missing (%)	-
Sex	
Male	50.6 (457)
Female	49.4 (446)
Missing	-

Childhood characteristics	
Wheezing patterns until 6 years (%)	
Never	45.8 (414)
Early	23.0 (208)
Late/Persistent	13.3 (120)
Missing	17.8 (161)
Age at follow up, y	9.8 (9.1 – 10.5)
Missing (%)	2.3 (21)
Body Mass Index at age 10 years (kg/m^2)	17.1 (2.2)
Missing (%)	3.4 (31)
Ever eczema at age 10 years (%)	
No	70.2 (634)
Yes	22.3 (201)
Missing	7.5 (68)
Inhalant allergic sensitization at age 10 years (%)	
No	56.7 (512)
Yes	25.2 (228)
Missing	18.1 (163)
Current Asthma (%)	
No	88.7 (801)
Yes	3.7 (33)
Missing	7.6 (69)
FEV_1 (L/s)	2.05 (0.29)
Missing (%)	13.1 (118)
FVC (L)	2.39 (0.35)
Missing (%)	13.1 (118)
FEV_1/FVC	0.86 (0.06)
Missing (%)	13.1 (118)

Values are means (standard deviation), medians (95% range) or valid percentages (absolute numbers). PI: pulsatility index, FEV_1: forced expiratory volume in the 1st second, FVC: forced vital

TABLE S3.3.2 | Characteristics of children and their mothers with and without follow-up data.

	Childhood outcome data available (n = 903)	Childhood follow-up data unavailable (n = 292)
Maternal characteristics		
Education (%)		
Low (no, primary, secondary education)	32.3 (292)	48.6 (142)†
High (higher education)	66.7 (602)	48.3 (141)†
Missing	1.0 (9)	3.1 (9)
Pre-pregnancy body mass index (kg/m²)	23.5 (4.0)	23.5 (4.3)
Missing (%)	14.7 (131)	15.4 (45)
Parity (%)		
Nullipara	62.7 (566)	53.8 (157)†
Multipara	37.1 (335)	45.9 (134)†
Missing	0.2 (2)	0.3 (1)
History of asthma or atopy (%)		
No	55.3 (499)	58.9 (172)
Yes	33.9 (306)	33.2 (97)
Missing	10.9 (98)	7.9 (23)
Smoking during pregnancy (%)		
No smoking throughout pregnancy	71.1 (642)	61.3 (179)†
Yes	18.9 (171)	32.2 (94)†
Missing	10.0 (90)	6.5 (19)†
Folic acid supplement use (%)		
No use	6.0 (54)	13.3 (39)†
Start within first 10 weeks of pregnancy	23.8 (215)	25.0 (73)†
Preconceptional start	52.7 (476)	45.2 (132)†
Missing	17.4 (156)	16.4 (48)
Pregnancy-induced complications (gestational hypertension/pre-eclampsia) (%)		
No	88.3 (797)	83.6 (244)
Yes	7.4 (67)	8.2 (24)
Missing	4.3 (39)	8.2 (24)
Third trimester fetal characteristics		
Gestational age at measurement, weeks	30.4 (28.5 – 32.7)	30.3 (28.4 – 32.5)
Missing	-	
Estimated fetal weight, grams	1632 (266)	1624 (275)
Missing (%)	0.7 (6)	1.0 (3)
Umbilical artery PI	0.97 (0.16)	0.98 (0.17)
Missing (%)	2.1 (19)	2.4 (7)
Middle cerebral artery PI	1.97 (0.33)	1.98 (0.34)
Missing (%)	2.8 (26)	2.1 (6)
Umbilical/Middle cerebral artery ratio	0.50 (0.11)	0.51 (0.12)
Missing (%)	5.0 (45)	4.1 (12)
Pulmonary artery time velocity integral	12.04 (1.83)	12.1 (1.73)
Missing (%)	12.8 (116)	15.4 (45)
Birth characteristics		
Gestational age at birth, weeks	40.3 (36.7 – 42.4)	40.1 (34.0 – 42.2)†
Missing (%)	-	
Birth weight, grams	3528 (509)	3472 (625)
Missing (%)	-	-
Sex		
Male	50.6 (457)	56.5 (165)
Female	49.4 (446)	43.6 (127)
Missing	-	-

	Childhood outcome data available (n = 903)	Childhood follow-up data unavailable (n = 292)
Childhood characteristics		
Wheezing patterns at age 6 years (%)		NA
Never	45.8 (414)	
Early	23.0 (208)	
Late/Persistent	13.3 (120)	
Missing	17.8 (161)	
Age at follow up, y	9.8 (9.1 – 10.5)	NA
Missing (%)	2.3 (21)	
Body Mass Index at age 10 years (kg/m^2)	17.1 (2.2)	NA
Missing (%)	3.4 (31)	
Ever eczema at age 10 years (%)		NA
No	70.2 (634)	
Yes	22.3 (201)	
Missing	7.5 (68)	
Inhalant allergic sensitization at age 10 years (%)		NA
No	56.7 (512)	
Yes	25.2 (228)	
Missing	18.1 (163)	
Current Asthma (%)		NA
No	88.7 (801)	
Yes	3.7 (33)	
Missing	7.6 (69)	
FEV_1 (L/s)	2.05 (0.29)	NA
Missing (%)	13.1 (118)	
FVC (L)	2.39 (0.35)	NA
Missing (%)	13.1 (118)	
FEV_1/FVC	0.86 (0.06)	NA
Missing (%)	13.1 (118)	

Values are means (standard deviation), medians (95% range) or valid percentages (absolute numbers). PI: pulsatility index, FEV_1: forced expiratory volume in the 1st second, FVC: forced vital capacity. NA: not applicable. Participants were compared using independent samples t-test for continuous variables and chi-square test for categorical variables. †P<0.01

TABLE S3.3.3 | Associations of third trimester fetal blood flow with wheezing patterns at age 6 years (basic model).

	Never wheezing **Odds Ratio (95% CI)** (n = 414)	**Early wheezing** **Odds Ratio (95% CI)** (n = 208)	**Late or persistent wheezing** **Odds Ratio (95% CI)** (n = 120)	**Overall wheezing** **Odds Ratio (95% CI)** (n = 386)
Umbilical artery PI (n = 884)	*Reference*	2.23 (0.76, 6.58)	1.84 (0.49, 6.96)	1.71 (0.85, 3.42)
Middle cerebral artery PI (n = 877)	*Reference*	0.75 (0.45, 1.26)	1.17 (0.62, 2.20)	1.13 (0.80, 1.60)
Umbilical/Middle cerebral artery ratio (n = 858)	*Reference*	3.32 (0.73, 15.18)	0.82 (0.12, 5.62)	0.96 (0.37, 2.50)
Pulmonary artery time velocity integral (n = 788)	*Reference*	1.01 (0.91, 1.12)	1.11 (0.98, 1.25)	1.02 (0.95, 1.09)

Values are odds ratios (95% confidence intervals) from logistic regression models and generalized estimating equation models (overall wheezing; wheezing on at least one time point). "n" represent number of cases. Models were adjusted gestational age at third trimester, estimated fetal weight and child sex.

TABLE S3.3.4 | Associations of third trimester fetal blood flow with lung function and asthma at age 10 years (basic model).

	FEV_1 Z-score (95% CI) (n = 785)	**FVC Z-score (95% CI)** (n = 785)	**FEV_1/FVC Z-score (95% CI)** (n = 785)	**Current asthma Odds Ratio (95% CI)** (n = 834)
Umbilical artery PI (n = 884)	-0.06 (-0.45, 0.33)	-0.23 (-0.60, 0.15)	0.33 (-0.09, 0.74)	2.48 (0.25, 24.80)
Middle cerebral artery PI (n = 877)	0.02 (-0.17, 0.21)	-0.08 (-0.26, 0.10)	0.19 (-0.01, 0.39)	0.66 (0.22, 1.93)
Umbilical/Middle cerebral artery ratio (n = 858)	-0.17 (-0.74, 0.40)	-0.26 (-0.79, 0.29)	0.16 (-0.44, 0.77)	1.41 (0.05, 39.44)
Pulmonary artery time velocity integral (n = 788)	0.01 (-0.03, 0.05)	0.02 (-0.01, 0.06)	-0.03 (-0.07, 0.01)	1.14 (0.92, 1.40)

Values are z-score differences or odds ratios (95% confidence intervals) and reflect the change in lung function or risk for asthma per change in fetal blood flow. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV1: forced expiratory volume in the 1st second, FVC: forced vital capacity. Models were adjusted for gestational age at third trimester, estimated fetal weight and child sex.

Chapter 3

Chapter 3.4

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

Kooijman MN, Bakker H, van der Heijden AJ, Hofman A, Franco OH, Steegers EAP, Taal HR, Jaddoe VWV

Adapted from: J Am Soc Nephrol. 2014;25(11):2616-24

Abstract

Background Impaired fetal abdominal blood flow may lead to smaller kidneys and subsequent impaired kidney function in later life.

Methods In a prospective cohort study among 923 pregnant women and their children, we measured fetal growth, kidney volumes, and umbilical and cerebral artery blood flow (gestational age of 30.3 weeks (95% range 28.5 – 32.7). We used a higher umbilical /cerebral artery pulsatility index ratio as an indicator of preferential fetal blood flow to the upper body parts at expense of the intra-abdominal organs. At a median age of 5.9 years (95% range 5.7–6.6), we measured childhood kidney volumes, creatinine and cystatin C blood levels, microalbuminuria and blood pressure, and estimated the glomerular filtration rate.

Results A preferential fetal blood flow to the upper body parts at expense of the intra-abdominal organs was only associated with a smaller combined kidney volume in childhood. Also, fetal combined kidney volume was positively associated with childhood combined kidney volume and estimated glomerular filtration rate, inversely associated with childhood creatinine and cystatin C levels (all p-values<0.05), but not associated with childhood microalbuminuria and blood pressure. Children within the highest tertile of fetal umbilical/cerebral ratio and lowest tertile of fetal combined kidney volume had the lowest estimated glomerular filtration rate (difference:-6.36ml/min per 1.73m^2, 95% confidence interval -11.78, -0.94 compared to children within the middle tertiles).

Conclusions and Relevance Impaired fetal blood to the abdominal organs and smaller fetal kidney size are associated with subclinical changes in kidney outcomes in school-age children.

Introduction

Third trimester of pregnancy is a critical period for fetal kidney development.[1] Nephrogenesis continues until 36 weeks of gestation, after which the induction of nephron numbers ceases.[2] A permanent reduction of kidney size and number of nephrons leads to a smaller glomerular filtration surface area, which might predispose the individual to decreased kidney function in childhood and subsequently to kidney disease and hypertension in adulthood.[3, 4] This hypothesis is supported by studies showing consistent associations of low birth weight with higher risks of kidney disease and hypertension in later life.[5, 6] Although the observed effect estimates from these studies were small, they are important from an etiological perspective.[5, 6] Also, post-mortem studies showed that nephron number is lower in hypertensive individuals than in normotensive controls,[7] and that nephron number is positively correlated with birth weight and kidney size.[8, 9] Animal studies demonstrated a reduction in nephron number due to vascular placental insufficiency.[10] Placental insufficiency is an important risk factor for fetal growth restriction and low birth weight.[11] We recently demonstrated that increased third trimester placental insufficiency is associated with a higher blood pressure in childhood.[12] Placental insufficiency is characterized by a preferential fetal blood flow to the brain at expense of the trunk.[13] This fetal blood flow redistribution is caused by a higher peripheral and lower cerebral arterial resistance,[11] and can be measured as a higher umbilical artery pulsatility index (PI) and lower cerebral artery PI, respectively. This combination leads to a higher ratio of these measures (higher U/C ratio).[14] Whether and to what extend impaired abdominal or more specifically, kidney blood flow and kidney growth restriction during fetal life lead to risk factors for kidney disease in later life is unknown.

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

Methods

Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, the Netherlands. All children were born between April 2002 and January 2006. Enrollment was aimed at early pregnancy at the routine fetal ultrasound examination in pregnancy but was allowed until birth of the child. In total, 9,778 mothers and their children were included in the study. More detailed assessments of fetal and postnatal growth and development were conducted in a random subgroup of 1,232 Dutch mothers and children (response 80%).[15] Twin pregnancies (n = 15) and pregnancies leading to perinatal death (n = 2)

were excluded from the analysis, leading to 1,215 singleton live births. Third trimester blood flow distribution and fetal kidney measurements were successfully performed in 1,201 singleton live born children, of whom 925 children (77%) visited the research center for follow up measurements at the median age of 5.9 (95% range 5.7 – 6.6) years. Childhood kidney measurements were successfully performed in 923 children (Flow chart is given in **Supplementary Material Figure S3.4.1**). Non-response analysis showed that as compared to mothers of children not included in the analysis, mothers of children included in the study were older, higher educated, smoked less frequently during pregnancy, were more frequently nulliparous and folic acid supplements users. Furthermore, children included in the study had a higher birth weight and gestational age at birth and were more often girls (**Supplementary Material Table S3.4.1**). The Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, has approved the study.

Third trimester fetal measurements

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

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

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

Fetal kidney dimensions: In a sagittal plane, the maximum longitudinal kidney lengths were measured placing the callipers on the outer edges of the caudal and cranial side.[21] Antero-

posterior and transverse kidney diameters were measured perpendicular to each other, outer tot outer, in an axial plane. The cross-sectional area in which the kidney appeared symmetrically round at its maximum width was used.[22] Kidney volume was calculated using the equation of an ellipsoid: volume (cm^3) = 0.523 x length (mm) x width (mm) x depth (mm).[23] Combined kidney volume was calculated by summing right and left kidney volume.

Childhood kidney outcomes

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

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

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

Covariates

Information on maternal age, pre-pregnancy body mass index, parity, educational level, smoking during pregnancy, folic acid use during pregnancy, gestational hypertensive complications was obtained by questionnaires and registries. Maternal height was measured without shoes and pre-pregnancy body mass index (BMI) was calculated (kg/m^2). Date of birth, infant sex and birth weight were obtained from midwife and hospital registries. At the age of 6 years, child height and weight were measured without shoes and heavy clothing, and body surface area was calculated.

Statistical analysis

First, we performed correlation analyses using scatterplots and Pearson correlation coefficients to explore the relation between gestational age with U/C ratio, fetal kidney volume and fetal kidney volume/estimated fetal weight. We also performed correlation analyses for the relations between fetal blood flow patterns, fetal combined kidney volume and childhood kidney outcomes. Second, the associations of fetal blood flow distribution and fetal combined kidney volume with childhood kidney outcomes were analyzed in a stepwise approach: We performed correlation analyses using scatterplots and Pearson correlation coefficients. Next we performed multiple linear regression models. The models were first adjusted for gestational age and estimated fetal weight at third trimester measurement, and child sex, current age and body surface area (Basic model). Analyses focused on eGFR were not further adjusted for body surface area since this is included in the Schwartz 2009 formula. These models were additionally adjusted for potential confounders including maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, pregnancy complications (pre-eclampsia and pregnancy induced hypertension), gestational age at birth and gestational age adjusted birth weight (Confounder model). We additionally adjusted the Confounder model for childhood combined kidney volume to explore whether any association was explained by childhood combined kidney volume (Childhood kidney volume model). Third, we tested potential combined effects and interactions between fetal blood flow, fetal combined kidney volume, and childhood combined kidney volume for the associations with childhood eGFR by performing stratified (tertiles) regression analyses. We performed a sensitivity analysis using gestational age adjusted birth weight as an additional index of final nephron number instead of fetal kidney volume. To reduce the possibility of potential bias associated with missing data (less than 17%), missing values in maternal, fetal and child covariates were imputed using the multiple imputations procedure with five imputations and these datasets were analyzed together. Further information about the methods of multiple imputations are given in the **Supplementary Material.** Observed data before multiple imputations are presented in the **Supplementary Material** (**Table S3.4.2**). All statistical analyses were performed using the Statistical Package for the Social Sciences version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Maternal, fetal and child characteristics are shown in **Table 3.4.1**. More descriptive data are given in the **Supplementary Material**. Subject characteristics according to tertiles of U/C ratio and fetal kidney volume are given in **Supplementary Material Tables S3.4.3** and **S3.4.4**, respectively. **Supplementary Material Figures S3.4.2** and **S3.4.3** give the correlations of gestational age and estimated fetal weight with fetal kidney volume/estimated fetal weight.

TABLE 3.4.1 | Subject characteristics (N = 923).

Maternal characteristics	
Age, y	32.2 (23.4 – 39.4)
Height, cm	171 (6.3)
Pre-pregnancy weight, kg	69.0 (13.0)
Pre-pregnancy body mass index, kg/m^2	23.6 (4.2)
Parity ≥1, %	37.7 (348)
Educational level, %	
Primary/secondary	34.1(315)
Secondary or higher	65.9 (608)
Smoking during pregnancy, %	
Yes	21.5 (198)
No	78.5 (745)
Folic acid supplement use, %	
Yes	90.9 (839)
No	9.1 (84)
Pregnancy induced hypertension, %	
Yes	5.6 (52)
No	94.4 (871)
Preeclampsia, %	
Yes	3.1 (29)
No	96.9 (894)
Fetal characteristics	
General	
Gestational age at measurement, wk	30.3 (28.5 – 32.7)
Estimated fetal weight, g	1634 (263)
Blood flow distribution	
Umbilical artery PI	0.97 (0.16)
Middle cerebral artery PI	1.97 (0.33)
U/C ratio middle cerebral artery	0.50 (0.11)
Fetal kidney biometrics	
Right kidney volume, cm^3	10.64 (3.07)
Left kidney volume, cm^3	9.94 (2.76)
Combined kidney volume, cm^3	20.56 (5.48)
Birth and infant characteristics	
Gestational age at birth, wk	40.3 (36.4 – 42.4)
Birth weight, g	3534 (509)
Sex boys, %	50.3 (464)
Childhood characteristics	
Age at follow up, y	5.9 (5.7 – 6.6)
Height, cm	119 (5.2)
Weight, kg	22.6 (3.2)
Body mass index, kg/m^2	15.9 (1.4)
Body surface area, m^2	0.86 (0.07)
Kidney volume left, cm^3	61.4 (12.6)
Kidney volume right, cm^3	59.5 (11.7)
Kidney volume combined, cm^3	120.9 (22.1)
Creatinine, µmol/l	36.8 (4.9)
Cystatin C, µg/l	790 (74)
Estimated glomerular filtration rate ml/min per 1.73m^2	120.2 (15.8)
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)
Microalbuminuria[a], %	7.1 (62)
Systolic blood pressure, mmHg	102.2 (7.7)
Diastolic blood pressure, mmHg	60.1 (6.3)

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

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

Fetal blood flow, fetal kidney volume and childhood kidney outcomes

Scatterplots for the correlations between fetal and childhood kidney volumes, fetal blood flow patterns, and childhood kidney function outcomes are shown in **Supplementary Material Figures S3.4.4, S3.4.5** and **S3.4.6.**

Table 3.4.2 shows that a higher third trimester fetal U/C ratio, which reflects a preferential blood flow to the upper body parts at expense of the intra-abdominal organs, was associated with a smaller childhood combined kidney volume (-2.46 cm^3 per 1 SDS increase in ratio, 95% Confidence Interval (CI) -3.89, -1.04, Basic model). This fetal blood flow pattern was not associated with childhood creatinine and cystatin C levels, eGFR, microalbuminuria or blood pressure. The effect estimates were only slightly modified by additional adjustment for confounders or childhood kidney size. **Table 3.4.3** shows that a larger third trimester fetal combined kidney volume was associated with larger childhood combined kidney volume (3.89 cm^3 per 1 SDS increase in volume, 95% CI 3.61, 4.16), lower creatinine levels (-1.18 µmol/l per 1 SDS increase in volume, 95% CI - 1.60, -0.77), lower cystatin C levels (-18 µg/l per 1 SDS increase in volume, 95% CI -25, -12) and higher eGFR (4.40 ml/min per 1.73m^2 per 1 SDS increase in volume, 95% CI 3.06, 5.75), but not with microalbuminuria and blood pressure at the age of 6 years. These effect estimates were similar after additional adjustment for potential confounders and combined childhood kidney volume. Additional results showing the effects of stepwise adjustment of the associations of third trimester fetal U/C ratio and fetal combined kidney volume with childhood kidney outcomes are given in the **Supplementary Material** (**Tables S3.4.5** and **S3.4.6**). Also, **Supplementary Material Tables S3.4.7** and **S3.4.8** give the R squares for the effect estimates for the associations of third trimester fetal U/C ratio and third trimester fetal combined kidney volume with childhood kidney outcomes. The R squares were all between 0.20 and 0.25.

Figure 3.4.1A shows the combined effects for fetal blood flow and fetal kidney volume on childhood eGFR. Children within the highest tertile of fetal U/C ratio and lowest tertile of fetal combined kidney volume had the lowest eGFR (difference: -6.36 ml/min per 1.73m^2, 95% confidence interval -11.78, -0.94, compared to children within the middle tertiles), whereas children within the lowest tertile of fetal U/C ratio and highest tertile of fetal combined kidney volume had the highest eGFR at school-age (p-value for interaction <0.05). **Figure 3.4.1B** gives the combined effects for fetal blood flow and childhood combined kidney volume on childhood eGFR. Children within the highest tertile of fetal U/C ratio and lowest tertile of childhood combined kidney volume had the lowest eGFR. This interaction was statistically not significant. Similarly, **Figure 3.4.1C** gives the combined effects for fetal combined kidney volume and childhood combined kidney volume on childhood eGFR. The lowest eGFR was observed in children within the lowest tertile of both fetal combined kidney volume and childhood combined kidney volume. This interaction was statistically not significant. Also, the interaction terms were statistically not significant for childhood cystatin C levels, micro-albuminuria, and blood pressure as outcomes (data not shown).

TABLE 3.4.2 | Associations of third trimester umbilical/cerebral artery resistance ratio with kidney outcomes at the age of 6 years (N = 879).

	Kidney size		**Kidney function**			**Blood pressure**	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
Umbilical/cerebral artery resistance ratio (SD = 0.11)							
Basic model	-2.46	0.14	4	-0.67	0.75	0.27	0.04
	(-3.89, -1.04)[b]	(-0.27, 0.55)	(-3, 10)	(-2.00, 0.68)	(0.55, 1.01)	(-0.26, 0.81)	(-0.41, 0.49)
Confounder model	-2.58	0.24	4	-1.03	0.71	0.30	0.05
	(-4.01, -1.14)[b]	(-0.18, 0.66)	(-3, 11)	(-2.39, 0.34)	(0.52, 0.97)[a]	(-0.25, 0.85)	(-0.41, 0.50)
Childhood kidney	NA	0.10	3	-0.29	0.72	0.21	-0.02
volume model		(-0.32, 0.52)	(-3, 10)	(-1.66, 1.08)	(0.52, 1.00)	(-0.37, 0.78)	(-0.50, 0.45)

eGFR, estimated glomerular filtration rate; BP, blood pressure

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

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

TABLE 3.4.3 | Associations of third trimester fetal kidney dimensions with kidney outcomes at the age of 6 years (N = 870).

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

eGFR, estimated glomerular filtration rate; BP, blood pressure

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight. Childhood kidney volume model is additionally adjusted for combined childhood kidney volume. [a]p <0.01

Results of the sensitivity analyses are given in the **Supplementary Material**. When we used gestational age adjusted birth weight as an index of final nephron number instead of fetal kidney volume, we observed similar results (**Supplementary Material Figure S3.4.7**). Lower gestational age adjusted birth weight was associated with smaller childhood kidneys with a lower eGFR. The interactions between U/C ratio and gestational age adjusted birth weight for the associations with childhood kidney outcomes were not significant. Also, when we used eGFR/cm3 kidney volume instead of eGFR, no differences in results were observed in the final models (data not shown).

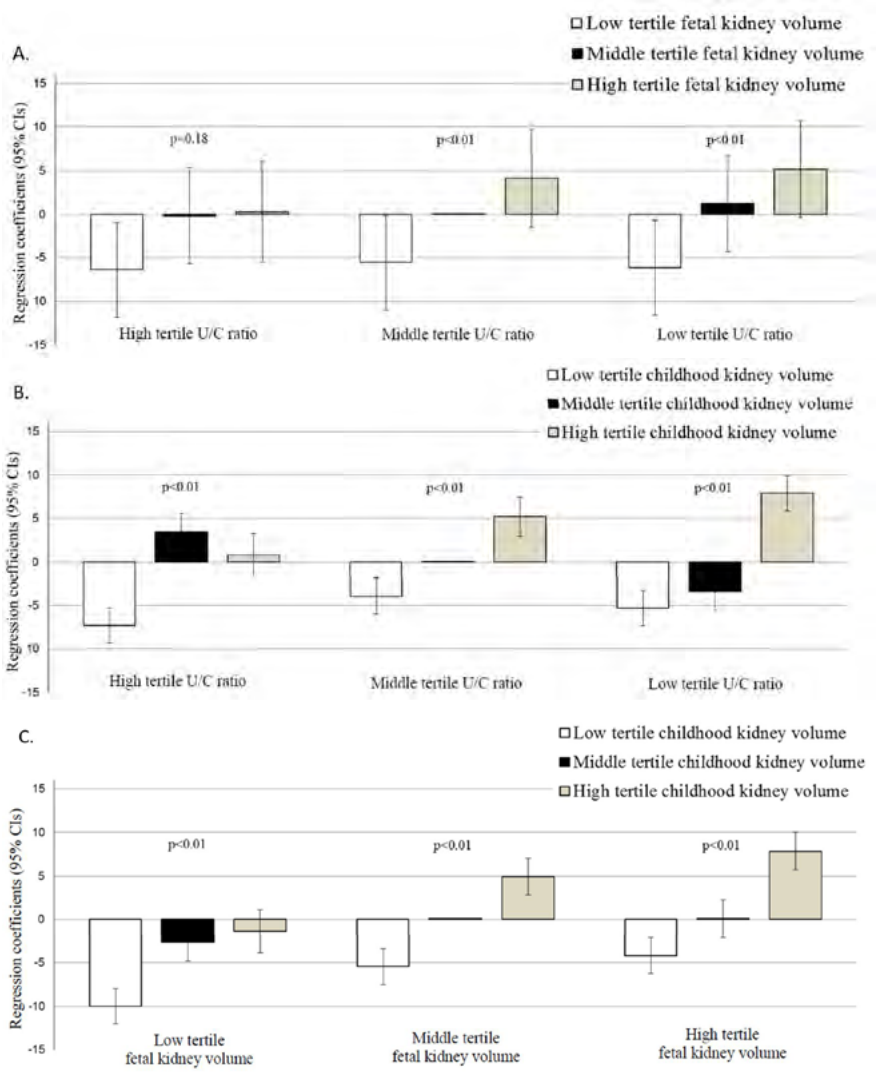

FIGURE 3.4.1 | Associations of fetal blood flow, fetal combined kidney volume and childhood kidney volume with childhood estimated glomerular filtration rate (N = 613).

Bars represent regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the associations of fetal blood flow and kidney volume (A), fetal blood flow and childhood kidney volume (B), and fetal and childhood kidney volume (C) (tertiles) with childhood estimated glomerular filtration rate. Models are adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, folic acid use during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, gestational age and gestational age adjusted birth weight, current age and body surface are. P-value <0.01 for interaction between fetal blood flow and fetal combined kidney volume for the association with childhood estimated glomerular filtration rate. P-value not significant for interaction between fetal blood flow and childhood combined kidney volume and for interaction between fetal combined kidney volume and childhood combined kidney volume.

Discussion

In a normal, low-risk population-based cohort study, third trimester fetal blood flow redistribution, at expense of the trunk and abdominal organs, was associated with smaller kidney volume, whereas a smaller third trimester fetal kidney size was associated with lower kidney function in school-age children. All differences were small and without clinical consequences at school-age. Fetal blood flow and fetal kidney size were not associated with childhood blood pressure.

An adverse fetal environment may lead to smaller kidneys with a reduced number of nephrons.[28] These kidney adaptations may lead to a reduced glomerular filtration surface area, hyperfiltration and eventually glomerular sclerosis and chronic kidney disease.[2, 3] Thus far, this hypothesis is mainly supported by studies showing associations of low birth weight with higher risks of kidney disease and hypertension in adulthood.[5, 6] Also, post-mortem studies showed that nephron number is lower in children with a low birth weight, or small kidney size, and in adults with primary hypertension.[7-9] Studies in rats showed that fetal growth restriction, induced by bilateral uterine artery ligation, leads to an increased risk of kidney disease and higher blood pressure in adulthood.[29, 30] In a study among 7,457 adults aged 20 to 30 years in Norway, intrauterine growth restriction was associated with low-normal kidney function.[31] Another study among 82 subjects born before 32 weeks of gestation in the Netherlands, observed no consistent associations of preterm birth in combination with intra-uterine growth restriction with kidney function in young adulthood.[32]

Animal studies showed a reduction in glomerular and nephron number due to vascular placental insufficiency.[10, 33] We observed in a previous study within the same cohort that fetal blood flow redistribution at expense of the abdominal organs, leads to smaller fetal kidneys.[21] In the current follow-up study, we observed that fetal blood flow redistribution is also associated with smaller kidneys in children. Surprisingly, we observed that fetal blood redistribution at expense of the abdominal organs was associated with a lower risk of microalbuminuria, independent of potential confounders and childhood kidney size. We could not explain this finding. Fetal blood flow redistribution was not associated with other kidney function outcomes. We measured fetal blood flow through the umbilical arteries, which reflects the arterial resistance and blood flow

to the intra-abdominal arteries, including the descending aorta and renal artery.[19] Future detailed studies focused on the directly measured renal artery blood flow might give more information about the fetal and childhood consequences of impaired fetal kidney blood flow.

A recent cross-sectional study, among 257 healthy children in Italy older than 6 months, indicated that childhood kidney size was inversely correlated with creatinine levels.[34] In line with that study, we observed an inverse association of fetal kidney size with creatinine and cystatin C levels in childhood, and a positive association with eGFR. Previously, we observed that small fetal kidney size tends to track throughout early childhood.[35] In the current study, we observed that the associations of fetal kidney volume with childhood kidney function outcomes were independent of childhood kidney size. These findings suggest that fetal kidney size may have permanent subclinical effects, independent of later kidney growth, on kidney function in later life. Fetal kidney size was not associated with childhood microalbuminuria or blood pressure. It might be that differences in these more clinical markers of kidney dysfunction appear at older ages.

The results from this study are important from an etiological perspective. They suggest that suboptimal abdominal blood flow and kidney growth in fetal life have persistent subclinical renal consequences. However, the observed effect estimates were small and reflect subclinical changes in kidney function in school-age children. None of the children had a known clinical kidney disease. Therefore, the results from the current study are without direct clinical consequence. Longitudinal studies reported tracking of risk factors for kidney and cardiovascular disease during childhood.[36, 37] Also, the consequences of impaired kidney growth might not yet be fully detectable in early childhood, but might become more evident in later life. It has been suggested that fetal adverse adaptations can be compensated for many years until for example hypertension occurs.[5]

The biological mechanisms underlying the observed associations may include other mechanisms than smaller kidneys with a lower number of nephrons.[4] Animal studies showed alterations in the renin angiotensin system in experimentally induced intrauterine growth restricted rats at adult age. These differences were not present at younger age.[38] Several markers of the renin angiotensin system were increased in intrauterine growth restricted subjects with hypertension.[38] An accumulating body of evidence suggests that an adverse intra-uterine environment might cause epigenetic alterations which in turn influence kidney growth and function.[4] Finally, a mismatch between fetal and postnatal growth may also lead to insufficient kidney function for an individual metabolic load. This might specifically be the case in children with fetal growth restriction and smaller fetal kidney's, but with a postnatal catch-up growth. Further studies are needed to examine the combined effects of impaired fetal kidney growth and postnatal catch-up growth on childhood kidney outcomes.

Some methodological considerations need to be discussed. The main strength of this study is the population-based prospective design from fetal life onwards. Follow-up measurements at the age of 6 years were obtained in 75% of the children. Mothers of children without follow-up measurements were more frequently multiparous, lower educated and smokers during

pregnancy. Their children had a lower birth weight. Thus, the remaining children with follow-up measurements may be healthier than the original study-population. Our results would be biased if the associations of fetal characteristics with childhood kidney outcomes would differ between those with and without follow-up measurements. This seems unlikely, but cannot be excluded. However, because of our relatively healthy population, it should be further studied whether the observed associations are generalizable to high-risk populations. We evaluated fetal blood flow and fetal kidney volume at one time point during late pregnancy. Although the intra- and interobserver variability are adequate and mean values are in line with previous studies, misclassification due to measurement error cannot be excluded. However, this would most likely have been due to random error, which reduces power of the study and may have led to an underestimation of the evaluated associations. We did not study abnormal blood flow distribution. Because our study was embedded in a low risk, healthy, population, our main focus was to study the renal consequences of variation in fetal blood flow within a normal range. Therefore, we used continuous data and tertiles of blood flow distribution. Fetal kidney volume was evaluated around 30 weeks of gestational age. Since nephrogenesis continues until 36 weeks gestational age, our measurements did not reflect final nephron number.[2] Evaluation of fetal kidney size until 36 weeks of gestational age, might have been more representative for final nephron number. When we used gestational age adjusted birth weight as a surrogate for final nephron number instead of third trimester kidney volume, we observed similar results. It is not known whether birth weight is a better proxy for fetal nephron number than fetal kidney size.[39] We used kidney size as a measure of kidney development, since nephron number cannot be studied in vivo. Kidney size is correlated with the number of glomeruli and can be used in epidemiological studies as measure of kidney development.[39] However, nephron enlargement due to hyperfiltration may attenuate the differences in childhood kidney volume and may lead to an underestimation of the associations of interest.[40] We estimated the glomerular filtration using blood creatinine levels. Blood cystatin C levels might be more accurate in estimating glomerular filtration. As compared to creatinine, cystatin C is freely filtered, produced more constantly and less dependent from children's body weight, height and sex.[41] In the current study, we observed similar results for creatinine and cystatin C levels. We used the urine albumin-creatinine ratio to evaluate albuminuria in a random urine sample.[42] Since the within subject variation in urinary albumin excretion is large, the variability would probably be lower if we collected first morning void samples instead of random during the day.[43] This was not possible in the current study. In the present study we evaluated multiple associations, this might have led to chance findings due to multiple testing. However, since the kidney related outcomes were correlated we did not adjust for multiple testing. Finally, although we had information about a large number of confounders, the influence of residual confounding should be considered, as in any observational study.

Conclusion and perspectives

In conclusion, fetal blood redistribution at expense of the intra-abdominal organs and smaller fetal kidney are associated with subclinical changes in kidney outcomes in a healthy population of school-age children. Although, these findings suggest that fetal kidney developmental adaptations affect kidney function throughout the life-course, the effect estimates were small and likely to be without clinical consequences in childhood. Further studies are needed to identify the underlying biological mechanisms and the long-term consequences of the observed associations.

References

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
2. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D. Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. Lab Invest. 1991;64(6):777-84.
3. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? Am J Hypertens. 1988;1(4 Pt 1):335-47.
4. Luyckx VA, Bertram JF, Brenner BM, Fall C, Hoy WE, Ozanne SE, et al. Effect of fetal and child health on kidney development and long-term risk of hypertension and kidney disease. Lancet. 2013.
5. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet. 2002;360(9334):659-65.
6. White SL, Perkovic V, Cass A, Chang CL, Poulter NR, Spector T, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Am J Kidney Dis. 2009;54(2):248-61.
7. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. The New England journal of medicine. 2003;348(2):101-8.
8. Hughson M, Farris AB, 3rd, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney international. 2003;63(6):2113-22.
9. Manalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney international. 2000;58(2):770-3.
10. Bassan H, Trejo LL, Kariv N, Bassan M, Berger E, Fattal A, et al. Experimental intrauterine growth retardation alters renal development. Pediatric nephrology (Berlin, Germany). 2000;15(3-4):192-5.
11. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.
12. Gaillard R, Steegers EA, Tiemeier H, Hofman A, Jaddoe VW. Placental Vascular Dysfunction, Fetal and Childhood Growth and Cardiovascular Development: The Generation R Study. Circulation. 2013.
13. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117(5):649-59.
14. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: a pulsed Doppler study. J Perinat Med. 1992;20(1):79-82.
15. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.
16. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.
17. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535-40.

18. Albaiges G, Missfelder-Lobos H, Parra M, Lees C, Cooper D, Nicolaides KH. Comparison of color Doppler uterine artery indices in a population at high risk for adverse outcome at 24 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21(2):170-3.
19. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
20. Ebbing C, Rasmussen S, Kiserud T. Middle cerebral artery blood flow velocities and pulsatility index and the cerebroplacental pulsatility ratio: longitudinal reference ranges and terms for serial measurements. Ultrasound Obstet Gynecol. 2007;30(3):287-96.
21. Verburg BO, Geelhoed JJ, Steegers EA, Hofman A, Moll HA, Witteman JC, et al. Fetal kidney volume and its association with growth and blood flow in fetal life: The Generation R Study. Kidney international. 2007;72(6):754-61.
22. Jeanty P, Dramaix-Wilmet M, Elkhazen N, Hubinont C, van Regemorter N. Measurements of fetal kidney growth on ultrasound. Radiology. 1982;144(1):159-62.
23. Geelhoed JJ, Taal HR, Steegers EA, Arends LR, Lequin M, Moll HA, et al. Kidney growth curves in healthy children from the third trimester of pregnancy until the age of two years. The Generation R Study. Pediatric nephrology (Berlin, Germany). 2010;25(2):289-98.
24. Geelhoed JJ, Kleyburg-Linkers VE, Snijders SP, Lequin M, Nauta J, Steegers EA, et al. Reliability of renal ultrasound measurements in children. Pediatric nephrology (Berlin, Germany). 2009.
25. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20(3):629-37.
26. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K, International Society for P, et al. ISPAD Clinical Practice Consensus Guidelines 2006-2007. Microvascular and macrovascular complications. Pediatr Diabetes. 2007;8(3):163-70.
27. Wong SN, Tz Sung RY, Leung LC. Validation of three oscillometric blood pressure devices against auscultatory mercury sphygmomanometer in children. Blood Press Monit. 2006;11(5):281-91.
28. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. British journal of obstetrics and gynaecology. 1992;99(4):296-301.
29. Schreuder MF, van Wijk JA, Delemarre-van de Waal HA. Intrauterine growth restriction increases blood pressure and central pulse pressure measured with telemetry in aging rats. J Hypertens. 2006;24(7):1337-43.
30. Schreuder MF, Van Wijk JA, Fodor M, Delemarre-van de Waal HA. Influence of intrauterine growth restriction on renal function in the adult rat. J Physiol Biochem. 2007;63(3):213-9.
31. Hallan S, Euser AM, Irgens LM, Finken MJ, Holmen J, Dekker FW. Effect of intrauterine growth restriction on kidney function at young adult age: the Nord Trondelag Health (HUNT 2) Study. Am J Kidney Dis. 2008;51(1):10-20.
32. Keijzer-Veen MG, Kleinveld HA, Lequin MH, Dekker FW, Nauta J, de Rijke YB, et al. Renal function and size at young adult age after intrauterine growth restriction and very premature birth. Am J Kidney Dis. 2007;50(4):542-51.
33. Moritz KM, Mazzuca MQ, Siebel AL, Mibus A, Arena D, Tare M, et al. Uteroplacental insufficiency causes a nephron deficit, modest renal insufficiency but no hypertension with ageing in female rats. J Physiol. 2009;587(Pt 11):2635-46.
34. Di Zazzo G, Stringini G, Matteucci MC, Muraca M, Malena S, Emma F. Serum creatinine levels are significantly influenced by renal size in the normal pediatric population. Clin J Am Soc Nephrol. 2011;6(1):107-13.
35. Geelhoed JJ, Verburg BO, Nauta J, Lequin M, Hofman A, Moll HA, et al. Tracking and determinants of kidney size from fetal life until the age of 2 years: the Generation R Study. Am J Kidney Dis. 2009;53(2):248-58.
36. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation. 2008;117(25):3171-80.
37. Singh A, Satchell SC. Microalbuminuria: causes and implications. Pediatr Nephrol. 2011;26(11):1957-65.
38. Grigore D, Ojeda NB, Robertson EB, Dawson AS, Huffman CA, Bourassa EA, et al. Placental insufficiency results in temporal alterations in the renin angiotensin system in male hypertensive growth restricted offspring. Am J Physiol Regul Integr Comp Physiol. 2007;293(2):R804-11.
39. Luyckx VA, Brenner BM. The clinical importance of nephron mass. J Am Soc Nephrol. 2010;21(6):898-910.
40. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. Current opinion in nephrology and hypertension. 2008;17(3):258-65.
41. Andersen TB, Eskild-Jensen A, Frokiaer J, Brochner-Mortensen J. Measuring glomerular filtration rate in children; can cystatin C replace established methods? A review. Pediatr Nephrol. 2009;24(5):929-41.

42. de Jong PE, Curhan GC. Screening, monitoring, and treatment of albuminuria: Public health perspectives. J Am Soc Nephrol. 2006;17(8):2120-6.
43. Miller WG, Bruns DE, Hortin GL, Sandberg S, Aakre KM, McQueen MJ, et al. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem. 2009;55(1):24-38.

Supplementary Material

Imputation procedure

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

TABLE S3.4.1 | Subject characteristics of participants with and without follow-up data (N = 1,201).

	Childhood data available (n=923)	Childhood data unavailable (n=278)
Maternal characteristics		
Age, y	32.2 (23.4 – 39.4)	31.1 (19.2 – 38.2)[a]
Height, cm	171 (6.3)	170 (6.4)
Pre-pregnancy weight, kg	69.3 (13.0)	67.1 (11.3)
Pre-pregnancy body mass index, kg/m²	23.6 (4.2)	23.1 (3.7)
Parity ≥1, %	37.7 (348)	44.2 (123)[a]
Missing	0.2 (2)	0.7 (2)
Educational level, %		
Primary/secondary	33.7 (311)	45.3 (126)[b]
Secondary or higher	65.3 (603)	51.1 (142)
Missing	1 (9)	3.6 (10)
Smoking during pregnancy, %		
Yes	19.4 (179)	35.3 (89)[b]
No	70.7 (653)	58.2 (171)
Missing	9.9 (91)	6.5 (18)
Folic acid, %		
Yes	76.6 (707)	69.4 (193)[b]
No	6.5 (60)	11.9 (33)
Missing	16.9 (156)	18.7 (52)
Pregnancy induced hypertension, %		
Yes	4.9 (45)	4.0 (11)
No	89.7 (828)	89.5 (249)
Missing	5.4 (50)	6.5 (18)
Preeclampsia, %		
Yes	2.5 (23)	1.8 (5)
No	89.7 (828)	89.5 (249)
Missing	7.8 (72)	8.7 (24)
Gestational age at measurement, wk	30.3 (28.5 – 32.7)	30.4 (28.1 – 32.5)
Estimated fetal weight, g	1634 (263)	1616 (285)

	Childhood data available (n=923)	Childhood data unavailable (n=278)
Blood flow distribution		
Umbilical artery PI	0.97 (0.16)	0.99 (0.18)
Middle cerebral artery PI	1.97 (0.33)	1.99 (0.33)
U/C ratio, middle cerebral artery	0.50 (0.11)	0.51 (0.12)
Fetal kidney biometrics		
Right kidney volume, cm^3	10.64 (3.07)	10.84 (3.13)
Left kidney volume, cm^3	9.94 (2.76)	10.01 (2.86)
Combined kidney volume, cm^3	20.56 (5.48)	20.85 (5.61)
Birth and infant characteristics		
Gestational age at birth, wk	40.3 (36.4 – 42.4)	40.0 (34.7 – 42.4)[b]
Birth weight, g	3534 (509)	3446 (628)[a]
Sex boys, %	50.3 (464)	57.6 (160)[a]
Childhood characteristics		
Age at follow up, y	5.9 (5.7 – 6.6)	NA
Height, cm	119 (5.2)	NA
Weight, kg	22.6 (3.2)	NA
Body mass index, kg/m^2	15.9 (1.4)	NA
Body surface area, m^2	0.86 (0.07)	NA
Kidney volume left, cm^3	61.4 (12.6)	NA
Kidney volume right, cm^3	59.5 (11.7)	NA
Kidney volume combined, cm^3	120.9 (22.1)	NA
Creatinine, µmol/l	36.8 (4.9)	NA
Cystatin C, µg/l	790 (74)	NA
Estimated glomerular filtration rate ml/min per $1.73m^2$	120.2 (15.8)	NA
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)	NA
Micro albuminuria[c], %	7.1 (62)	NA
Systolic blood pressure, mmHg	102.2 (7.7)	NA
Diastolic blood pressure, mmHg	60.1 (6.3)	NA

Values are means (SD), medians (95% range), or % (numbers). Participants were compared using independent samples t-test for continuous variables and chi-square test for categorical variables. [a]p<0.05, [b]p<0.01, [c]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

TABLE S3.4.2 | Subject characteristics of observed and imputed data (N = 923).

	Observed data	Imputed data
Maternal characteristics		
Age, y	32.2 (23.4 – 39.4)	32.2 (23.4 – 39.4)
Height, cm	171 (6.3)	171 (6.3)
Pre-pregnancy weight, kg	69.3 (13.0)	69.0 (13.0)
Pre-pregnancy body mass index, kg/m^2	23.6 (4.2)	23.6 (4.2)
Parity ≥1, %	37.7 (348)	37.7 (348)
Missing	0.2 (2)	
Educational level, %		
Primary/secondary	33.7 (311)	34.1(315)
Secondary or higher	65.3 (603)	65.9 (608)
Missing	1 (9)	
Smoking during pregnancy, %		
Yes	19.4 (179)	21.5 (198)
No	70.7 (653)	78.5 (745)
Missing	9.9 (91)	

	Observed data	Imputed data
Folic acid, (%)		
Yes	76.6 (707)	90.9 (839)
No	6.5 (60)	9.1 (84)
Missing	16.9 (156)	
Pregnancy induced hypertension, %		
Yes	4.9 (45)	5.6 (52)
No	89.7 (828)	94.4 (871)
Missing	5.4 (50)	
Preeclampsia, %		
Yes	2.5 (23)	3.1 (29)
No	89.7 (828)	96.9 (894)
Missing	7.8 (72)	
Fetal characteristics		
General		
Gestational age at measurement, wk	30.3 (28.5 – 32.7)	30.3 (28.5 – 32.7)
Estimated fetal weight, g	1634 (263)	1634 (263)
Blood flow distribution		
Umbilical artery PI	0.97 (0.16)	Not imputed
Middle cerebral artery PI	1.97 (0.33)	Not imputed
U/C ratio, middle cerebral artery	0.50 (0.11)	Not imputed
Fetal kidney biometrics		
Right kidney volume, cm^3	10.64 (3.07)	Not imputed
Left kidney volume, cm^3	9.94 (2.76)	Not imputed
Combined kidney volume, cm^3	20.56 (5.48)	Not imputed
Birth and infant characteristics		
Gestational age at birth, wk	40.3 (36.4 – 42.4)	40.3 (36.4 – 42.4)
Birth weight, g	3534 (509)	3534 (509)
Sex boys, %	50.3 (464)	50.3 (464)
Childhood characteristics		
Age at follow up, y	5.9 (5.7 – 6.6)	5.9 (5.7 – 6.6)
Height, cm	119 (5.2)	119 (5.2)
Weight, kg	22.6 (3.2)	22.6 (3.2)
Body mass index, kg/m^2	15.9 (1.4)	15.9 (1.4)
Body surface area, m^2	0.86 (0.07)	0.86 (0.07)
Kidney volume left, cm^3	61.4 (12.6)	Not imputed
Kidney volume right, cm^3	59.5 (11.7)	Not imputed
Kidney volume combined, cm^3	120.9 (22.1)	Not imputed
Creatinine, µmol/l	36.8 (4.9)	Not imputed
Cystatin C, µg/l	790 (74)	Not imputed
Estimated glomerular filtration rate ml/min per $1.73m^2$	120.2 (15.8)	Not imputed
Estimated glomerular filtration rate/cm^3 kidney volume	1.02 (0.19)	Not imputed
Micro albuminuria[a], %	7.1 (62)	Not imputed
Systolic blood pressure, mmHg	102.2 (7.7)	Not imputed
Diastolic blood pressure, mmHg	60.1 (6.3)	Not imputed

Values are means (SD), medians (95% range), or % (numbers) [a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls). Missing values for continuous maternal characteristics are: age (n=0), height (n=0), pre-pregnancy weight (n=138), pre-pregnancy body mass index (n=138), for fetal characteristics: gestational age at measurement (n=0), estimated fetal weight (n=6), for infant characteristics: gestational age at birth (n=0), birth weight (n=0), for childhood characteristics: age (n=0), height (n=1), weight (n=1), body mass index (n=1), body surface area (n=1).

TABLE S3.4.3 | Subject characteristics in tertiles of U/C ratio (N=879).

	High tertile U/C ratio (N = 297)	Middle tertile U/C ratio (N = 285)	Low tertile U/C ratio (N = 297)	P-value for trend
Maternal characteristics				
Age, y	31.8 (24.1, 39.5)	32.3 (23.6, 40.6)	32.2 (21.9, 39.0)	P=0.43
Height, cm	170.9 (6.2)	171.4 (6.3)	170.8 (6.1)	P=0.53
Pre-pregnancy weight, kg	68.0 (12.7)	69.5 (13.6)	69.6 (13.3)	P=0.39
Pre-pregnancy body mass index, kg/m^2	23.4 (4.1)	23.6 (4.2)	23.9 (4.4)	P=0.35
Parity ≥1, %	32.0 (95)	40.7 (116)	41.8 (124)	P<0.05
Educational level, %				
Primary/secondary	28.6 (85)	36.1 (103)	35.7 (106)	P=0.12
Secondary or higher	71.4 (212)	63.9 (182)	64.6 (191)	
Smoking during pregnancy, %				
Yes	19.9 (59)	21.8 (62)	22.2 (66)	P=0.74
No	80.1 (238)	78.2 (223)	77.8 (231)	
Folic acid supplement use, %				
Yes	89.9 (267)	93.3 (266)	87.9 (261)	P=0.10
No	10.1 (30)	6.7 (19)	12.1 (36)	
Pregnancy induced hypertension, %				
Yes	6.1 (18)	5.6 (16)	4.7 (14)	P=0.74
No	93.9 (279)	94.4 (269)	96.3 (283)	
Preeclampsia, %				
Yes	3.4 (10)	3.2 (9)	2.2 (6)	P=0.69
No	96.6 (287)	96.8 (276)	97.8 (291)	
Fetal characteristics				
Gestational age at measurement, wk	30.2 (28.5, 32.5)	30.4 (28.6, 32.5)	30.4 (28.4, 33.1)	P<0.05
Estimated fetal weight, g	1580 (237)	1647 (259)	1675 (279)	P<0.01
Umbilical artery PI	1.08 (0.16)	0.97 (0.12)	0.86 (0.12)	P<0.01
Middle cerebral artery PI	1.73 (0.28)	1.98 (0.26)	2.21 (0.28)	P<0.01
U/C ratio middle cerebral artery	0.63 (0.08)	0.49 (0.03)	0.39 (0.04)	P<0.01
Right kidney volume, cm^3	10.3 (3.1)	10.7 (2.9)	11.0 (3.2)	P<0.05
Left kidney volume, cm^3	9.5 (2.7)	10.1 (2.7)	10.4 (2.8)	P<0.01
Combined kidney volume, cm^3	19.8 (5.5)	20.8 (5.2)	21.3 (5.7)	P<0.01
Birth and infant characteristics				
Gestational age at birth, wk	40.4 (35.6, 42.6)	40.3 (37.0, 42.6)	40.3 (36.9, 42.1)	P=0.36
Birth weight, g	3450 (506)	3568 (510)	3601 (474)	P<0.01
Sex boys, %	47.1 (140)	52.3 (149)	52.5 (156)	P=0.34
Childhood characteristics				
Height, cm	118.3 (5.2)	119.4 (5.1)	119.4 (5.1)	P<0.05
Weight, kg	22.1 (3.1)	22.9 (3.6)	22.8 (3.0)	P<0.01
Age at follow up, y	5.9 (5.6, 6.8)	5.9 (5.7, 6.5)	5.9 (5.7, 6.8)	P=0.69
Body mass index, kg/m^2	15.8 (1.37)	16.0 (1.62)	15.9 (1.28)	P=0.12
Body surface area, m^2	0.85 (0.07)	0.87 (0.08)	0.87 (0.07)	P<0.01
Kidney volume left, cm^3	59.2 (11.5)	61.7 (12.7)	63.4 (13.2)	P<0.01
Kidney volume right, cm^3	56.8 (11.4)	60.5 (12.0)	61.3 (11.5)	P<0.01
Kidney volume combined, cm^3	115.9 (20.8)	122.1 (22.5)	124.8 (22.6)	P<0.01
Creatinine, µmol/l	36.8 (5.2)	36.8 (4.6)	37.0 (5.0)	P=0.91
Cystatin C, µg/l	793 (77)	794 (77)	784 (70)	P=0.32
Estimated glomerular filtration rate ml/min per 1.73m^2	119.4 (15.5)	120.4 (15.2)	120.4 (16.8)	P=0.75
Estimated glomerular filtration rate/cm^3 kidney volume	1.06 (0.19)	1.01 (0.18)	0.97 (0.17)	P<0.01

	High tertile U/C ratio (N = 297)	Middle tertile U/C ratio (N = 285)	Low tertile U/C ratio (N = 297)	P-value for trend
Microalbuminuria[a], %	4.0 (12)	9.1 (26)	7.1 (21)	P=0.05
Systolic blood pressure, mmHg	101.9 (8.5)	102.7 (7.4)	101.8 (7.1)	P=0.28
Diastolic blood pressure, mmHg	59.8 (6.9)	60.3 (5.7)	60.0 (6.3)	P=0.64

Means were compared using ANOVA for continuous variables and chi-square test for categorical variables. [a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

TABLE S3.4.4 | Subject characteristics in tertiles of fetal kidney volume (N=870).

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

	Low tertile kidney volume (N = 302)	Middle tertile kidney volume (N = 292)	High tertile kidney volume (N = 276)	P-value for trend
Childhood characteristics				
Age at follow up, y	5.9 (5.7, 6.7)	5.9 (5.7, 6.6)	6.0 (5.7, 6.7)	P=0.19
Height, cm	118.3 (5.4)	118.7 (5.1)	120.2 (4.9)	P<0.01
Weight, kg	22.2 (3.4)	22.3 (2.9)	23.3 (3.4)	P<0.01
Body mass index, kg/m^2	15.8 (1.5)	15.8 (1.2)	16.1 (1.5)	P<0.05
Body surface area, m^2	0.85 (0.08)	0.86 (0.07)	0.88 (0.07)	P<0.01
Kidney volume left, cm^3	58.9 (11.9)	61.4 (12.6)	63.9 (12.6)	P<0.01
Kidney volume right, cm^3	56.5 (11.1)	59.3 (11.2)	62.8 (12.1)	P<0.01
Kidney volume combined, cm^3	115.4 (21.0)	120.7 (21.6)	126.7 (22.4)	P<0.01
Creatinine, µmol/l	37.8 (4.7)	36.4 (4.8)	36.1 (5.1)	P<0.01
Cystatin C, µg/l	812 (74)	780 (75)	776 (71)	P<0.01
Estimated glomerular filtration rate ml/min per 1.73m^2	116.0 (14.0)	121.3 (15.2)	124.0 (17.1)	P<0.01
Estimated glomerular filtration rate/cm^3 kidney volume	1.04 (0.01)	1.02 (0.01)	1.00 (0.01)	P=0.16
Microalbuminuria[a], %	6.6 (20)	5.1 (15)	8.3 (23)	P=0.62
Systolic blood pressure, mmHg	102.2 (8.2)	101.9 (7.7)	102.4 (7.2)	P=0.88
Diastolic blood pressure, mmHg	60.3 (6.2)	60.2 (6.5)	59.8 (6.0)	P=0.70

Means were compared using ANOVA for continuous variables and chi-square test for categorical variables.
[a]Defined as levels between 2.5-25.0 mg/mmol (boys) and 3.5-25.0 mg/mmol (girls)

TABLE S3.4.5 | Associations of third trimester umbilical/cerebral artery resistance ratio and covariates with kidney outcomes at the age of 6 years (N = 879).

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

	Kidney size		Kidney function			Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
+ preeclampsia	-3.86 (-5.43, 2.30)[b]	-0.04 (-0.45, 0.38)	4 (-3, 10)	-0.83 (-1.93, 0.75)	0.75 (0.56, 1.01)	0.14 (-0.40, 0.68)	0.06 (-0.39, 0.50)
+ gestational age at measurement	-3.86 (-5.43, -2.30)[b]	0.00 (-0.42, 0.42)	4 (-3, 10)	-0.71 (-2.05, 0.63)	0.74 (0.55, 1.00)	0.17 (-0.37, 0.71)	0.05 (-0.39, 0.50)
+ third trimester fetal weight	-3.52 (-5.08, -1.97)[b]	0.01 (-0.40, 0.43)	3 (-3, 10)	-0.63 (-1.97, 0.72)	0.75 (0.56, 1.01)	0.19 (-0.09, 0.47)	0.03 (-0.39, 0.46)
+ gestational age at birth	-3.89 (-5.45, -2.33)[b]	-0.03 (-0.45, 0.39)	4 (-2, 11)	-0.62 (-1.95, 0.72)	0.75 (0.55, 1.00)	0.15 (-0.39, 0.69)	0.06 (-0.39, 0.50)
+ SDS birth weight	-3.34 (-4.90, -1.77)[b]	0.05 (-0.36, 0.47)	4 (-3, 10)	-0.68 (-2.03, 0.67)	0.74 (0.55, 0.99)	0.13 (-0.42, 0.68)	0.01 (-0.44, 0.46)
+ child sex	-3.80 (-5.35, -2.25)[b]	-0.04 (-0.46, 0.38)	4 (-2, 10)	-0.58 (-1.91, 0.75)	0.75 (0.56, 1.01)	0.12 (-0.41, 0.66)	0.04 (-0.40, 0.48)
+ age at 6	-3.71 (-5.25, -2.17)[b]	0.00 (-0.41, 0.41)	4 (-2, 11)	-0.62 (-1.95, 0.71)	0.75 (0.55, 1,00)	0.17 (-0.37, 0.70)	0.07 (-0.38, 0.51)
+ child body surface area	-2.58 (-3.99, -1.16)[b]	0.12 (-0.29, 0.53)	4 (-2, 11)	-0.41 (-1.75, 0.94)	0.74 (0.56, 0.98)[a]	0.30 (-0.23, 0.83)	0.10 (-0.35, 0.54)

eGFR, estimated glomerular filtration rate; BP, blood pressure

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

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

TABLE S3.4.6 | Associations of third trimester fetal kidney size and covariates with kidney outcomes at the age of 6 years (N = 870).

	Kidney size		Kidney function			Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per 1.73m^2)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
Fetal kidney volume (SD = 5.48)	5.22 (3.71, 6.73)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.24, 4.68)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.44)	-0.25 (-0.67, 0.18)
+ maternal age	5.21 (3.70, 6.72)[b]	-0.70 (-1.08, -0.31)[b]	-16 (-22, -10)[b]	3.44 (2.21, 4.66)[b]	1.07 (0.82, 1.39)	-0.08 (-0.61, 0.45)	-0.23 (-0.66, 0.20)
+ pre-pregnancy body mass index	5.22 (3.71, 6.73)[b]	-0.72 (-1.10, -0.33)[b]	-16 (-22, -10)[b]	3.48 (2.26, 4.71)[b]	1.08 (0.83, 1.40)	-0.14 (-0.67, 0.39)	-0.27 (-0.70, 0.16)
+ parity	5.23 (4.46, 6.00)[b]	-0.71 (-0.90, -0.51)[b]	-16 (-19, -13)[b]	3.46 (2.84, 4.08)[b]	1.08 (0.94, 1.23)	-0.09 (-0.36, 0.18)	-0.25 (-0.47, 0.03)

	Kidney size		Kidney function			Blood pressure	
	Combined kidney volume difference (95%CI) (cm^3)	Creatinine difference (95%CI) (µmol/l)	Cystatin C difference (95%CI) (µg/l)	eGFR difference (95%CI) (ml/min per $1.73m^2$)	Microalbuminuria Odds ratio (95%CI)	Systolic BP difference (95%CI) (mmHg)	Diastolic BP difference (95%CI) (mmHg)
+ maternal educational level	5.20 (3.73, 6.70)[b]	-0.70 (-1.33, -0.14)[b]	-16 (-19, -13)[b]	3.42 (2.21, 4.63)[b]	1.07 (0.83, 1.39)	-0.08 (-0.61, 0.45)	-0.24 (-0.66, 0.19)
+ maternal smoking	5.22 (3.73, 6.71)[b]	-0.71 (-1.09, -0.33)[b]	-16 (-22, -10)[b]	3.45 (2.23, 4.68)[b]	1.08 (0.83, 1.40)	-0.08 (-0.62, 0.45)	-0.24 (-0.67, 0.19)
+ maternal folic acid use	5.24 (3.74, 6.74)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.44 (2.22, 4.67)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.45)	-0.24 (-0.67, -0.19)
+ gestational hypertension	5.22 (3.57, 5.87)[b]	-0.71 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.24, 4.68)[b]	1.08 (0.84, 1.39)	-0.09 (-0.62, 0.44)	-0.25 (-0.46, -0.03)
+ preeclampsia	5.21 (3.70, 6.71)[b]	-0.74 (-1.09, -0.32)[b]	-16 (-22, -10)[b]	3.46 (2.29, 4.63)[b]	1.08 (0.83, 1.40)	-0.09 (-0.62, 0.44)	-0.25 (-0.46, -0.03)
+ gestational age at measurement	5.85 (4.26, 7.44)[b]	-0.91 (-1.32, -0.50)[b]	-17 (-23, -11)[b]	4.22 (2.93, 5.51)[b]	1.08 (0.82, 1.43)	-0.18 (-0.74, 0.39)	-0.27 (-0.73, 0.18)
+ third trimester fetal weight	4.96 (3.29, 6.63)[b]	-1.02 (-1.44, -0.59)[b]	-17 (-23, -11)[b]	4.21 (2.85, 5.56)[b]	1.04 (0.90, 1.21)	-0.25 (-0.84, 0.35)	-0.26 (-0.72, 0.19)
+ gestational age at birth	5.22 (3.71, 6.73)[b]	-0.70 (-1.09, -0.31)[b]	-16 (-22, -10)[b]	3.43 (2.20, 4.65)[b]	1.08 (0.83, 1.40)	-0.08 (-0.62, 0.45)	-0.25 (-0.68, 0.18)
+ SDS birth weight	4.41 (2.86, 5.96)[b]	-0.87 (-1.27, -0.48)[b]	-16 (-22, -10)[b]	3.71 (2.45, 4.96)[b]	1.09 (0.83, 1.43)	-0.08 (-0.63, 0.48)	-0.24 (-0.63, 0.27)
+ child sex	-3.22 (-6.21, -0.24)[a]	-0.71 (-1.10, -0.33)[b]	-16 (-22, -11)[b]	3.47 (2.24, 4.69)[b]	1.07 (0.82, 1.39)	-0.02 (-0.55, 0.51)	-0.19 (-0.62, 0.24)
+ age at 6	4.97 (3.47, 6.48)[b]	-0.82 (-1.19, -0.44)[b]	-16 (-22, -11)[b]	3.61 (2.39, 4.84)[b]	1.09 (0.84, 1.41)	-0.14 (-0.67, 0.40)	-0.26 (-0.69, 0.17)
+ child body surface area	3.52 (2.14, 4.89)[b]	-0.89 (-1.27, -0.51)[b]	-17 (-23, -11)[b]	3.33 (2.10, 4.57)[b]	1.09 (0.84, 1.42)	-0.34 (-0.87, 0.20)	-0.30 (-0.73, 0.14)

eGFR, estimated glomerular filtration rate; BP, blood pressure

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and Odds ratio's (95% CI) for microalbuminuria based on logistic regression models and reflect the difference for each outcome for fetal blood flow characteristics. [a]p<0.05, [b]p<0.01

TABLE S3.4.7 | Associations of third trimester umbilical/cerebral artery resistance ratio with childhood kidney volume at the age of 6 years (N = 834).

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

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the difference for kidney volume for fetal blood flow characteristics. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight. [a]p<0.01

TABLE S3.4.8 | Associations of third trimester fetal kidney volume with childhood kidney volume at the age of 6 years (N = 799).

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

Values are regression coefficients (95% Confidence interval (CI)) based on multiple regression models and reflect the difference for kidney volume for fetal kidney volume. Basic model is adjusted for gestational age at third trimester measurement, third trimester estimated fetal weight, child sex, current age and body surface area. Confounder model is additionally adjusted for maternal age, parity, educational level, pre-pregnancy body mass index, smoking status during pregnancy, maternal pregnancy complications (hypertension, preeclampsia), folic acid use during pregnancy, gestational age and gestational age adjusted birth weight. [a]p<0.01

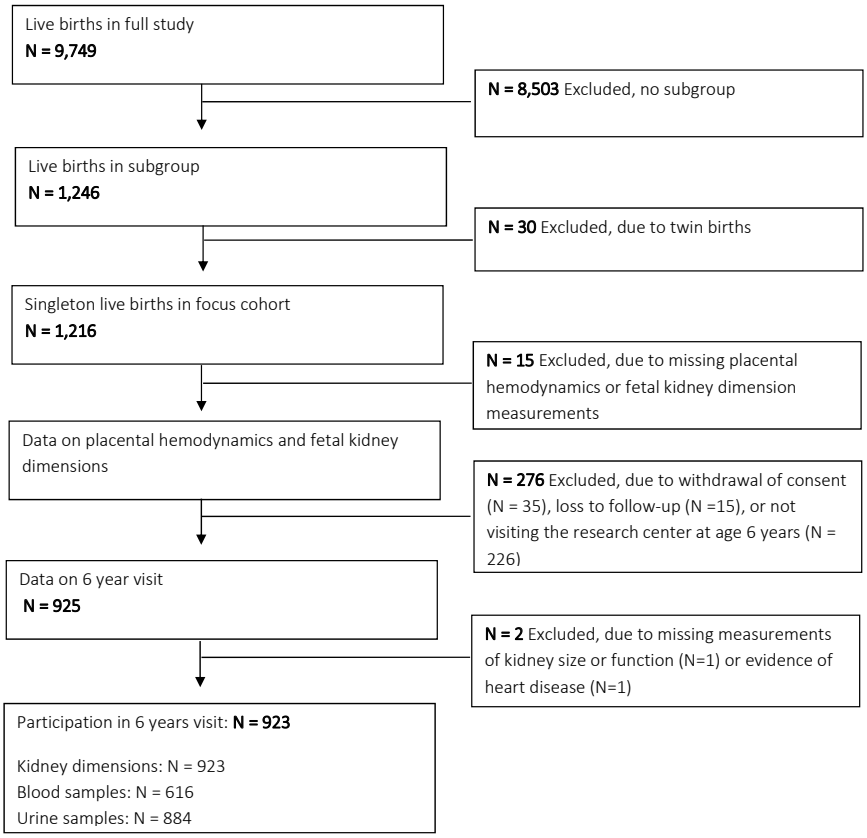

FIGURE S3.4.1 | Flow chart of participants included in the analysis.

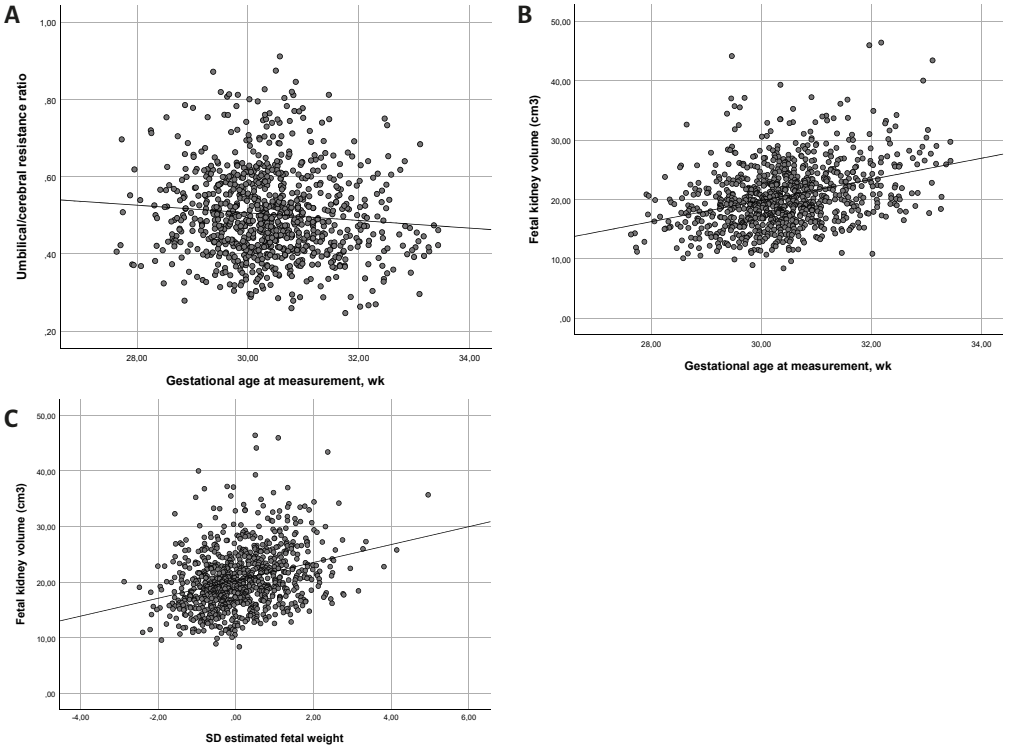

FIGURE S3.4.2 | Scatterplots: gestational age, estimated fetal weight and fetal kidney outcomes (N=879).

3.4

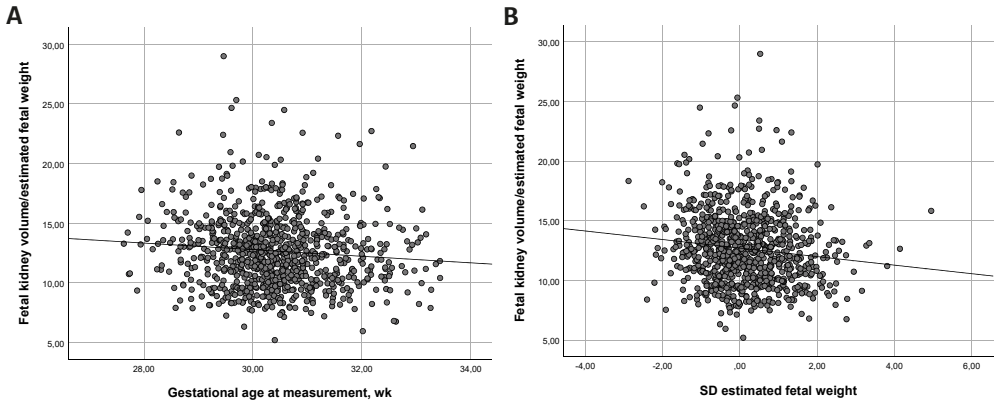

FIGURE S3.4.3 | Scatterplots: gestational age, estimated fetal weight and fetal kidney volume/estimated fetal weight (N=870).

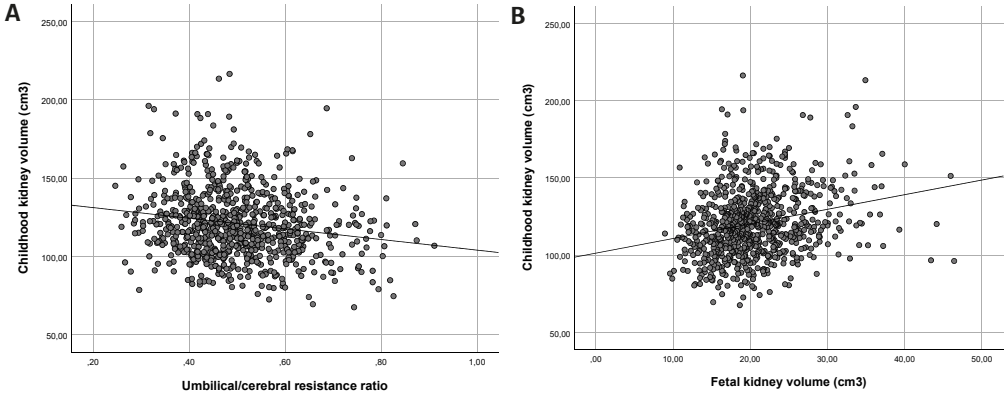

FIGURE S3.4.4 | Scatterplots: third trimester umbilical/cerebral resistance ratio, fetal kidney volume and childhood kidney volume (n=923).

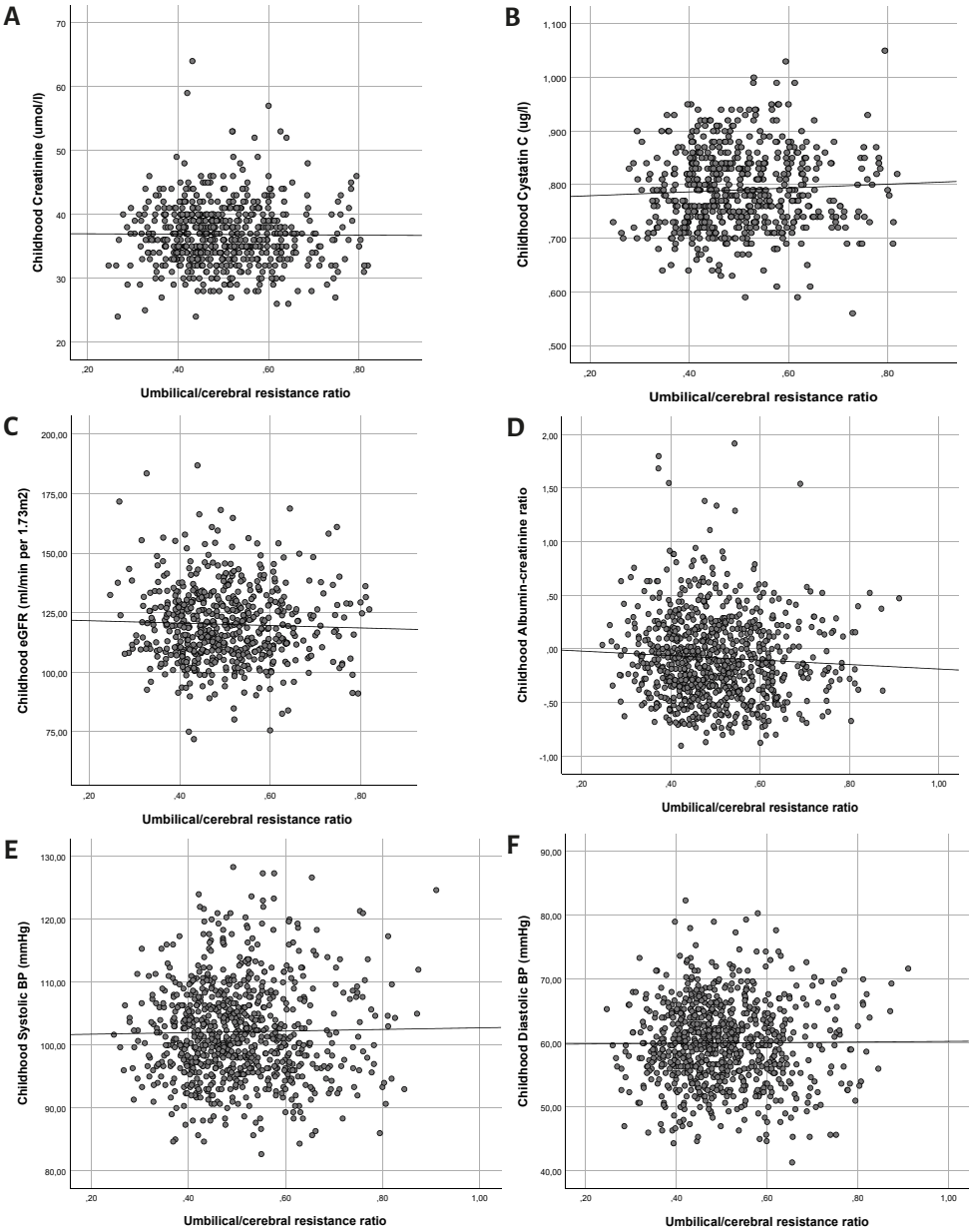

FIGURE S3.4.5 | Scatterplots: third trimester umbilical/cerebral resistance ratio and childhood kidney outcomes (n=879).

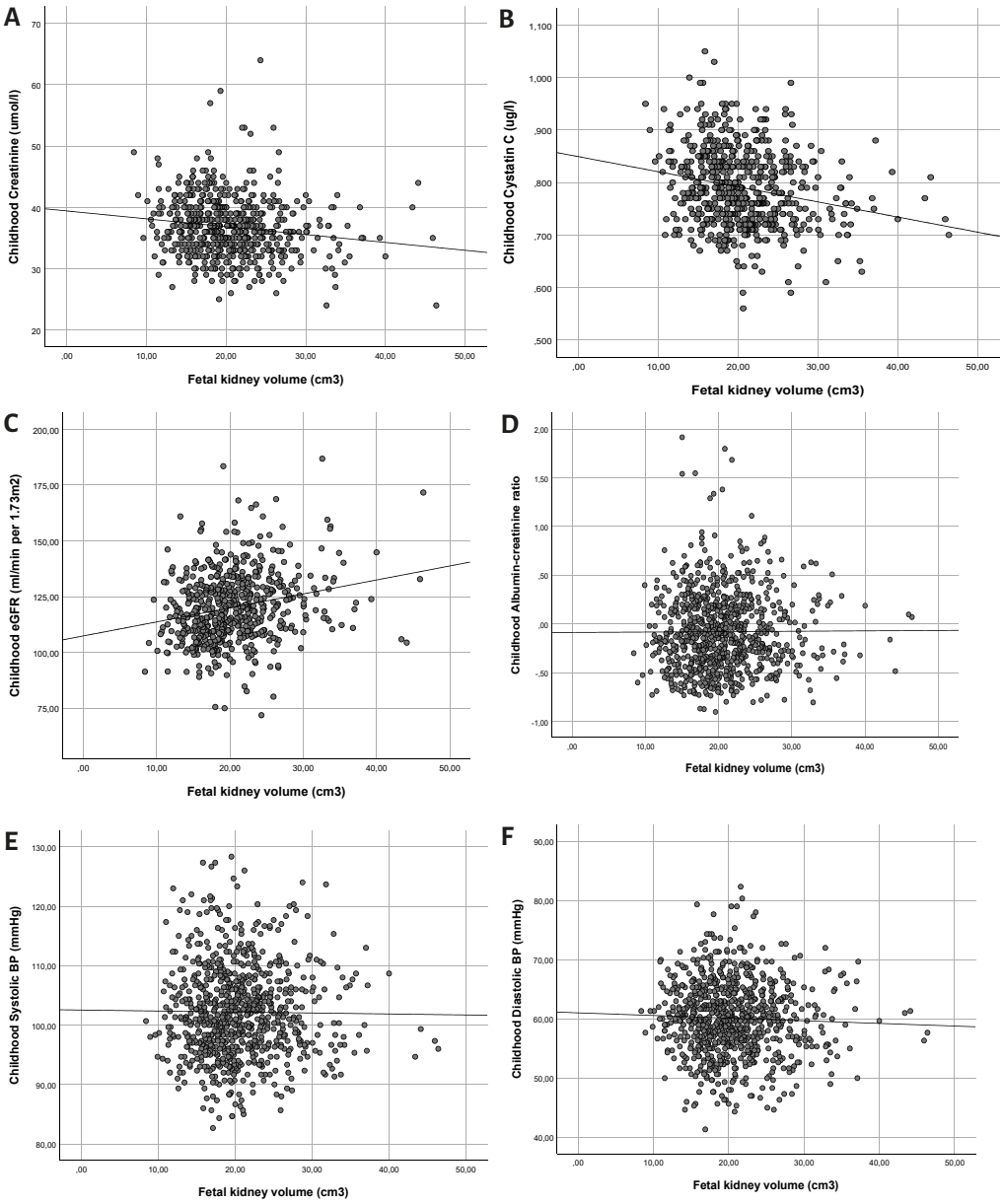

FIGURE S3.4.6 | Scatterplots: fetal kidney volume and childhood kidney outcomes (N=870).

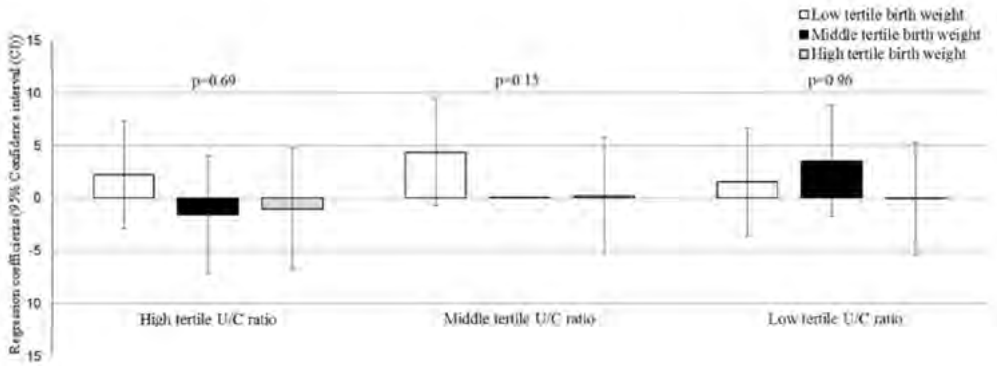

FIGURE S3.4.7 | Associations of gestationa.l age adjusted birth weight with childhood estimated glomerular filtration rate (N = 613).

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

Chapter 4

Discussion

Chapter 4

Chapter 4.1

General discussion: Maternal and offspring health consequences of placental and fetal hemodynamic alterations throughout pregnancy

Kooijman MN, Gaillard R

Partly adapted from narrative review, submitted

Introduction

The placenta is the interface between the maternal and fetal blood circulation, and responsible for the maternal to fetal transfer of essential nutrients for fetal growth and development.[1] Accumulating evidence suggests that an impaired placental development in early-pregnancy affects subsequent placental and fetal vascular formation and blood flow patterns.[2] During pregnancy, already from 13 weeks of gestation onwards, placental and fetal blood flow can be assessed by Doppler ultrasound, which provides detailed information of feto-placental hemodynamic function and its adaptations during pregnancy. With the continuing advancements in ultrasound techniques, feto-placental hemodynamic measurements are increasingly used in clinical practice and research settings to identify fetuses at increased risk of adverse birth and long-term adverse health outcomes.

In this chapter, we discuss novel insights of the influence of maternal factors on feto-placental hemodynamic measures and the associations of feto-placental hemodynamic measures with pregnancy complications and long-term adverse health outcomes in the offspring. Finally, we discuss potential underlying mechanisms and challenges for future research.

Placenta and fetal hemodynamics

Early in pregnancy, placental tissue develops by trophoblast shell plugs invasion of the utero-placental vessels.[3] After 10 weeks of gestation, these plugs are regressed and blood flows into the intervillous space to exchange oxygen and nutrient with the developing fetus.[4] From the uterine arteries maternal blood enters the intervillious space in the placenta through the spiral arteries. Blood flows from the umbilical cord blood through the ductus venosus, which shunts the blood to the inferior vena cava. From the vena cava blood travels to the right atrium of the heart. In the fetus most of the blood flows through the foramen oval directly into the left atrium from the right atrium, thus bypassing pulmonary circulation. The continuation of this blood flow is into the left ventricle, and from there it is pumped through the aorta into the body. Some of the blood moves from the aorta through the internal iliac arteries to the umbilical arteries, and re-enters the placenta, where carbon dioxide and other waste products from the fetus are taken up and enter the maternal circulation.[5] **Figure 4.1.1** shows the placental and fetal circulation.

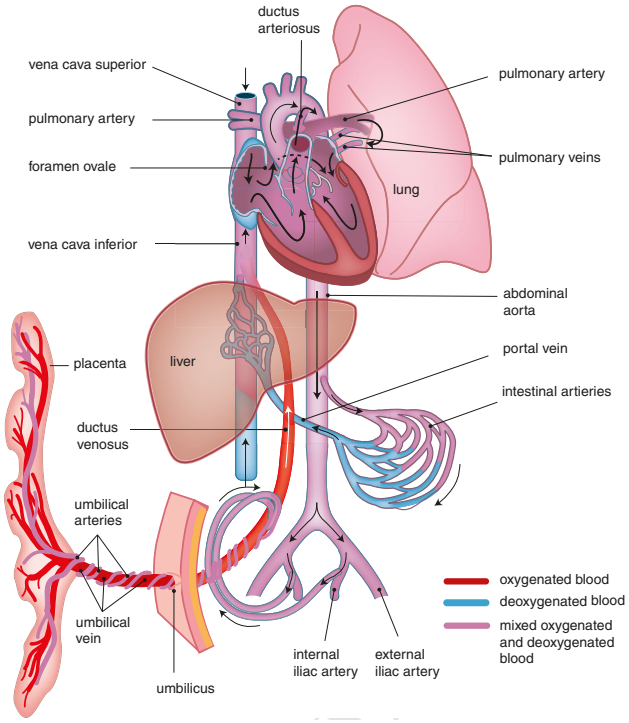

FIGURE 4.1.1 | Placental and fetal circulation[6] (*Printed with approval*).

Doppler ultrasound is used for non-invasive assessment of the utero- and feto-placental circulation, which can be performed from 13 weeks onwards. An overview of the measurements which can be performed of the placenta and fetal cerebral, cardiac, pulmonary and body hemodynamics are described in more detail in **Table 4.1.1**. The formula's which can be used to quantify placenta and fetal hemodynamic are described in **textbox 4.1.1**. The most commonly used Doppler ultrasound examinations measures are the vascular resistance indexes of placental and fetal cerebral blood flow. A higher pulsatility index (PI) and resistance index (RI) are indications of an increased vascular resistance and a decrease in blood flow.[7,8] The PI of the fetal cerebral arteries provide insight into fetal cerebral blood flow patterns. Reductions in middle cerebral artery PI are valid indicators of the brain-sparing effect and fetal blood flow redistribution.[9,10] Fetal blood flow redistribution in favor to the brain at expense of the trunk is indicated by an increased ratio between the umbilical (U) artery PI and the cerebral (C) artery PI.[11]

TABLE 4.1.1 | Placental and fetal hemodynamic measures.

		Hemodynamic alterations in complicated pregnancies	
Placental hemodynamics			
Uterine artery RI	Utero-placental vascular resistance, parameter primarily of the maternal circulation	↑	A higher uterine artery RI is an indication of an increased vascular resistance and a decrease in blood flow [7, 8]
Umbilical artery PI	Feto-placental vascular resistance, parameter primarily of the fetal circulation	↑	A higher umbilical artery PI is an indication of an increased vascular resistance and a decrease in blood flow [7, 8]
Umbilical vein volume flow	Volume of fluid that passes a given cross-sectional area per time unit	↓	Reduced flow indicator of a suboptimal fetal environment
Fetal cerebral hemodynamics			
Middle cerebral artery PI	Fetal cerebral blood flow	↓	Reductions in middle cerebral artery PI are valid indicators of the brain-sparing effect and fetal blood flow redistribution [9, 10]
U/(middle)C artery PI ratio	Fetal blood flow redistribution	↑	Fetal blood flow redistribution in favor to the brain at expense of the trunk is indicated by an increased ratio between the umbilical artery PI and the cerebral artery PI [11]
Fetal cardiac hemodynamics			
Aorta ascendens diameter	Inner diameter of the aorta	↓	A smaller diameter in fetuses with abnormal placental or fetal hemodynamics was seen
Aorta ascendens PSV	Cardiac function and vascular compliance of the aorta	↓	A low PSV can be a sign of reduced cardiac function, raised afterload or decreased vascular compliance [12, 13]
Aorta ascendens TVI	Cardiac function and vascular compliance of the aorta	↓	A low TVI can be a sign of reduced cardiac function, raised afterload or decreased vascular compliance [12, 13]
Left cardiac output		↓	A reduced cardiac output is a sign of deterioration of cardiac function [14]
Mitral valve E wave	Cardiac flow-velocity waveforms at the level of the mitral valves. Measure of early (E) passive ventricular filling	↓↑	
Mitral valve A wave	Cardiac flow-velocity waveforms at the level of the mitral valves. Measure of active (A) filling during atrial contraction	↓↑	
Mitral valve E/A ratio	Index for ventricular diastolic function and expresses both cardiac compliance and preload conditions	↓↑	Reductions and increases in E/A wave has been shown in complicated pregnancies [15]

			Hemodynamic alterations in complicated pregnancies
Fetal pulmonary hemodynamics			
Pulmonary artery TVI	Pulmonary vascular resistance	↑	A higher pulmonary artery TVI indicated higher pulmonary vascular resistance, this might also be a sign of underdevelopment of the fetal airways, such as fewer but larger alveoli and impaired growth of the airways and lungs [16,17]
Fetal body hemodynamics			
Ductus venosus PI	Flow assessment at the level of the ductus venosus	↑	A raised PI can be a sign of suboptimal arterial contraction an preload conditions [18]

RI = resistance index, PI = pulsatiliy index, U/C = umbilical/cerebral, PSV = peak systolic velocity, TVI = time-velocity integral

TEXTBOX 4.1.1 | Formula's to quantify placenta and fetal hemodynamics.

$$\text{Resistance Index (RI)} = \frac{\text{Peak systolic velocity}}{\text{Peak systolic velocity+Lowest diastolic velocity}}$$

$$\text{Pulsatiliy index (PI)} = \frac{\text{Peak systolic velocity-lowest diastolic velocity}}{\text{Mean peak systolic velocity}}$$

$$\text{Umbilical (U) /(middle) Cerebral (C) artery PI ratio} = \frac{\text{PI umbilical artery}}{\text{PI middle cerebral artery}}$$

$$\text{Time-velocity integral (TVI)} = \int \text{of each point under the curve with time}$$

$$\text{Left cardiac output} = \text{Vessel area} * \text{TVI} * \text{Fetal heart rate}$$

$$\text{Early (E) passive ventricular filling/Active (A) filling ratio} = \frac{\text{E wave}}{\text{A wave}}$$

Maternal pre-existing conditions and metabolic concentrations

Accumulating evidence suggests that both placental and fetal hemodynamics are influenced by a variety of maternal determinants, including maternal pre-existing conditions, metabolic concentrations and lifestyle factors during pregnancy. Most studies have focused on the influence of these maternal characteristics on birth weight or placental weight, as proxy measures of feto-placental vascular function during pregnancy.[19] Only a few studies are focused on the influence of these maternal factors on directly measured placental and fetal hemodynamics.

The most common studied maternal pre-existing conditions are maternal age, parity, and weight or body mass index. Most of these studies were among small groups of women and used strongly different measures of placental and fetal hemodynamics.[20-31] Results from the Generation R Study, a large population-based cohort study among 9,778 women and their children, showed that a higher maternal age was associated with a higher third trimester RI of the uterine arteries and lower risk of notching.[22] No effects of maternal age in second trimester or on the umbilical artery PI were shown.[22] Results from the same study cohort also showed that among multiparous women, in second and third trimester, the RI of the uterine arteries was increased as compared to nulliparous women.[31] However the PI of the umbilical artery was decreased in multiparous women.[31] A low-risk cohort among 608 women showed that nulliparous women had a higher fetal middle cerebral artery PI compared to the parous women, but no changes in the umbilical arteries were shown.[30] Both maternal underweight as well as overweight and obesity also seem to have an adverse impact of the feto-placental circulation. From a review among 94 studies it was concluded that maternal undernutrition leads to decrease or increase in placental weight, changes in morphology, diminished vascular development and transport of essential nutrients.[21] In 193 obese women, it was demonstrated that obesity was associated with an increased rate of placental vascular supply abnormalities, with severe effects on the fetal circulation, independent of the metabolic status of the mother.[20] It was also shown that higher maternal pre-pregnancy weight was associated with an increased second, but not third-trimester, uterine artery RI.[22] Among 10 low and high-risk pregnant women it was shown that after an oral glucose tolerance test, the vascular resistance of the uterine artery increases, whereas no changes in the umbilical artery were seen.[25] In line with findings from this study, several studies among healthy pregnant women, also showed that vascular resistance of the fetal cerebral arteries decreases after an oral glucose tolerance test, but no differences in the umbilical artery vascular resistance are present.[23, 24, 29] In this thesis we found that an adverse maternal metabolic profile, especially higher cholesterol and triglyceride concentrations, are associated with increased fetal cerebral vascular resistance and cardiac hemodynamics, but not with placental vascular resistince indices. Two other small studies have demonstrated that higher maternal total- and LDL-cholesterol concentrations, but not triglyceride concentrations, are correlated with lower sensitivity of the umbilical vein rings and reactivity, a phenomena that is likely due to endothelial dysfunction.[32, 33] When these associations were explored among more high risk groups, such as pregnant women with familial hypercholesterolemia it was shown that the physiological decrease of uterine artery RI during the second half of pregnancy was not present among women with high cholesterol levels.[26] Together, these small studies seem to suggest that maternal pre-existing conditions and metabolic characteristics have some effects on placental hemodynamics, but their effects on fetal hemodynamic measures are not well-studied and less clear (**Table 4.1.2**).

TABLE 4.1.2 | Maternal pre-existing conditions, metabolic concentrations and placental and fetal hemodynamic measures.

	Placental hemodynamics		Fetal cerebral hemodynamics	Fetal cardiac hemodynamics
	Uterine artery RI	Umbilical artery PI		
Maternal age	+	+/-	NA	NA
Maternal parity	+	+/-	-	NA
Maternal underweight	+	+	NA	NA
Maternal overweight	+	+	NA	NA
Maternal metabolic concentrations	+	+/-	+/-	+

+ = increase in index, +/- = no change in index or inconsistent results, - = decrease in index, NA = no studies applicable

Maternal lifestyle characteristics

The influence of maternal lifestyle factors on placental and fetal hemodynamics has been studied in more detail. A systematic review which included 12 original articles concluded that smoking was associated with an alternated placental vasculature and increases the vascular resistance of the uterine, umbilical and fetal cerebral arteries.[34] Also the effects of a dose response were studied; smoking more than ten cigarettes a day increases the RI of the middle cerebral artery.[35] Furthermore, passive smoking is associated with an increased resistance of the uterine and umbilical arteries and adaptive changes in the cerebral-placental circulation for maintaining fetal cerebral circulation.[36] A narrative review among the effects of prenatal alcohol exposure on fetal cerebrovascular function concluded that the majority of studies showed a decrease in fetal cerebral artery blood flow.[37] A large prospective study among 781 healthy pregnant women from the Pregnancy, Infection and Nutrition Study, demonstrated opposite effects of physical activity; recreational activity was associated with a higher uterine artery PI, while work activity was associated with a lower uterine artery PI. No effects of physical activity on the umbilical artery circulation were shown.[38] Results from the Generation R Study, among 5,993 pregnant women, showed that maternal folic acid supplementation use was associated with lower uterine artery, but not with umbilical artery vascular resistance.[39] In line with human studies, also findings from animals suggest an influence of lifestyle factors on placental and fetal hemodynamics. These studies have mainly focused on the associations of alcohol exposure and nutrition status with adaptations in placental and fetal cerebral blood flow; those studies showed positive [40], negative [41,42] or no associations with fetal blood flow measures.[43] Thus, lifestyle factors might be associated with placental and fetal cerebral hemodynamic changes, although limited studies are available and results are inconsistent (**Table 4.1.3**).

TABLE 4.1.3 | Maternal lifestyle factors and placental and fetal hemodynamic measures.

	Placental hemodynamics		Fetal cerebral hemodynamics
	Uterine artery RI	Umbilical artery PI	
Smoking	+	+	+
Alcohol	NA	NA	+
Physical activity	+/-	+/-	NA
Folic acid use	-	+/-	NA

+ = increase in index, +/- = no change in index or inconsistent results, - = decrease in index, NA = no studies applicable

Maternal pregnancy complications

Most research has focused on the influence of impaired feto-placental vascular function on the development of pregnancy complications. Also in clinical practice, placental and fetal hemodynamic parameters are most commonly used to identify women at increased risk of maternal and fetal pregnancy complications. The most commonly studied maternal pregnancy complications are gestational hypertension, pre-eclampsia and gestational diabetes. However, these conditions at least partly originate from a different pathophysiology and may have different effects on feto-placental vascular development. Impaired early placental vascular development seems to increase the risk of pre-eclampsia, whereas gestational hypertension and gestational diabetes may mainly negatively affect feto-placental vascular development.[44, 45]

Multiple studies have focused on the associations of maternal uterine artery vascular resistance throughout pregnancy with the risk of preeclampsia, and its predictive accuracy. A meta-analysis and two reviews concluded that an abnormal first trimester uterine artery blood flow, defined as increase in the resistance index or pulsatility index; or the presence of notching, is associated with an increased risk of early-onset pre-eclampsia and can aid in prediction of preeclampsia from early-pregnancy onwards.[46-48] Studies focused on the second trimester of pregnancy showed that altered uterine blood flow patterns with a diastolic notch at 20-24 weeks of gestation are more often present in patients who later develop pre-eclampsia, whereas no clear changes in umbilical blood flow patterns were detectable among women with pre-eclampsia.[49] Together with other maternal characteristics, also second trimester uterine artery hemodynamic measurements may improve prediction of preeclampsia.[50] Based on small studies it seems that no clear differences in fetal blood flow to the brain, liver and kidneys are present in fetuses from women with pre-eclampsia compared to normal pregnancy.[51, 52] Similar to findings related to preeclampsia, an observational study showed that an increase first trimester uterine resistance index is associated with gestational hypertension.[53] Other observational studies, focusing on second and third trimester, have shown that among women with gestational hypertension higher trimester uterine and umbilical artery vascular resistances indices are present.[54-57] However, these hemodynamic parameters do not strongly aid in prediction of gestational hypertension, in addition to other maternal characteristics.[58] Next to the associations with utero-placental and feto-placental

vascular function, gestational hypertension is associated with a decreased resistance of the fetal middle cerebral artery and proximal descending aorta in the second and third trimester in small studies but associations with other fetal body hemodynamic parameters are not well studied.[55, 59]

Fewer studies have been conducted focused on the associations of gestational diabetes with feto-placental hemodynamic measures and these studies showed less consistent results than those for pre-eclampsia and gestational hypertension. Gestational diabetes is not consistently associated with alterations in uterine or umbilical artery vascular resistance, but might be associated with alterations in fetal middle cerebral artery PI and cardiac hemodynamic measures, although the direction of these associations are inconsistent.[60-63]

Thus, a higher uterine artery vascular resistance, and to a lesser extent a higher umbilical artery vascular resistance are associated with increased risks of preeclampsia and gestational hypertension from the end of the first trimester onwards. No consistent associations of placental and fetal hemodynamics with the risk of gestational diabetes are present. No consistent of associations of these maternal pregnancy complications with fetal cerebral and body hemodynamic parameters are present (**Table 4.1.4**).

TABLE 4.1.4 | Maternal pregnancy complications and placental and fetal hemodynamic measures.

	Placental hemodynamics	Fetal cerebral hemodynamics	Fetal body hemodynamics
Pre-eclampsia	+	+/-	NA
Gestational hypertension	+	+/-	+/-
Gestational diabetes	+/-	+/-	+/-

+ = increase in index, +/- = no change in index or inconsistent results, - = decrease in index, NA = no studies applicable

Birth outcomes

Most studies focused on the influence of placental and fetal hemodynamics on fetal complications used preterm birth, small for gestational age and admission to a neonatal care unit as outcomes. A cohort study among 2,340 normally grown fetuses demonstrated that an increased fetal middle cerebral artery PI between 28-32 weeks was associated with a higher risk of preterm birth, while no correlations with the umbilical artery were found.[64] However, three other studies found that a reduction in the PI of the middle cerebral artery was associated with increased risk of preterm birth, but also no correlations with waveforms of the uterine and umbilical artery were found.[65-67] An increase in the umbilical artery PI was associated with a higher risk of being born small for gestational age, no associations with changes in the cerebral artery PI were shown.[67] A cross-sectional and a longitudinal study among 292 fetuses demonstrated an increase in the umbilical artery and a decrease in the cerebral artery PI in small for gestational age fetuses.[68] It was also seen, among small for gestational age infants, that early in gestation changes in Doppler abnormalities of the umbilical artery PI were present, followed by changes in the PI of cerebral

arteries.[69] In 179 pregnant women it was demonstrated that a reduction in Doppler waveforms of the ductus venosus was associated with the risk of delivering a small for gestational age fetus.[70] A large retrospective study among 2,518 pregnancies found an increase in third trimester umbilical artery and a decrease in the middle cerebral artery PI in babies requiring neonatal unit admission.[71] It was also demonstrated that the ratio between the umbilical and middle cerebral artery PI was an independent predictor of admission to the neonatal unit in term born fetuses.[72, 73] Among 45 small for gestational age fetuses it was shown that the PI of the ductus venosus was increasing.[74] However, no relation was observed between small for gestational age and the atrioventricular flow velocities.[74] In high risk pregnancy's it was shown that abnormal blood flow through the ductus venosus was associated with fetal structural cardiac abnormalities.[75] Thus, changes in placental, fetal cerebral and body hemodynamics might influence the risk of small for gestational age birth and admission to the neonatal care, but associations with preterm birth and cardiac abnormalities are less consistent across studies (**Table 4.1.5**).

TABLE 4.1.5 | Placental and fetal hemodynamic measures and birth outcomes.

	Preterm birth	Small for gestational age	Admission to neonatal care	Cardiac abnormalities
Placental hemodynamics	+/-	+	+	NA
Fetal cerebral hemodynamics	+/-	+	+	NA
Fetal cardiac hemodynamics	NA	+/-	NA	+/-
Fetal body hemodynamics	NA	+	NA	+

+ = increase in risk, +/- = no change in risk or inconsistent results, - = decrease in risk, NA = no studies applicable

Childhood outcomes

Impaired placental and fetal hemodynamic function during pregnancy may not only increase the risks of short-term adverse outcomes, but may also affect long-term offspring health outcomes. The long-term follow up on neurocognitive outcome was described in two systematic reviews. Ylijoki et al included 15 studies among umbilical artery or cerebral blood flow and neurocognitive outcome; 8 of these papers found, in growth restricted fetuses only, that changes in fetal blood flow were associated with abnormal cognitive development.[76] Another systematic review among 38 studies also concluded that intrauterine growth restricted infants with fetal blood flow redistribution had severe neurodevelopmental impairment, which consisted of cognitive, language, motor and behavior development.[77] Results of the Generation R Study demonstrated among 6,716 mothers and their children an association of an increased third trimester umbilical artery vascular resistance, but not that of the uterine artery, with a higher childhood BMI, fat mass, systolic blood pressure and a lower left ventricular mass.[78] In this thesis, we demonstrated that among a subgroup of 1,195 children of this study, no association of the ratio between the umbilical and middle cerebral artery PI with body and fat mass at age 6 years.[67] However, this blood flow

redistribution was associated with a smaller head, length and weight growth from third trimester onwards with persistent effects until the age of 6 years.[67] Fetal blood flow redistribution was not associated with childhood cardiac measures, but the fetal aorta ascendens diameter and left cardiac output were positively associated with aortic root diameter and left ventricular mass at the age of 6.[79] We found that an increased umbilical artery resistance and higher fetal left cardiac output were associated with a larger ventricular ejection fraction and ventricular mass at the age of 10 years.[80] One other study investigated the long term follow-up of an absent or reverse end-diastolic blood flow in the umbilical artery among 32 fetuses with intrauterine growth restriction and showed that at the age of 7 years those children had a higher blood pressure, lower stiffness in the aorta and endothelial-dependent vasodilatory response.[81] Furthermore, we demonstrated in this subgroup of the Generation R study that a higher fetal vascular resistance in the pulmonary artery and middle cerebral artery was related to a higher risk of wheezing at age 6, and a slightly lower lung function at age 10, respectively.[82] Finally we showed, that the ratio between the umbilical and middle cerebral artery PI was associated with smaller kidney volume.[83] Although limited studies investigated the associations of placental and fetal blood hemodynamics with development in childhood, it can be concluded that changes in placenta and fetal hemodynamics might lead to subclinical changes in childhood growth and fat distribution, childhood neuro and cardiovascular development, lung function and kidney outcomes (**Table 4.1.6**).

TABLE 4.1.6 | Placental and fetal hemodynamic measures and childhood outcomes.

	Childhood neuro development	Childhood growth	Childhood cardiac development	Childhood lung function	Childhood kidney outcomes
Placenta hemodynamics	-	+	+/-	+/-	-
Fetal cerebral hemodynamics	-	-	+/-	+/-	-
Fetal cardiac hemodynamics	NA	NA	+	NA	NA
Fetal pulmonary hemodynamics	NA	NA	NA	+	NA

+ = increase in growth or organ development, +/- = no change in in growth or organ development, - = decrease in growth or organ development, NA = no studies applicable

Underlying mechanisms

The mecha.determinants and pregnancy and childhood outcomes are poorly understood. An important developmental stage in early pregnancy is human placentation characterized by remodeling of the spiral arteries during which the vessels lose their elastic lamina and smooth muscle, and consequently their sensibility to circulating vasoactive compounds.[84] The goal of these vascular changes is to optimize the distribution of maternal blood into a low-resistance uterine vascular network. On the fetal side the trophoblast cells start to form the placenta; from the primary villi, mesodermal cells grow into the direction of the maternal endometrium and mesodermal cells start to differentiation into bloods cells and vessels.[6] Later in pregnancy the

distance between the intervillous space with maternal blood and fetal vessels gets smaller, due to disappearing of the cytotrophoblast cells; and subsequently facilitating an adequate exchange of nutrients and oxygen.[6]

A defect in early trophoblast invasion, marked by an insufficiency to fully convert the spiral-arteries into low-resistance channels, results in retention of smooth muscle cells within their walls, and vasoreactivity in the placental vascular bed.[85, 86] This may lead to diminished perfusion of the intervillous space and transient hypoxia.[87] An adverse physiological condition in pregnant women might lead to arterial lesions and thrombotic infarctions in the placental vessels.[88, 89] However, suboptimal arterial remodeling and maternal vascular lesions are also seen in normal pregnancies.[90] Arterial lesions can restrict the quality of the utero-placental vessels, exacerbate the thrombotic lesions, and thus decreasing the blood flow entering the placenta which can cause an infarction with increased risk of adverse birth outcomes.[91] Furthermore, abnormal placentation and oxidative stress are able to stimulate the release of pro-inflammatory cytokines and angiogenic regulators which in turn lead to activation of the maternal endothelium and hence signs of pre-eclampsia.[92] Also, impaired placentation and abnormal placental perfusion might diminish fetal blood vessel development.[93] An adequate fetal development depends on an optimal placental and fetal blood flow to deliver nutrients and oxygen to the developing fetus. The placenta is able to respond to supply signals arising from the mother and demand signals from the fetus.[94] The efficiency of placental exchange is a complex interplay of many different components including rates of placental blood flow.[95] This interplay is orchestrated by maternal, placental and fetal hormones, and under favorable conditions ensures an adequate supply to the fetus without over depletion of maternal reserves.[95]

To conclude, impaired remodeling of the spiral arteries and trophoblast invasion early in pregnancy may affect subsequent placental development and fetal blood vessel development, leading to alterations in feto-placental blood flow. Subsequently, this might lead to adverse fetal growth and development and finally to increased risks of adverse birth outcomes and long-term health outcomes.

Further research

The current evidence suggest that placental and fetal hemodynamic changes might be influenced by maternal factors and influence the risk of adverse birth outcomes and long term offspring development. However, there remain import issues to be addressed.

First, studies presented in this review show inconsistent results and mostly small effects, especially studies addressing the long-term consequences of placental and fetal hemodynamic changes. Limited research to the long-term consequences of placental and fetal hemodynamic changes are available and future studies are needed to replicate findings in diverse populations. Large long-term follow-up of prenatal cohort studies with detailed measures of placental and

fetal hemodynamics and childhood outcomes in both low and high-risk populations are needed to gain better insight into the consequences of suboptimal placental and fetal hemodynamics throughout the life-course.

Secondly, the mechanisms underlying the described associations are poorly understood. Although impaired remodeling of the spiral arteries seems to be a critical factor, it is not well-understood how impaired remodeling of spiral arteries leads to a suboptimal feto-placental vascular development and function. Even among healthy pregnancies without complications impaired remodeling of spiral arteries has been shown, which suggests that other factors leading to complications are likely to also be involved.[90] Recently, high-resolution, three-dimensional ultrasound techniques and virtual reality systems are developed.[96] These techniques will enable us to visualize and quantify placental vasculature development in first trimester.[97] Furthermore, advanced biomarker measurements during pregnancy such as angiogenesis markers, markers of oxidative stress and markers of endothelial dysfunction might provide further insight into the role of feto-placental angiogenesis and hypoxic damage on fetal hemodynamic parameters and adverse offspring health outcomes. At birth, placental bed biopsies in cohort studies could be used to study vascular development and signs of hypoxic damage in uncomplicated and complicated pregnancies. These results could be linked to placental and fetal hemodynamic parameters for a better understanding of these associations and subsequently long-term follow up of childhood development and health outcomes.

Third, whether and which placenta and fetal hemodynamic measures must be used in the clinical management of complicated pregnancy must be established. Nowadays, evaluation of the umbilical and fetal cerebral artery blood flow is often part of clinical-follow up in growth restricted fetuses.[98] It must be established whether these measurements must be incorporated in routinely screening of all pregnant women, by identifying placental and fetal hemodynamic measures which are related to adverse pregnancy, birth and long-term outcomes across the general population.

To conclude, further research must be focused on identifying the underlying mechanisms, replicating our findings and establish whether placenta and fetal hemodynamic measures should be implemented in routine care.

Conclusion

Impaired placental and fetal vascular development may adversely influence fetal growth and organ development predisposing to increased risks of adverse birth outcomes and potential long-term adverse health outcomes. Due to continuing advancements in ultrasound technology, there is increasing interest in using feto-placental hemodynamic measurements to identify fetuses at increased risk of adverse health outcomes. The most commonly used Doppler ultrasound examinations are focused on measuring uterine, umbilical and fetal middle cerebral

artery vascular resistance. Current evidence suggest that maternal pre-pregnancy overweight and obesity, smoking, and alcohol consumption negatively affect placental and fetal cerebral vascular function. Thus far most studies have focused on the influence of these feto-placental hemodynamics measurements with the risks of pregnancy complications and showed that that higher uterine and umbilical artery vascular resistance are associated with increased risk of preeclampsia and gestational hypertension, but not with gestational diabetes. Only a few studies examined long-term consequences of impaired placental and fetal hemodynamics on childhood outcomes, but suggest that they may impact childhood growth, fat distribution, cardiovascular and neurological development. The limited available research showed relatively small effect estimates and reflect mainly subclinical consequences in childhood. However, these findings are important from an etiological point of view and provide a starting point for further research to elucidate the importance and relations of placental and fetal hemodynamic alterations with childhood development and health outcomes.

References

1. Araujo JR, Keating E, Martel F. Impact of gestational diabetes mellitus in the maternal-to-fetal transport of nutrients. Curr Diab Rep. 2015;15(2):569.
2. Longtine MS, Nelson DM. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. Semin Reprod Med. 2011;29(3):187-96.
3. Carter AM, Enders AC, Pijnenborg R. The role of invasive trophoblast in implantation and placentation of primates. Philos Trans R Soc Lond B Biol Sci. 2015;370(1663):20140070.
4. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. Thromb Res. 2004;114(5-6):397-407.
5. Whitaker KB. Comprehensive Perinatal and Pediatric Respiratory Care: Delmar Thomson Learning; 2001.
6. Editors, Steegers EAP, Fauser BCJM, Hilders CGJM, Jaddoe VWV, Massuger LFAG, et al. Textbook of Obstetrics and Gynaecology - A life course approach: bohn stafleu van loghum; 2019.
7. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67-80.
8. Albaiges G, Missfelder-Lobos H, Parra M, Lees C, Cooper D, Nicolaides KH. Comparison of color Doppler uterine artery indices in a population at high risk for adverse outcome at 24 weeks' gestation. Ultrasound Obstet Gynecol. 2003;21(2):170-3.
9. van den Wijngaard JA, Groenenberg IA, Wladimiroff JW, Hop WC. Cerebral Doppler ultrasound of the human fetus. Br J Obstet Gynaecol. 1989;96(7):845-9.
10. Wladimiroff JW, vd Wijngaard JA, Degani S, Noordam MJ, van Eyck J, Tonge HM. Cerebral and umbilical arterial blood flow velocity waveforms in normal and growth-retarded pregnancies. Obstet Gynecol. 1987;69(5):705-9.
11. Scherjon SA, Kok JH, Oosting H, Wolf H, Zondervan HA. Fetal and neonatal cerebral circulation: A pulsed Doppler study. J PERINAT MED. 1992;20(1):79-82.
12. Gardiner H, Brodszki J, Marsal K. Ventriculovascular physiology of the growth-restricted fetus. Ultrasound Obstet Gynecol. 2001;18(1):47-53.
13. Severi FM, Rizzo G, Bocchi C, D'Antona D, Verzuri MS, Arduini D. Intrauterine growth retardation and fetal cardiac function. Fetal Diagn Ther. 2000;15(1):8-19.
14. Rizzo G, Arduini D. Fetal cardiac function in intrauterine growth retardation. Am J Obstet Gynecol. 1991;165(4 Pt 1):876-82.
15. Godfrey ME, Messing B, Cohen SM, Valsky DV, Yagel S. Functional assessment of the fetal heart: a review. Ultrasound Obstet Gynecol. 2012;39(2):131-44.

16. Duijts L. Fetal and infant origins of asthma. Eur J Epidemiol. 2012;27(1):5-14.
17. Maritz GS, Cock ML, Louey S, Suzuki K, Harding R. Fetal growth restriction has long-term effects on postnatal lung structure in sheep. Pediatr Res. 2004;55(2):287-95.
18. Kiserud T. Physiology of the fetal circulation. Semin Fetal Neonatal Med. 2005;10(6):493-503.
19. Catalano PM, Kirwan JP. Maternal factors that determine neonatal size and body fat. Curr Diab Rep. 2001;1(1):71-7.
20. Bar J, Kovo M, Schraiber L, Shargorodsky M. Placental maternal and fetal vascular circulation in healthy non-obese and metabolically healthy obese pregnant women. Atherosclerosis. 2017;260:63-6.
21. Belkacemi L, Michael Nelson D, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod. 2010;83(3):325-31.
22. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.
23. Gillis S, Connors G, Potts P, Hunse C, Richardson B. The effect of glucose on Doppler flow velocity waveforms and heart rate pattern in the human fetus. EARLY HUM DEV. 1992;30(1):1-10.
24. Haugen G, Bollerslev J, Henriksen T. Human umbilical and fetal cerebral blood flow velocity waveforms following maternal glucose loading: A cross-sectional observational study. Acta Obstet Gynecol Scand. 2016;95(6):683-9.
25. Jaffe R, Friedman Z. Changes in uterine artery doppler velocimetry in pregnant patients undergoing glucose tolerance test may predict adverse outcome in later pregnancy: A preliminary study. Fetal Diagn Ther. 1998;13(4):241-3.
26. Khoury J, Amundsen AL, Tonstad S, Henriksen T, Ose L, Retterstol K, et al. Evidence for impaired physiological decrease in the uteroplacental vascular resistance in pregnant women with familial hypercholesterolemia. Acta Obstet Gynecol Scand. 2009;88(2):222-6.
27. Leiva A, Fuenzalida B, Barros E, Sobrevia B, Salsoso R, Sáez T, et al. Nitric oxide is a central common metabolite in vascular dysfunction associated with diseases of human pregnancy. Curr Vasc Pharmacol. 2016;14(3):237-59.
28. Leiva MC, Tolosa JE, Binotto CN, Weiner S, Huppert L, Denis AL, et al. Fetal cardiac development and hemodynamics in the first trimester. Ultrasound Obstet Gynecol. 1999;14(3):169-74.
29. Pardo J, Orvieto R, Rabinerson D, Bar J, Hod M, Kaplan B. Fetal middle-cerebral and umbilical artery flow assessments after glucose challenge test. International Journal of Gynecology & Obstetrics. 1999;65(3):255-9.
30. Prior T, Mullins E, Bennett P, Kumar S. Influence of parity on fetal hemodynamics and amniotic fluid volume at term. Ultrasound Obstet Gynecol. 2014;44(6):688-92.
31. Rurangirwa AA, Gaillard R, Steegers EA, Hofman A, Jaddoe VW. Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women; the Generation R study. Am J Hypertens. 2012;25(8):892-9.
32. Leiva A, de Medina CD, Salsoso R, Saez T, San Martin S, Abarzua F, et al. Maternal hypercholesterolemia in pregnancy associates with umbilical vein endothelial dysfunction: role of endothelial nitric oxide synthase and arginase II. Arterioscler Thromb Vasc Biol. 2013;33(10):2444-53.
33. Leiva A, Salsoso R, Saez T, Sanhueza C, Pardo F, Sobrevia L. Cross-sectional and longitudinal lipid determination studies in pregnant women reveal an association between increased maternal LDL cholesterol concentrations and reduced human umbilical vein relaxation. Placenta. 2015;36(8):895-902.
34. Pintican D, Poienar AA, Strilciuc S, Mihu D. Effects of maternal smoking on human placental vascularization: A systematic review. Taiwanese J Obstet Gynecol. 2019;58(4):454-9.
35. Albuquerque CA, Smith KR, Johnson C, Chao R, Harding R. Influence of maternal tobacco smoking during pregnancy on uterine, umbilical and fetal cerebral artery blood flows. Early Hum Dev. 2004;80(1):31-42.
36. Yildiz S, Sezer S, Boyar H, Cece H, Ziylan SZ, Vural M, et al. Impact of passive smoking on uterine, umbilical, and fetal middle cerebral artery blood flows. Jap J Rad. 2011;29(10):718-24.
37. Bukiya AN, Dopico AM. Fetal Cerebral Circulation as Target of Maternal Alcohol Consumption. Alcohol Clin Exp Res. 2018;42(6):1006-18.
38. Nguyen NC, Evenson KR, Savitz DA, Chu H, Thorp JM, Daniels JL. Physical activity and maternal-fetal circulation measured by Doppler ultrasound. J Perinatol. 2013;33(2):87-93.

39. Timmermans S, Jaddoe VW, Silva LM, Hofman A, Raat H, Steegers-Theunissen RP, et al. Folic acid is positively associated with uteroplacental vascular resistance: the Generation R study. Nutr Metab Cardiovasc Dis. 2011;21(1):54-61.
40. Tobiasz AM, Duncan JR, Bursac Z, Sullivan RD, Tate DL, Dopico AM, et al. The Effect of Prenatal Alcohol Exposure on Fetal Growth and Cardiovascular Parameters in a Baboon Model of Pregnancy. Reprod Sci. 2018;25(7):1116-23.
41. Szostak-Wegierek D. Intrauterine nutrition: Long-term consequences for vascular health. Int J Womens Health. 2014;6(1):647-56.
42. Frias AE, Morgan TK, Evans AE, Rasanen J, Oh KY, Thornburg KL, et al. Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. Endocrinology. 2011;152(6):2456-64.
43. Newnham JP, Kelly RW, Patterson L, James I. The influence of maternal undernutrition in ovine twin pregnancy on fetal growth and Doppler flow-velocity waveforms. J DEV PHYSIOL. 1991;16(5):277-82.
44. Davison JM, Homuth V, Jeyabalan A, Conrad KP, Karumanchi SA, Quaggin S, et al. New aspects in the pathophysiology of preeclampsia. J Am Soc Nephrol. 2004;15(9):2440-8.
45. Sibai BM, Ross MG. Hypertension in gestational diabetes mellitus: pathophysiology and long-term consequences. J Matern Fetal Neonatal Med. 2010;23(3):229-33.
46. Velauthar L, Plana MN, Kalidindi M, Zamora J, Thilaganathan B, Illanes SE, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. Ultrasound Obstet Gynecol. 2014;43(5):500-7.
47. Carbillon L. First trimester uterine artery Doppler for the prediction of preeclampsia and foetal growth restriction. J Matern Fetal Neonatal Med. 2012;25(7):877-83.
48. Townsend R, Khalil A, Premakumar Y, Allotey J, Snell KIE, Chan C, et al. Prediction of pre-eclampsia: review of reviews. Ultrasound Obstet Gynecol. 2019;54(1):16-27.
49. Zahumensky J. Doppler flowmetry in preeclampsia. Bratislava Med J. 2009;110(7):432-5.
50. Pedroso MA, Palmer KR, Hodges RJ, Costa FDS, Rolnik DL. Uterine Artery Doppler in Screening for Preeclampsia and Fetal Growth Restriction Doppler das arterias uterinas no rastreamento para pre-eclampsia e restricao do crescimento fetal. Rev Bras Ginecol Obstet. 2018;40(5):287-93.
51. Addley S, Ali A, Ong S. An Assessment of Fetal Cerebral and Hepatic Perfusion in Normal Pregnancy and Pre-Eclampsia Using Three-Dimensional Ultrasound. Ulster Med J. 2017;86(1):10-4.
52. Ali A, Addley S, Ong S. Three-dimensional indices of renal perfusion in normal pregnancy and pre-eclampsia. Ir J Med Sci. 2019;188(1):173-7.
53. Gyselaers W, Vonck S, Staelens AS, Lanssens D, Tomsin K, Oben J, et al. Gestational hypertensive disorders show unique patterns of circulatory deterioration with ongoing pregnancy. Am J Physiol Regul Integr Comp Physiol. 2019;316(3):R210-R21.
54. Perry H, Lehmann H, Mantovani E, Thilaganathan B, Khalil A. Correlation between central and uterine hemodynamics in hypertensive disorders of pregnancy. Ultrasound Obstet Gynecol. 2019;54(1):58-63.
55. Gupta S, Misra R, Ghosh UK, Gupta V, Srivastava D. Comparison of foetomaternal circulation in normal pregnancies and pregnancy induced hypertension using color doppler studies. Indian J Physiol Pharmacol. 2014;58(3):282-7.
56. Arakaki T, Hasegawa J, Takita H, Nakamura M, Hamada S, Kawashima A, et al. Can umbilical artery Doppler findings at 36 weeks' gestation predict maternal hypertension at later gestation? J Matern -Fetal Neonatal Med. 2017;30(2):177-80.
57. Mitsui T, Masuyama H, Maki J, Tamada S, Hirano Y, Eto E, et al. Differences in uterine artery blood flow and fetal growth between the early and late onset of pregnancy-induced hypertension. J Med Ultrason. 2016;43(4):509-17.
58. Erkamp JS, Jaddoe VWV, Duijts L, Reiss IKM, Mulders A, Steegers EAP, et al. Population screening for gestational hypertensive disorders using maternal, fetal and placental characteristics: A population-based prospective cohort study. Prenat Diagn. 2020;40(6):746-57.
59. Guedes-Martins L, Cunha A, Saraiva J, Rita-Gaio A, Cerdeira AS, Macedo F, et al. Foetal aortic flow velocity waveforms in healthy and hypertensive pregnant women. Cardiovasc Ultrasound. 2014;12(1).
60. Li J, Chen YP, Dong YP, Yu CH, Lu YP, Xiao XM, et al. The impact of umbilical blood flow regulation on fetal development differs in diabetic and non-diabetic pregnancy. Kidney Blood Press Res. 2014;39(4):369-77.

61. Shabani Zanjani M, Nasirzadeh R, Fereshtehnejad SM, Yoonesi Asl L, Alemzadeh SAP, Askari S. Fetal cerebral hemodynamic in gestational diabetic versus normal pregnancies: A Doppler velocimetry of middle cerebral and umbilical arteries. Acta Neurol Belg. 2014;114(1):15-23.
62. Rizzo G, Arduini D, Capponi A, Romanini C. Cardiac and venous blood flow in fetuses of insulin-dependent diabetic mothers: Evidence of abnormal hemodynamics in early gestation. AM J OBSTET GYNECOL. 1995;173(6):1775-81.
63. Moradian M, Tabib A, Ghasempour A, Alaee N, Akbarzadeh Z. Comparing ventricular function in fetuses of diabetic and non-diabetic mothers using tissue doppler imaging. Res Cardiovasc Med. 2016;5(4).
64. Morales-Rosello J, Khalil A, Salvi S, Townsend R, Premakumar Y, Perales-Marin A. Abnormal Middle Cerebral Artery Doppler Associates with Spontaneous Preterm Birth in Normally Grown Fetuses. Fetal Diagnosis and Therapy. 2016;40(1):41-7.
65. Rizzo G, Capponi A, Arduini D, Turri E, Romanini C. Uterine and fetal blood flows in pregnancies complicated by preterm labor. GYNECOL OBSTET INVEST. 1996;42(3):163-6.
66. Ghezzi F, Ghidini A, Romero R, Gomez R, Galasso M, Cohen J, et al. Doppler velocimetry of the fetal middle cerebral artery in patients with preterm labor and intact membranes. J ULTRASOUND MED. 1995;14(5):361-6.
67. Kooijman MN, Gaillard R, Reiss IKM, Hofman A, Steegers EAP, Jaddoea VWV. Influence of fetal blood flow redistribution on fetal and childhood growth and fat distribution: The generation R study. BJOG Int J Obstet Gynaecol. 2016;123(13):2104-12.
68. Harrington K, Thompson MO, Carpenter RG, Nguyen M, Campbell S. Doppler fetal circulation in pregnancies complicated by pre-eclampsia or delivery of a small for gestational age baby: 2. Longitudinal analysis. Br J Obstet Gynaecol. 1999;106(5):453-66.
69. Morales-Rosello J, Khalil A, Fornes-Ferrer V, Alberola-Rubio J, Hervas-Marin D, Llorens NP, et al. Progression of Doppler changes in early-onset small for gestational age fetuses. How frequent are the different progression sequences? Journal of Maternal-Fetal & Neonatal Medicine. 2018;31(8):1000-8.
70. Nakata M. Doppler-velocity waveforms in ductus venosus in normal and small-for-gestational-age fetuses. J Obstet Gynaecol Res. 1996;22(5):489-96.
71. Khalil AA, Morales-Rosello J, Elsaddig M, Khan N, Papageorghiou A, Bhide A, et al. The association between fetal Doppler and admission to neonatal unit at term. Am J Obstet Gynecol. 2015;213(1):57.e1-.e7.
72. Khalil AA, Morales-Rosello J, Morlando M, Hannan H, Bhide A, Papageorghiou A, et al. Is fetal cerebroplacental ratio an independent predictor of intrapartum fetal compromise and neonatal unit admission? Am J Obstet Gynecol. 2015;213(1):54.e1-.e10.
73. Turner JM, Flatley C, Kumar S. A low fetal cerebroplacental ratio confers a greater risk of intrapartum fetal compromise and adverse neonatal outcomes in low risk multiparous women at term. Eur J Obstet Gynecol Reprod Biol. 2018;230:15-21.
74. Van Splunder P, Stijnen T, Wladimiroff JW. Fetal atrioventricular, venous, and arterial flow velocity waveforms in the small for gestational age fetus. PEDIATR RES. 1997;42(6):765-75.
75. Sau A, Sharland G, Simpson J. Agenesis of the ductus venosus associated with direct umbilical venous return into the heart - Case series and review of literature. Prenat Diagn. 2004;24(6):418-23.
76. Ylijoki MK, Ekholm E, Ekblad M, Lehtonen L. Prenatal Risk Factors for Adverse Developmental Outcome in Preterm Infants-Systematic Review. Front Psychol. 2019;10:595.
77. Murray E, Fernandes M, Fazel M, Kennedy SH, Villar J, Stein A. Differential effect of intrauterine growth restriction on childhood neurodevelopment: A systematic review. BJOG Int J Obstet Gynaecol. 2015;122(8):1062-72.
78. Gaillard R, Steegers EA, Tiemeier H, Hofman A, Jaddoe VW. Placental Vascular Dysfunction, Fetal and Childhood Growth and Cardiovascular Development: The Generation R Study. Circulation. 2013;128(20):2202-10.
79. Kooijman MN, De Jonge LL, Steegers EAP, Van Osch-Gevers L, Verburg BO, Hofman A, et al. Third trimester fetal hemodynamics and cardiovascular outcomes in childhood: The Generation R study. J Hypertens. 2014;32(6):1275-82.
80. Toemen L, Jelic G, Kooijman MN, Gaillard R, Helbing WA, van der Lugt A, et al. Third Trimester Fetal Cardiac Blood Flow and Cardiac Outcomes in School-Age Children Assessed By Magnetic Resonance Imaging. J Am Heart Assoc. 2019;8(16):e012821.

81. Morsing E, Liuba P, Fellman V, Maršál K, Brodszki J. Cardiovascular function in children born very preterm after intrauterine growth restriction with severely abnormal umbilical artery blood flow. Eur J Prev Cardiol. 2014;21(10):1257-66.
82. Kooijman MN, van Meel ER, Steegers EAP, Reiss IKM, de Jongste JC, Jaddoe VWV, et al. Fetal umbilical, cerebral and pulmonary blood flow patterns in relation to lung function and asthma in childhood. The Generation R Study. Pediatr Allergy Immunol. 2019;30(4):443-50.
83. Kooijman MN, Bakker H, Van Der Heijden AJ, Hofman A, Franco OH, Steegers EAP, et al. Childhood kidney outcomes in relation to fetal blood flow and kidney size. J Am Soc Nephrol. 2014;25(11):2616-24.
84. Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update. 2006;12(6):747-55.
85. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. Br J Obstet Gynaecol. 1994;101(8):669-74.
86. Labarrere CA, Althabe OH. Inadequate maternal vascular response to placentation in pregnancies complicated by preeclampsia and by small-for-gestational-age infants. Br J Obstet Gynaecol. 1987;94(11):1113-6.
87. Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. Am J Pathol. 2001;159(3):1031-43.
88. Sheppard BL, Bonnar J. The ultrastructure of the arterial supply of the human placenta in pregnancy complicated by fetal growth retardation. Br J Obstet Gynaecol. 1976;83(12):948-59.
89. Salafia CM, Minior VK, Pezzullo JC, Popek EJ, Rosenkrantz TS, Vintzileos AM. Intrauterine growth restriction in infants of less than thirty-two weeks' gestation: Associated placental pathologic features. AM J OBSTET GYNECOL. 1995;173(4):1049-57.
90. Aardema MW, Oosterhof H, Timmer A, van Rooy I, Aarnoudse JG. Uterine artery Doppler flow and uteroplacental vascular pathology in normal pregnancies and pregnancies complicated by pre-eclampsia and small for gestational age fetuses. Placenta. 2001;22(5):405-11.
91. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BMJ. 2019;366:l2381.
92. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350(7):672-83.
93. Regnault TR, Galan HL, Parker TA, Anthony RV. Placental development in normal and compromised pregnancies--a review. Placenta. 2002;23 Suppl A:S119-29.
94. Burton GJ, Fowden AL. Review: The placenta and developmental programming: balancing fetal nutrient demands with maternal resource allocation. Placenta. 2012;33 Suppl:S23-7.
95. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond , B, Biol Sci. 2015;370(1663):20140066.
96. Reijnders IF, Mulders A, Koster MPH, Koning AHJ, Frudiger A, Willemsen SP, et al. New imaging markers for preconceptional and first-trimester utero-placental vascularization. Placenta. 2018;61:96-102.
97. Reus AD, Klop-van der Aa J, Rifouna MS, Koning AHJ, Exalto N, van der Spek PJ, et al. Early pregnancy placental bed and fetal vascular volume measurements using 3-D virtual reality. Ultrasound Med Biol. 2014;40(8):1796-803.
98. Hernandez-Andrade E, Benavides Serralde JA, Cruz-Martinez R. Can anomalies of fetal brain circulation be useful in the management of growth restricted fetuses? Prenat Diagn. 2012;32(2):103-12.

Chapter 4

Chapter 4.2

Methodological considerations

General methodological considerations regarding selection bias, information bias, confounding, causality and external validity are discussed in the following paragraph.

Selection bias

If the association between the determinant and outcome of interest is different in subjects who participate in the study and those who were eligible for the study, selection bias at baseline may occur. In the Generation R Study 61% of all eligible children at birth participated. The non-response seems not to be at random. Participating women had a higher socioeconomic status and were more often of the Dutch ethnicity than expected from the overall population in Rotterdam.[1] Participating women had also less pregnancy complications and outcomes than expected from population figures. Detailed assessments of fetal growth and development were performed in a random subgroup of 1,232 Dutch mothers and their children. Especially in this subgroup, the women and children tended to be relatively more affluent and healthy. This selection might have led to reduced statistical power because of the lower prevalence rates of adverse outcomes and might have affected the generalizability of our results to other less healthy and affluent populations. However, it has been shown that in cohort studies associations are not strongly influenced by selective non-participation at baseline.[2, 3]

Selection bias may also occur due to selective loss to follow-up. Selective loss to follow-up will lead to bias if associations between those included in the analyses and those loss to follow-up would be different. In the Dutch subgroup of Generation R only 2% of the participants were loss at follow-up during pregnancy or at birth. The response rates at the age of 6 and 10 years in this Dutch subgroup were approximately 75%. Mothers of children without follow-up measurements were more often multiparous, lower educated and smokers and their children had a lower birth weight. Thus the children with follow-up measurements might be more healthier than those without follow-up measures and this might have biased our effect estimates. However, this bias is difficult to quantify.

Information bias

Misclassification of determinant or outcome measures results in information bias.[4] Differential misclassification (not at random) involves misclassification which is related to the exposure and outcome of interest. This might lead to under- or overestimation of the effect estimates. Non-differential misclassification (random) involves misclassification which the exposure is unrelated to the outcome and vice versa. Non-differential misclassification leads to an underestimation of the effect estimates. In this thesis, maternal metabolic factors and fetal hemodynamics were the exposures. These data were collected before assessment of the outcomes, and the participants

and research employees were unaware of the specific research questions. This makes differential misclassification unlikely. It seems also unlikely that the outcome measures were influenced by differential misclassification, because the research employees involved in data collection were unaware of the fetal hemodynamics of the participant. However, non-differential misclassification may have occurred in the study which used self-reported pre-pregnancy weight. Self-reported weight tends to be underestimated, especially in cases with a higher weight. This might have led to an underestimation of the observed effects of maternal pre-pregnancy BMI. The outcome measures were assessed by well-trained research employees and with standardized assessments. Therefore non-differential misclassification of the outcome is unlikely.

Confounding

A confounder is a variable which is associated with the exposure and the outcome, and this variable is not an intermediate in the causal pathway. If the confounder is not taken into account effect estimates might be biased because the observed effect estimates might be attributed to the exposure while the effect estimates are caused by the confounder. In the studies presented in this thesis we used several approaches to deal with confounding in the studied associations. All our analyses were adjusted for multiple potential confounders. These variables were selected based on literature and the change in effect estimates. Although we adjusted for many potential confounders, residual confounding might still be an issue, as in all observational studies. Also, most information about confounding variables was obtained by questionnaire and this might have led to measurement error.

Causality

The observed associations we described in this thesis were based on observational studies and causality cannot be established from observational studies. A randomized control trial is the preferred study design to establish causality, but is difficult to perform for the exposures we studied within this thesis. Only limited intervention studies on the effects on maternal lifestyle on placental and fetal hemodynamics are available. Two randomized trials compared the effects of a low-cholesterol diet with a normal diet and showed that in the intervention group the gestational age decrease in the umbilical artery PI was more pronounced between 24 and 30 weeks.[5, 6] A randomized controlled trial among 60 women with abnormal umbilical blood flow demonstrated that low-dose aspirin use improved blood flow.[7] However, among 43 pregnant women at risk of pregnancy complications no effects on blood flow measurements of the uterine arteries and the umbilical cord were seen after using low-dose aspirin.[8] Thus far, no further adequate methods are available to improve placental and fetal hemodynamic function and to assess its effects on

pregnancy and childhood outcomes. In observational studies, more advanced study designs can be used as an aid to further explore causality, which include sibling comparisons studies, maternal-paternal comparison studies and Mendelian Randomization studies. With regards to placental research, sibling comparisons studies can be used to obtain further insight into the role of confounding in these observed associations.[9, 10] These studies allow for control of shared characteristics among siblings, such as environmental and genotype of the parents.[10] Thus far, no sibling comparison studies have focused on placental and fetal hemodynamics. A study in human twins with the twin-twin syndrome showed that the recipient fetus had increased aortic and pulmonary velocities compared with the donor co-twins.[11] Also elevated left ventricular filling pressure and a decrease in systolic function were seen in the recipient fetus.[12] Hecher et al. concluded that impaired neurodevelopment affect the donor and recipient twins; whereas cardiovascular structures more often lead to complications in the recipient twin.[13] Mendelian randomization is a method of the use of variation in genes which are associated with the exposure and when this exposure is associated with the outcome, the variation in genes must be associated with the outcome.[14] Up to now no studies that applied Mendelian randomization using variations in genes and placental or fetal hemodynamics were available.

External validity

External validity is the degree to which results of a study can be applied to different populations. The mothers and children from the Generation R subgroup are higher educated in comparison with the general population in Rotterdam and from Dutch origin only. Although there is a selection towards a more healthy and higher educated population, we assume that results from this thesis could be applied to other populations. However, replications of our associations in lower educated and non-Dutch populations must be performed before conclusions can be made which are more generalizable and related to other populations.

Summary

Altogether, common limitations which apply for all cohort studies do also apply to our study. Although our results suggest that alterations in placental and fetal hemodynamics have subclinical consequences for childhood growth, cardiovascular development, lung function and kidney outcomes, the role of these methodological limitations needs to be further studied.

References

1. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.
2. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? Epidemiology. 2006;17(4):413-8.
3. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23(6):597-608.
4. Rothman KJ. Epidemiology: An introduction. New york: Oxford university press 2002.
5. Odibo AO. Can maternal dietary intervention modify Doppler parameters of the fetoplacental circulation? Am J Obstet Gynecol. 2007;196(6):497-8.
6. Khoury J, Haugen G, Tonstad S, Frøslie KF, Henriksen T. Effect of a cholesterol-lowering diet during pregnancy on maternal and fetal Doppler velocimetry: the CARRDIP study. Am J Obstet Gynecol. 2007;196(6):549.e1-.e7.
7. Ali MK, Abbas AM, Yosef AH, Bahloul M. The effect of low-dose aspirin on fetal weight of idiopathic asymmetrically intrauterine growth restricted fetuses with abnormal umbilical artery Doppler indices: a randomized clinical trial. J Matern Fetal Neonatal Med. 2018;31(19):2611-6.
8. Erdmann M, Paulus WE, Flock F, Herget I, Terinde R, Grab D. Haemodynamic measurements of the utero- and fetoplacental circulation during low-dose Aspirin treatment. Z Geburtshilfe Neonatol. 1999;203(1):18-23.
9. Gaillard R, Felix JF, Duijts L, Jaddoe VW. Childhood consequences of maternal obesity and excessive weight gain during pregnancy. Acta Obstet Gynecol Scand. 2014;93(11):1085-9.
10. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol. 2015;30(11):1141-52.
11. Karatza AA, Wolfenden JL, Taylor MJ, Wee L, Fisk NM, Gardiner HM. Influence of twin-twin transfusion syndrome on fetal cardiovascular structure and function: prospective case-control study of 136 monochorionic twin pregnancies. Heart. 2002;88(3):271-7.
12. Wohlmuth C, Boudreaux D, Moise KJ, Jr., Johnson A, Papanna R, Bebbington M, et al. Cardiac pathophysiology in twin-twin transfusion syndrome: new insights into its evolution. Ultrasound Obstet Gynecol. 2018;51(3):341-8.
13. Hecher K, Gardiner HM, Diemert A, Bartmann P. Long-term outcomes for monochorionic twins after laser therapy in twin-to-twin transfusion syndrome. Lancet Child Adolesc Health. 2018;2(7):525-35.
14. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003;32(1):1-22.

Chapter 5

Summary/Samenvatting

Summary

In **Chapter 1.1** the background and rationale of the studies presented in this thesis were described. The placenta is responsible for the maternal to fetal transfer of nutrients essential for fetal growth and development. Suboptimal placental development leads to placental and fetal hemodynamic alterations, which are important mechanisms for the fetus to protect the most important organs such as the brain and heart from an adverse fetal environment. Fetal blood flow redistribution is characterized by an increase in the umbilical artery resistance and a decreases of the fetal cerebral artery resistance, known as brainsparing. Fetal blood flow measurements of different placenta and fetal hemodynamics may be of prognostic value in predicting fetal outcome. Population based studies have demonstrated associations of low birth weight with diseases in later life, however birth weight is not the causal factor per se leading to diseases in later life. Limited studies have suggested that changes in placental and fetal hemodynamics affect fetal organ development and may have long term consequences. Identifying more detailed measures of placental and fetal hemodynamic alterations might give further insight in long-term consequences of an unfavorable fetal environment. Therefore, studies presented in this thesis were designed to identify alterations in placental and fetal hemodynamics which are associated with childhood growth, cardiovascular development, lung function and kidney outcomes. In **Chapter 1.2** we present the overall design of the Generation R Study, a population based prospective study form fetal life until adulthood. The study is designed to identify early environmental and genetic causes and causal pathways leading to normal and abnormal growth, development and health from fetal life, childhood and young adulthood. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study.

In **Chapter 2** we examined whether an maternal adverse metabolic profile in early pregnancy was associated with placental, fetal cerebral and cardiac hemodynamic development. We measured maternal BMI, blood pressure, cholesterol, triglyceride and glucose concentrations. Maternal obesity and subsequently metabolic disturbances are major risk factors for pregnancy outcomes and adverse cardiovascular outcomes in offspring. The mechanisms by which these factors lead to adverse fetal and childhood outcomes might involve suboptimal early placental developmental leading to placental and fetal cerebral and cardiac hemodynamic alterations. We demonstrated that an adverse maternal early-pregnancy metabolic profile, especially higher maternal cholesterol and triglycerides concentrations, were associated with increased fetal cerebral vascular resistance and increased fetal cardiac hemodynamics, but not with placental vascular resistance indices. These associations were not explained by maternal BMI.

In **Chapter 3** we studied whether placental and fetal hemodynamics were associated with childhood development. In **Chapter 3.1** we examined whether fetal blood flow redistribution is associated with birth outcomes and repeatedly measured fetal and childhood growth and fat mass measures. It was previously shown that fetal blood flow redistribution was associated with asymmetrical fetal growth restriction, characterized by a relatively larger head size than body

size at birth. This might lead to a different body composition and increased percentages of body fat in later life. We found that an increase in fetal blood flow redistribution was associated with increased risks of preterm birth and small size for gestational age. The longitudinal growth analyses showed that an increase in fetal blood flow redistribution was associated with persistently lower head circumference, length and weight from third trimester fetal life until childhood. Fetal blood flow redistribution was not associated with total body and abdominal fat measures at age 6 years. In **Chapter 3.2** we examined the associations of placental, fetal cerebral and cardiac hemodynamic development with cardiovascular outcomes at the age of 6 years. Low birth weight is associated with higher risks of cardiovascular disease. The mechanisms underlying these associations might include hemodynamic adaptations in the placental and fetal circulation. The early hemodynamic adaptations may have persistent influences on cardiovascular structure and functional development. In this study we observed that fetal hemodynamics were not associated with childhood blood pressure and carotid-femoral pulse wave velocity. The fetal aorta ascendens diameter and left cardiac output were positively associated with childhood aortic root diameter and left ventricular mass. Fetal left ventricular diastolic filling pattern was negatively associated with childhood aortic root diameter and left ventricular mass. In **Chapter 3.3** we examined the associations of fetal umbilical, cerebral and pulmonary blood flow with wheezing patterns, lung function and asthma in childhood. In later fetal life the small airways and alveoli are formed. An adverse intrauterine environment with fetal blood flow adaptations might have persistent influences on respiratory health and disease in later life. A non-significant tendency towards associations for fetal blood flow redistribution with higher risks of early wheezing were found. A higher pulmonary vascular resistance was associated with higher risk of late/persistent wheezing. An increase in the middle cerebral vascular resistance index was associated with a higher FEV_1/FVC. The associations were not explained by birth parameters, current BMI, or allergic predisposition. No other consistent associations of changes in fetal umbilical, cerebral, or pulmonary blood flow with wheezing patterns until age 6 years, or lung function and asthma at age 10 years, were found. In **Chapter 3.4** we examined the associations of third trimester fetal blood flow redistribution and fetal kidney size with kidney size and function in school-aged children. Nephrogenesis continues until 36 weeks of gestation, after which the induction of nephron numbers ceases. A decrease in number of nephrons leads to a smaller glomerular filtration surface area, which might predispose the individual to a decreased kidney function. It is unknown whether and to what extent impaired abdominal or kidney blood flow and kidney growth restriction during fetal life lead to risk factors for kidney disease in later life. Fetal blood flow redistribution was associated with smaller childhood kidney volume, whereas an increase fetal kidney size was associated with lower levels of creatinine and cystatin C, and a higher eGFR in school-aged children, independent of childhood kidney size.

In **Chapter 4.1** a general discussion of the studies described in this thesis are presented in a wider context. In **Chapter 4.2** methodological considerations are discussed.

In conclusion, findings from this thesis suggest that placental and fetal hemodynamic changes

might be influenced by maternal factors and influence the risk of adverse birth outcomes and long term offspring health outcomes. Although, the observed associations were relatively small and mainly reflect subclinical consequences in childhood, these findings are important from an etiological point of view and provide a starting point for further research to elucidate the importance and relations of placental and fetal hemodynamic alterations with childhood development and health outcomes.

Samenvatting

In **Hoofdstuk 1** zijn de achtergrond en de reden van de studies die in dit proefschrift staan beschreven. De placenta is verantwoordelijk voor de overdracht van voedingsstoffen van moeder naar foetus die essentieel zijn voor de groei en ontwikkeling van de foetus. Suboptimale placentale ontwikkeling leidt tot placentale en foetale hemodynamische veranderingen, dit zijn belangrijke mechanismen voor de foetus om de belangrijkste organen zoals de hersenen en het hart te beschermen tegen een nadelige foetale omgeving. Herverdeling van de foetale bloedstroom wordt gekenmerkt door een toename van de weerstand van de navelstreng en een afname van de weerstand van de hersenader, dit staat ook wel bekend als 'brainsparing'. Metingen van de foetale bloedstroming van verschillende placentale en foetale hemodynamische veranderingen kunnen van voorspellende waarde zijn om de foetale uitkomst te voorspellen. Populatie studies hebben associaties aangetoond van een laag geboortegewicht met ziekten op latere leeftijd, maar geboortegewicht op zichzelf is niet de oorzakelijke factor die leidt tot ziekten op latere leeftijd. Beperkte studies hebben gesuggereerd dat veranderingen in de placentale en foetale hemodynamiek de foetale ontwikkeling van organen beïnvloeden en op de lange termijn gevolgen kunnen hebben. Het identificeren van meer gedetailleerde metingen van placentale en foetale hemodynamische veranderingen kunnen verder inzicht geven in de lange termijn gevolgen van een ongunstige foetale omgeving. De studies die in dit proefschrift staan beschreven zijn ontworpen om veranderingen in de placentale en foetale hemodynamiek te identificeren die verband houden met de groei van kinderen, cardiovasculaire ontwikkeling, longfunctie en nier uitkomsten. In **Hoofdstuk 1.2** beschrijven we de algemene opzet van de Generation R studie, een populatie gebaseerde prospectieve cohort studie vanaf het foetale level tot de volwassenheid. De studie is opgezet om vroege omgevings- en genetische oorzaken en causale mechanismen te identificeren die leiden tot normale en abnormale groei, ontwikkeling en gezondheid van het foetale leven, de kindertijd en de jonge volwassenheid. In totaal zijn 9,778 moeders met een bevallingsdatum tussen April 2002 en Januari 2006 geïncludeerd in de studie.

In **Hoofdstuk 2** hebben we onderzocht of een maternaal nadelig metabolisch profiel in de vroege zwangerschap geassocieerd was met de placenta, foetale hersen en cardiale hemodynamische ontwikkeling. We hebben de BMI van de moeder, bloeddruk, cholesterol, triglyceride en glucose concentraties gemeten. Maternale obesitas en bijbehorende metabolische stoornissen zijn belangrijke risicofactoren voor zwangerschapsuitkomsten en nadelige cardiovasculaire uitkomsten bij kinderen. De mechanismen waardoor deze factoren leiden tot nadelige uitkomsten bij de foetus en kinderen zou gerelateerd kunnen zijn aan suboptimale vroege placentale ontwikkeling die leidt tot veranderingen in de placenta, foetale hersen en cardiale hemodynamiek. We hebben aangetoond dat een nadelig maternaal metabolisch profiel in de vroege zwangerschap, met name hogere cholesterol- en triglyceride concentraties, geassocieerd zijn met verhoogde foetale cerebrale vasculaire weerstand en foetale cardiale hemodynamiek, maar niet met de vasculaire weerstand van de placenta. Deze associaties werden niet verklaard door de BMI van moeder.

In **Hoofdstuk 3** hebben we onderzocht of de placentale en foetale hemodynamiek geassocieerd is met de ontwikkeling van kinderen. In **Hoofdstuk 3.1** onderzochten we of de herverdeling van de foetale bloedstroom geassocieerd is met geboorte uitkomsten, groei van de foetus en kinderen en de vetmassa. Eerder werd aangetoond dat herverdeling van de foetale bloedstroom geassocieerd is met asymmetrische foetale groeivermindering, gekenmerkt door een relatief groot hoofd in vergelijking met het lichaam bij de geboorte. Dit kan leiden tot een andere lichaamssamenstelling en verhoogde percentages lichaamsvet op latere leeftijd. We vonden dat een toename van de herverdeling van de foetale bloedstroom geassocieerd was met een verhoogd risico op vroeggeboorte en een laag geboortegewicht voor de zwangerschapsduur. De longitudinale groeianalyses toonden aan dat een toename van de herverdeling van de foetale bloedstroom is geassocieerd met een aanhoudende kleinere hoofdomtrek, lengte en lager gewicht vanaf de derde trimester in het foetale leven tot aan de kinderleeftijd. Herverdeling van de foetale bloedstroom is niet geassocieerd met lichaams- en buikvetmetingen op de leeftijd van 6 jaar. In **Hoofdstuk 3.2** onderzochten we de associaties van placentale, foetale hersen en cardiale hemodynamische ontwikkeling met cardiovasculaire uitkomsten op de leeftijd van 6 jaar. Een laag geboortegewicht is geassocieerd met hogere risico's op hart- en vaatziekten. De onderliggende mechanismen hiervan kunnen hemodynamische veranderingen in de placentale en foetale circulatie zijn. Deze vroege hemodynamische veranderingen kunnen blijvend invloed hebben op de structurele en functionele cardiovasculaire ontwikkeling. In deze studie vonden we dat de foetale hemodynamiek niet geassocieerd was met bloeddruk en de carotis-femorale polsgolfsneldheid op de kinderleeftijd. De diameter van de foetale aorta asendens en de linker cardiale uitstroom was positief geassocieerd met de diameter van de aorta en de linker ventrikel massa. Het foetale linker ventrikel diastolisch vullingspatroon was negatief geassocieerd met de diameter van de aorta en de linker ventrikel massa. In **Hoofdstuk 3.3** onderzochten we de associaties tussen de placentale, foetale hersen en long bloed doorstroming met een piepende ademhaling, longfunctie en astma op de kinderleeftijd. In het latere foetale leven worden de kleine luchtwegen en longblaasjes gevormd. Een ongunstige intra-uteriene omgeving, met herverdeling van de foetale bloeddoorstroming, kan blijvende invloed hebben op de gezondheid van de luchtwegen en ziekte op de latere leeftijd. We zagen een niet significante trend voor associaties tussen een herverdeling van de foetale bloeddoorstroming en een hoger risico op een piepende ademhaling vroeg in de kindertijd. Een hogere pulmonale vaatweerstand is geassocieerd met een hoger risico op een piepende ademhaling later of aanhoudend in de kindertijd. Een toename van de vaatweerstand in de middelste hersen arterie was geassocieerd met een hogere FEV_1/FVC. De associaties werden niet verklaard door geboorte parameters, het huidige BMI of een allergische aanleg. Geen andere consistente associaties van veranderingen in de placentale, foetale hersen of long hemodynamiek met een piepende ademhaling tot de leeftijd van 6 jaar, longfunctie of astma op 10 jarige leeftijd werden gevonden. In **Hoofdstuk 3.4** hebben we de associaties van de herverdeling van de foetale bloeddoorstroming en nier grootte in het derde trimester in verband gebracht met de nier grootte en functie bij schoolgaande kinderen. Nefrogenese gaat door tot

36 weken zwangerschap, hierna stopt de toename van nieuwe nefronen. Een afname van het aantal nefronen leidt tot een kleiner filtratie oppervlak, en dit kan leiden tot een verminderde nierfunctie. Het is onbekend of en in welke mate een verminderde doorbloeding van de buik of nier en beperking van de niergroei tijdens het foetale leven leiden tot het risico op nier ziekten later in het leven. Herverdeling van de foetale bloedstroom is geassocieerd met een kleiner niervolume bij kinderen, terwijl een toegenomen foetale niergrootte is geassocieerd met lagere creatine en cystatine C levels, en een hogere eGFR bij schoolgaande kinderen, onafhankelijk van de niergrootte. In **Hoofdstuk 4.1** worden de in dit proefschrift beschreven studies beschouwd in een bredere context. In **Hoofdstuk 4.2** worden de methodologische afwegingen bediscussieerd.

Concluderend suggereren de bevindingen van dit proefschrift dat placentale en foetale hemodynamische veranderingen beïnvloedt kunnen worden door maternale factoren en het risico op nadelige geboorte uitkomsten en lange termijn gezondheid van de kinderen kunnen beïnvloeden. Hoewel de waargenomen associaties relatief klein waren, en hoofdzakelijk niet klinische consequenties in de kindertijd representeren, zijn de bevindingen belangrijk vanuit een etiologisch standpunt en zijn ze een startpunt voor verder onderzoek om het belang en relaties van placentale en foetale hemodynamische veranderingen met ontwikkelingen en gezondheidsuitkomsten in de kindertijd te ontrafelen.

Chapter 6

Authors' affiliations

The Generation R Study Group, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Bakker H, Felix JF, Gaillard R, Hofman A, Jaddoe VWV, de Jonge LL, Kooijman MN, Kruithof CJ, van Meel ER, Taal HR

Department of Epidemiology, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
van Duijn CM, Felix JF, Franco OH, Gaillard R, Hofman A, Jaddoe VWV, de Jonge LL, Kooijman MN, Kruithof CJ

Department of Pediatrics, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Bakker H, Duijts L, Felix JF, Gaillard R, van der Heijden AJ, Helbing WA, Jaddoe VWV, de Jonge LL, de Jongste JC, Kooijman MN, Kruithof CJ, van Meel ER, Moll HA, van Osch-Gevers L, Helbing WA, Reiss IKM, Rings EH, Taal HR

Division of Respiratory Medicine and Allergology, Department of Pediatrics, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Duijts L, de Jongste JC, van Meel ER

Division of Neonatology, Department of Pediatrics, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Duijts L, Reiss IKM, Taal HR

Center for Child and Family Studies, Leiden University, Leiden, The Netherlands
van IJzendoorn MH

Department of Psychology, Education and Child Studies, Erasmus University Rotterdam, Rotterdam, The Netherlands
van IJzendoorn MH

Department of Ophthalmology, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Klaver CC

Department of Radiology, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
van der Lugt A

Department of Public Health, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Mackenbach JP, Raat H

Department of Internal Medicine, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Peeters RP, Rivadeneira F, Uitterlinden AG

Department of Otolaryngology, Head and Neck Surgery, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
van der Schroeff MP

Department of Obstetrics and Gynecology, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Steegers EAP, Verburg BO

Department of Child and Adolescent Psychiatry, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Tiemeier H, Verhulst FC

Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands
Wolvius E

Publication list

1. Toemen L, Jelic G, **Kooijman MN**, Gaillard R, Helbing W, van der Lugt A et al. *Third trimester fetal cardiac blood flow and cardiac outcomes in school-age children assessed by magnetic resonance imaging*. J Am Heart Assoc. 2019;8(16):e012821
2. **Kooijman MN**, van Meel ER, Steegers EAP, Reiss IKM, de Jongste JC, Jaddoe VWV, Duijts L. *Fetal umbilical, cerebral and pulmonary blood flow patterns in relation to lung function and asthma in childhood. The Generation R Study*. Pediatr Allergy Immunol. 2019;30(4):443-450
3. Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, Bacelis J, Peng S, Hao K, Feenstra B, Wood AR, Mahajan A, Tyrrell J, Robertson NR, Rayner NW, Qiao Z, Moen GH, Vaudel M, Marsit CJ, Chen J, Nodzenski M, Schnurr TM, Zafarmand MH, Bradfield JP, Grarup N, **Kooijman MN** et al. *Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors.* Nat Genet. 2019;51(5):804-814
4. Tachmazidou I, Süveges D, Min JL, Ritchie GRS, Steinberg J, Walter K, Iotchkova V, Schwartzentruber J, Huang J, Memari Y, McCarthy S, Crawford AA, Bombieri C, Cocca M, Farmaki AE, Gaunt TR, Jousilahti P, **Kooijman MN** et al. *Whole-genome sequencing coupled to imputation discovers genetic signals for anthropometric traits*. Am J Hum Genet. 2017;100(6):865-884
5. **Kooijman MN**, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IJzendoorn MH et al. *The Generation R Study: design and cohort update 2017*. Eur J Epidemiol. 2016;31(12):1243-1264
6. **Kooijman MN**, Gaillard R, Reiss I, Hofman A, Steegers E, Jaddoe VWV. *Influence of fetal blood flow redistribution on fetal and childhood growth and fat distribution: the Generation R Study*. BJOG. 2016;123(13):2104-2112
7. Horikoshi M*, Beaumont RN*, Day FR*, Warrington NM*, **Kooijman MN***, Fernandez-Tajes J* et al. *Genome-wide associations for birth weight and correlations with adult disease*. Nature. 2016;538(7624):248-252
8. **Kooijman MN***, Bakker H*, Franco OH, Hofman A, Taal HR, Jaddoe VW.V *Fetal smoke exposure and kidney outcomes in school-aged children*. Am J Kidney Dis. 2015;66(3):412-20
9. van der Valk RJ*, Kreiner-Møller E*, **Kooijman MN***, Guxens M*, Stergiakouli E*, Sääf A et al. *A novel common variant in DCST2 is associated with length in early life and height in adulthood*. Hum Mol Genet. 2015;24(4):1155-68
10. Kruithof CJ, **Kooijman MN**, van Duijn CM, Franco OH, de Jongste JC, Klaver CC et al. *The Generation R Study: Biobank update 2015*. Eur J Epidemiol. 2014;29(12):911-27

11. **Kooijman MN***, Bakker H*, van der Heijden AJ, Hofman A, Franco OH, Steegers EAP et al. *Childhood kidney outcomes in relation to fetal blood flow and kidney size*. J Am Soc Nephrol. 2014;25(11):2616-24

12. Bakker H, **Kooijman MN**, van der Heijden AJ, Hofman A, Franco OH, Taal HR, Jaddoe VWV. *Kidney size and function in a multi-ethnic population-based cohort of school-age children*. Pediatr Nephrol. 2014;29(9):1589-98

13. **Kooijman MN**, de Jonge LL, Steegers EAP, van Osch-Gevers L, Verburg BO, Hofman A et al. *Third trimester fetal hemodynamics and cardiovascular outcomes in childhood: the Generation R Study*. J Hypertens. 2014;32(6):1275-82

14. Wijtzes AI*, **Kooijman MN***, Kiefte-de Jong JC, de Vries SI, Henrichs J, Jansen W et al. *Correlates of physical activity in 2-year-old toddlers: the Generation R Study*. J Pediatr. 2013;163(3):791-9.e1-2

Submitted

15. **Kooijman MN**, Jaddoe VWV, Steegers EAP, Gaillard R. *Associations of maternal metabolic profile with placental, and fetal cerebral and cardiac hemodynamics*

16. **Kooijman MN**, Gaillard R. *Maternal and offspring health consequences of placental and fetal hemodynamic alterations throughout pregnancy: a narrative review*

* These authors contributed equally

About the author

Marjolein Kooijman was born on December 29th, in Cromstrijen, the Netherlands. In 2006 she finished high school at the RSG Hoeksche Waard in Oud-Beijerland. With the completion of het Bachelor degree in Human movement technology at The Hague university of applied sciences, she continued with a Master in Human Movement Sciences at the Vrije Universiteit Amsterdam and graduated in 2012. After her graduation she started as a datamanager within the Generation R Study. In August 2013 she started with her Master in Health Sciences, specialization Epidemiology, at the Netherlands Institute for Health Sciences. In 2015 she expanded her research project entitled 'Determinants and consequences of placental and fetal hemodynamic alterations' under supervision of Prof. Dr. V.W.V. Jaddoe (Department of Pediatrics), Prof. Dr. E.A.P. Steegers (Department of Obstetrics and Gynecology) and Dr. R. Gaillard (Department of Pediatrics). The results of this work are presented in this dissertation.

PhD portfolio

Summary PhD training

Name PhD Student	Marjolein N. Kooijman
Erasmus MC Department	Generation R Study
Promotors	Prof. dr. V.W.V. Jaddoe, Prof. dr. E.A.P. Steegers
Co-promotor	Dr. R. Gaillard
PhD period	2015 - 2020

	Year	Workload (ECTS)
1. PhD training		
General and advanced courses		
Master Health Sciences, specialization Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2013-2017	
Study design		4.3
Biostatical Methods I: Basic Principles		5.7
Biostatical Methods II: Classical Regression Models		4.3
Methodologic Topics in Epidemiologic Research		1.4
Principles of Research in Medicine and Epidemiology		0.7
Methods of Public Health Research		0.7
Introduction to Public Health		0.7
Primary and Secondary Prevention Research		0.7
Social Epidemiology		0.7
Fundamentals of Medical Decision Making		0.7
Clinical Epidemiology		5.7
Repeated Measurements in Clinical Research		1.4
Missing Values in Clinical Research		0.7
Women's Health		0.9
Conceptual Foundation of Epidemiologic Research		0.7
Cohort Studies		0.7
History of Epidemiologic Ideas		0.7
Markers and Prognostic Research		0.7
Logistic Regression		1.4
Principles of Epidemiologic Data-analysis		0.7
Quality of Life Measurement		0.9
Other		
Course SNP's and Human Diseases, Molecular Medicine Postgraduate School, Rotterdam, the Netherlands	2012	1.4
Research skills		
Scientific Integrity for PhD students, Erasmus MC, Rotterdam, the Netherlands	2017	0.3
BROK (Good Clinical Practice), Erasmus MC, Rotterdam, the Netherlands	2016	1.0

(Inter)national congresses and presentations		
Health sciences research day, Rotterdam, the Netherlands	2019	0.7
Poster presentation		
3rd Paula Rantakallio Symposium on Birth Cohorts and Longitudinal Studies, Oulu, Finland	2018	0.7
Poster presentation		
Development Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands	2017	0.7
Oral presentation		
Early Nutrition, The Power of Programming, Munich, Germany	2016	0.7
Poster presentation		
Development Origins of Health and Disease (DOHaD), Singapore	2013	0.7
Poster presentation		
Sophia Research day, Rotterdam, the Netherlands	2013	0.7
Poster Presentation		
Development Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands	2012	0.7
Poster presentation		
Generation R, Research meetings, Rotterdam, the Netherlands	2012-2019	1.0
(Inter)national meetings research projects		
LifeCycle Project – Consortium meetings,	2017-2019	
LifeCycle Project - Datashield workshops/meetings	2017-2019	
Rotterdam, the Netherlands; Copenhagen, Denmark; Oulu, Finland		
Grants		
Vereniging Trustfonds Erasmus Universiteit, 3 travel grants	2013 -2018	
2. Other		
Klankbordsessie zwangerschapsregister pREGnant, Utrecht, The Netherlands	2019	
DOHaD conference organizing committee, Rotterdam, The Netherlands	2017	
Development Optio, new logistic system Generation R, klankbordgroep	2016-2020	
METC application for Generation R *Next*	2016-2020	
METC application for Generation R – Focus op Tieners	2015-2020	
Datamanagement Generation R	2012-2020	
Reviewed articles for Eur J Epidemiology		

Dankwoord

De afgelopen jaren zijn er toch met enige regelmaat momenten geweest waarop ik dacht dat ik nooit het laatste hoofdstuk van mijn proefschrift zou kunnen schrijven. Nu is eindelijk het moment daar, ook ik heb mijn proefschrift afgerond. Het schrijven hiervan zou niet gelukt zijn zonder de kinderen en hun ouders die al jaren meedoen aan Generation R. Dank voor jullie bezoeken aan het onderzoekscentrum en het invullen van de enorme hoeveelheid vragenlijsten.

Graag wil ik mijn promoteren, Prof. Jaddoe en Prof. Steegers bedanken. Beste Vincent, 8 jaar geleden heb jij mij aangenomen in de rol als datamanager en daarnaast ook de optie gegeven om onderzoek te kunnen doen. Heel veel dank voor alle mogelijkheden die je mij hebt gegeven, je vertrouwen, en alles wat je mij geleerd hebt op wetenschappelijk gebied. Ook heb ik veel geleerd van je adviezen over hoe om te gaan met deadlines, prioriteiten en overvolle mailboxen. Beste Eric, ook u wil ik bedanken voor de betrokkenheid bij mijn promotietraject. Mijn co-promotor, dr Gaillard. Beste Romy, enorm bedankt voor je betrokkenheid, enthousiasme en motiverende woorden tijdens, vooral de laatste loodjes van, dit promotietraject. Ik had 8 jaar geleden, toen wij elkaar leerden kennen in de studenten kamer en we samen op huisbezoek gingen, nooit kunnen denken dat je mijn co-promotor zou worden. Je bent een geweldige wetenschapper en ik heb enorm veel van je geleerd. Zonder jou had dit boek nooit afgekomen.

De kleine commissie, Prof. dr. Reiss, Prof dr. Franx en Prof. dr. de Groot, hartelijk dank voor het beoordelen van dit proefschrift en jullie bereidheid om plaats te nemen in de kleine commissie. De grote commissie, dr. Duijts, dr. van Rijn en dr. Roest ook jullie wil ik hartelijk danken voor jullie bereidheid om plaats te nemen in de grote commissie.

Geen enkel onderzoeksteam functioneert zonder een geweldig secretariaat. Beste Patricia, Maaike en Rose, jullie zijn de basis van Generation R. Heel veel dank voor het versturen van al die brieven, opnemen van telefoontjes, jullie adviezen, praktische tips en de gezelligheid. De focus dames én Ronald, dank voor jullie inzet bij de dataverzameling en het uitvoeren van de minder leuke, maar daarom niet minder belangrijke, 'klusjes' die gedaan moeten worden wanneer het focus centrum tijdelijk dicht is. Een belangrijke, maar minder zichtbare rol speelt het IT-team. Eric en Edward, ook al klagen we nog al is over de beperkingen van GeneratoR/Optio, jullie inzet zorgt er mede voor dat er écht al veel geautomatiseerd verloopt in Generation R. Beste Janine, de afgelopen jaren hebben we veel samengewerkt, van het schrijven van papers, het opstellen van PIFs, kaderprotocollen tot het oplossen van 'incidentjes'. Heel veel dank voor je (persoonlijke) interesse, enthousiasme en bereidheid om mee te denken. Beste Jolien, ook met jou heb ik veel samengewerkt aan alles om Generation R 'draaiende' te houden. Naast deze fijne samenwerking was het ook altijd erg gezellig om tussen de werkzaamheden door even bij te kletsen en lekkere koffie te halen. Bovenal heel veel dank aan mijn mede-datamanagers. Lieve Claudia, vanaf werkdag 1 zit ik bij jou op de kamer en heb je me (geduldig ;)) alle ins en outs van het datamanagement bijgebracht. De afgelopen jaren heb

ik je leren kennen als iemand met een gouden hart die altijd voor me klaar staat! Lieve Eline en Annemiek, een paar jaar geleden zijn jullie ons komen versterken. Ik kan me niet meer indenken wat Generation R zonder jullie zou moeten.

Ook dank aan al mijn coauteurs voor de bijdrage aan verschillende manuscripten. In het bijzonder dank aan Anne, Layla en Hanneke voor de samenwerking, jullie uitleg en geduld met het schrijven van mijn eerste papers.

Al mijn (oud)collega's van Generation R, inmiddels teveel om op te noemen, wil ik bedanken. Ik heb de afgelopen jaren veel mooie, leuke en super slimme mensen leren kennen. De altijd gezellige koffie-, lunch-, en tussendoor snack momentjes waren altijd een welkome onderbreking gedurende de werkdag. Niet te vergeten ook de leuke (en leerzame) werk/congres uitstapjes naar onder andere Singapore München en Oulou! Annemarijne, Carlijn, Claire, Elise, Evelien, Florianne, Laura, Sanne, Simone, Suzanne en Sylvie dank voor jullie gezelligheid in de afgelopen jaren! Gaan we dat weekend Antwerpen nu nog is over doen?

Lieve Florianne, vanaf dag 1 bij Generation R heb ik aan jou een super fijne collega gehad! Naast het harde werken ben je ook altijd in voor goed advies en een hoop gezelligheid. Ik ben enorm blij dat ik je heb leren kennen, je nu mijn paranimf wilt zijn en dat je zo een goede vriendin bent geworden.

De 'weekendcie – on tour': lieve Anne, Marieke, Denise, Gwen en Luis. De afgelopen jaren is er een super vriendschap ontstaan, zijn er vele borrelavonden geweest, hebben we een mooi reisje naar Portugal ondernomen en natuurlijk een geweldig Safari weekend beleefd. An en Mariek, ik vind het echt onwijs jammer dat jullie het Schiebroekse hebben verlaten, kom maar weer snel terug. ☺ Bedankt voor alles en ik hoop dat er nog vele gezellige uitstapjes zullen volgen.

Lieve Ellen, Nelianneke, Roos en Sanne. Mijn volwassen avontuur begon met jullie. Neli, ik weet nog goed hoe we elkaar 14 (?) jaar geleden voor het eerst tegenkwamen onderweg naar Den Haag. Ellen, Roos en Sanne met jullie erbij was het een heerlijke studietijd, waarin we feestjes bezochten, reisjes maakten ook nog is wat studeerden. We zien elkaar niet zoveel meer als vroeger maar iedere keer als we afspreken is het weer als vanouds. Ik ben blij dat jullie ook deze mijlpaal weer met mij meevieren.

Familie Taal veel dank voor jullie belangstelling, support en mooie vakanties de afgelopen jaren.

Mijn lieve zusje en broertje, Heleen en Kasper. Ik ben dankbaar dat ik met jullie ben opgegroeid en de goede band die we nog steeds hebben. Lieve Heleen ik vind het geweldig dat je mijn paranimf bent en naast me staat op 19 oktober.

Lieve pap en mam, door jullie vertrouwen en alles wat jullie mij meegegeven hebben ben ik geworden wie ik ben. Jullie staan altijd voor ons klaar en het is mooi om te zien hoe trots jullie altijd zijn. De rol van opa en oma vervullen jullie met enorm veel toewijding, enthousiasme en liefde. Het is fantastisch om jullie zo te zien.

Allerliefste Rob, ik weet niet of het me was gelukt om zonder jou support dit proefschrift af te ronden. Bedankt voor je eindeloze geduld, aanmoediging en vertrouwen. Ik heb veel bewondering voor hoe je alles combineert, je ambities, nooit stress lijkt te hebben en altijd rustig blijft. Bedankt voor alle mooie momenten, de toekomst lacht ons tegemoet! Lieve Maud en Guus, jullie liefde, ondeugende streken, te zien hoe jullie je ontwikkelen en opgroeien relativeert al het andere. Ik hou van jullie!